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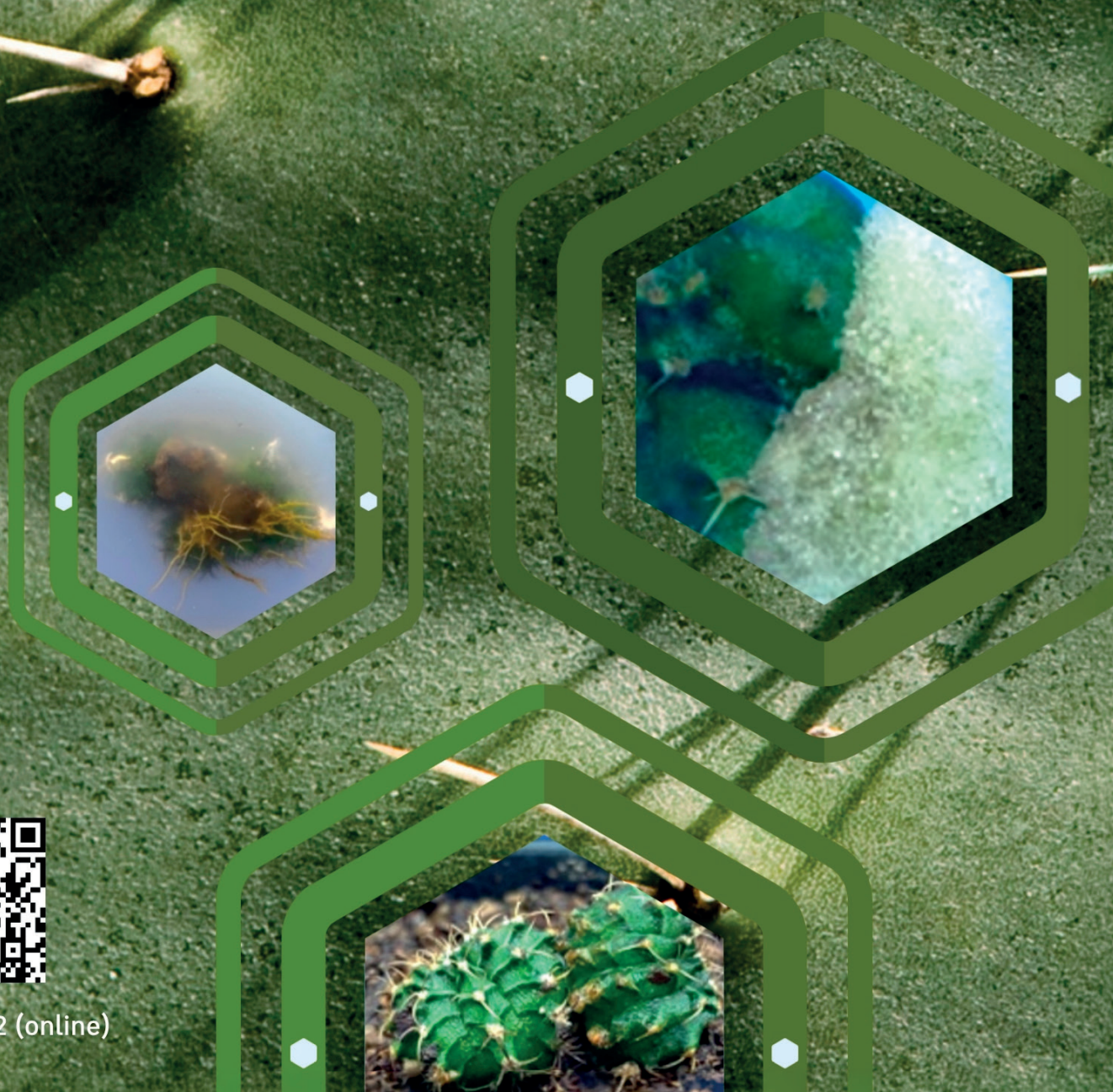
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# Development of a Rework-Based Inventory Policy for Ordering Water Sports Equipment Bags: A Case Study of a Manufacturing Packaging Department

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**Abstract:** Inventory management plays a critical role in reducing operational costs and maintaining efficiency in competitive manufacturing environments. In the production of water sports equipment, packaging bags are essential supporting materials; however, the absence of structured ordering policies often leads to uncontrolled purchasing, excessive inventory, and increased sunk costs. This study aims to develop an improved inventory policy for packaging bags by comparing three ordering approaches: the Traditional Ordering Method, the Fixed Time Period System (FTPS), and a proposed rework-based inventory policy that incorporates rework rate and rework time into inventory planning. The research was conducted at a water sports equipment manufacturing company in Chonburi, Thailand, focusing on eleven models of packaging bags used in the packaging process. Production and inventory data were collected over three months, and the performance of each ordering method was evaluated based on average inventory level, sunk cost, and service reliability. The results indicate that the Traditional Ordering Method generated the highest inventory cost of 353,307 Baht due to excessive overstocking. The FTPS approach, which applies periodic review and safety stock principles, reduced inventory costs to 200,691 Baht and improved stock control, although inventory levels remained higher than actual demand. In contrast, the proposed rework-based inventory policy achieved the lowest average cost of 57,209 Baht by incorporating production variability through defect rates and rework cycle times. This method reduced costs by approximately 300,000 Baht compared with the traditional method and by 146,000 Baht compared with FTPS, while preventing stockouts during the observation period. These findings demonstrate that integrating rework-related variability into inventory planning can significantly improve cost efficiency and operational reliability. The study contributes to the inventory management literature by bridging theoretical inventory models with practical industrial implementation and by highlighting the potential of rework-sensitive policies as an effective framework for managing packaging materials in manufacturing systems.

**Keywords:** Inventory management; fixed time period system; rework rate; packaging process; manufacturing efficiency

## 1. Introduction

The global water sports equipment manufacturing industry has experienced increased competition driven by rising consumer demand, technological innovation, and growing interest in recreational water activities. As markets become more saturated and customer expectations continue to rise, manufacturers must improve operational efficiency and product quality to remain competitive. Previous studies have demonstrated that effective quality management and inventory control practices play a critical role in reducing operational costs while improving customer satisfaction in manufacturing environments [1–3]. Inventory management is particularly important in industries where production and packaging processes involve variability in demand, customization, and quality performance. Inadequate inventory policies often lead to overstocking, excessive holding costs, inefficient use of warehouse space, and reduced operational profitability [4, 5]. Conversely, well-structured inventory systems that incorporate operational data such as demand variability, defect rates, and production lead times can improve material flow, reduce excess stock, and optimize resource utilization [6, 7].

In the case study company examined in this research, an internal operational review revealed that packaging materials, specifically water-sports equipment bags, were frequently over-ordered due to the absence of systematic stock-monitoring and verification procedures. Employees tended to place orders based on perceived demand rather than actual inventory levels or production requirements. As a result, the packaging department accumulated excessive inventory, increasing sunk costs and creating inefficiencies in warehouse space utilization. To address this problem, this study investigates alternative inventory control strategies for managing packaging bag inventory in the production process. Three ordering approaches are evaluated: the Traditional Ordering Method currently used by employees, the Fixed Time Period System (FTPS) based on periodic inventory review, and a proposed inventory policy that incorporates rework rate and rework time into inventory planning. By integrating production variability into inventory control, the proposed approach aims to provide a more adaptive and realistic ordering framework. Despite the extensive literature on safety stock and periodic inventory review systems, limited studies have examined how rework-related variability can be incorporated into inventory planning for packaging materials in real manufacturing environments.

The objective of this research is to develop an improved ordering policy for packaging materials in the manufacturing packaging department to reduce excess inventory and associated sunk costs while maintaining reliable service levels. This study contributes to the literature in three ways. First, it integrates rework rate and rework time into a periodic inventory control framework for packaging materials. Second, the proposed model is validated using real operational data from a manufacturing packaging department. Third, the results demonstrate that incorporating rework-related variability into inventory planning can significantly reduce costs while maintaining service reliability.

## 2. Research literature

Effective inventory management has long been recognized as a critical factor in maintaining manufacturing competitiveness, ensuring customer satisfaction, and improving operational efficiency. In the context of packaging materials, particularly for specialized products such as water sports equipment bags, balancing stock availability with cost control poses unique operational challenges. These challenges become even more complex when quality-related factors, such as rework rate and rework time, influence production lead times and material availability. To develop an effective ordering policy that reflects these operational realities, it is necessary to examine prior research on safety stock management, periodic review systems, and inventory models that incorporate imperfect quality and rework conditions. Table 1 summarizes key studies on inventory control approaches, including economic order quantity (EOQ) models, periodic review or base-stock systems, safety stock strategies, and inventory models that account for rework or imperfect production quality. The table highlights how different studies have addressed various aspects of inventory control and provides a foundation for identifying research gaps relevant to this study.

**Table 1.** Summary of Previous Studies and Identified Research Gaps



| No. | Reference                   | EOQ (Economic Order Quantity) | Periodic Review / Base-stock | Safety Stock Models | Rework / Imperfect Quality |
|-----|-----------------------------|-------------------------------|------------------------------|---------------------|----------------------------|
| 1   | Gonçalves et al. [8]        |                               |                              | ✓                   |                            |
| 2   | Barros et al. [9]           |                               |                              | ✓                   |                            |
| 3   | Demiray Kırmızı et al. [10] |                               |                              | ✓                   |                            |
| 4   | Tagaras & Vlachos [11]      |                               | ✓                            |                     |                            |
| 5   | Chiang [12]                 |                               | ✓                            |                     |                            |
| 6   | Sonntag & Kiesmüller [13]   |                               |                              |                     | ✓                          |
| 7   | Alamri et al. [14]          | ✓                             |                              |                     | ✓                          |
| 8   | Cárdenas-Barrón et al. [15] | ✓                             |                              |                     | ✓                          |
| 9   | Lin et al. [16]             | ✓                             |                              |                     | ✓                          |
| 10  | Haque et al. [17]           | ✓                             |                              |                     | ✓                          |

A review of the existing literature indicates that substantial progress has been made in developing safety stock strategies and periodic-review inventory models. Several studies have focused on analytical approaches for determining safety stock levels and managing uncertainty in supply chains [8–10]. Other studies have examined periodic review systems and replenishment strategies, including emergency ordering policies and multi-supply modes designed to improve inventory responsiveness under uncertain demand conditions [11, 12]. In addition, research has investigated inventory models that incorporate imperfect production processes, including defective items and rework activities. These models extend classical EOQ frameworks by accounting for the impact of defective units and repair processes on production and inventory planning [13–16]. More recent studies have also explored sustainable manufacturing systems that integrate remanufacturing or environmentally conscious production processes within inventory decision models [17].

Despite these advances, several important research gaps remain. First, most existing studies focus on finished products or general manufacturing environments, with limited attention given to packaging materials that support production processes. Second, although some research considers defective items or rework, the combined effect of rework rate and rework time variability is rarely incorporated into periodic inventory review systems. Third, many existing models are primarily theoretical and lack empirical validation using real operational data from manufacturing environments. To address these gaps, this study develops an improved ordering policy for packaging materials, specifically water sports equipment bags, by integrating rework rate, rework time, and demand variability within a periodic review inventory framework. By applying the proposed approach to real operational data from a manufacturing packaging department, this research aims to bridge the gap between theoretical inventory models and practical industrial implementation. The findings provide both theoretical insights and practical guidance for improving inventory management in manufacturing systems where rework-related variability affects production flow.

### 3. Research Methodology

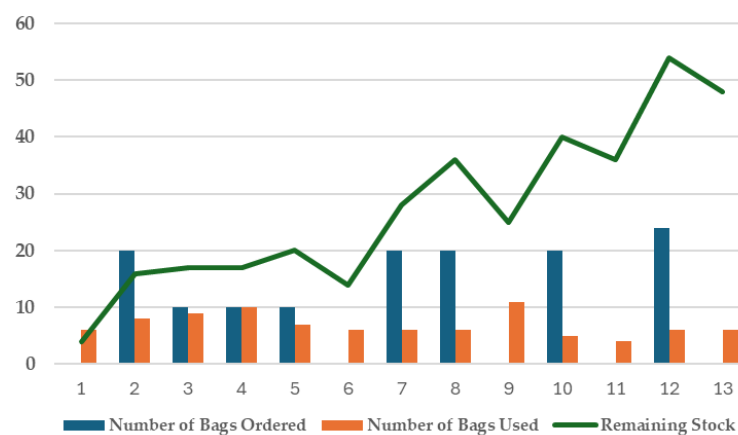
#### 3.1 Study of the Production Process of Water Sports Equipment Bags.

A water-sports equipment manufacturing company in Chonburi Province, Thailand, specializes in producing a variety of composite-material products, including stand-up paddleboards (SUPs), surfboards, windsurfers, and motorized boards. The overall production process consists of 11 stages: (1) design and form development, (2) mold making, (3) foam cutting, (4) fiberglass lamination, (5) resin application, (6) color base coating, (7) surface finishing, (8) graphic application, (9) clear coat finish, (10) quality inspection, and (11) packaging. This study focuses on the packaging stage, where finished boards are wrapped in plastic, protected with cardboard, placed into packaging bags, inspected for packaging-related issues, and sealed for customer delivery. The packaging inventory consists of 11 types of water sports equipment bags, with an average unit value of 1,990 THB, as shown in Table 2.

**Table 2.** Average Unit Prices of Water Sports Equipment Bags

| Product Name | Average Unit Price (Thai Baht) |
|--------------|--------------------------------|
| FL1          | 1,886                          |
| FL2          | 1,725                          |
| FL3          | 1,577                          |
| FL4          | 1,422                          |
| AR1          | 1,700                          |
| AR2          | 1,792                          |
| AW1          | 2,034                          |
| JF1          | 2,615                          |
| JF2          | 2,761                          |
| RD1          | 2,096                          |
| RD2          | 2,282                          |

Excessive stocking of these bags results in higher sunk costs because they can also be sold separately from finished water sports equipment. Therefore, holding excess packaging inventory represents an opportunity cost and increases the company's overall inventory burden. To illustrate the problem, the study first examined the ordering behavior associated with the FL1 bag model. The initial stock level was 10 units. Analysis of daily inventory records showed that employees repeatedly ordered more bags than needed, leading to a persistent increase in inventory over time.



**Figure 2.** Daily Inventory Level of FL1 Bag Model

The figure shows that inventory levels for the FL1 bag model increased steadily during the observation period despite fluctuations in daily usage. The initial stock level was relatively low; however, repeated ordering without regard for actual consumption led to continuous inventory growth. By Day 11, inventory exceeded 50 units, well above the initial 10-unit level. This pattern indicates the absence of an effective inventory verification system and suggests that ordering decisions were not aligned with actual demand. To extend the analysis beyond the FL1 case, the inventory levels of all 11 bag models were examined under the traditional ordering approach, as summarized in Table 3.

**Table 3.** Inventory Levels of 11 Models of Water Sports Equipment Bags.

| Product Code | Product Name | Average Inventory Level (Traditional Ordering Method) |
|--------------|--------------|-------------------------------------------------------|
| 12125639     | FL1          | 45                                                    |
| 12126499     | FL2          | 28                                                    |
| 12144353     | FL3          | 17                                                    |
| 12125642     | FL4          | 10                                                    |
| 12156832     | AR1          | 15                                                    |
| 12149837     | AR2          | 19                                                    |
| 12125153     | AW1          | 12                                                    |
| 12153261     | JF1          | 12                                                    |
| 12153262     | JF2          | 8                                                     |
| 12143298     | RD1          | 9                                                     |
| 12142614     | RD2          | 10                                                    |

As shown in Table 3, the average inventory levels of all 11 bag models were higher than operationally necessary. This finding confirms the existence of excess stock in the packaging department. The data used in this study were collected over a three-month observation period.

**3.1.1 Packaging Department Workflow:** The company's workflow begins with the production planning department, which generates production schedules based on customer orders. Once products complete stage 10, they proceed to final quality inspection (QC-Final), where both functional and aesthetic characteristics are assessed. At this stage, multiple defect types may be detected, each requiring a different amount of rework time. Table 4 summarizes the ten most frequently observed defects and their average correction times.

**Table 4.** Characteristics of Defects and Time to Fix

| Item | Symptom                                | Time to Fix (Days) | Inspection Point |
|------|----------------------------------------|--------------------|------------------|
| 1    | Peeling paint                          | 3                  | QC-Final         |
| 2    | Scratches not caused by heat           | 2                  | QC-Final         |
| 3    | Surface scratches                      | 2                  | QC-Final         |
| 4    | Paint bubbling                         | 3                  | QC-Final         |
| 5    | Incorrect paint, bubble, or glue color | 3                  | QC-Final         |
| 6    | Dust or foreign objects in the coating | 2                  | QC-Final         |
| 7    | Surface adhesion issues                | 2                  | QC-Final         |
| 8    | Improper finishing                     | 3                  | QC-Final         |
| 9    | Incorrect assembly alignment           | 2                  | QC-Final         |
| 10   | Dirt contamination                     | 2                  | QC-Final         |

**3.1.2 Ordering and Warehouse Practices:** Due to uncertainty caused by defects and rework, employees tend to order more packaging bags than required and do so without a formal stock verification system. This practice, combined with a daily ordering policy, contributes to stock imbalance and overstocking. The warehouse department supplies bags daily, with a replenishment lead time of approximately 1 day. Based on these observations, this study proposes applying a Fixed Time Period System (Periodic Review Policy) to better align bag inventory with actual production demand and rework variability. Such an approach has been widely recommended in the inventory control literature for balancing service levels, lead times, and delays due to defects [11-12, 15].

**3.1.3 Final Quality Control (QC-Final):** The QC-Final stage is responsible for inspecting both the structural and aesthetic quality of each product. Because the types and severities of defects cannot be predicted in advance, production flow is disrupted, and a strict First-In, First-Out (FIFO) sequence cannot always be maintained. On average, defect correction takes approximately 3 days, consistent with prior studies that highlight the impact of rework on production scheduling [13-14].



3.1.4 Packaging Process: After quality inspection, products proceed to the packaging stage. Because the production sequence may be delayed by rework, employees often place orders for packaging bags without knowing the exact timing of the final output. In the absence of a stock verification system, this practice leads to excessive ordering and increased holding costs [1, 10].

3.1.5 Warehouse Department: Upon receiving requisitions from the production department, the warehouse supplies the required bags daily. Although this practice ensures immediate material availability, it does not prevent over-ordering. Therefore, this study proposes an improved inventory control framework based on a Fixed Time Period Inventory System to synchronize ordering with actual production demand and rework variability [11-12, 15].

### 3.2 Analysis of Problems

This study analyzes the problem of excess inventory in the packaging department and evaluates alternative solutions by comparing three ordering approaches for water sports equipment bags.

3.2.1 Traditional Ordering Method. Under the traditional approach, employees order bags based on perceived demand without verifying stock levels. This practice results in excessive replenishment and high average inventory levels across the 11 bag models, as shown in Table 4. Such behavior is consistent with the literature, which indicates that ad hoc ordering increases holding costs and ties up working capital in unnecessary stock [1, 5].

3.2.2 Ordering with Inventory Control: Fixed Time Period System. The analysis of the packaging department workflow indicates that current employee ordering behavior already resembles a Fixed Time Period (Periodic Review) System, in which orders are placed at regular intervals. However, the system lacks formal target inventory levels. In this study, the Fixed Time Period System is used to calculate target inventory levels for each bag model, maintaining service reliability while reducing excess inventory. The target inventory level is calculated using Equation (1). [11-12]

$$\text{Target Inventory Level (T)} = \bar{d}(P + LT) + Z \sigma_d \sqrt{P + LT} \quad (1)$$

Where

|            |                                      |
|------------|--------------------------------------|
| $\bar{d}$  | = average daily demand               |
| $\sigma_d$ | = standard deviation of daily demand |
| $P$        | = ordering period (days)             |
| $LT$       | = lead time (days)                   |
| $Z$        | = service level factor               |

A service level of 99% ( $Z=2.33$ ) was selected to minimize the risk of stockouts in the packaging process, where product availability is critical for shipment reliability. Because packaging bags are required at the final stage before delivery, even a minor shortage can disrupt shipment schedules and degrade customer service performance. Therefore, a relatively high service level was considered appropriate for this case study. For the FL1 model (12125639), the input data were as follows:

The average daily demand: 8 units per day  
 The standard deviation : 3 units per day  
 Ordering period: 1 day  
 Lead time: 1 day  
 Desired service level: 99% ( $Z = 2.33$ )

$$\begin{aligned} \text{Target Inventory Level (T)} &= (8)(1 + 1) + (2.33)(3)\sqrt{1 + 1} \\ &= 26 \text{ units} \end{aligned}$$

**Table 5.** Inventory Target

| No. | Product Code | Product Name | Inventory Target (Target) |
|-----|--------------|--------------|---------------------------|
| 1   | 12125639     | FL1          | 26                        |
| 2   | 12126499     | FL2          | 21                        |
| 3   | 12144353     | FL3          | 5                         |
| 4   | 12125642     | FL4          | 8                         |
| 5   | 12156832     | AR1          | 7                         |
| 6   | 12149837     | AR2          | 7                         |
| 7   | 12125153     | AW1          | 10                        |
| 8   | 12153261     | JF1          | 6                         |
| 9   | 12153262     | JF2          | 5                         |
| 10  | 12143298     | RD1          | 5                         |
| 11  | 12142614     | RD2          | 5                         |

Table 5 shows that the proposed Fixed Time Period System allocates inventory targets according to demand magnitude and variability. Products in the FL series require the highest target inventory levels, particularly FL1 and FL2, due to their relatively high daily demand and variability. In contrast, lower-demand models such as JF2, RD1, and RD2 require smaller target inventories. This approach replaces subjective ordering decisions with data-driven inventory control, helping reduce unnecessary stock while maintaining the desired service level. To further compare the existing and proposed approaches, Table 6 summarizes the key differences between the traditional ordering method and the Fixed Time Period System.

**Table 6.** Comparison of the Traditional Ordering Method and Fixed Time Period System

| Criteria                | Traditional Ordering Method                                                               | Fixed Time Period System (Proposed)                                                            |
|-------------------------|-------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Average Inventory Level | High (FL1 = 45 units; overall average > 20 units across models)                           | Lower (calculated targets: FL1 = 26 units; overall average < 15 units)                         |
| Cost Impact             | Increased holding costs and sunk costs due to excess stock. Storage space inefficiencies. | Reduced inventory holding cost, optimized working capital, and better warehouse utilization.   |
| Ordering Practice       | Ad-hoc ordering without verification. Employees tend to over-order daily.                 | Periodic review with target stock levels calculated using demand, variance, and service level. |
| Service Level           | Not explicitly defined; often leads to a stock surplus rather than a balance.             | Defined at 99% service level using statistical safety stock calculation.                       |
| Risk of Stockouts       | Low (due to over-ordering), but at a high cost.                                           | Controlled by the safety stock factor ( $Z = 2.33$ ), balancing service level with cost.       |
| System Alignment        | Reactive and employee-dependent.                                                          | Structured and data-driven, aligned with inventory control theory.                             |

In summary, the Fixed Time Period System offers a more structured, cost-efficient approach than the traditional ordering method. By aligning target stock levels with demand variability and service requirements, the system reduces excess inventory while maintaining high operational reliability.

### 3.2.3 Ordering with inventory control based on rework rate and rework time analysis

In real manufacturing environments, production imperfections are unavoidable, and defective items requiring rework can significantly affect both production scheduling and inventory requirements. To better reflect these operational conditions, this study incorporates rework rate and rework time into the ordering policy for packaging bags. This approach enables inventory decisions to account for both the proportion of defective items and the timing of reworked items' return to the production process. The FL1 model (12125639) was selected as a representative case for analyzing rework behavior. Table 7 presents inspection data over 15

days, including the number of inspected items, the number of passed items, the number of reworked items, the rework time, and the percentage reworked.

**Table 7.** Rework Rate Behavior of Water Sports Equipment Production Using FL1 Model Bags (12125639)

| Date  | Items Inspected | Items Passed | Items Reworked | Rework Time (Days) | Percentage Reworked |
|-------|-----------------|--------------|----------------|--------------------|---------------------|
| 01.11 | 11              | 6            | 5              | 0                  | 45%                 |
| 02.11 | 7               | 7            | 0              | 0                  | 0%                  |
| 03.11 | 8               | 8            | 0              | 0                  | 0%                  |
| 04.11 | 9               | 9            | 0              | 0                  | 0%                  |
| 05.11 | 6               | 6            | 0              | 0                  | 0%                  |
| 06.11 | 8               | 8            | 0              | 0                  | 0%                  |
| 07.11 | 8               | 5            | 3              | 0                  | 38%                 |
| 08.11 | 11              | 11           | 0              | 0                  | 0%                  |
| 09.11 | 8               | 4            | 4              | 0                  | 50%                 |
| 10.11 | 8               | 8            | 0              | 0                  | 0%                  |
| 11.11 | 9               | 9            | 0              | 0                  | 0%                  |
| 12.11 | 8               | 8            | 0              | 0                  | 0%                  |
| 13.11 | 8               | 8            | 0              | 0                  | 0%                  |
| 14.11 | 6               | 6            | 0              | 0                  | 0%                  |
| 15.11 | 3               | 1            | 2              | 0                  | 67%                 |

Note: In Table 7, rework time is recorded as 0 because the table reports the day on which defects were identified, while the average repair cycle time is presented separately in Table 5 and discussed in the workflow analysis.

From Table 7, the rework rate for FL1 varies considerably across days, ranging from 0% to 67%, with an average rework rate of 20%. This indicates that one-fifth of inspected items required rework before they could proceed to packaging. The analysis was then extended to all bag models.

**Table 8.** Rework Rate Behavior of Selected Water Sports Equipment Bag Models.

| No. | Product Code | Product Name | Rework Rate |
|-----|--------------|--------------|-------------|
| 1   | 12125639     | FL1          | 20%         |
| 2   | 12126499     | FL2          | 16%         |
| 3   | 12144353     | FL3          | 17%         |
| 4   | 12125642     | FL4          | 15%         |
| 5   | 12156832     | AR1          | 9%          |
| 6   | 12149837     | AR2          | 22%         |
| 7   | 12125153     | AW1          | 19%         |
| 8   | 12153261     | JF1          | 23%         |

The results indicate that rework rates differ substantially across product models. Models with higher rework rates require more conservative inventory policies to compensate for delays caused by defect correction and ensure continuity in the packaging process. To integrate rework information into inventory planning, this study adopts a stepwise procedure for determining the withdrawal plan and safety stock requirements.

1. Adjustment of Shipment Plan by Rework Rate. The production planning department issues a weekly shipment plan based on a 5-day workweek. Because not all produced units are immediately available for packaging, the initial withdrawal quantity must be adjusted to account for the expected number of defective items. The initial withdrawal quantity is defined as equation (2).

$$W = S - D \quad (2)$$

Where

S = shipment plan target (units)

D = expected number of defective units

W = withdrawal quantity required for packaging

The expected number of defective units is estimated using the observed rework rate as shown in Equation (3).

$$D = S \times r \quad (3)$$

Where

D = expected number of defective units requiring rework

S = shipment plan target (units)

r = rework rate (proportion of items requiring correction)

This formulation assumes that the number of defective items is proportional to the production quantity and the observed rework rate. Similar approaches are commonly used in inventory models under imperfect quality conditions. Table 9 provides an example of the weekly shipment plan for the FL1 model.

**Table 9.** Example of Packaging Targets (Shipment Plan)

| Date | 3.10 | 4.10 | 5.10 | 6.10 | 7.10 | Total |
|------|------|------|------|------|------|-------|
| FL1  | 5    | 5    | 5    | 5    | 5    | 25    |

Using Table 9, the shipment plan target for FL1 is 25 units per week. With an average rework rate of 20%, the expected number of defective items is:

$$D = S \times r = 25 \times 0.20 = 5$$

Accordingly, the adjusted withdrawal plan is:

$$W = S - D = 25 - 5 = 20$$

However, defective items are not permanently removed from the system. After rework is completed, these items re-enter the production process and become available for packaging. Since the average rework time is approximately three days, the effective withdrawal plan must account for both the immediately available units and the return of reworked items. Therefore, the adjusted withdrawal quantity is represented as equation (4).

$$W = S(1 - r) + R_t \quad (4)$$

Where

S = shipment plan target (units)

r = rework rate (proportion of items requiring rework)

S(1-r) = effective number of items immediately available for packaging

R<sub>t</sub> = number of items returning from the rework process after t days

W = adjusted withdrawal quantity

Equation (2) represents the initial withdrawal estimate after excluding expected defective units, whereas Equation (4) extends the formulation by incorporating the return flow of reworked items. This reflects the actual production environment, in which defective units are repaired and reintroduced into the system rather than discarded [14,15].

2. Determination of Safety Stock. To mitigate discrepancies between planned withdrawals and actual production output, a safety stock buffer is required. Table 10 compares the packaging plan and actual output over the same period.

**Table 10.** Comparison of Bagging Plans and Actual Output of Water Sports Equipment.

| Date          | 3.10 | 4.10 | 5.10 | 6.10 | 7.10 | Total |
|---------------|------|------|------|------|------|-------|
| Bagging Plan  | 4    | 4    | 5    | 5    | 5    | 23    |
| Actual Output | 4    | 6    | 4    | 6    | 3    | 22    |
| Difference    | 0    | -2   | 0    | -1   | 2    | -2    |



Table 10 shows that actual output does not always match the packaging plan. These deviations justify the inclusion of safety stock to absorb variability from rework and maintain service reliability without excessive overstocking. In summary, the proposed rework-based approach provides a more realistic and adaptive basis for managing packaging bag inventory. By adjusting the shipment plan to account for returned, reworked items and incorporating safety stock, the proposed method aligns inventory decisions more closely with actual production conditions, helping reduce excess inventory while preventing shortages.

## 4. Results

The primary objective of this study was to reduce excess inventory and associated sunk costs in the packaging process by applying inventory control techniques to determine more appropriate stock levels. To evaluate the effectiveness of alternative ordering approaches, three methods were compared: (1) the Traditional Ordering Method, (2) the Fixed Time Period System (FTPS), and (3) the proposed Rework-Based Ordering Method.

### 4.1 Traditional Ordering Method.

The first analysis examined employees' existing ordering behavior in the packaging department. Data collected during November showed substantial overstocking of packaging bags, as the quantities ordered frequently exceeded actual usage demand. As a result, a considerable amount of capital was tied up in inventory. The average inventory cost for each bag model under the traditional ordering method is presented in Table 11.

**Table 11.** Average Inventory Cost Using the Traditional Ordering Method

| No. | Product Code | Product Name | Quantity Ordered | Product Value (Baht) | Cost Using Traditional Method (Baht) |
|-----|--------------|--------------|------------------|----------------------|--------------------------------------|
| 1   | 12125639     | FL1          | 45               | 1,886                | 84,870                               |
| 2   | 12126499     | FL2          | 28               | 1,725                | 48,300                               |
| 3   | 12144353     | FL3          | 17               | 1,577                | 26,809                               |
| 4   | 12125642     | FL4          | 10               | 1,422                | 14,220                               |
| 5   | 12156832     | AR1          | 15               | 1,700                | 25,500                               |
| 6   | 12149837     | AR2          | 19               | 1,792                | 34,048                               |
| 7   | 12125153     | AW1          | 12               | 2,034                | 24,408                               |
| 8   | 12153261     | JF1          | 12               | 2,615                | 31,380                               |
| 9   | 12153262     | JF2          | 8                | 2,761                | 22,088                               |
| 10  | 12143298     | RD1          | 9                | 2,096                | 18,864                               |
| 11  | 12142614     | RD2          | 10               | 2,282                | 22,820                               |

The results indicate that the traditional ordering method yielded an average inventory cost of 353,307 Baht. This high cost was mainly caused by excessive stock accumulation beyond operational requirements, confirming the inefficiency of employee-based ad hoc ordering without inventory verification.

### 4.2 Fixed Time Period System Ordering Method.

To address inefficiencies in the traditional method, the Fixed Time Period System (FTPS) was implemented. This method determines a target inventory level for each bag model based on average demand, demand variability, lead time, and safety stock considerations. Table 12 compares the traditional order quantities with the FTPS target inventory levels and the corresponding inventory costs.

**Table 12.** Comparison of Inventory Levels and Costs Between the Traditional Ordering Method and the Fixed Time Period System

| Product Code | Product Name | Traditional Method Order Quantity | Fixed Time Period System Target Inventory Level | Product Value (Baht) | Method 1 Traditional Ordering (Average Inventory Level) | Method 2 Inventory Target | Cost Reduction (Baht) |
|--------------|--------------|-----------------------------------|-------------------------------------------------|----------------------|---------------------------------------------------------|---------------------------|-----------------------|
| 12125639     | FL1          | 45                                | 26                                              | 1,886                | 84,870                                                  | 49,036                    | 35,834                |
| 12126499     | FL2          | 28                                | 21                                              | 1,725                | 48,300                                                  | 36,225                    | 12,075                |
| 12144353     | FL3          | 17                                | 5                                               | 1,577                | 26,809                                                  | 7,885                     | 18,924                |
| 12125642     | FL4          | 10                                | 8                                               | 1,422                | 14,220                                                  | 11,376                    | 2,844                 |
| 12156832     | AR1          | 15                                | 7                                               | 1,700                | 25,500                                                  | 11,900                    | 13,600                |
| 12149837     | AR2          | 19                                | 7                                               | 1,792                | 34,048                                                  | 12,544                    | 21,504                |
| 12125153     | AW1          | 12                                | 10                                              | 2,034                | 24,408                                                  | 20,340                    | 4,068                 |
| 12153261     | JF1          | 12                                | 6                                               | 2,615                | 31,380                                                  | 15,690                    | 15,690                |
| 12153262     | JF2          | 8                                 | 5                                               | 2,761                | 22,088                                                  | 13,805                    | 8,283                 |
| 12143298     | RD1          | 9                                 | 5                                               | 2,096                | 18,864                                                  | 10,480                    | 8,384                 |
| 12142614     | RD2          | 10                                | 5                                               | 2,282                | 22,820                                                  | 11,410                    | 11,410                |
|              |              |                                   |                                                 | Total                | 353,307                                                 | 200,691                   | 152,616               |

The application of FTPS resulted in a substantial reduction in target inventory levels for most bag models compared with the traditional ordering method. In financial terms, the total inventory cost decreased from 353,307 Baht under the traditional method to 200,691 Baht under FTPS, representing a cost reduction of 152,616 Baht. Importantly, no stockouts were observed during the trial period, indicating that the inventory reduction did not compromise operational reliability. These findings suggest that FTPS provides a more systematic and cost-efficient ordering approach than the traditional method by replacing subjective ordering behavior with statistically determined inventory targets.

#### 4.3 Rework Rate and Rework Time-Based Inventory Control.

To further improve inventory performance under actual production conditions, the third method incorporated rework rate and rework time into the inventory planning process. This method adjusts inventory requirements based on the proportion of defective items and the timing of their return to the production process. Table 13 compares the average inventory levels obtained under the three methods using December data.

**Table 13.** Comparison of Average Inventory Levels in 3 Methods (December)

| No. | Product Code | Product Name | Traditional Method Inventory Level | Fixed Time Period System Inventory Level | Rework Rate-Based Inventory Level |
|-----|--------------|--------------|------------------------------------|------------------------------------------|-----------------------------------|
| 1   | 12125639     | FL1          | 45                                 | 26                                       | 2                                 |
| 2   | 12126499     | FL2          | 28                                 | 21                                       | 3                                 |
| 3   | 12144353     | FL3          | 17                                 | 5                                        | 2                                 |
| 4   | 12125642     | FL4          | 10                                 | 8                                        | 4                                 |
| 5   | 12156832     | AR1          | 15                                 | 7                                        | 2                                 |
| 6   | 12149837     | AR2          | 19                                 | 7                                        | 2                                 |
| 7   | 12125153     | AW1          | 12                                 | 10                                       | 4                                 |
| 8   | 12153261     | JF1          | 12                                 | 6                                        | 2                                 |
| 9   | 12153262     | JF2          | 8                                  | 5                                        | 2                                 |
| 10  | 12143298     | RD1          | 9                                  | 5                                        | 3                                 |
| 11  | 12142614     | RD2          | 10                                 | 5                                        | 3                                 |

The results show that the rework-based method consistently produced the lowest inventory levels across all product models. This indicates that incorporating rework-related variability enables inventory to be more closely aligned with actual production requirements than either the traditional ordering method or FTPS.

To evaluate the financial impact of this method, Table 14 compares the average inventory costs of all three approaches.

**Table 14.** Comparison of Average Inventory Costs Using Three Methods

| No. | Product Code | Product Name | Product Value (Baht) | Method 1 Cost (Baht) | Method 2 Cost (Baht) | Method 3 Cost (Dec) (Baht) |
|-----|--------------|--------------|----------------------|----------------------|----------------------|----------------------------|
| 1   | 12125639     | FL1          | 1,886                | 84,870               | 49,036               | 3,772                      |
| 2   | 12126499     | FL2          | 1,725                | 48,300               | 36,225               | 5,658                      |
| 3   | 12144353     | FL3          | 1,577                | 26,809               | 7,885                | 3,772                      |
| 4   | 12125642     | FL4          | 1,422                | 14,220               | 11,376               | 7,544                      |
| 5   | 12156832     | AR1          | 1,700                | 25,500               | 11,900               | 3,772                      |
| 6   | 12149837     | AR2          | 1,792                | 34,048               | 12,544               | 3,772                      |
| 7   | 12125153     | AW1          | 2,034                | 24,408               | 20,340               | 7,554                      |
| 8   | 12153261     | JF1          | 2,615                | 31,380               | 15,690               | 3,772                      |
| 9   | 12153262     | JF2          | 2,761                | 22,088               | 13,805               | 3,772                      |
| 10  | 12143298     | RD1          | 2,096                | 18,864               | 10,480               | 5,658                      |
| 11  | 12142614     | RD2          | 2,282                | 22,820               | 11,410               | 5,658                      |

Based on the cost comparison, Method 3 achieved the lowest overall inventory cost. The proposed rework-based method reduced inventory cost by 298,613 Baht compared with the traditional method and by 145,997 Baht compared with FTPS. These results demonstrate that including rework rate and rework time in inventory planning can significantly improve cost efficiency while maintaining operational continuity. To assess the robustness of the proposed method, inventory levels under Method 3 were monitored over three consecutive months from December to February. The comparative results are shown in Table 15.

**Table 15.** Inventory Levels Under the Three Ordering Methods Across Three Consecutive Months

| No. | Product Code | Product Name | Traditional Method Inventory Level | Fixed Time Period System Inventory Level | Rework Rate-Based Inventory Level (Dec) | Rework Rate-Based Inventory Level (Jan) | Rework Rate-Based Inventory Level (Feb) |
|-----|--------------|--------------|------------------------------------|------------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| 1   | 12125639     | FL1          | 45                                 | 26                                       | 2                                       | 3                                       | 3                                       |
| 2   | 12126499     | FL2          | 28                                 | 21                                       | 3                                       | 3                                       | 3                                       |
| 3   | 12144353     | FL3          | 31                                 | 24                                       | 2                                       | 3                                       | 3                                       |
| 4   | 12125642     | FL4          | 10                                 | 8                                        | 4                                       | 3                                       | 3                                       |
| 5   | 12156832     | AR1          | 15                                 | 7                                        | 2                                       | 3                                       | 3                                       |
| 6   | 12149837     | AR2          | 19                                 | 10                                       | 2                                       | 3                                       | 3                                       |
| 7   | 12125153     | AW1          | 9                                  | 10                                       | 4                                       | 3                                       | 3                                       |
| 8   | 12153261     | JF1          | 8                                  | 10                                       | 2                                       | 3                                       | 3                                       |
| 9   | 12153262     | JF2          | 5                                  | 5                                        | 2                                       | 3                                       | 3                                       |
| 10  | 12143298     | RD1          | 5                                  | 5                                        | 3                                       | 3                                       | 3                                       |
| 11  | 12142614     | RD2          | 5                                  | 5                                        | 3                                       | 3                                       | 3                                       |

The results indicate that the rework-based method maintained low and stable inventory levels throughout the three-month observation period without causing stock shortages. This finding suggests that the proposed method is not only cost-efficient but also operationally reliable over time. To further compare the cost implications across the observation period, Table 16 summarizes the average inventory costs for the three methods.

**Table 16.** Summary of Average Inventory Costs

| No.          | Product Code | Product Name | Product Value (Baht) | Method 1 Cost (Baht) | Method 2 Cost (Baht) | Method 3 Cost (Baht) (Dec) | Method 3 Cost (Baht) (Jan) | Method 3 Cost (Baht) (Feb) |
|--------------|--------------|--------------|----------------------|----------------------|----------------------|----------------------------|----------------------------|----------------------------|
| 1            | 12125639     | FL1          | 1,886                | 84,870               | 49,036               | 3,772                      | 5,658                      | 5,658                      |
| 2            | 12126499     | FL2          | 1,725                | 48,300               | 36,225               | 5,658                      | 5,658                      | 5,658                      |
| 3            | 12144353     | FL3          | 1,577                | 26,809               | 7,885                | 3,772                      | 3,772                      | 3,772                      |
| 4            | 12125642     | FL4          | 1,422                | 14,220               | 11,376               | 7,544                      | 7,544                      | 5,658                      |
| 5            | 12156832     | AR1          | 1,700                | 25,500               | 11,900               | 3,772                      | 5,658                      | 5,658                      |
| 6            | 12149837     | AR2          | 1,792                | 34,048               | 12,544               | 3,772                      | 5,658                      | 5,658                      |
| 7            | 12125153     | AW1          | 2,034                | 24,408               | 20,340               | 7,554                      | 7,544                      | 7,544                      |
| 8            | 12153261     | JF1          | 2,615                | 31,380               | 15,690               | 3,772                      | 5,658                      | 3,772                      |
| 9            | 12153262     | JF2          | 2,761                | 22,088               | 13,805               | 3,772                      | 3,772                      | 3,772                      |
| 10           | 12143298     | RD1          | 2,096                | 18,864               | 10,480               | 5,658                      | 5,658                      | 5,658                      |
| 11           | 12142614     | RD2          | 2,282                | 22,820               | 11,410               | 5,658                      | 5,658                      | 5,658                      |
| <b>Total</b> |              |              |                      | <b>353,307</b>       | <b>200,691</b>       | <b>54,694</b>              | <b>60,352</b>              | <b>56,580</b>              |

The comparative analysis confirms the superiority of the rework rate and rework time-based approach. While the traditional ordering method resulted in the highest inventory cost of 353,307 Baht, FTPS reduced it to 200,691 Baht; the proposed Method 3 achieved the lowest average cost of approximately 57,209 Baht across the three months. More importantly, Method 3 maintained service reliability by preventing stock shortages across all product categories during the observation period. Overall, the results highlight two important findings. First, incorporating production variability through rework rate and rework time enables more realistic and efficient inventory decisions. Second, the proposed model provides a stronger alignment between inventory planning and actual production conditions than conventional approaches. Therefore, the rework-based method offers a practical and effective framework for managing packaging inventory in manufacturing environments.

## 5. Discussion

The findings of this study demonstrate that different inventory control policies produce substantially different outcomes in terms of cost efficiency and service reliability. The traditional ordering method led to excessive inventory accumulation and high sunk costs, confirming earlier studies that have highlighted the risks of uncontrolled, experience-based ordering practices in manufacturing environments [1, 4]. Although this approach reduced the likelihood of stock shortages, it did so at the expense of tying up excessive capital in inventory. The Fixed Time Period System (FTPS) provided a more structured and theoretically grounded alternative. By incorporating demand averages, demand variability, and safety stock into the calculation of target inventory levels, FTPS reduced total inventory costs by more than 152,000 Baht compared with the traditional method. This result is consistent with prior studies on periodic review systems and safety stock policies, which suggest that statistically determined replenishment rules can improve cost control and inventory discipline [8, 11]. However, the results also show that FTPS alone was not sufficient to fully reflect the operational realities of the packaging process, particularly when rework-related disruptions affected the timing of product availability. The most important contribution of this study lies in the proposed rework-based inventory approach, which explicitly integrates rework rate and rework time into inventory planning. This method outperformed both the traditional method and FTPS by achieving the lowest average inventory cost while maintaining service reliability and preventing stockouts. The superior performance of this approach indicates that production variability caused by defects and rework should not be treated as a peripheral issue, but rather as a central factor in inventory decision-making. In this respect, the findings support earlier studies on imperfect quality and rework in production-inventory systems, particularly those of Sonntag and Kiesmüller [13], Alamri et al. [14], and Lin et al. [16], which emphasize the importance of incorporating



production imperfections into inventory models. From a practical standpoint, the results suggest that inventory policies for packaging materials should be closely aligned with actual production behavior rather than based solely on average demand or fixed replenishment intervals. In manufacturing systems where rework delays are common, an inventory policy that accounts for defect rates and rework cycle times can provide a more realistic balance between cost reduction and service continuity. Therefore, the proposed method offers not only theoretical relevance but also managerial value for firms seeking to improve inventory efficiency under imperfect production conditions. Overall, this study demonstrates that although EOQ, periodic review, and safety stock models provide a useful analytical foundation, more adaptive policies are required when production systems are affected by rework and quality variability. By integrating these operational factors into inventory planning, the proposed framework bridges the gap between theoretical inventory control models and practical implementation in manufacturing environments.

## 6. Conclusion

This study compared three inventory management approaches for water sports equipment bags in the packaging department: the Traditional Ordering Method, the Fixed Time Period System (FTPS), and the proposed rework rate and rework time-based inventory control method. The results showed that the traditional ordering method led to substantial overstocking and the highest inventory cost, amounting to 353,307 Baht. The FTPS improved inventory control and reduced total cost to 200,691 Baht; however, it still maintained inventory levels above actual operational requirements. In contrast, the proposed rework-based inventory approach produced the most effective results. By incorporating rework rate and rework time into inventory planning, the method achieved the lowest average inventory cost of 57,209 Baht over the three-month observation period while preventing stockouts across all product categories. These findings demonstrate that inventory policies can be significantly improved when operational variability is explicitly incorporated into planning decisions rather than relying solely on demand averages or fixed review cycles.

From a managerial perspective, the study suggests that manufacturing firms should adopt more adaptive inventory systems that reflect actual production conditions, particularly in environments affected by defects and rework. Integrating rework-related data into inventory decisions helps reduce excess capital investment, improve material availability, and strengthen operational reliability. Therefore, the proposed approach offers practical value not only for water sports equipment manufacturing but also for other industries characterized by rework-related uncertainty, such as automotive, electronics, and customized production systems. Despite its contributions, this study has several limitations. First, the analysis was based on a single case company, which may limit the generalizability of the findings. Second, the observation period covered only three months and may not fully capture seasonal demand variation or long-term operational changes. Third, the proposed model assumes relatively stable rework rates and rework times, whereas these factors may vary in practice due to workforce performance, technological conditions, or material quality. Future research should extend the analysis to multiple industrial settings and longer study periods, and incorporate stochastic modeling more effectively better to reflect uncertainty in both demand and production quality. Further investigation into sustainability outcomes, such as waste reduction and environmental impact, would also enhance the relevance of rework-based inventory policies in modern manufacturing.

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# Effect of Plant Growth Regulators on *In Vitro* Shoot and Root Induction and Acclimatization of *Gymnocalycium mihanovichii* LB2178 Agua Dulce (Cactaceae)

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**Abstract:** *Gymnocalycium mihanovichii* LB2178 Agua Dulce is a popular cactus still in demand, known for its distinctive shape and ease of growth. It can be propagated by tissue culture to produce strong, disease-free plants. Therefore, the aim of this research was 1) to study the effect of BA and IBA hormones and types of explants on the growth rate of *G. mihanovichii* LB2178 Agua Dulce cactus. and 2) to study suitable planting materials for survival and growth of *G. mihanovichii* LB2178 Agua Dulce cactus under natural conditions. After 3 months of culture, the results showed that the explant sectioned longitudinally were cultured on MS medium supplemented with 0 and 2 mg/L BA gave the highest survival, 100%, and shoot induction, 86.67%, the explant sectioned transversally were cultured on MS medium supplemented with 1 mg/L BA gave the highest number of shoot, 4.20 shoots/explant, and shoot length, 0.36 mm respectively, significant difference at  $p \leq 0.05$ . Following three months of cultivation, the findings indicated that shoot explants cultured on MS medium supplemented with 1 mg/L IBA showed the highest root induction (91.67%), average number of roots (20.47 roots/explant), and average root length (7.63 mm), with significant differences at  $p \leq 0.05$ . After 3 months, the rooted plants were removed from the tissue culture room and transferred to sterile plastic pots. The result showed that *G. mihanovichii* LB2178 Agua Dulce grown in potting soil: sand, showed the highest survival rate of 100%.

**Keywords:** *Gymnocalycium mihanovichii* LB2178 Agua Dulce; benzyladenine; indole-3-butyric acid; acclimatization; *in vitro*

## 1. Introduction

Cacti are one of the plant groups with high morphological diversity, with 127 genera and approximately 1,430-2,000 species reported worldwide [1]. Most of them are densely distributed in South America, particularly Mexico, and in North America, including the United States and Canada [2]. Most cacti are cultivated as ornamental plants with high market value [3]. In addition, some cacti are used as food plants, eaten as fruits, and used in the food industry for livestock [4]. They are used as ingredients in food menus or processed foods, and local people in Mexico like to raise hummingbirds (*Mellisuga helenae*) to help pollinate cacti and increase fruiting rates, helping secure the local people's

food source. Cacti are slow-growing plants that can thrive in a variety of environments, especially in desert areas, making them among the most beautiful desert plants in the world.

Cactus has special characteristics. It has its own unique characteristics: a succulent stem that is round, cylindrical, or serrated, growing as a single stem or a clumping stem; and leaves that have evolved into thorns to reduce water loss. It also has a wide variety of thorn types, including needle-like, ribbed, paper, and hairy thorns. In addition, cacti are plants with beautiful flowers in various shapes, such as funnel-, bell-, dish-, and tubular-shaped flowers, as well as stems and flowers in various colors, such as yellow, purple, pink, red, white, and cream [5]. Because of these unique qualities, cacti are now among the most widely used ornamental plants in homes and offices. As a result, 130 species of cacti are imported and exported to 182 countries, with an average annual market value of 11–60 billion US dollars. The value is continuously increasing every year [6, 7].

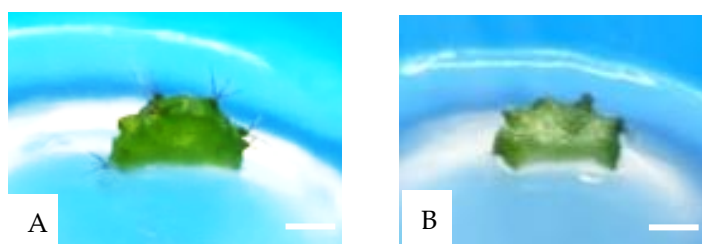
Cacti are among the most popular ornamental plants grown and cultivated in Thailand. *Gymnocalycium mihanovichii* is one of the *Gymnocalycium* genus's cactus family, which is ranked first in popularity (average 55%) among cactus lovers compared to other cactus species (45%). It has a total value of up to 2 million baht [3]. This is because the plants have unique stem characteristics, stem color, thorn characteristics, and highly diverse flower shape and color. Consumers, especially those interested in new plant varieties with specific qualities that differ from the original varieties, frequently influence market trends in ornamental plants and the cactus family. As a result, the market value of the new plant varieties increases several times. Furthermore, since *G. mihanovichii* LB2178 Agua Dulce is a lovely decorative plant that may be multiplied by dividing the shoots or, if a large quantity is required, by seed, it is also much sought after in the market. However, the propagation process is slow and susceptible to plant diseases [8]. Currently, researchers are using plant tissue culture techniques to propagate plantlets and increase the number of plants in a short period of time. According to the report by Rittirong *et al.* [9], 0.5 cm shoots of *Gymnocalycium* spp. were cultured on MS medium with different concentrations of NAA (0.0, 1.0, 2.0, 3.0, and 4.0 mg/L) and BA (0.0, 1.0, 2.0, 3.0, and 4.0 mg/L). After 3 months of culture, the efficiency of the tissue culture approach for producing a large number of *G. mihanovichii* LB2178 Agua Dulce. The application of cytokinin can awaken previously dormant axillary buds from the constraints of apical dominance [10]. Regarding shoot rooting, auxins are the most commonly used exogenous phytohormones for root development, and IBA is more effective than NAA alone or in combination with IBA [11].

We cannot find any reports on *in vitro* shoot and root induction in *G. mihanovichii* LB2178 Agua Dulce, or on the acclimatization and hardening of rooted plants. In commercial plantations of this species, *ex vitro* propagation using stem cuttings takes approximately 1 month to induce root growth, although the exact duration varies across cultivation stages. In this research, the effect of BA and IBA hormones and types of explants on the increase of *G. mihanovichii* LB2178 Agua Dulce cactus was studied, as well as the planting materials that are suitable for the survival and growth of *G. mihanovichii* LB2178 Agua Dulce cactus.

## 2. Materials and Methods

### 2.1 Plant materials

The 1 cm seedlings of *G. mihanovichii* LB2178 Agua Dulce were cut from the shoot and divided into two types of parts: a longitudinal cut and a cross-sectional piece measuring 3–4 mm. In the tissue culture room at the science center in Nakhon Si Thammarat Rajabhat University.



**Figure 1.** The 1 cm seedlings of *G. mihanovichii* LB2178 Agua Dulce were cut off the shoot and divided into two types of parts (bar = 1 cm) (A) a longitudinal cut (B) a cross-sectional cut



## 2.2 Effect of different explant fragmentation and different concentrations of Benzyladenine (BA) on shoot induction of *G. mihanovichii* LB2178 Agua Dulce

The 0.5 cm shoot explants were cultured on MS medium containing 3 BA concentrations (0, 1, and 2 mg/L). All culture media were supplemented with 30 g/L sucrose and 8 g/L agar. The media were adjusted to a pH of 5.7. For three months, the cultures were subcultured every 4 weeks on the same medium component, kept at 25±2 °C under 3000 lux light intensity for 14 hours per day. The survival rate, shoot induction percentage, number of shoots per explant, and shoot length were evaluated after 3 months of culture.

## 2.3 Effect of different concentrations of Indole-3-butyric acid (IBA) on root induction of *G. mihanovichii* LB2178 Agua Dulce

The shoot explants from experiment 2.2 were cultured on MS medium containing 5 IBA concentrations (0, 0.5, 1, 1.5, and 2 mg/L). All culture media were supplemented with 30 g/L sucrose and 8 g/L agar. The media were adjusted to a pH of 5.7. For three months, the cultures were subcultured every 4 weeks on the same medium component, kept at 25±2 °C under 3000 lux light intensity for 14 hours per day. Root induction, average number of roots, and average root length were evaluated after 3 months of culture.

## 2.4 Acclimatization and hardening of rooted plants

After 3 months, the rooted plants were removed from the tissue culture room and adjusted to room temperature for 2 days. Plantlets with well-developed roots were thoroughly washed in tap water and then transplanted into sterile plastic pots filled with a 1:1 mixture of three sterilized soil formulas: potting soil and pumice, potting soil and sand, and potting soil, sand, and pumice. Put 25 grams of each soil formula in 15 pots. Plastic bags were placed over the pots. After adjusting it with a rubber band for seven days, move the cactus to the greenhouse to acclimate. Observe and record the percentage survival after 1 month in culture.

## 2.5 Statistical analysis

The experimental design and statistical analysis were conducted using a fully randomized design with three replicates, each containing 10 explants. Using Duncan's Multiple Range Test (DMRT), the mean differences were compared after ANOVA.

# 3. Results and Discussion

## 3.1 Effect of different explant fragmentation and different concentrations of Benzyladenine (BA) on shoot induction of *G. mihanovichii* LB2178 Agua Dulce

The 0.5 cm shoot explants were cultured on MS medium containing 3 BA concentrations (0, 1, and 2 mg/L). The result found that the explant sectioned longitudinally, cultured on MS medium containing concentrations of BA (0 and 2 mg/L), gave the highest survival, 100%, followed by the explant sectioned transversally, which was cultured on MS medium containing concentrations of BA (0 and 2 mg/L), and the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (1 mg/L). The explant sections transversally were cultured on MS medium containing concentrations of BA (1 mg/L), 86.67%, 80.00%, and 73.33%, respectively, with a significant difference at  $p \leq 0.05$ . Shoot induction, the result found that the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (2 mg/L) gave the highest shoot induction, 86.67%, followed by the explant sectioned transversally were cultured on MS medium containing concentrations of BA (1 and 2 mg/L) and the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (1 mg/L), 76.67% and 56.57% respectively, significant difference at  $p \leq 0.05$ . Number of shoot, the result found that the explant sectioned transversally were cultured on MS medium containing concentrations of BA (1 mg/L) gave the highest number of shoot, 4.20 shoots/explant, followed by the explant sectioned transversally were cultured on MS medium containing concentrations of BA (2 mg/L), the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (2 mg/L) and the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (1 mg/L), 2.20 shoots/explant, 2.13 shoots/explant and 1.33 shoots/explant respectively, significant difference at  $p \leq 0.05$ . Shoot length, the result found that the explant sectioned transversally, cultured on MS medium containing concentrations of BA (1 mg/L), gave the highest shoot length, 0.36 cm, followed by the explant sectioned longitudinally were cultured on MS medium containing

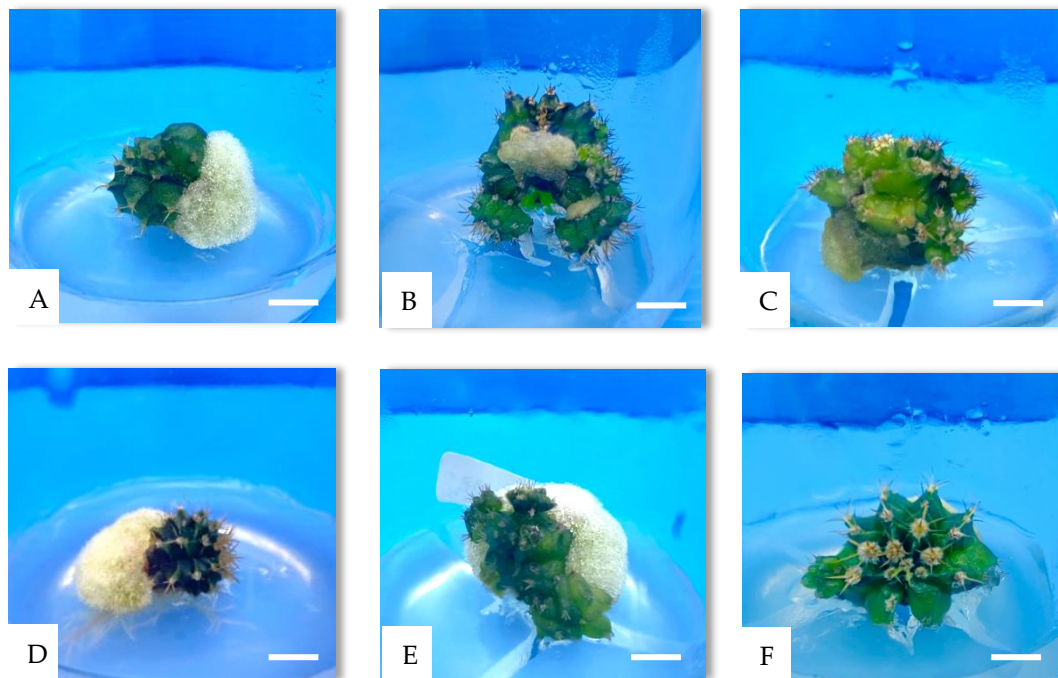


concentrations of BA (2 mg/L), the explant sectioned transversally, which was cultured on MS medium containing concentrations of BA (2 mg/L). The explant sections longitudinally, which were cultured on MS medium containing concentrations of BA (1 mg/L), 0.19 cm, 0.17 cm, and 0.14 cm, respectively, showed a significant difference at  $p \leq 0.05$ . (Table 1 and Figure 2)

The study found that the induction of shoots of *G. mihanovichii* LB2178 Agua Dulce is affected by high BA concentrations and explant fragmentation. Because it has been observed that changing explant polarity can lead to faster growth responses, the explant orientation data presented here contradict these findings. A greater area of contact with the culture medium, its nutrients, and the PGRs it contains could be one factor contributing to explants in vertical posture having higher %E values [12]. According to Bonga [13], the findings of this study are consistent with the observation that very small explants frequently do not grow at all, thereby lowering statistical multiplication rates. Other techniques, such as fragmentation and different explant orientations in the culture media, can also be considered to boost *in vitro* shoot output and reduce the number of original plants used. When PGRs such as auxins and cytokinins are used, the number of shoots often increases. [14] According to Silva [15], the longitudinal cuts of *Melocactus glaucescens* cultured on MS medium supplemented with different concentrations of BA and NAA showed higher growth and shoot induction than the transverse cuts. When BA or kinetin (KIN) was combined with naphthalene acetic acid (NAA), BAP produced more shoots than KIN, according to an analysis of the morphogenic responses of *M. glaucescens* explants to PGRs. Nevertheless, branches with BAP concentrations of 4.44, 8.88, and 17.76  $\mu\text{M}$  differed morphologically from the control plants. According to Masakee *et al.* [16] *Haworthia* callus cultured on MS medium supplemented with 1.0 mg/L BA showed 75.00% green spot formation and 48.75 green spots. Similarly, Marhri *et al.* [17] reported that the concentration of BAP growth hormone had a direct effect on shoot proliferation, as indicated by analysis of variance, which showed significant differences in the means of the four groups. When compared to the other doses studied, BAP alone produced the most efficient number of regenerated shoots from a single wild prickly pear shoot, averaging 19.42 shoots at a concentration of 5 mg/L. Furthermore, BA hormone is crucial for plant regeneration and shoot induction, as well as for cell division and growth, lateral bud formation and development, seed germination, and protein synthesis. The effects of hormone concentration revealed that, whereas intermediate doses often permitted increased shoot formation, higher concentrations were less effective. It is well known that reactions to exogenous hormones vary depending on the species and explant source. In purple pitahaya (*Hylocereus costaricensis*), higher hormone concentrations have been shown to affect calluses while blocking shoot development. [18]. The *Turbinicarpus mombergeri* shoots were cut longitudinally, and the segments were transferred to media containing 2.22 or 4.44  $\mu\text{M}$  benzyladenine to induce shooting. The generated shoots were highly hydrated. [19] According to Huong [20], all explants in the medium culture with BA added showed improved shoot formation after 6 weeks of culture, with 100% shoot induction. As the concentration of BA grew from 0.5 to 1.0 mg/L, the number of shoots per explant and shoot height increased. However, as the concentration of BA climbed to higher concentrations (1.5 and 2.0 mg/L), it gradually declined. The trial with MS media supplemented with 1.0 mg/L BA yielded the highest number of shoots, shoot height, and shoot regeneration rate (43 shoots per explant, 9.4 mm, and 100%, respectively).

**Table 1.** Effect of different explant fragmentation and different concentrations of BA on the shoot induction of *G. mihanovichii* LB2178 Agua Dulce after 3 months of culture

| Culture medium    | Explant fragmentation    | Survival (%)        | Shoot induction (%) | Number of shoots (shoots/explant) | Shoot Length (cm) |
|-------------------|--------------------------|---------------------|---------------------|-----------------------------------|-------------------|
| MS                | Sectioned transversally  | 86.67 <sup>ab</sup> | 0.00 <sup>c</sup>   | 0.00 <sup>c</sup>                 | 0.00 <sup>c</sup> |
|                   | Sectioned longitudinally | 100.00 <sup>a</sup> | 0.00 <sup>c</sup>   | 0.00 <sup>c</sup>                 | 0.00 <sup>c</sup> |
| MS +<br>BA 1 mg/L | Sectioned transversally  | 73.33 <sup>b</sup>  | 76.67 <sup>ab</sup> | 4.20 <sup>a</sup>                 | 0.36 <sup>a</sup> |
|                   | Sectioned longitudinally | 80.00 <sup>ab</sup> | 56.57 <sup>b</sup>  | 1.33 <sup>bc</sup>                | 0.14 <sup>b</sup> |
| MS +<br>BA 2 mg/L | Sectioned transversally  | 86.67 <sup>ab</sup> | 76.67 <sup>ab</sup> | 2.20 <sup>b</sup>                 | 0.17 <sup>b</sup> |
|                   | Sectioned longitudinally | 100.00 <sup>a</sup> | 86.67 <sup>a</sup>  | 2.13 <sup>b</sup>                 | 0.19 <sup>b</sup> |
|                   | F-test                   | *                   | *                   | *                                 | *                 |
|                   | C.V.(%)                  | 36.87               | 60.57               | 14.87                             | 11.06             |

\* significant difference at  $p \leq 0.05$ **Figure 2.** Characteristics of shoot formation of *G. mihanovichii* LB2178 Agua Dulce were cultured on MS medium containing 3 different concentrations of BA after culturing for 3 months (bar = 1 cm) (A) MS: Sectioned transversally (B) MS: Sectioned longitudinally (C) MS+1 mg/L BA: Sectioned transversally (D) MS+1 mg/L BA: Sectioned longitudinally (E) MS+2 mg/L BA: Sectioned transversally (F) MS+2 mg/L BA: Sectioned longitudinally

### 3.2 Effect of different concentrations of Indole-3-butyric acid (IBA) on root induction of *G. mihanovichii* LB2178 Agua Dulce

The shoot explants from experiment 2.2 were cultured on MS medium containing 5 IBA concentrations (0, 0.5, 1, 1.5, and 2 mg/L). The result found that the shoot explant were cultured on MS medium containing concentrations of IBA (1 mg/L) gave the highest root induction, 91.67%, followed by the shoot explant were cultured on MS medium containing concentrations of IBA (0.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (1.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (2 mg/L). The shoot explants were cultured on MS medium containing

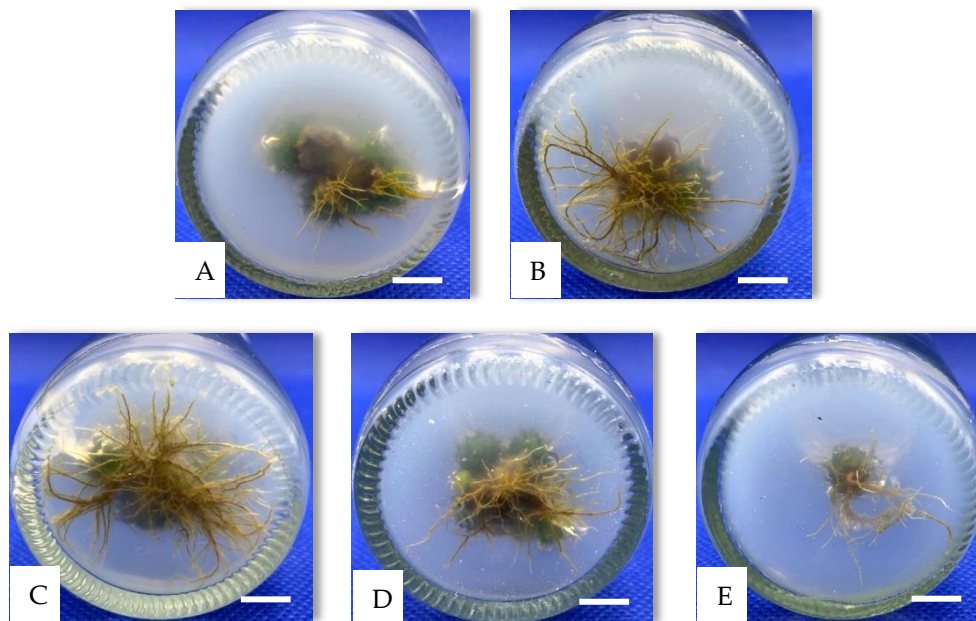
concentrations of IBA (0 mg/L), 83.33%, 81.67%, 75.00%, and 70.00%, respectively, with a significant difference at  $p \leq 0.05$ . Average number of root, the result found that the shoot explant were cultured on MS medium containing concentrations IBA (1 mg/L) gave the highest average number of root, 20.47 roots/explant, followed by the shoot explant were cultured on MS medium containing concentrations of IBA (0.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (1.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (2 mg/L). The shoot explants were cultured on MS medium containing concentrations of IBA (0 mg/L), 14.73 roots/explant, 10.47 roots/explant, 7.87 roots/explant, and 4.87 roots/explant, respectively, with a significant difference at  $p \leq 0.05$ . Average length of root, the result found that the shoot explant were cultured on MS medium containing concentrations of IBA (1 mg/L) gave the highest average length of root, 7.63 mm, followed by the shoot explant were cultured on MS medium containing concentrations of IBA (0.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (1.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (2 mg/L) and the shoot explant were cultured on MS medium containing concentrations of IBA (0 mg/L), 6.57 mm, 5.38 mm, 4.62 mm and 4.03 mm respectively, significant difference at  $p \leq 0.05$ . (Table 2 and Figure 2)

The study found that IBA concentration affects root induction in *G. mihanovichii* LB2178 Agua Dulce. Since IBA is a member of the auxin family of plant hormones and a component of many commercial horticulture plant rooting products. In addition to hastening root production, it is applied to a variety of crops to promote fruit growth and flower development. In plant tissue culture, IBA and other auxins are used to initiate root formation *in vitro*. Regarding shoot rooting, auxins are the most commonly used exogenous phytohormones for root development, and IBA is more effective than NAA alone or in combination with IBA [11]. In the current investigation, kinetin was utilized solely to direct organogenesis toward root development. The optimal outcome was achieved with kinetin at 1.5 mg/L, yielding an average of 9.08 roots. The percentage of rooted explants reached 100% when harvested, and regenerated *Selenicereus megalanthus* shoots were added to rooting media supplemented with 0.5 mg/L IBA; this percentage decreased progressively with higher IBA concentrations (1.0–2.0 mg/L). The maximum percentage of rooted explants, number of roots per explant, and root length were seen on a half-strength MS medium supplemented with 0.5, 1.0, 1.5, and 2.0 mg/L IBA. Every culture was incubated at  $25 \pm 2^\circ\text{C}$  with a 16-hour photoperiod ( $10\text{--}15 \mu\text{Em}^{-2}\text{s}^{-1}$ ). After a week of culture, IBA 1.0 mg/L showed signs of rooting. 100% of the shoots had rooted two weeks following transplant [21]. The best rooting response for the *Haworthia retusa* shoot was observed in half-strength MS medium supplemented with 0.5 mg/L IBA. This medium formulation produced the largest mean root number (5 roots/explant) and the highest rooting rate (100%) [22]. Auxins such as IBA can be added to the culture medium to aid root development in succulent plants. It is important to strike a balance between the auxin concentration and the succulent species' requirements to achieve successful rooting [23]. Furthermore, even in the absence of growth hormones, the organ developed sufficiently after three weeks, suggesting that this species does not require root development stimulation. In actuality, plants have a natural ability to regenerate missing sections after damage, and the agar media further promotes the growth of adventitious roots [24, 18].

**Table 2.** Effect of different concentrations of IBA on root induction of *G. mihanovichii* LB2178 Agua Dulce after 3 months of culture

| Culture medium    | Root induction (%)  | Average number of roots (roots/explant) | Average length of root (cm) |
|-------------------|---------------------|-----------------------------------------|-----------------------------|
| MS                | 70.00 <sup>b</sup>  | 4.87 <sup>e</sup>                       | 4.03 <sup>d</sup>           |
| MS + IBA 0.5 mg/L | 83.33 <sup>ab</sup> | 14.73 <sup>b</sup>                      | 6.57 <sup>b</sup>           |
| MS + IBA 1 mg/L   | 91.67 <sup>a</sup>  | 20.47 <sup>a</sup>                      | 7.63 <sup>a</sup>           |
| MS + IBA 1.5 mg/L | 81.67 <sup>ab</sup> | 10.47 <sup>c</sup>                      | 5.38 <sup>c</sup>           |
| MS + IBA 2 mg/L   | 75.00 <sup>ab</sup> | 7.87 <sup>d</sup>                       | 4.62 <sup>cd</sup>          |
| F-test            | *                   | *                                       | *                           |
| C.V.(%)           | 29.00               | 30.00                                   | 23.00                       |

\*significant difference at  $p \leq 0.05$



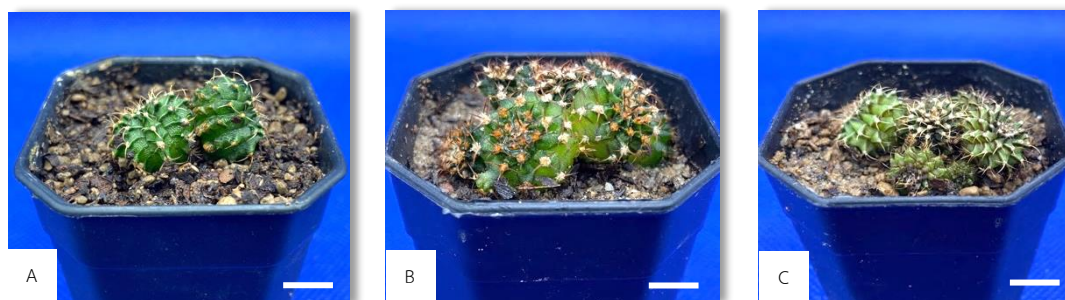
**Figure 3.** Characteristics of root formation of *G. mihanovichii* LB2178 Agua Dulce were cultured on MS edium supplemented with different concentrations of IBA after culturing for 3 months (bar = 1 cm) (A) MS (B) MS+0.5 mg/L IBA (C) MS+1 mg/L IBA (D) MS+1.5 mg/L IBA (E) MS+2 mg/L IBA

### 3.3 Acclimatization and hardening of rooted plants

After 3 months, the rooted plants were removed from the tissue culture room and placed in sterile plastic pots filled with a 1:1 mixture of three sterilized soil formulas: potting soil:pumice, potting soil: sand, or potting soil:sand:pumice. These pots were left for seven days before being moved to the greenhouse for a month of acclimatization. According to the findings, *G. mihanovichii* LB2178 Agua Dulce grown in potting soil: sand showed the highest survival rate of 100%, followed by potting soil:sand:pumice with 80% survival rate and potting soil: pumice with 75% survival rate. It was also found that a potting soil:sand ratio of 1:1 gave the highest average height of *G. mihanovichii* LB2178 Agua Dulce plants, 2.4 cm, after 2 months of growth (Figure 4). The study found that *G. mihanovichii* LB2178 Agua Dulce grown in potting soil (sand) showed the highest survival rate of 100%. This success can be ascribed to the Opuntiaceae's well-developed root structure, which permits a high capacity for rehydration. Desiccation can also be prevented by employing a progressive acclimatization technique, which involves applying a plastic cover for 2-3 weeks to raise external humidity and address anomalies. [17] According to the healthy regenerated *Caralluma diffusa* (Wight) N.E.Br. plantlets were removed from the growth tubes and placed in tiny plastic cups filled with sand, fertile soil, and vermicompost (1:1:1) in a controlled environment. The plantlets were then moved to large pots and allowed to acclimate to outdoor conditions over 4 weeks. Compared with their parents, *in vitro*-grown plants did not exhibit any discernible differences in physical traits, and over 98% of the plantlets were effectively acclimated. [25] After 4 weeks, the roots of *Selenicereus megalanthus* were removed from the culture media, properly cleaned with autoclaved, double-distilled water, and placed in sterile plastic containers with a 1:1 ratio of sterilized soil to sand. After being wrapped in polythene bags for ten to twelve days, the cups were moved to the greenhouse to acclimate. After 2 months, established plants were transferred to containers. After 2 months, 95% of the transferred plants survived. [21] In addition, because the planting material is an important factor in raising all species of cactus, "*Gymnocalycium*" requires soil with sufficient nutrients and good drainage, and the soil must not be too compact, as this can prevent the plant's roots from expanding into the bottom of the pot. Consequently, airy planting material is required. Most planters use a pot filled with crushed volcanic rock/cooking charcoal, cactus soil mixed with small volcanic rock, crab claw soil, perlite, coarse sand, Osmocote fertilizer, and Stargle. You can adjust the proportions and soil formulas to suit your specific



cultivation needs. The materials can also be adjusted according to what is available in each area. However, regardless of the formula used, the soil must drain well so it does not hold too much water [26]. According to the Digital Agriculture Database System and Sarapancactus, cacti should be planted in sandy loam soil, use fertile planting materials, or use mixed soil with abundant nutrients, with easy water flow, not water-retentive, and good moisture retention, and receive sufficient sunlight for cactus growth. This may be due to the limitation of the study period of cactus growth, so no difference in the diameter of the cactus stems was seen. This is evident in the low percentage of cactus seed survival. If the study period is extended, the percentage of survival may increase, leading to differences in the diameter of the cactus stems.



**Figure 4.** After culturing for a month, the seedlings of *G. mihanovichii* LB2178 Agua Dulce emerged in various growing media. (A) Soil: pumice ratio 1:1 (B) Soil: sand ratio 1:1 (C) Soil:sand:pumice ratio 1:1:1 (bar = 1 cm)

#### 4. Conclusions

*In vitro* methods can be used to propagate *G. mihanovichii* LB2178 Agua Dulce. The longitudinally sectioned explants cultivated on MS media containing doses of BA (0 and 2 mg/L) exhibited the best survival rate (100%) and shoot induction rate (86.67%) after 3 months of culture, according to the data. The transversally sectioned explants grown on MS medium containing concentrations of BA (1 mg/L) exhibited the greatest shoot length (0.36 cm) and shoot number (4.20 shoots/explant). The shoot explants cultivated on MS medium containing concentrations of IBA (1 mg/L) produced the maximum root induction (91.67%), average number of roots (20.47 roots/explant), and average root length (7.63 cm) after 3 months of culture. The rooted plants were taken out and placed in sterile plastic pots for acclimation and hardening. The maximum survival rate of 100% was demonstrated by *G. mihanovichii* LB2178 Agua Dulce cultivated in a potting soil: sand mixture. Additionally, these methods can help meet commercial demands, thereby lessening the strain on *G. mihanovichii* LB2178 Agua Dulce natural populations. Other possibilities for the quick growth of *G. mihanovichii* LB2178 Agua Dulce include BA and IBA hormones.

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# *In vitro* Degradability and Rumen Microbial Dynamics in Goats Supplemented with *Pleurotus* Spent Mushroom Substrate at Different Inclusion Levels

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**Abstract:** This study evaluated the effects of *Pleurotus* spent mushroom substrate (PSMS) supplementation on *in vitro* degradability and rumen microbial dynamics in goats (*Capra hircus*). It aimed to determine the degradability of PSMS and its influence on gas production, nutrient degradability, and microbial composition. A Randomized Complete Block Design was employed with treatments: T0 – 0% PSMS (control), T1 – 3% PSMS, and T2 – 6% PSMS, all mixed with Napier grass. Parameters measured included *in vitro* gas production (IVGP), dry matter degradability (IVDMD), crude protein degradability (IVCPD), neutral detergent fiber degradability (IVNDFD), and acid detergent fiber degradability (IVADFD). Data were analyzed using Analysis of Variance (ANOVA) in SAS OnDemand, with differences determined using Tukey's Honestly Significant Difference (HSD) test at  $\alpha = 0.05$ . Microbial analysis showed the highest bacterial and protozoal populations in T1, whereas fungal populations were not detected in PSMS-supplemented treatments. This observation may be associated with the presence of phenolic compounds in PSMS, which originate from lignin degradation during mushroom cultivation and have been reported to exert antimicrobial or inhibitory effects on rumen microorganisms. Methodological factors, such as sampling rumen fluid and reliance on culture-based detection, may also have influenced fungal recovery. IVGP was numerically highest in T1 (52.39 cc), indicating enhanced fermentability. During the first digestion stage, the highest IVDMD occurred in T2 (54.85%), while IVCPD peaked in T1 (60.29%) but declined in T2 (37.60%), suggesting possible inhibitory effects at higher inclusion levels. IVNDFD and IVADFD were also higher in PSMS treatments, with IVADFD showing a significant linear increase ( $p=0.0294$ ). In the second stage simulating abomasal digestion, DM degradability remained similar across treatments, while ADF degradability declined significantly in T2 ( $p=0.0035$ ). Overall, 3% PSMS improved fermentability, digestibility, and microbial activity, indicating its potential as a sustainable ruminant feed supplement.

**Keywords:** *in vitro* degradability; *in vitro* gas production; *pleurotus* spent mushroom substrates; rumen microbial analysis

## 1. Introduction

Spent mushroom substrates (SMS) are the residual materials remaining after the harvest of edible mushroom fruiting bodies. These substrates are commonly composed of lignocellulosic agricultural by-products such as rice

straw, corn cobs, cottonseed hulls, bagasse, and other fibrous materials used as growth media during mushroom cultivation [1]. After cultivation, SMS retains appreciable amounts of nutrients, including crude protein, amino acids, minerals, partially degraded lignocellulosic components, and residual fungal biomass [1, 2]. Despite their potential value, large quantities of SMS are often incinerated or improperly disposed of, contributing to environmental problems such as pollution and eutrophication [2, 3, 4]. Therefore, the valorization of SMS as livestock feed has gained increasing attention as a sustainable strategy to recycle agricultural residues while improving feed resource availability.

Among edible mushrooms, species of the genus *Pleurotus* are widely cultivated due to their strong lignocellulose-degrading capacity. These fungi produce extracellular enzymes such as laccases, manganese peroxidases, and cellulolytic enzymes that break down lignin, cellulose, and hemicellulose in the substrate during cultivation [5]. As a result, *Pleurotus* spent mushroom substrate (PSMS) often contains partially degraded fiber fractions that may enhance microbial accessibility and digestibility when used as ruminant feed. Previous studies have reported that SMS supplementation can influence rumen fermentation, gut microbial diversity, animal performance, and nutrient utilization [1, 3, 4]. However, SMS also contains bioactive compounds such as  $\beta$ -glucans, chitin, flavonoids, and phenolic compounds that may exert stimulatory or inhibitory effects on rumen microorganisms depending on their concentration [6]. These phenolic compounds originate from lignin degradation during fungal growth and may potentially influence rumen microbial populations and fermentation patterns.

Although several studies have evaluated the use of SMS in ruminant diets, limited information is available regarding the fermentability and microbial effects of *Pleurotus*-derived SMS specifically under controlled *in vitro* rumen conditions. Moreover, the optimal inclusion level of PSMS for improving rumen degradability without negatively affecting microbial activity remains unclear. Therefore, this study evaluated the effects of graded levels of PSMS supplementation (3% and 6%) on *in vitro* rumen fermentation characteristics, gas production, nutrient degradability, and microbial population dynamics in goats (*Capra hircus*). The selected inclusion levels were designed to represent low and moderate supplementation levels commonly used in exploratory feed evaluation trials. The study hypothesized that moderate inclusion of PSMS would enhance rumen fermentability and nutrient degradability by promoting partial lignocellulose degradation during mushroom cultivation.

## 2. Materials and Methods

### 2.1 Experimental Animals

The experimental animals consisted of three mature female Philippine native goats maintained at the Small Ruminant Project of Visayas State University. The animals served solely as donors of rumen fluid for the *in vitro* fermentation assays. The use of multiple donors allowed the rumen inoculum to represent biological variation among animals while maintaining consistent management conditions. All animals used in this study were handled in accordance with standard animal welfare practices and institutional guidelines for the care and use of livestock in research. The experimental procedures involved routine feeding and non-invasive rumen fluid collection, and no procedures causing pain or distress were performed.

### 2.2 Preparation of Feeding Materials

The basal feed, particularly Napier grass (*Pennisetum purpureum*), was collected from the Department of Animal Science pasture area at Visayas State University. Forage was collected every morning, chopped, and freshly fed to match the animal's voluntary feed intake. On the other hand, the SMS was obtained from a mushroom farm in Dagohoy, Bohol, Philippines. To improve the palatability of SMS, a considerable amount of molasses was added and thoroughly mixed into the feeding materials.

### 2.3 Feeding and Management of Experimental Animals

Before rumen fluid collection, the donor animals were maintained under controlled feeding conditions for a 14-day adaptation period. During this period, goats were provided Napier grass as the basal forage and small quantities of PSMS to familiarize the animals with the substrate and avoid abrupt dietary exposure. Feeding was conducted twice daily (morning and afternoon), and fresh water was provided *ad libitum*.



The adaptation period was implemented to stabilize rumen microbial activity before rumen fluid collection. However, it is acknowledged that exposure of donor animals to PSMS may influence the microbial composition of the rumen inoculum. Consequently, the results of the present study should be interpreted primarily as an evaluation of the fermentability of PSMS under controlled *in vitro* conditions rather than as a direct simulation of animals maintained on a strictly basal diet.

#### 2.4 Collection of Rumen Fluids

Rumen fluid was collected in the morning, 1 hour before feeding, to prevent contamination with saliva. The rumen fluid collection method was conducted using an oral stomach tube [7]. The rumen fluids were carefully stored in a clean, air-tight flask. A volume of 100-150 ml of rumen fluid was collected from each of the animals. Moreover, it was filtered through a 2-layer cheesecloth, followed by the determination of rumen fluid pH using a pH meter.

#### 2.5 Buffer Solution

The rumen fluids used in the *in vitro* degradability of the substrates were added with a buffer solution to neutralize the rumen fluid pH. A 1:2 ratio was used upon neutralizing the rumen fluids, and the buffer solution used was McDougall's buffer or phosphate-carbonate buffer.

#### 2.6 Two-step *in vitro* Digestibility of Spent Mushroom Substrates

Two-step *in vitro* digestibility was determined using the method of Tilley and Terry with minor modifications [8]. Ground samples (3 g) of each treatment were incubated in fermentation vials containing 10 mL rumen fluid and 20 mL McDougall's buffer solution under anaerobic conditions. Carbon dioxide was flushed into each vial before sealing to maintain anaerobiosis. The vials were incubated in a shaking water bath maintained at 39–41°C for 24 h to simulate ruminal fermentation. A total of 18 incubation vials were prepared (six per treatment). After the first incubation phase, *in vitro* gas production was measured using a glass syringe. Rumen fluid samples (2 mL) were withdrawn from selected vials for microbial analysis. Following the first digestion phase, residues were recovered for the determination of ruminal degradability parameters. The remaining samples underwent the second digestion stage using acid-pepsin solution for 48 h to simulate post-ruminal digestion. The recovered residues were oven-dried at 105°C for 12 h before determination of degradability indices, including IVDMD, IVCPD, IVNDFD, and IVADFD [8, 9].

#### 2.7 Proximate Analysis of SMS

Proximate analysis was conducted to determine the nutritional value of PSMS. Analyses on dry matter, crude protein, crude fiber, ash, and crude fat were conducted at the Visayas State University- Department of Animal Science - Animal Nutrition Laboratory.

#### 2.8 Rumen Microbial Analysis

The withdrawn rumen fluids from each vial after the first digestion phase were stored in sterile, sealed test tubes. Analysis of the rumen microbial population resulting from incubation with varying treatments was conducted through a series of laboratory processes. Hence, in the analysis of microbial population dynamics, a total of 9 rumen fluid samples from the three treatments, with three replicates each, underwent gross microscopy to determine and differentiate microbial shapes. The identified rumen bacteria were subjected to Gram staining to facilitate thorough isolation from the diverse consortium. Furthermore, the bacterial species were counted as colony-forming units (CFU/ml), while protozoal and fungal species were counted as cells/ml.

#### 2.9 Experimental Design and Treatment

The study used a Randomized Complete Block Design (RCBD), in which rumen fluid from each donor goat served as a biological block to account for variation among rumen inoculum sources. Three dietary treatments were evaluated:

T0: 0% PSMS (control; Napier grass only)

T1: 3% PSMS supplementation

T2: 6% PSMS supplementation

Each treatment was incubated in six fermentation vials, yielding 18 incubation units in total. The selected inclusion levels (3% and 6%) were chosen to represent low and moderate supplementation levels commonly used in exploratory feed evaluation studies to assess the potential effects of novel feed resources without drastically altering substrate composition.

## 2.10 Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) in SAS OnDemand, with differences determined by Tukey's Honestly Significant Difference Test (HSD) at  $\alpha=0.05$ . The present study utilized rumen fluid obtained from three donor goats, which represents a relatively small number of biological sources. While this approach is commonly used in preliminary *in vitro* fermentation studies, the limited number of donors may limit statistical power and the generalizability of the results. Furthermore, donor animals were exposed to PSMS during the adaptation period, which may have influenced the rumen inoculum's microbial composition. Future studies should consider using a larger number of donor animals maintained on a uniform basal diet to minimize potential confounding effects and improve the robustness of the findings.

## 3. Results and Discussion

### 3.1 Chemical Composition of PSMS

Displayed in Table 1 is the chemical composition of the *Pleurotus* spent mushroom substrate (PSMS). The material exhibited a relatively high dry matter content (80.38%), while crude protein (1.79%) and ether extract (4.74%) were comparatively low. Notably, the ash content reached 61.59%, which is substantially higher than values commonly reported for most forages and agricultural by-products. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were 19.77% and 18.49%, respectively.

**Table 1.** Chemical composition of PSMS

| Material | Composition (%) |      |      |       |       |       |
|----------|-----------------|------|------|-------|-------|-------|
|          | DM              | EE   | CP   | Ash   | NDF   | ADF   |
| PSMS     | 80.38           | 4.74 | 1.79 | 61.59 | 19.77 | 18.49 |

The crude protein level observed in the present study was lower than values reported in other SMS studies [10]. This difference may be attributed to the composition of the substrate materials used in the mushroom production process. In the present study, the PSMS was primarily composed of rice straw (approximately 75%), supplemented with sawdust, rice bran, molasses, and agricultural lime. These materials are inherently low in nitrogen, and the available nitrogen may have been further utilized by the growing fungal mycelium during mushroom cultivation. Previous studies have reported that nitrogen present in mushroom substrates can be incorporated into fungal biomass during fruiting body formation, thereby reducing the residual crude protein content of the spent substrate [11]. The ash content recorded in this study (61.59%) was considerably higher than values typically reported for lignocellulosic feed materials. This unusually high ash fraction may partly reflect the accumulation of inorganic residues in the substrate during mushroom cultivation. The addition of agricultural lime during substrate preparation likely increased the substrate's mineral content. At the same time, the dominance of rice straw as a substrate component may also have contributed significant silica content, which is known to occur at relatively high levels in rice straw [12]. Additionally, progressive degradation and fungal metabolism of organic matter may concentrate the remaining inorganic components in the spent substrate. However, it should be noted that the present study did not include a detailed mineral fraction analysis (e.g., silica or individual mineral determination), and therefore, the precise contributors to the elevated ash fraction remain uncertain.

The fiber fractions of PSMS were relatively low compared with those typically reported for raw lignocellulosic materials. The NDF and ADF values were also very close to each other, suggesting a minimal hemicellulose fraction (approximately 1.3%). Such a narrow difference between NDF and ADF may indicate extensive hemicellulose degradation during mushroom cultivation. *Pleurotus* species are known to secrete lignocellulolytic enzymes such as laccases, lignin peroxidases, manganese peroxidases, and cellulases, which

collectively degrade lignin and associated polysaccharides in lignocellulosic substrates [13]. Through this enzymatic process, hemicellulose and other easily degradable structural carbohydrates may be partially hydrolyzed during fungal colonization, potentially explaining the reduced fiber fractions observed in the present study. Nevertheless, the possibility of analytical variation in fiber determination cannot be completely excluded. It should also be noted that lignin concentration was not determined in the present study because acid detergent lignin (ADL) analysis was not included in the proximate evaluation. Since lignin plays a critical role in determining the structural characteristics and degradability of lignocellulosic substrates, future studies should include detailed fiber fractionation (NDF, ADF, and ADL) to characterize better the structural carbohydrate composition of PSMS and its implications for rumen fermentation.

### 3.2 Rumen Microbial Analysis

The data presented in Table 2 show the observed dynamics in rumen microbial populations following incubation with different levels of PSMS. Three major microbial groups typically present in the rumen, which include bacteria, protozoa, and fungi, were evaluated. Bacterial populations were quantified as colony-forming units (CFU/mL), whereas protozoal and fungal populations were expressed as cells/mL. Morphological observations were conducted using microscopy and Gram staining to characterize general microbial features. As shown in Table 2, bacterial populations were numerically higher than protozoal and fungal populations across treatments, consistent with the general microbial hierarchy in the rumen ecosystem, in which bacteria are the dominant microbial group. Microscopic observations indicated that most bacterial isolates were Gram-negative and predominantly rod-shaped or coccoid forms. Similar observations have been reported in rumen microbial ecology, where Gram-negative anaerobic bacteria dominate the microbial community and degrade structural and non-structural carbohydrates [14].

**Table 2.** Dynamics in rumen microbial population

| Treatment | Rumen Microorganism |                     |                  | Description                                                                                                                                   |
|-----------|---------------------|---------------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
|           | Bacteria (cfu/ml)   | Protozoa (cells/ml) | Fungi (cells/ml) |                                                                                                                                               |
| 0% PSMS   | 6267.00             | 4000.00             | 3000.00          | Bacteria: Gram-negative (long rod-shaped, short rod-shaped, coccus-shaped)<br>Protozoa: Not indicated<br>Fungi: Bi-flagellate fungi zoospores |
| 3% PSMS   | 21866.67            | 5750.00             | 0.00             | Bacteria: Gram-negative (long rod-shaped)<br>Protozoa: Not indicated<br>Fungi: Not detected                                                   |
| 6% PSMS   | 2233.33             | 3000.00             | 0.00             | Bacteria: Gram-negative (Streptobacillus) and Gram-positive<br>Protozoa: Not indicated<br>Fungi: Not detected                                 |

Numerically, the treatment receiving 3% PSMS (T1) showed the highest bacterial and protozoal counts compared with the control and the 6% supplementation level. In contrast, microbial counts declined in T2, suggesting that higher PSMS inclusion may not favor microbial proliferation under the present incubation conditions. However, it should be noted that no statistical comparisons were performed for microbial population counts; therefore, the observed differences should be interpreted only as descriptive trends rather than statistically validated treatment effects. The absence of detectable fungal cells in T1 and T2 may be associated with several factors. One possible explanation is the presence of phenolic compounds in PSMS. Phenolic metabolites can be generated during lignin degradation by ligninolytic enzymes such as laccases and peroxidases produced by *Pleurotus* species during mushroom cultivation [15, 16]. Phenolic compounds have been reported to exert antimicrobial effects and may influence rumen microbial populations under certain conditions [17, 18]. Nevertheless, this explanation remains speculative, as phenolic concentrations were not quantified in the present

study. Alternative explanations must also be considered. Rumen anaerobic fungi are known to strongly attach to feed particles during colonization and fiber degradation [19, 20]. Since rumen fluid samples were collected from the liquid phase of the incubation vials while avoiding feed residues, fungal structures attached to the substrate particles may not have been adequately captured during sampling. Consequently, the apparent absence of fungi in PSMS treatments may reflect sampling limitations rather than the complete absence of fungal populations.

It is also important to acknowledge that microbial characterization in this study relied exclusively on culture-dependent techniques, including colony counting, Gram staining, and microscopic observation. While these methods provide useful preliminary information on culturable microbial populations, they capture only a small fraction of the total rumen microbiota, as a large proportion of rumen microorganisms are not readily culturable under standard laboratory conditions. Therefore, the microbial results presented here should be interpreted with caution, and future studies employing culture-independent molecular techniques (e.g., 16S rRNA sequencing or metagenomic analysis) would provide a more comprehensive characterization of microbial community dynamics associated with PSMS supplementation. Overall, the observed microbial patterns suggest that moderate PSMS inclusion (3%) may support microbial proliferation under *in vitro* fermentation conditions, possibly due to the availability of partially degraded lignocellulosic components. However, given the methodological limitations described above, these findings should be regarded as preliminary observations requiring confirmation through more comprehensive microbial analyses.

### 3.3 *In vitro* Gas Production (IVGP)

Presented in Table 3 are the *in vitro* gas production (IVGP) values obtained from the different treatments after the first digestion phase. The control treatment (T0) produced a mean gas volume of 46.90 cc, while treatments supplemented with PSMS produced slightly higher gas volumes of 52.39 cc (T1) and 51.54 cc (T2). Statistical analysis indicated no significant differences among treatments ( $p=0.4542$ ). Linear and quadratic contrasts were also not significant.

**Table 3.** *In vitro* gas production

| Parameter | Treatment |         |         | SEM    | p-value | p-value |           |
|-----------|-----------|---------|---------|--------|---------|---------|-----------|
|           | 0% PSMS   | 3% PSMS | 6% PSMS |        |         | Linear  | Quadratic |
| IVGP (cc) | 46.90     | 52.39   | 51.54   | 3.6421 | 0.4542  | 0.3359  | 0.4377    |

*In vitro* gas production is commonly used as an indirect indicator of rumen substrate fermentability and microbial activity, as gas is produced during microbial degradation of carbohydrates and other fermentable feed components [21]. Although the treatments supplemented with PSMS showed numerically higher gas production compared to the control, these differences were not statistically significant, indicating that the inclusion of PSMS at 3% and 6% did not significantly alter fermentation activity under the conditions of this experiment. Previous studies have reported an increase in *in vitro* gas production from lignocellulosic substrates treated with *Pleurotus* species, attributed to fungal degradation of structural carbohydrates, such as cellulose, hemicellulose, and lignin, during solid-state fermentation [21, 22]. Such degradation can increase microbial accessibility to fermentable substrates. However, in the present study, the lack of significant treatment effects suggests that PSMS supplementation at the evaluated inclusion levels did not substantially modify the fermentability of the basal diet. Gas produced during *in vitro* fermentation generally consists primarily of carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>), which are generated during microbial fermentation processes in the rumen. While higher gas production may reflect increased fermentative activity, *in vitro* gas volume alone does not distinguish between CO<sub>2</sub> and methane production and therefore cannot be directly interpreted as an indicator of enteric methane emissions without additional measurements. Consequently, the environmental implications of the observed gas production in the present study remain uncertain and would require further investigation using methods specifically designed to quantify methane production.

### 3.4 *In vitro* Dry Matter Degradability (IVDMD)

Table 4 presents the IVDMD of the treatments at the 24-hour *in vitro* 1st-stage nutrient digestibility. The results show no significant differences among the treatments ( $p=0.6988$ ), with IVDMD ranging from 51%

to 54%. These results suggest that treatments have a similar degree of DM degradability and are sufficiently degradable by rumen microbes. Although statistically the same, T2 showed a slightly higher IVDMD (54.58%), followed by T1 (51.59%) and T0 (51.45%). The increase in IVDMD with PSMS inclusion may be attributed to its pre-digested nature, as indicated by its NDF and ADF values, which have been degraded by fungal biomass. Residual ligninolytic enzymes present in PSMS [23] may also have contributed to increased substrate accessibility for microbial degradation. Overall, the results suggest that PSMS supplementation did not significantly affect DM degradability of the basal feed. The second-stage IVDMD, simulating abomasal digestion (Table 5), also showed no significant differences ( $p=0.8061$ ). In this stage, T0 had the highest IVDMD (65.30%), followed by T1 (62.54%) and T2 (59.26%), indicating that overall DM degradability remains comparable across treatments after the acid-pepsin phase.

### 3.5 *In vitro* Crude Protein Degradability (IVCPD)

The IVCPD of the first-stage *in vitro* digestion (Table 4) was highest in T1 (60.29%), followed by T0 (40.07%) and T2 (37.60%). The lower IVCPD in T2 than in T1 may be associated with a higher inclusion rate of PSMS. Lignocellulosic residues such as spent mushroom substrate may contain bioactive compounds, including phenolic constituents derived from plant materials or fungal metabolism, which have been reported to interact with digestive enzymes and potentially influence protein degradability. However, the phenolic content of the PSMS used in this study was not determined; therefore, this explanation should be considered speculative and requires further investigation. After the second-stage *in vitro* digestion (Table 5), IVCPD values were relatively stable across treatments (~53–55%), reflecting the effect of pepsin and HCl on enzymatic digestion at the abomasal phase. The observed pattern suggests that crude protein degradability in the substrates is relatively robust to PSMS inclusion, even though structural fiber fractions may vary.

### 3.6 *In vitro* Neutral Detergent Fiber Degradability (IVNDFD)

The IVNDFD (Tables 4 and 5) was slightly lower in the first stage (rumen simulation) than in the second stage. No significant differences were observed (1st stage  $p=0.7795$ ; 2nd stage  $p=0.6530$ ). Although statistically the same, T1 showed higher IVNDFD in both stages (30.78% in stage 1 and 38.69% in stage 2) than T0 (21.05% and 26.52%) and T2 (22.30% and 22.26%). Higher degradability during the first stage can be attributed to active rumen microbial fermentation before the addition of HCl and pepsin. The inclusion of PSMS, with its partially degraded fiber components, likely increased the accessibility of cellulose and hemicellulose to microbial breakdown, enhancing NDF degradability. In the second stage, the addition of acid-pepsin may have contributed to slightly lower fiber degradability due to partial degradation of microbial cells.

### 3.7 *In vitro* Acid Detergent Fiber Degradability (IVADFD)

First-stage IVADFD (Table 4) showed a non-significant trend ( $p=0.0525$ ) but a significant linear increase with PSMS inclusion: T0 = 45.46%, T1 = 46.34%, T2 = 54.84%. This may reflect the effect of residual ligninolytic enzymes in PSMS, which degrade lignified fiber fractions, thereby increasing ADF digestibility during ruminal simulation [22]. In contrast, second-stage IVADFD (Table 5) showed a significant quadratic effect ( $p=0.0035$ ), with T0 = 61.33%, T1 = 60.33%, and T2 = 43.33%. The decline in IVADFD at the highest PSMS inclusion may be related to bioactive compounds present in PSMS that interact with fiber-degrading enzymes under acidic conditions. However, the exact mechanism remains hypothetical due to the absence of measured phenolic content. Differences in the simulated digestive environments may explain the contrasting patterns across stages. The first stage represents ruminal fermentation under near-neutral pH (~6.8), where microbial cellulolytic activity predominates, and PSMS inclusion may improve fiber accessibility. The second stage simulates abomasal digestion under acidic conditions (pH 2–4), where enzyme activity and interactions with potential bioactive compounds may differ, leading to decreased ADF degradation at higher PSMS inclusion levels. Finally, the reduction in second-stage IVADFD at T2 may contribute to the slight numerical decline in second-stage IVDMD, since the ADF fraction represents the more resistant structural components of plant cell walls. In contrast, IVCPD remained relatively stable across treatments, indicating that the protein fraction was less affected by changes in structural fiber degradation.



**Table 4.** 1<sup>st</sup> stage *in vitro* nutrient degradability of substrates

| Parameter (%) | Treatment |         |         | SEM     | p-value | p-value |           |
|---------------|-----------|---------|---------|---------|---------|---------|-----------|
|               | 0% PSMS   | 3% PSMS | 6% PSMS |         |         | Linear  | Quadratic |
| DM            | 51.45     | 51.59   | 54.85   | 3.0677  | 0.6988  | 0.4778  | 0.6987    |
| CP            | 40.07     | 60.29   | 37.60   | 0.2845  | <0.0001 | 0.0004  | <0.0001   |
| NDF           | 21.05     | 30.75   | 22.30   | 10.4559 | 0.7795  | 0.9350  | 0.5096    |
| ADF           | 45.46     | 46.34   | 54.84   | 2.0750  | 0.0525  | 0.0294  | 0.1946    |

**Table 5.** 2<sup>nd</sup> stage *in vitro* nutrient degradability of substrates

| Parameter (%) | Treatment |         |         | SEM     | p-value | p-value |           |
|---------------|-----------|---------|---------|---------|---------|---------|-----------|
|               | 0% PSMS   | 3% PSMS | 6% PSMS |         |         | Linear  | Quadratic |
| DM            | 65.30     | 62.54   | 59.26   | 6.9175  | 0.8061  | 0.5373  | 0.9747    |
| CP            | 55.48     | 52.95   | 55.38   | 6.9142  | 0.9584  | 0.9918  | 0.7842    |
| NDF           | 26.52     | 38.69   | 22.65   | 12.1485 | 0.6530  | 0.8325  | 0.3967    |
| ADF           | 61.33     | 60.33   | 43.33   | 18.7098 | 0.0035  | 0.0021  | 0.0221    |

#### 4. Conclusions

The study indicates that supplementation of PSMS at moderate levels (3% of the diet) does not significantly alter overall ruminal fermentation, *in vitro* gas production, or nutrient degradability in goats. While some numerical trends, such as slightly higher bacterial and protozoal counts and marginal increases in IVGP and degradability parameters at 3% PSMS, were observed, these differences were not statistically significant. Therefore, claims of enhanced fermentation or nutrient utilization based solely on numerical trends should be interpreted cautiously. Notably, the second-stage IVADFD showed a significant decrease at the highest PSMS inclusion (6%), suggesting that higher levels of PSMS may reduce the degradability of structural fiber components, potentially due to bioactive compounds such as phenolics. This is consistent with the observed absence of rumen fungi at 6% PSMS, indicating that excessive inclusion may adversely affect specific microbial groups involved in fiber degradation. Based on these findings, PSMS supplementation up to 3% of the diet appears safe and unlikely to affect rumen microbial populations or nutrient degradability negatively, whereas higher levels may inhibit fiber-degrading microbes and enzymatic activity. The mineral-rich composition and partially pre-digested nature of PSMS make it a potentially valuable feed ingredient, but care should be taken to avoid exceeding the suggested inclusion rate. Overall, PSMS can be considered a supplemental feed resource for small ruminants at moderate inclusion levels. Its effects on rumen fermentation, microbial dynamics, and fiber degradability warrant further investigation, particularly with direct measurements of phenolic compounds and their interaction with rumen microbial enzymes.

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# Whole-Genome Analysis of MR219 and Its Drought-Tolerant Mutant, NMR151 to Elucidate Drought Resistance Mechanisms

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**Abstract:** Background: Drought is among the most severe abiotic stresses limiting global rice (*Oryza sativa* L.) productivity and threatening food security, particularly in high-yielding but stress-sensitive cultivars such as MR219. Understanding the genomic basis of drought tolerance is essential to support the development of resilient rice varieties. This study aimed to identify genome-wide variants and drought-responsive genes associated with induced drought tolerance in the mutant line NMR151 derived from MR219. Methods: Whole-genome resequencing was performed on the drought-tolerant mutant NMR151 and its parental line MR219. High-quality paired-end reads totaling 92.1 million (MR219) and 78.5 million (NMR151) were mapped to the *O. sativa* cv. Nipponbare reference genome with an alignment efficiency of 91.4% and coverages of 35.4× and 29.6×, respectively. Variant calling, functional annotation using SnpEff, and visualization through the Integrative Genomics Viewer (IGV) were employed to detect and characterize single-nucleotide polymorphisms (SNPs) and insertions/deletions (InDels). Results: A total of 4.27 million and 4.18 million polymorphisms were identified in MR219 and NMR151, dominated by SNPs and InDels with a transition-to-transversion ratio of 2.4. Comparative analysis revealed 3.01 million shared and 1.17 million unique SNPs in NMR151, indicating mutation-driven allelic diversification. Most variants occurred in intergenic (33.2 %), upstream (29.1 %), and downstream (24.6 %) regions, while six drought-responsive genes (*OsDREB2A*, *OsNAC6*, *OsLEA3-1*, *OsAPX2*, *OsCPK21*, and *OsP5CS1*) were uniquely mutated in NMR151. Conclusions: The drought resilience of NMR151 results primarily from regulatory fine-tuning rather than extensive coding alterations. These findings provide valuable molecular insights and genomic markers for breeding climate-resilient rice varieties through mutation and marker-assisted selection.

**Keywords:** Drought-responsive genes; MR219; NMR151; SNPs; whole-genome resequencing

## 1. Introduction

Rice (*Oryza sativa* L.) belongs to the kingdom Plantae, order Poales, family Poaceae, sub-family Oryzoideae, and genus *Oryza*. It is the staple food

for more than half of the world's population, particularly in Asia, making its reliable and sustainable production critical for global food security [1]. Globally, rice is cultivated on over 160 million hectares and produces more than 500 million metric tons annually, with Asia accounting for nearly 90 % of both production and consumption [2]. The relatively compact and well-annotated rice genome has established it as a model cereal species for genomics research, enabling unprecedented advances in functional genetics and molecular breeding [3]. Nevertheless, rice production is continually threatened by abiotic stresses such as drought, salinity, and cold, as well as by biotic pressures from pests, weeds, and pathogens. Among these, drought is one of the most severe abiotic constraints; recent reviews estimate yield losses of 20–30 % or more in rain-fed systems, and this threat is increasing under climate change scenarios [4, 5]. In Malaysia, drought stress is a recurrent challenge, particularly within granary regions such as Kedah, Perlis, and Kelantan, where seasonal water shortages have led to major reductions in grain yield and crop failure during critical reproductive stages [6]. The recurrent drought events associated with climate change pose a rising threat to Malaysia's rice self-sufficiency (currently around 70 %) [7].

To safeguard rice production, the development of drought-tolerant varieties has become a national priority. However, conventional breeding approaches face limitations when addressing drought tolerance, a complex, quantitatively inherited trait strongly influenced by genotype  $\times$  environment interactions [8,9]. Although marker-assisted breeding has been deployed to pyramid quantitative trait loci (QTLs) associated with drought tolerance (e.g., qDTYs) into elite cultivars, the process is often slow, resource-intensive, and yields only partial gains under severe field conditions [10]. These challenges underscore the need for complementary approaches, such as mutation breeding, to generate novel allelic variation within well-adapted genetic backgrounds without disrupting their elite traits. In Malaysia, gamma irradiation was applied to the widely grown high-yielding cultivar MR219 to induce new genetic variation. This effort produced promising mutant lines, including NMR151 (MR219-4) and NMR152 (MR219-9), which demonstrated enhanced tolerance to drought stress in greenhouse and field evaluations [11]. Among these, NMR151 stands out for its superior agronomic performance, including enhanced tillering, more panicles per plant, and higher grain yield potential, with reported yields reaching  $\approx 15 \text{ t ha}^{-1}$  under optimized conditions, compared with the national average of  $\approx 4.2 \text{ t ha}^{-1}$  [12, 13]. NMR151 has been officially registered (Plant Breeder's Certificate PBR0159, 2020), highlighting its agronomic and economic importance.

Despite these advances, the molecular mechanisms conferring drought tolerance in NMR151 remain poorly understood. With the advent of next-generation sequencing (NGS), whole-genome resequencing (WGRS) has enabled the genome-wide identification of single-nucleotide polymorphisms (SNPs), insertions/deletions (InDels), and structural variants, which are crucial resources for dissecting complex traits such as drought tolerance [14, 15]. Recent bioinformatic studies of drought-tolerant versus susceptible rice genotypes have identified differential variant and regulatory profiles, particularly in promoter regions, transcription-factor binding sites, and cis-regulatory elements [16]. However, a comprehensive genome-wide characterization of drought-associated variants in the MR219/NMR151 mutant background is still lacking. Therefore, this study aims to perform a whole-genome comparative analysis of MR219 and its drought-tolerant mutant NMR151. Specifically, we seek to (i) identify high-confidence SNPs and InDels associated with drought tolerance, (ii) annotate functional variants linked to drought-response pathways, and (iii) provide molecular resources to enhance marker-assisted and genomic selection for drought-resilient rice improvement in Malaysia. Collectively, our findings will elucidate the genetic basis of induced drought tolerance in NMR151 and contribute to the sustainable development of climate-resilient rice cultivars.

## 2. Materials and Methods

### 2.1 Plant material and DNA extraction

Seeds of the parental cultivar MR219 (a widely grown high-yielding Malaysian indica rice) were obtained from the Rice Genebank of the Malaysian Agricultural Research and Development Institute (MARDI). The Malaysian Nuclear Agency supplied seeds of the gamma-irradiation-derived drought-tolerant mutant line NMR151. Both genotypes were germinated under controlled growth chamber conditions (28 °C/16 h light – 24 °C/8 h dark, 70% relative humidity). Genomic DNA was extracted from young leaf tissue harvested



from 14–21-day-old seedlings using the QIAGEN DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's protocol. DNA quality and concentration were assessed with a NanoDrop spectrophotometer (Thermo Scientific, USA) and quantified using a Qubit fluorometer (Thermo Fisher, USA). DNA integrity was confirmed by electrophoresis on a 1% agarose gel.

## 2.2 Whole-genome sequencing

High-quality genomic DNA from each genotype (MR219 and NMR151) was submitted to Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) for paired-end sequencing. Libraries were prepared using the Illumina DNA Prep Kit (Illumina, USA) with an average insert size of ~350 bp. Sequencing was conducted on an Illumina NovaSeq 6000 platform to generate 150 bp paired-end reads, targeting ~30× depth per genotype. Raw reads were delivered in FASTQ format and are deposited in the NCBI Sequence Read Archive (SRA) under accession numbers SRR21043964 and SRR20995190 [17].

## 2.3 Read preprocessing and mapping

Adaptor trimming and quality filtering were performed using Trimmomatic v0.39 (leading/trailing: 3; sliding-window: 4:30; minlen: 50) [18]. Reads with Phred quality score < 30 or containing > 10% ambiguous bases (N) were removed. Cleaned reads were aligned to the reference genome of *Oryza sativa* ssp. japonica (IRGSP-1.0, Nipponbare; RefSeq: GCF\_001433935.1) using BWA-MEM v0.7.17 [19]. SAM files were converted to sorted BAM and indexed using SAMtools v1.14 [20]. Duplicate reads were marked with Picard v2.27.1 (Broad Institute). Mapping statistics (total reads, mapped reads, properly paired reads, singleton reads, and coverage) were calculated using SAMtools flagstat and coverage metrics (see Section 3.1 for details).

## 2.4 Variant calling and filtering

Variant detection (SNPs and small InDels) was performed using the Genome Analysis Toolkit (GATK v4.3.0; HaplotypeCaller) and BCFtools v1.14 (mpileup + call) to provide cross-validation of calls [21,22]. Raw variant calls from both pipelines were filtered using the following thresholds: minimum read depth (DP) ≥ 10, mapping quality (MQ) ≥ 30, variant quality (QUAL) ≥ 50, genotype quality (GQ) ≥ 20, and allelic support (alternate-allele ratio) ≥ 0.9. Variants concordant between both pipelines were retained for downstream analysis. Variant positions were output in VCF format and converted to tabular form using custom Python scripts. The number of SNPs and InDels, along with size distributions and substitution types, were summarized (see Sections 3.2 and 3.3).

## 2.5 Functional annotation of variants

Variants passing quality filters were annotated using ANNOVAR [23] or SnpEff v5.0 [24] against rice gene models from RAP-DB [3] and MSU Rice Genome Annotation Project v7.0. Variants were classified by genomic context (exonic, intronic, UTR, upstream, downstream, intergenic) and by effect (synonymous, missense, nonsense, frameshift, splice region). Identified variants were also cross-referenced with known drought-tolerance quantitative trait loci (qDTY) and candidate gene lists derived from recent resequencing studies of drought- and salt-tolerant rice [25, 26]. Summary statistics and high-impact variant counts are reported in Section 3.5.

## 2.6 Candidate drought-response gene annotation and visualization

To identify potentially functional polymorphisms within drought-responsive genes, annotated VCFs and the corresponding BAM alignments were visualized in the Integrative Genomics Viewer (IGV v2.12.3) [27]. Gene loci of interest (e.g., DREB, NAC, bZIP transcription factor families; osmoprotectant biosynthesis enzymes; ABA signaling components) were queried within the RAP-DB and Gramene databases for variant presence or absence between genotypes. Unique SNPs and InDels identified in NMR151 (absent in MR219) were flagged as candidate functional polymorphisms and further categorized (Section 3.6).

# 3. Results and Discussion

## 3.1 Whole-Genome Read Mapping and Variant Detection

After quality filtering, a total of 92.1 million reads for MR219 and 78.5 million reads for NMR151 were obtained and successfully aligned to the *Oryza sativa* cv. Nipponbare reference genome, achieving mapping

efficiencies of 91.9% and 91.5%, with mean coverages of 35.4× and 29.6×, respectively. These high mapping rates indicate robust sequencing quality and reliable genome representation in both genotypes (Table 1). Such depth and accuracy are comparable to those reported in high-coverage rice resequencing projects [14,4], ensuring sufficient sensitivity for variant discovery.

**Table 1.** Mapping statistics of short reads from MR219 and NMR151 mutant rice varieties aligned to the *Oryza sativa* Nipponbare reference genome.

| Reference genome               | Mapping statistics  | MR219      | NMR151     |
|--------------------------------|---------------------|------------|------------|
| <i>Oryza sativa</i> Nipponbare | Total reads         | 92,097,778 | 78,541,072 |
|                                | Mapped reads        | 90,190,469 | 76,295,108 |
|                                | Properly paired     | 84,659,706 | 71,826,370 |
|                                | Singleton           | 5,404,008  | 3,947,014  |
|                                | Sequencing coverage | 35.4       | 29.6       |

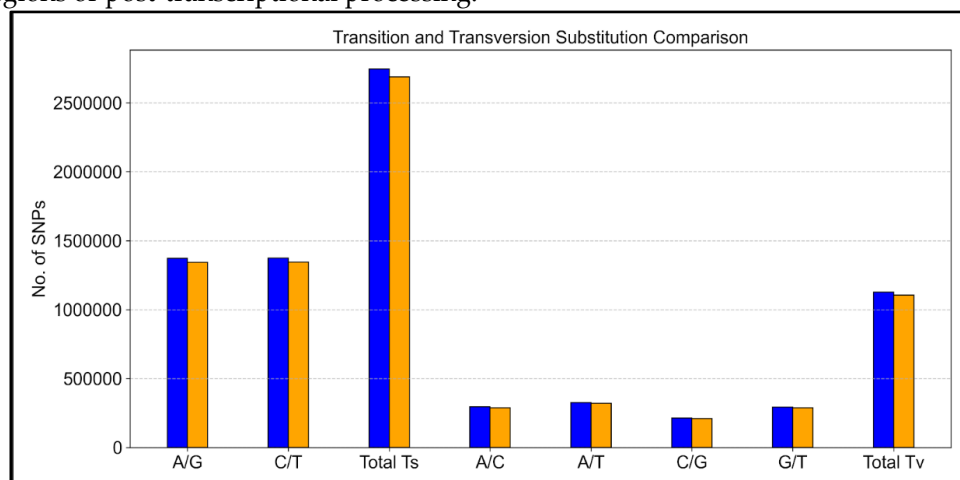
Variant calling revealed 4.27 million and 4.18 million polymorphisms in MR219 and NMR151, respectively, dominated by single-nucleotide polymorphisms (SNPs) and short insertions/deletions (InDels) (Table 2). The slightly lower variant density in NMR151 suggests that gamma irradiation induced localized point mutations without large-scale genomic rearrangements. The observed transition-to-transversion ratio (Ts/Tv  $\approx$  2.4) for both genomes further confirms high variant call quality, consistent with typical values for well-assembled *Oryza sativa* datasets [14].

**Table 2.** Number of SNPs and INDELs identified in MR219 and NMR151 mutant rice varieties.

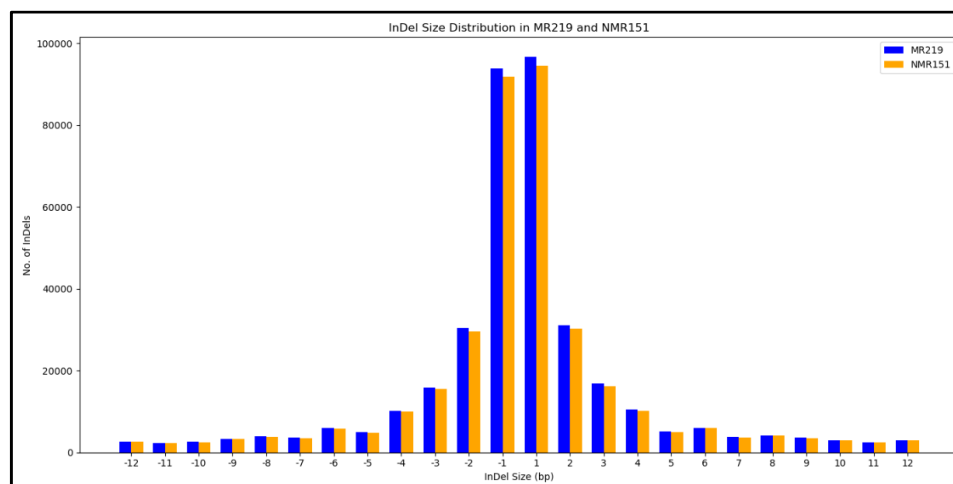
| Reference genome               | Variant    | MR219     | NMR151    |
|--------------------------------|------------|-----------|-----------|
| <i>Oryza sativa</i> Nipponbare | SNPs       | 3,864,470 | 3,785,451 |
|                                | Insertions | 195,700   | 190,000   |
|                                | Deletions  | 211,500   | 207,700   |
|                                | Total      | 4,271,679 | 4,183,151 |

### 3.2 Variant Distribution and Functional Implications

Both genotypes exhibited comparable SNP and InDel distributions when aligned to the reference genome (Figures 1 and 2). Transitions (A $\leftrightarrow$ G and C $\leftrightarrow$ T) were more frequent than transversions (A $\leftrightarrow$ C, A $\leftrightarrow$ T, C $\leftrightarrow$ G, G $\leftrightarrow$ T), exceeding 2.7 million substitutions per genome. The InDel length spectrum ranged from -12 bp to +12 bp, with 1 bp InDels most abundant—a typical pattern previously reported in rice resequencing studies [14]. NMR151 showed a slightly higher proportion of short deletions (-1 to -3 bp), which may influence gene-regulatory regions or post-transcriptional processing.

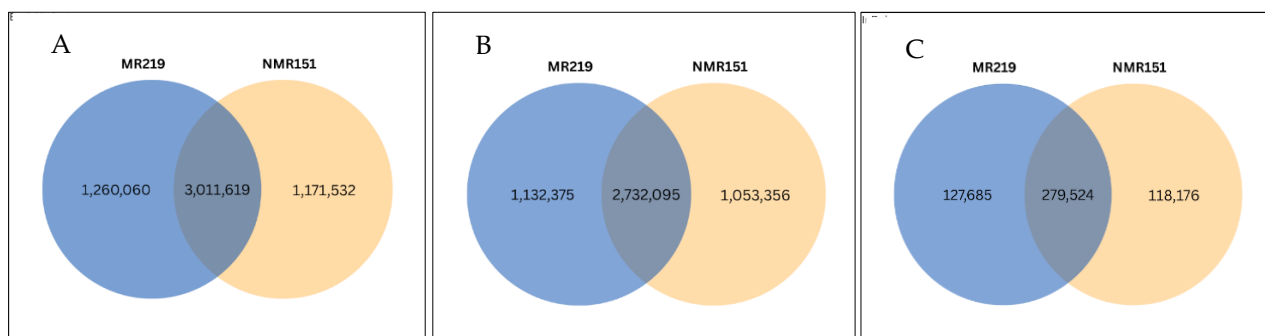


**Figure 1.** Distribution of nucleotide substitution types in MR219 (blue) and NMR151 (orange) aligned to the *Oryza sativa* Nipponbare reference genome. Transition substitutions (A $\leftrightarrow$ G, C $\leftrightarrow$ T) predominated over transversions, resulting in a Ts/Tv ratio of approximately 2.4 in both genotypes.



**Figure 2.** The graph shows the number of InDels ranging from -12 bp to +12 bp in the MR219 and NMR151 Mutant varieties using the *Oryza sativa* Nipponbare reference genome.

Comparative analysis revealed 3.01 million SNPs shared by both genomes, 1.26 million unique to MR219, and 1.17 million unique to NMR151, while 279,524 InDels were common, with 127,685 and 118,176 unique to MR219 and NMR151, respectively (Figure 3). The high proportion of shared variants confirms the genetic closeness of the mutant to its parental line, whereas the presence of private variants in NMR151 indicates irradiation-driven allelic diversification rather than hybridization-derived divergence [11].



**Figure 3.** Number of shared variants (A), unique SNPs (B), and unique InDels (C) between MR219 and NMR151 aligned to the *Oryza sativa* Nipponbare reference genome

Functional annotation using SnpEff detected 78,341 high-confidence variants. The majority occurred in intergenic (33.2 %), upstream (29.1 %), and downstream (24.6 %) regions, with only 2.7 % in exons. Among exonic variants, missense (0.86 %), synonymous (0.76 %), and splice-region (0.11 %) substitutions were most common. This regulatory-region predominance supports the view that drought adaptation in rice is largely governed by cis-regulatory modulation rather than pervasive coding-sequence changes [15,4]. Previous greenhouse and field evaluations demonstrated that NMR151 maintained higher grain yield, tiller number, and survival rate under reproductive-stage drought compared to MR219 [11]. Physiological assessments showed improved relative water content and osmotic adjustment capacity, supporting its enhanced drought resilience.

### 3.3 Identification of Drought-Responsive Genes

Integrative Genomics Viewer (IGV) analysis identified six drought-responsive genes harboring unique polymorphisms in NMR151: *OsDREB2A*, *OsNAC6/SNAC2*, *OsLEA3-1*, *OsAPX2*, *OsCPK21*, and *OsP5CS1* (Table 3). These genes represent complementary roles in transcriptional regulation, antioxidative defense, signal transduction, and osmoprotectant biosynthesis.

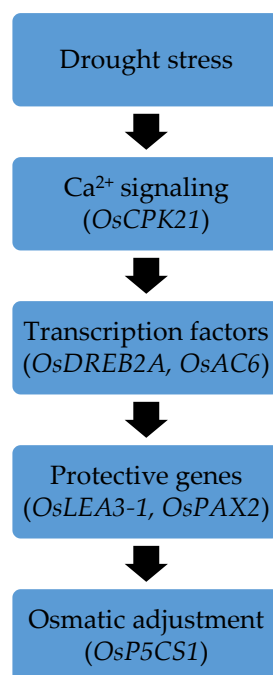
**Table 3.** Functional effects of selected high- and moderate-impact variants predicted by SnpEff in MR219 and NMR151.

| Gene ID             | Variant type | Chromosome position      | Predicted effect | Functional class | Remarks                                                                                                                                                                                                                                                                                                                     |
|---------------------|--------------|--------------------------|------------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Os01g0718300</i> | SNP          | Chr 1: 28,450,376 (A→G)  | Missense         | Moderate impact  | Alters AP2/ERF DNA-binding domain in <i>OsDREB2A</i>                                                                                                                                                                                                                                                                        |
| <i>Os03g0327800</i> | SNP          | Chr 3: 9,612,844 (C→T)   | Missense         | Moderate impact  | Substitution in the NAC-domain region of <i>OsNAC6</i>                                                                                                                                                                                                                                                                      |
| <i>Os02g0782500</i> | SNP          | Chr 2: 31,040,782 (G→A)  | Synonymous       | Low impact       | Silent mutation in the <i>OsLEA3-1</i> coding sequence                                                                                                                                                                                                                                                                      |
| <i>Os05g0477700</i> | InDel        | Chr 5: 23,511,904 (+T)   | Frameshift       | High impact      | Causes reading-frame change in <i>OsCPK21</i>                                                                                                                                                                                                                                                                               |
| <i>Os09g0527200</i> | SNP          | Chr 9: 17,788,605 (C→A)  | Missense         | Moderate impact  | Substitution in the catalytic domain of <i>OsP5CS1</i>                                                                                                                                                                                                                                                                      |
| <i>Os12g0630200</i> | SNP          | Chr 12: 19,501,342 (G→T) | Stop-gained      | High impact      | A premature stop codon was detected in <i>OsAPX2</i> . While such mutations are typically associated with loss-of-function, compensatory upregulation of paralogous APX genes or altered regulatory feedback may contribute to stress adaptation. Functional validation is required to clarify its biological consequences. |

*OsDREB2A* and *OsNAC6* act as key transcriptional hubs controlling ABA-independent and ABA-dependent drought responses, respectively [28,4]. *OsLEA3-1* and *OsAPX2* contribute to stress protection through desiccation tolerance and reactive oxygen species (ROS) detoxification [29,30]. *OsCPK21* functions in Ca<sup>2+</sup>-mediated ABA signaling, whereas *OsP5CS1* catalyzes proline biosynthesis for osmotic adjustment. The presence of NMR151-specific mutations in these loci indicates potential functional modulation; however, further transcriptomic, physiological, and functional validation studies are required to confirm their direct contribution to drought tolerance.

### 3.4 Integrative Interpretation and Breeding Implications

The combined variant analyses and functional annotation reveal that NMR151's enhanced drought tolerance is mainly derived from transcriptional and regulatory fine-tuning rather than large-scale coding disruptions. The prevalence of non-coding variants supports adaptive modulation of gene expression, while the identified genes form a coherent stress-response cascade encompassing perception (*OsCPK21*), regulation (*OsDREB2A*, *OsNAC6*), protection (*OsLEA3-1*, *OsAPX2*), and osmotic balance (*OsP5CS1*). These results complement previous genomic and QTL-based studies, which show that stable yield under drought is achieved through the integration of multiple regulatory networks [10,26]. The precise identification of candidate polymorphisms in an elite Malaysian background extends current drought-genomics knowledge and provides immediate targets for marker-assisted and haplotype-informed breeding. Future research incorporating transcriptomic and methylomic analyses, along with CRISPR or near-isogenic line validation, will further elucidate the causal role of these allelic variants in enhancing drought resilience. Gamma irradiation typically induces point mutations and small insertions/deletions rather than large chromosomal rearrangements. Some mutations may be neutral or even mildly deleterious; therefore, the drought-tolerant phenotype of NMR151 likely results from a combination of beneficial regulatory modifications and background genomic buffering. The proposed interactions among the key drought-responsive genes identified in this study are summarized in Figure 4.



**Figure 4.** Proposed regulatory framework of drought tolerance in NMR151. Drought stress triggers Ca<sup>2+</sup>-mediated signaling (OsCPK21), which activates key transcription factors (OsDREB2A, OsNAC6), leading to induction of protective genes (OsLEA3-1, OsAPX2) and osmotic adjustment via proline biosynthesis (OsP5CS1). The model represents a conceptual interpretation based on genomic variant analysis.

#### 4. Conclusions

Whole-genome resequencing of the drought-tolerant mutant NMR151 and its parental cultivar MR219 identified over 4.2 million high-quality variants, revealing irradiation-induced allelic diversification associated with drought resilience. Six drought-responsive genes (OsDREB2A, OsNAC6, OsLEA3-1, OsAPX2, OsCPK21, OsP5CS1) carried unique mutations in NMR151, highlighting key roles in transcriptional regulation, signaling, and oxidative protection. The data suggest that drought tolerance in NMR151 may be associated with regulatory fine-tuning of stress-responsive pathways, although functional validation is necessary to confirm causative relationships.

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**Author Contributions:** Conceptualization, writing—original draft preparation, Hasan, N.A; methodology and software, Kamaruddin, A.A and Kamaruzzaman, A.S.; Ahmad, F.; supervision and validation, Frary, A.; Hasan, A.A.; Harun, A.R.

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# Factors Influencing the Use of Herbal Medicines in Public Health

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**Abstract:** To foster independence and cut costs, Thai traditional medicine and herbs are becoming more well-known within Thailand's healthcare system. Nonetheless, Pathum Thani Province's use of herbal medicines remains lower than the national average, highlighting a discrepancy between policy and reality, especially in areas such as Keha Rangsit Khlong Hok. Factors associated with the use of herbal medicines for self-care include personal factors, predisposing factors, enabling factors, and reinforcing factors. A survey study was carried out. A survey with an Item-Objective Congruence index of 0.92 was used to randomly select 318 residents (aged 20–59) from Keha Rangsit Khlong Hok, Pathum Thani Province. Chi-square tests and descriptive statistics were used to assess the data; (3) Results: Use of herbal medicine was low (mean 1.55), despite moderate to high attitudes (mean 3.44) and knowledge (46% high). There was a substantial ( $p < 0.05$ ) correlation between usage and occupation, marital status, and gender; (4) Conclusions: Effective promotion strategies are needed to bridge the gap between the community's knowledge and attitudes on herbal medicine and its actual use.

**Keywords:** Behaviour; herbal medicine; healthcare

## 1. Introduction

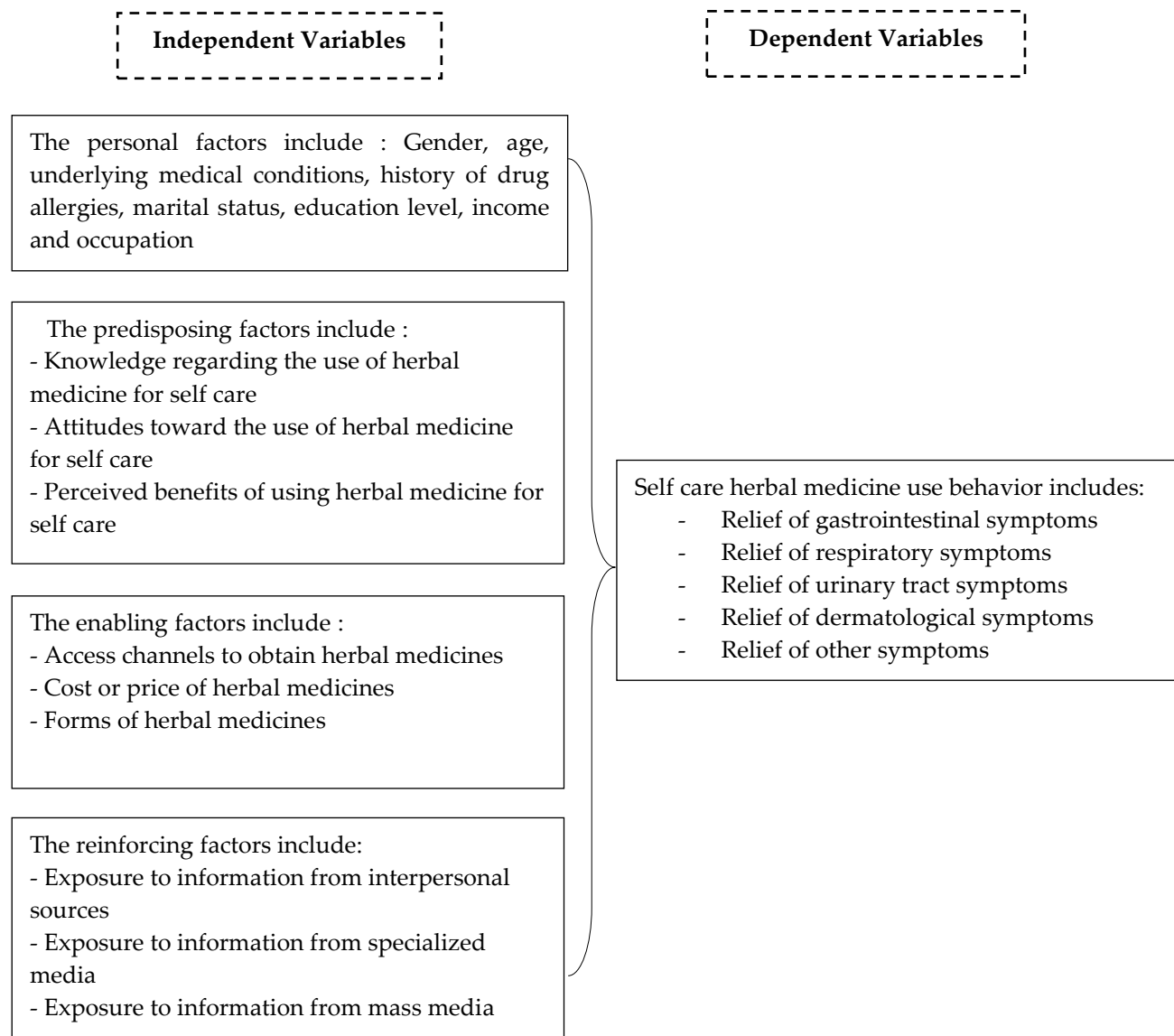
Thai traditional medicine and herbs, which have deep roots in Thai society, are a body of health knowledge that is increasingly being recognized in the nation's current healthcare system, particularly in relation to health self-reliance, a significant trend in health promotion for the twenty-first century (Ministry of Public Health [1]. According to the National Health Commission Office [2], the usage of herbs is considered a vital tool that helps people take basic care of themselves, reduce healthcare expenses, and lessen dependency on the modern medical system in non-essential situations. In an attempt to incorporate herbs into the mainstream healthcare system, government policy consistently promotes their use. It sets a goal for public health facilities to use medicines made from Thai herbs, with a minimum of 6% of total medicine use (Department of Thai Traditional and Alternative Medicine [3]). The rate of herbal medicine use in Health Region 4, which includes Pathum Thani Province, is still lower than the national average, at 3.60% compared to 5.53%, according to data from the Ministry of Public Health's Health Data Center [4]. This highlights a disconnect between public health behavior and policy.

Herbal medicine use behavior among the population is a health-related behavior influenced by multiple factors, including both personal and environmental determinants. Therefore, explaining such behavior requires applying relevant concepts and theories that comprehensively account for its underlying causes. The PRECEDE Model, developed by Lawrence W. Green and Marshall W. Kreuter, provides an appropriate framework for this purpose. This model is widely recognized in public health research for its strength in providing a systematic and stepwise approach to problem analysis. It begins with an assessment of health issues at the societal level, then links these to related behavioral and environmental factors, and further considers key determinants such as knowledge, attitudes, resources, and social support, all of which influence individual behavior. The model emphasizes the analysis of three major categories of factors affecting health behavior: predisposing factors, enabling factors, and reinforcing factors [5]. In the context of herbal medicine use, predisposing factors such as knowledge of herbal medicines, beliefs about their safety, and attitudes toward traditional Thai medicine influence individuals' decisions to use herbal remedies. Enabling factors, including access to herbal medicines or traditional Thai medical services, facilitate more convenient use. Meanwhile, reinforcing factors such as recommendations from healthcare professionals, family members, or peers serve as supportive influences that encourage continued and more confident use of herbal medicines.

In Pathum Thani Province, the Keha Rangsit Khlong Hok hamlet is situated in a semi-rural, semi-urban environment. The aging population is steadily growing, and the majority of people are workers with moderate to low average household incomes. According to data from the Pathum Thani Provincial Administrative Organization [6], 26% of the population is elderly, a demographic that is frequently at higher risk of health problems and in need of safe, affordable healthcare. Additionally, 82% of the population makes no more than 15,000 baht per month. Therefore, encouraging the usage of herbs is a good strategy for this community's circumstances. Additionally, it has been noted that some locals use plants daily, such as aloe vera for wound care, ginger, galangal, and lemongrass boiled for drinking to relieve cold symptoms, and Fah Talai Jone (*Andrographis paniculata*) during the COVID-19 epidemic. However, there are still prejudices or a lack of faith in the effectiveness of herbs compared to modern treatments, the selection process is not systematic, and thus, herbal use behavior lacks accurate knowledge and a systematic understanding [7]. As a result, researching the population's use of herbal medicines for self-care in this region is essential for developing community-level public health policies and providing baseline data for effective, long-lasting herbal health promotion initiatives.

To better understand the context, requirements, and constraints of the local population, it is crucial to investigate the self-care herbal medicine use behavior of residents in the Keha Rangsit Khlong Hok village, Pathum Thani Province. Given the lack of empirical evidence systematically explaining the causal factors influencing herbal medicine use in this urban community context and the absence of a clear theoretical framework, the application of the PRECEDE Model is highly important. This model enables a comprehensive analysis of the determinants of herbal medicine use behavior at both the individual and environmental levels, thereby contributing to the development of appropriate and safe strategies for promoting the use of herbal medicines. To develop policies or guidelines that support the appropriate use of herbal medicines in alignment with the community's way of life and available resources, thereby leading to a sustainable improvement in the population's quality of life.

## 1.1 Conceptual Framework



## 2. Materials and Methods

### 2.1 Study design and sample

This study explores the factors associated with the population's use of herbal medicine for self-care. There are 1,042 residents of Rangsit Klong Rok Housing Community in Pathum Thani Province aged 20 to 59. The sample group included 318 people living in the Rangsit Klong Rok Housing Community, Pathum Thani Province. The sample size was calculated using Taro Yamane's formula with a 5% margin of error. A simple random sample was used to select participants from the Keha Rangsit Khlong Hok village in Pathum Thani Province who use herbal medicine for self-care.

The formula used for calculation is:

$$n = \frac{N}{1 + Ne^2}$$

**When:**

- n represents the sample size
- N represents the population size
- e represents the acceptable margin of error, 5%



**Substitute the variable**

$$\frac{1,042}{1+1,042(0.05)^2}$$

Therefore, the calculated sample size was 289 participants. However, to account for potential data loss or participant attrition, the sample size was increased by 10%. Thus, the final sample size was 318 participants.

**Inclusion Criteria for Volunteers**

1. People aged 20-59 years old who live in the area of Rangsit Housing Community, Klong Hok, Pathum Thani Province.
2. People who have taken care of their personal health by using herbal medication.
3. People without hearing or speech disabilities.
4. People who are fully cognizant and capable of thinking.
5. People who can answer the interview questions and who can communicate in Thai.
6. People who have lived in the Keha Rangsit Khlong Hok community area in Pathum Thani Province for at least six months.

**Exclusion Criteria for Volunteers**

1. People who are unable to respond to the survey.
2. People who are unable to take part in the research project.

**2.2 Ethical considerations**

This study was approved by the Rajamangala University of Technology Thanyaburi Research Ethical Committee (RMUTT\_REC No. COA 90/67). It was designed to adhere to established clinical study guidelines, including the CIOMS Guidelines, the Belmont Report, the Declaration of Helsinki, and the International Conference on Harmonization Good Clinical Practice (ICH GCP) [8],[9]. Participants in the study who qualified were selected after ethical approval. Before any study-related procedures began, each participant signed an informed consent form.

**2.3. Data collection**

After the research project was considered and approved by the Human Research Ethics Committee of Rajamangala University of Technology Thanyaburi, the researcher wrote a letter requesting permission to collect data to the head of the Rangsit Housing Community Group, Klong Hok, Pathum Thani Province. To obtain permission to conduct research and clarify the volunteers participating in the project regarding the purpose of the research, as well as to protect their rights and sign the Rights Protection Form. In the consent form to participate in the research, answer the questionnaire and complete it. The researchers then used the data to assess accuracy and completeness before using it for statistical analysis.

**2.4 Research instruments and Data analysis****2.4.1 Research instruments**

This study used a tool to collect data across 5 episodes, developed based on the PRECEDE Model. The questionnaire was sent to 3 experts. Consider checking the accuracy, coverage, and appropriateness of the language used in each question. The index of item (Objective Congruence: IOC) is analyzed and found to be 0.92 [10]. The questionnaire was then improved and modified, and its reliability was tested on the sample (Try-Out) at the 95% confidence level using the coefficient method based on Cronbach's Alpha [11], yielding a coefficient of 0.82.

Part 1: General information questionnaire, including gender, age, medical conditions, and history of drug allergies: status, education level, income, and occupation. The questionnaire is a checklist and has 8 questions. It is a closed-ended questionnaire.

Part 2: Questionnaire on the knowledge and understanding of the use of herbal medicine based on the application of the PRECEDE Model, specifically the predisposing factors. The questionnaire has 14 questions, each with an estimation scale of 3 levels: Yes, No, and Unknown. The scoring criteria are as follows: 1 point for correct answers. Wrong answer and do not know 0 points. There are 3 levels of

knowledge classification: low, medium, and high. Bloom's [12] criteria, knowledge levels were divided into three categories: low knowledge (0–59.99%), moderate knowledge (60–79.99%), and high knowledge (80–100%).

Part 3: Questionnaire about attitudes and benefits in the use of herbal medicine. Based on the application of the PRECEDE Model, specifically the predisposing factors. There are 10 questions, and the messages have both positive and negative sides, with 5 rating-scale questions according to Likert's design [13]. The researcher used the criteria for classifying the score of Best [14] as follows: low level has an average of 1.00–2.33, medium level has an average of 2.34–3.66, and high level has an average of 3.67–5.00.

Part 4: Questionnaire on Herbal Medicine Use Behavior. Based on the application of the PRECEDE Model, specifically the enabling factors. The question is a rating scale with 3 levels: practice every time. Practice sometimes and never practice: Rate 3, 2, and 1, respectively. The criteria for classifying the score of Best [14] are as follows: Low level has an average of 1.00 – 1.66, medium level has an average of 1.67 2.33, and high level has an average of 2.34 – 3.00. Also inquire about the channels for obtaining herbs, their cost, and the form of the herbs. The question is multiple-choice. If you answer "yes", give 2 points; if you answer "no", give 1 point. The researcher used the criteria for classifying the scores of Best [14] as follows: Low level has an average of 1.00 – 1.66, medium level has an average of 1.67 2.33, and high level has an average of 2.34 – 3.00.

Part 5: Evaluation Form on Information and Suggestions About the Use of Herbal Medicines from Different Sources. Based on the application of the PRECEDE Model, specifically the reinforcing factors. The question will be an evaluation scale. There are 3 answers to choose from: ever, never, and don't answer. If ever, give 1 point, never, and don't answer 0 points. The researcher used criteria to classify the scores. As follows: Low level has an average of 0 - 0.5, high level has an average of 0.51 - 1 .00

#### 2.4.2 Data analysis

Descriptive statistics were used to analyze the general characteristics of the study population, including gender, age, underlying medical conditions, history of drug allergies, marital status, educational level, income, and occupation, using frequency and percentage distributions. Data on predisposing, enabling, and reinforcing factors, as well as behaviors related to the use of herbal medicine for self-care, were analyzed by calculating the mean and standard deviation. The relationships between personal factors and herbal medicine use behaviors were examined using the Chi-square test.

### 3. Results and Discussion

#### 3.1 Results

The purpose of this survey study is to examine the use of herbal medicine in public health care and to examine the relationship between individual characteristics, predisposing factors, enabling factors, and reinforcing factors in the behavior of using herbal medicine for public health care. Thus, the following arrangement is how the researcher would like to display the data:

Part 1: Respondents general data, It was found that most of them were female, 60 percent, 63 percent are in the 50–59 age range, 60 percent have no underlying medical conditions, 96 percent have never had a drug allergy, 63 percent are married, 45 percent have most Primary School Graduates, 31 percent income between 7,001 and 9,000 baht per month, and 54 percent are Trading profession.

Part 2: Knowledge of the use of herbal medicines in health care of the people in the Keha Rangsit Khlong Hok community in Pathum Thani Province, 46 percent have a high degree, followed by moderate at 38 percent and low at 16 percent of understanding about the use of herbal medicine for public health care, as indicated in Table 1.

Part 3. Attitudes and perceptions of the benefits of using herbal medicine in health care. Moderate consent overall ( $\bar{x}$  = 3.44, S.D. = 0.71). When classified by item, it was found that using herbal medicine to relieve symptoms and get better, would you recommend or pass it on to others? It has the highest mean ( $\bar{x}$  = 4.56, S.D. = 0.60). This is followed by supporting the use of herbal medicine in disease treatment ( $\bar{x}$  = 4.42, S.D. = 0.69) and using herbal medicine to treat the disease or relieve its symptoms has no toxicity to the body, especially herbal medicine with information confirmed by the Ministry of Public Health ( $\bar{x}$  = 4.24, S.D. = 0.90), according to Table 2.

Part 4: Behavior of Using Herbal Medicine for Public Health Care in the Keha Rangsit Khlong Hok community, Pathum Thani Province. Overall low levels ( $\bar{x} = 1.55$ , S.D. = 0.53). When classified by aspect, the behavior of using herbal medicine for respiratory illnesses has the highest average ( $\bar{x} = 2.19$ , S.D. = 0.71). Secondly, other illnesses ( $\bar{x} = 1.76$ , S.D. = 0.39), skin illnesses ( $\bar{x} = 1.60$ , S.D. = 0.23), gastrointestinal illnesses ( $\bar{x} = 1.39$ , S.D. = 0.42) and urinary illnesses ( $\bar{x} = 1.35$ , S.D. = 0.69) respectively. If classified by clause, it is found that behavior of using herbal medicine for respiratory illnesses is mostly consuming emblica when experiencing sore throat, cough, and phlegm symptoms had the highest average ( $\bar{x} = 2.54$ , S.D. = 0.61), followed by *Andrographis paniculata* ( $\bar{x} = 2.24$ , S.D. = 0.72) and lemon juice ( $\bar{x} = 2.16$ , S.D. = 0.54), according to the item classification. The next, behavior of using herbal medicine for other illnesses is mostly using phlai to treat muscle pain had the highest average ( $\bar{x} = 2.51$ , S.D. = 0.68), followed by eating boraphet to reduce fever and increase appetite ( $\bar{x} = 1.63$ , S.D. = 0.56) and treating lice with a mixture of liquor and custard apple leaves ( $\bar{x} = 1.51$ , S.D. = 0.75), according to the item classification. The behavior of using herbal medicine for skin illnesses is mostly treating burns with aloe vera ( $\bar{x} = 2.50$ , S.D. = 0.59), followed by treating burns with gotu kola ( $\bar{x} = 2.00$ , S.D. = 0.82), and treating bug bite rashes with *Clinacanthus nutans* ( $\bar{x} = 1.73$ , S.D. = 0.60), according to the item classification. The behavior of using herbal medicine for gastrointestinal illnesses is mostly. Consuming turmeric for symptoms of indigestion, bloating, flatulence, and stomach disease had the highest average ( $\bar{x} = 2.00$ , S.D. = 0.65), followed by neem leaves for improving food intake when feeling famished ( $\bar{x} = 1.98$ , S.D. = 0.58), and senna leaves for constipation ( $\bar{x} = 1.97$ , S.D. = 0.67), according to the item classification. Finally, the behavior of using herbal medicine for urinary illnesses is mostly consuming roselle juice for dysuria symptoms, which had the highest average ( $\bar{x} = 1.61$ , S.D. = 0.61), followed by consuming red sugarcane ( $\bar{x} = 1.28$ , S.D. = 0.49), and consuming lemongrass juice ( $\bar{x} = 1.27$ , S.D. = 0.48), according to the item classification, as indicated in Table 3.

Part 5: Information Reception About the Use of Herbal Medicine for Health Care of the Public in the Keha Rangsit Khlong Hok village in Pathum Thani Province. Overall low levels ( $\bar{x} = 0.34$ , S.D. = 0.47). When classified by aspect, it is found that reception of information from the mass media has the highest average ( $\bar{x} = 0.36$ , S.D. = 0.54). Secondly, specific media ( $\bar{x} = 0.36$ , S.D. = 0.54) and personal media ( $\bar{x} = 0.32$ , S.D. = 0.35) respectively. If classified by clause, it is found that information reception from the mass media is mostly television ( $\bar{x} = 0.87$ , S.D. = 0.33), websites ( $\bar{x} = 0.81$ , S.D. = 0.39), and radio ( $\bar{x} = 0.59$ , S.D. = 0.49) are the main sources of information for people on the use of herbal medicine for self-care. The next, information reception from the specific media is mostly pamphlets ( $\bar{x} = 0.50$ , S.D. = 0.50), books or manuals ( $\bar{x} = 0.43$ , S.D. = 0.49), and leaflets ( $\bar{x} = 0.34$ , S.D. = 0.47). Finally, information reception from the personal media is mostly from friends ( $\bar{x} = 0.77$ , S.D. = 0.42), family or relatives ( $\bar{x} = 0.85$ , S.D. = 0.35), and community health volunteers ( $\bar{x} = 0.42$ , S.D. = 0.49), according to the item classification, as indicated in Table 4.

The results of a Chi-Square analysis examining the association between personal characteristics and this behavior showed that gender, marital status, and occupation are all associated with the use of herbal medicine for health care at the 0.05 level of statistical significance, as indicated. When examined in detail, the findings indicate that gender is significantly associated with herbal medicine use behavior. Females tend to use herbal medicines for self-care more frequently than males. Regarding marital status, married individuals show a higher tendency to use herbal medicines compared to single individuals, which may reflect their roles in family health care. In terms of occupation, those engaged in trade or self-employment are more likely to use herbal medicines than those working as government officials or employees of state enterprises, as shown in Table 5.

Chi-Square analysis of the relationship between predisposing factors and the use of herbal medicine for health care revealed that, at the 0.05 significance level, attitudes toward the use of herbal medicine for self-care are associated with the use of herbal medicine for health care, as indicated in Table 6. The channels for obtaining herbal medicine and the forms of herbal medicine are related to the behavior of using herbal medicine for health care at a statistically significant level of 0.05, according to the results of a Chi-Square analysis that tested the relationship between enabling factors and the behavior of using herbal medicine for health care, as indicated in Table 7. As indicated in Table 8, the results of a Chi-Square analysis of the

relationship between reinforcing factors and the behavior of using herbal medicine for health care revealed that, at the 0.05 significance level, reinforcing factors are not related to this behavior.

### 3.2 Discussion

The study on herbal medicine use behavior for self-care among residents of the Keha Rangsit Khlong Hok community in Pathum Thani Province found that the population had a high level of knowledge about herbal medicines (46%). This finding is inconsistent with the study by Kritadech Mingmai [15], which examined knowledge, attitudes, and behaviors regarding herbal medicine use among elderly individuals with chronic diseases in Tha Ruea District, Phra Nakhon Si Ayutthaya Province. That study reported that the majority of participants had a moderate level of knowledge (57.5). This finding is also inconsistent with the study by Wilaiwan Chainarong [16], which examined factors associated with herbal medicine use for primary care among residents in Thanyaburi District, Pathum Thani Province, and reported that the majority of participants had a moderate level of knowledge (45.0%). Furthermore, it is inconsistent with the study by Somkiatyos Woradet et al. [17], which investigated factors related to self-care herbal medicine use among village health volunteers in Phatthalung Province and found that most participants had a moderate level of knowledge (68.8%). The findings revealed that residents of the Keha Rangsit Khlong Hok community in Pathum Thani Province had a high level of knowledge regarding the use of herbal medicines. This differs from previous studies conducted across various settings, which generally reported moderate levels of knowledge. Contextual factors may explain this discrepancy, as the study area is a semi-urban community with greater access to health-related information, particularly through mass media and digital platforms. This has enabled the population to receive continuous, diverse information about herbal medicines, compared with earlier studies, thereby contributing to a higher overall level of knowledge. In addition, government policies promoting the integration of herbal medicines into the healthcare system, such as the implementation of herbal medicine services in subdistrict health-promoting hospitals and the dissemination of knowledge through public health agencies, have played a significant role in enhancing public knowledge in the area. These factors may explain the inconsistency between this study's findings and those of previous research.

When considering the levels of attitudes and perceived benefits regarding the use of herbal medicines for self-care, the findings indicated that the majority of participants had moderate attitudes (mean 3.44). This is consistent with the study by Kritadech Mingmai [15], which investigated knowledge, attitudes, and behaviors related to herbal medicine use among elderly individuals with chronic diseases in Tha Ruea District, Phra Nakhon Si Ayutthaya Province, and found that most participants had moderate attitudes (58.2%). Similarly, this finding aligns with the study by Jaruwan Srisroi et al. [18], which examined factors influencing the consumption of herbal decoctions among residents in Khok Lai Village, Sakon Nakhon Province, and reported that the majority of participants had moderate attitudes (78.7%). It is also consistent with the study by Sanga Yookhong [19], which explored knowledge, attitudes, and behaviors related to herbal medicine use among village health volunteers in Yasothon Province and found that most participants had moderate attitudes (63.7%). Although most people perceive herbal medicines as beneficial, safe, and a viable option for self-care, there remain certain concerns and limitations. These include inconvenience in use, uncertainty regarding their effectiveness compared to modern medicine, and the perception that herbal medicines are traditional or outdated. The coexistence of both positive and negative factors contributes to attitudes that are neither distinctly high nor distinctly low, remaining at a moderate level. Furthermore, the current healthcare system, which integrates both modern medicine and herbal medicine, encourages individuals to adopt a "situational use" approach rather than relying on herbal medicine as a primary option. This, in turn, prevents attitudes from becoming strongly positive. In addition, influences from personal experiences and social information sources, such as word of mouth and mass media, which may include both supportive and skeptical perspectives on herbal medicine, also help maintain attitudes at a moderate level.

When considering the use of herbal medicine, the findings indicated that it was low (mean 1.55). This is inconsistent with the study by Kritadech Mingmai [15], which examined knowledge, attitudes, and behaviors related to herbal medicine use among elderly individuals with chronic diseases in Tha Ruea



District, Phra Nakhon Si Ayutthaya Province, and reported that the level of herbal medicine use behavior was moderate (54.7%). It is also inconsistent with the study by Wilaiwan Chainarong [16], which investigated factors associated with herbal use behavior for primary care among residents in Thanyaburi District, Pathum Thani Province, and found that the majority of participants had a moderate level of behavior (33.40%). Furthermore, it does not align with the study by Sukit Chaichompoo et al. [20], which explored factors related to herbal medicine use behavior among the population in Public Health Region 11, and reported that most participants demonstrated a moderate level of behavior (36.2%). This finding is also inconsistent with the study by Phitsanee Wijantruk [21], which investigated knowledge, attitudes, and behaviors related to the use of herbal products among residents of Nong Bua Sala Village, Mueang District, Nakhon Ratchasima Province, and reported that herbal product use behavior was at a moderate level (90.74%). It is likewise inconsistent with the study by Rosnanee Hemtrakunwong et al. [22], which examined factors associated with self-care herbal medicine use among residents in Yala Municipality, Sateng Subdistrict, Mueang District, Yala Province, and found that herbal medicine use behavior was at a moderate level (66.1%). Furthermore, it does not align with the study by Saroj Phetmanee et al. [23], which investigated factors related to herbal use behavior among residents of Pratu Chang Tok Community, Thaiburi Subdistrict, Tha Sala District, Nakhon Si Thammarat Province, and reported that the population demonstrated a moderate level of herbal medicine use behavior (84.6%). The findings revealed that the population's herbal medicine use behavior was low, unlike most previous studies, which reported a moderate level. Differences in contextual factors, population characteristics, and current determinants of health behavior may explain this discrepancy. As Keha Rangsit is a semi-urban community, residents have greater access to healthcare services and modern medicine, making herbal medicine less likely to be a primary option for self-care. Although the participants demonstrated a high level of knowledge and a moderate level of attitudes toward herbal medicine, these were not sufficient to translate into actual practice. This may be due to factors such as ease of use, habitual reliance on modern medicine, and confidence in treatment effectiveness. These findings contrast with those from rural settings or studies involving elderly populations, where individuals may rely more heavily on herbal medicine in their daily lives.

The analysis of the relationship between personal factors and herbal medicine use behavior for self-care revealed that gender, marital status, and occupation were significantly associated with herbal medicine use behavior ( $p < 0.05$ ). This finding is consistent with the study by Punyanpat Chaimail et al. [24], which reported that occupation influences the likelihood of herbal medicine use, with individuals engaged in trade or hired labor being more likely to use herbal medicines than those in other occupational groups. This finding is inconsistent with the study by Wilaiwan Chainarong [16], which reported that age and educational level had a significant effect on basic herbal medicine use behavior. It is also inconsistent with the study by Wirinya Muangchang [25], which investigated factors associated with self-care herbal medicine use among residents in Mae Chai District, Phayao Province, and found that gender and occupation were not significantly associated with herbal medicine use behavior ( $p = 0.473$  and  $p = 0.535$ , respectively). The findings revealed that gender, marital status, and occupation were significantly associated with herbal medicine use behavior. From an academic perspective, these personal factors can be interpreted as reflecting differences in "social roles and lifestyles," which influence health-related decision-making. Regarding gender, the observed association with behavior may be explained by the fact that females often assume greater responsibility for their own health and that of family members. As a result, they are more likely to acquire knowledge about and use herbal medicines more frequently than males. Regarding marital status, particularly among individuals with families, their behavior is often influenced by close family members, such as spouses or elderly relatives, who serve as sources of traditional knowledge on herbal medicine. This influence increases the likelihood of herbal medicine use. In terms of occupation, which was found to be significantly associated with behavior, this can be explained by differences in work characteristics and lifestyles that affect both access to and choices regarding herbal medicine use. For example, individuals engaged in trade or hired labor may have more flexible schedules, greater access to herbal products, and broader social networks that facilitate the exchange of information. Consequently, they are more likely to use



herbal medicines compared to those in other occupational groups. This finding is consistent with the study by Punyanpat Chaimail et al. [24].

The analysis of the relationship between predisposing factors and herbal medicine use behavior for self-care revealed that attitudes toward herbal medicine use were significantly associated with herbal medicine use behavior ( $p < 0.05$ ). This finding is consistent with the study by Wirinya Muangchang [25], which examined factors related to the use of self-care herbal medicine among residents in Mae Chai District, Phayao Province. The study reported that predisposing factors, namely knowledge regarding the use of herbal medicine for self-care, attitudes toward herbal medicine use, and perceived benefits of herbal medicine, were significantly associated with herbal medicine use behavior, as determined using Pearson's correlation coefficient. Additionally, this result is in agreement with the study by Sansanee Rittongpitak [26], which found that attitudes were significantly related to herbal medicine use. The findings of this study are consistent with the conceptual framework of the PRECEDE Model, which explains that predisposing factors, particularly attitudes, beliefs, and perceived benefits, are key determinants of health behavior. This is also in line with health behavior theories, which suggest that individuals' perceptions of value and confidence in a treatment approach directly influence their behavioral choices. At the same time, although knowledge is an essential foundation, it alone may not be sufficient to generate behavior if individuals lack confidence or have unclear attitudes. Therefore, individuals who possess knowledge but lack positive attitudes may be less likely to use herbal medicines. In contrast, those with favorable attitudes, even with only a moderate level of knowledge, are more likely to engage in herbal medicine use behavior.

The analysis of the relationship between enabling factors and herbal medicine use behavior for self-care revealed that access channels to obtain herbal medicines and the forms of herbal medicines were significantly associated with herbal medicine use behavior ( $p < 0.05$ ). This finding is consistent with the study by Wirinya Muangchang [25], which examined factors related to the use of self-care herbal medicine among residents in Mae Chai District, Phayao Province. The study reported that enabling factors, including access channels to obtain herbal medicines, cost or price, forms of herbal medicines, and promotional activities, were significantly associated with herbal medicine use behavior, as determined using Pearson's Product-Moment Correlation Coefficient. These findings support the study by Sukit Chaichompoo [20], which reported that ease of access to herbal sources is significantly associated with the use of herbal medicine. The results are also consistent with the PRECEDE Model, which identifies enabling factors such as access to resources and services as key components that facilitate health behaviors. Furthermore, the findings align with previous research indicating that accessibility of herbal medicines and the characteristics of herbal products are significantly associated with their utilization.

The analysis of the relationship between reinforcing factors and herbal medicine use behavior for self-care revealed no significant association ( $p > 0.05$ ). This finding is inconsistent with the study by Wirinya Muangchang [25], which examined factors related to the use of self-care herbal medicine among residents in Mae Chai District, Phayao Province. That study reported that reinforcing factors, including exposure to information about herbal medicine use from interpersonal sources, specialized media, and mass media, were significantly associated with herbal medicine use behavior, as determined using Pearson's Product-Moment Correlation Coefficient. This finding partially contradicts the study by Somkiatys Woradet [27], which reported that social support was positively associated with herbal medicine use. This discrepancy may be attributed to differences in community characteristics or variations in support systems within the study area. When considered alongside the findings that predisposing and enabling factors were significantly associated with behavior, it can be inferred that decisions to use herbal medicines in this context may depend more on internal factors (e.g., attitudes) and accessibility (e.g., availability of herbal sources and product forms) than on external factors such as social support. Therefore, it can be concluded that the lack of association between reinforcing factors and herbal medicine use behavior may be due to the nature of the data and the relatively weak level of social support. In addition, differences in the social context of the study area compared with other settings may have contributed to the inability of reinforcing factors to significantly influence herbal medicine use behavior in this sample.

### 3.3 Figures, Tables, and Schemes

**Table 1.** The level of knowledge regarding the use of herbal medicines for self-care among the population (n=318)

| Knowledge regarding the use of herbal medicines for self-care among the population | Frequency  | %          |
|------------------------------------------------------------------------------------|------------|------------|
| High level                                                                         | 146        | 46         |
| Moderate level                                                                     | 121        | 38         |
| Low level                                                                          | 51         | 16         |
| <b>Total</b>                                                                       | <b>318</b> | <b>100</b> |

**Table 2.** Attitudes and perceptions of the benefits of using herbal medicine in health care.

| Topic                                                                                                                                                    | Mean        | S.D.        | Interpretation  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|-------------|-----------------|
| 1. The use of herbal medicine to relieve symptoms has a complicated and inconvenient procedure                                                           | 3.44        | 1.00        | Moderate        |
| 2. The use of herbal medicines to relieve symptoms has fewer side effects than the use of modern medicines.                                              | 3.79        | 0.76        | High            |
| 3. The use of herbal medicine to relieve symptoms makes it feel unfashionable, backward, and archaic                                                     | 3.64        | 0.94        | High            |
| 4. The use of herbal medicines to relieve symptoms is not toxic to the body, especially herbs that have been confirmed by the Ministry of Public Health. | 4.24        | 0.90        | High            |
| 5. Looking at the characteristics, color, smell, and taste of herbal medicine, it is considered an obstacle to using herbal medicine.                    | 2.39        | 1.01        | Moderate        |
| 6. Herbal medicine is more beneficial than modern medicine.                                                                                              | 3.71        | 0.68        | High            |
| 7. Herbal medicine today is more effective than before.                                                                                                  | 3.90        | 0.64        | High            |
| 8. There is confidence that herbal medicine is highly safe.                                                                                              | 3.97        | 0.65        | High            |
| 9. You will support the use of herbal medicine in disease treatment.                                                                                     | 4.42        | 0.69        | High            |
| 10. If you use herbal medicine to relieve symptoms and get better, would you recommend or pass it on to others?                                          | 4.56        | 0.60        | High            |
| <b>Total</b>                                                                                                                                             | <b>3.44</b> | <b>0.71</b> | <b>Moderate</b> |

**Table 3.** Behavior of Using Herbal Medicine for Public Health Care

| Topic                                                                                        | Mean | S.D. | Interpretation |
|----------------------------------------------------------------------------------------------|------|------|----------------|
| <b>1. Gastrointestinal illnesses</b>                                                         |      |      |                |
| <b>1.1 Herbal medicine used to treat stomach diseases</b>                                    |      |      |                |
| 1.1.1 Turmeric relieves bloating, indigestion, and fullness, and treats stomach diseases.    | 2.00 | 0.65 | Moderate       |
| 1.1.2 Raw banana to treat stomach disease or mild diarrhea.                                  | 1.23 | 0.46 | Low            |
| <b>1.2 Herbal medicine used to relieve symptoms of flatulence, bloating, and indigestion</b> |      |      |                |
| 1.2.1 You consume garlic when you have symptoms of flatulence, bloating, and indigestion.    | 1.66 | 0.69 | Low            |
| 1.2.2 You consume basil when you have symptoms of flatulence, bloating, or indigestion.      | 1.88 | 0.62 | Moderate       |
| 1.2.3 You consume pepper when you have symptoms of flatulence, bloating, or indigestion.     | 1.78 | 0.61 | Moderate       |
| 1.2.4 You consume fingerroot when you have symptoms of flatulence, bloating, or indigestion. | 1.35 | 0.62 | Low            |
| <b>1.3 Herbal medicine used to relieve constipation</b>                                      |      |      |                |
| 1.3.1 You consume senna when you have constipation.                                          | 1.07 | 0.29 | Low            |

**Table 3.** Behavior of Using Herbal Medicine for Public Health Care (continue)

| Topic                                                                                                                                    | Mean        | S.D.        | Interpretation  |
|------------------------------------------------------------------------------------------------------------------------------------------|-------------|-------------|-----------------|
| 1.3.2 You consume Senna alexandrina when you have constipation.                                                                          | 1.97        | 0.67        | Moderate        |
| 1.3.3 You consume basil seeds when you have constipation.                                                                                | 1.20        | 0.44        | Low             |
| <b>1.4 Herbal medicine used to relieve diarrhea</b>                                                                                      |             |             |                 |
| 1.4.1 You consume raw guava when you have diarrhea.                                                                                      | 1.13        | 0.39        | Low             |
| 1.4.2 You consume pomegranate peel when you have diarrhea.                                                                               | 1.10        | 0.36        | Low             |
| 1.4.3 You consume mangosteen peel when you have diarrhea.                                                                                | 1.08        | 0.33        | Low             |
| <b>1.5 Herbal medicine used to relieve nausea and vomiting</b>                                                                           |             |             |                 |
| 1.5.1 You consume ginger when you have nausea, vomiting, or motion sickness.                                                             | 1.64        | 0.57        | Low             |
| 1.5.2 You consume a YA HOM THEP PA JIT POWDER when you have dizziness.                                                                   | 1.06        | 0.31        | Low             |
| <b>1.6 Herbal medicine used to treat parasitic worms</b>                                                                                 |             |             |                 |
| 1.6.1 You consume Diospyros mollis to treat parasitic diseases.                                                                          | 1.08        | 0.30        | Low             |
| 1.6.2 You consume Quisqualis indica to treat parasitic diseases.                                                                         | 1.02        | 0.14        | Low             |
| 1.6.3 You consume artocarpus lacucha to treat parasitic diseases.                                                                        | 1.05        | 0.21        | Low             |
| 1.6.4 You consume pumpkin seeds to treat parasitic diseases.                                                                             | 1.05        | 0.26        | Low             |
| <b>1.7 Herbal medicine used to relieve a toothache</b>                                                                                   |             |             |                 |
| 1.7.1 When you have a toothache or tooth decay, you use streblus asper to temporarily relieve the toothache before seeing a dentist.     | 1.34        | 0.49        | Low             |
| 1.7.2 When you have a toothache or tooth decay, you use Spilanthes acmella to temporarily relieve the toothache before seeing a dentist. | 1.06        | 0.27        | Low             |
| <b>1.8 Herbal medicine that relieves loss of appetite and helps improve appetite</b>                                                     |             |             |                 |
| 1.8.1 You consume bitter melon when you feel a loss of appetite because it will make you eat.                                            | 1.92        | 0.56        | Moderate        |
| 1.8.2 You consume neem when you feel a loss of appetite because it will make you eat.                                                    | 1.98        | 0.58        | Moderate        |
| <b>Total</b>                                                                                                                             | <b>1.39</b> | <b>0.42</b> | <b>Low</b>      |
| <b>2. Respiratory illnesses</b>                                                                                                          |             |             |                 |
| 2.1 You consume lemon juice when you have symptoms of a sore throat, cough, and phlegm.                                                  | 2.16        | 0.54        | Moderate        |
| 2.2 You consume emblica when you have symptoms of a sore throat, cough, and phlegm.                                                      | 2.54        | 0.61        | High            |
| 2.3 You consume Solanum trilobatum when you have symptoms of a sore throat, cough, and phlegm.                                           | 2.05        | 0.84        | Moderate        |
| 2.4 You consume Solanum indicum when you have symptoms of sore throat, cough, and phlegm                                                 | 1.98        | 0.84        | Moderate        |
| 2.5 You consume Andrographis paniculata when you have symptoms of sore throat, cough, and phlegm.                                        | 2.24        | 0.72        | Moderate        |
| <b>Total</b>                                                                                                                             | <b>2.19</b> | <b>0.71</b> | <b>Moderate</b> |
| <b>3. Urinary Illnesses</b>                                                                                                              |             |             |                 |
| 3.1 You consume lemongrass juice when you have symptoms of dysuria.                                                                      | 1.27        | 0.48        | Low             |
| 3.2 You consume pineapple core when you have symptoms of dysuria.                                                                        | 1.24        | 1.16        | Low             |
| 3.3 You consume roselle juice when you have symptoms of dysuria.                                                                         | 1.61        | 0.61        | Low             |
| 3.4 You consume red sugarcane when you have symptoms of dysuria.                                                                         | 1.28        | 0.49        | Low             |
| <b>Total</b>                                                                                                                             | <b>1.35</b> | <b>0.69</b> | <b>Low</b>      |

**Table 3.** Behavior of Using Herbal Medicine for Public Health Care (continue)

| Topic                                                                                      | Mean        | S.D.        | Interpretation |
|--------------------------------------------------------------------------------------------|-------------|-------------|----------------|
| <b>4. Skin Illnesses</b>                                                                   |             |             |                |
| <b>4.1 Treat ringworm/tinea versicolor</b>                                                 |             |             |                |
| 4.1.1 You use White crane flower when you have ringworm/tinea versicolor.                  | 1.50        | 0.56        | Moderate       |
| <b>4.2 Herbal medicine used to treat Tinea Capitis</b>                                     |             |             |                |
| 4.2.1 You use Sapindus rarak to treat Tinea Capitis on the head or relieve an itchy scalp. | 1.43        | 0.53        | Moderate       |
| <b>4.3 Herbal medicine used to treat burns</b>                                             |             |             |                |
| 4.3.1 You use coconut oil to treat burns.                                                  | 1.68        | 0.60        | Moderate       |
| 4.3.2 You use aloe vera to treat burns.                                                    | 2.50        | 0.59        | Moderate       |
| 4.3.3 You use gotu kola to treat burns.                                                    | 2.00        | 0.82        | Low            |
| <b>4.4 Herbal medicine used to treat abscesses and blisters</b>                            |             |             |                |
| 4.4.1 You use Gynura pseudochina when you have abscesses or blisters.                      | 1.05        | 0.29        | Low            |
| 4.4.2 You use Garden Balsam when you have abscesses or blisters.                           | 1.01        | 0.10        | Low            |
| <b>4.5 Herbal medicine used to relieve inflammation from insect bites</b>                  |             |             |                |
| 4.5.1 You use barleria lupulina when you have rashes from insect bites.                    | 1.73        | 0.60        | Low            |
| 4.5.2 You use coccinia grandis when you have rashes from insect bites.                     | 1.69        | 0.59        | Low            |
| <b>4.6 Herbal medicine used to treat urticaria</b>                                         |             |             |                |
| 4.6.1 You use betel pepper when you have urticaria.                                        | 1.56        | 0.55        | Low            |
| <b>4.7 Herbal medicine used to treat herpes/shingles</b>                                   |             |             |                |
| 4.7.1 When you have herpes/shingles, you use Clinacanthus nutans to heal.                  | 1.52        | 0.55        | Low            |
| <b>Total</b>                                                                               | <b>1.60</b> | <b>0.23</b> | <b>Low</b>     |
| <b>5. Other illnesses</b>                                                                  |             |             |                |
| 5.1 You use Zingiber montanum for muscle pain.                                             | 2.51        | 0.68        | Moderate       |
| 5.2 You consume tinospora cordifolia to relieve fever and stimulate appetite.              | 1.63        | 0.56        | Low            |
| 5.3 You consume Thai Copper Pod when you have insomnia.                                    | 1.39        | 0.58        | Low            |
| 5.4 When you have lice, you use custard apple leaves mixed with liquor to treat them.      | 1.51        | 0.75        | Low            |
| <b>Total</b>                                                                               | <b>1.76</b> | <b>0.39</b> | <b>Low</b>     |
| <b>Overall Total</b>                                                                       | <b>1.55</b> | <b>0.53</b> | <b>Low</b>     |

**Table 4.** Information Reception About the Use of Herbal Medicine for the Health Care of the Public

| Topic                        | Mean        | S.D.        | Interpretation |
|------------------------------|-------------|-------------|----------------|
| <b>Personal Media</b>        |             |             |                |
| 1. Family /relatives         | 0.85        | 0.35        | High           |
| 2. Friends/neighbors         | 0.77        | 0.42        | High           |
| 3. Public health personnel   | 0.16        | 0.36        | Low            |
| 4. Teachers                  | 0.17        | 0.37        | Low            |
| 5. Traditional healers       | 0.08        | 0.27        | Low            |
| 6. Village health volunteers | 0.42        | 0.49        | Low            |
| 7. Community leaders         | 0.10        | 0.30        | Low            |
| 8. Others                    | 0.05        | 0.21        | Low            |
| <b>Total</b>                 | <b>0.32</b> | <b>0.35</b> | <b>Low</b>     |

**Table 4.** Information Reception About the Use of Herbal Medicine for the Health Care of the Public (continue)

| Topic                               | Mean        | S.D.        | Interpretation |
|-------------------------------------|-------------|-------------|----------------|
| <b>Specific media</b>               |             |             |                |
| 1. Books/Manuals                    | 0.43        | 0.49        | Low            |
| 2. Billboards/Posters               | 0.22        | 0.41        | Low            |
| 3. Leaflets                         | 0.50        | 0.50        | Low            |
| 4. Flyer                            | 0.34        | 0.47        | Low            |
| 5. Journals                         | 0.08        | 0.27        | Low            |
| 6. Herbal exhibitions               | 0.16        | 0.36        | Low            |
| 7. Participate in training on herbs | 0.33        | 0.47        | Low            |
| 8. Others                           | 0.06        | 0.23        | Low            |
| <b>Total</b>                        | <b>0.36</b> | <b>0.54</b> | <b>Low</b>     |
| <b>Mass media</b>                   |             |             |                |
| 1. Radio                            | 0.59        | 0.49        | High           |
| 2. Television                       | 0.87        | 0.33        | High           |
| 3. Internet/Website                 | 0.81        | 0.39        | High           |
| 4. Village broadcast system         | 0.38        | 0.48        | Low            |
| 5. Magazines                        | 0.07        | 0.25        | Low            |
| 6. Newspapers                       | 0.22        | 0.41        | Low            |
| 7. Others                           | 0.03        | 0.17        | Low            |
| <b>Total</b>                        | <b>0.36</b> | <b>0.54</b> | <b>Low</b>     |
| <b>Overall Total</b>                | <b>0.34</b> | <b>0.47</b> | <b>Low</b>     |

**Table 5.** Relationship between Personal Factors and Herbal Medicine Use Behavior for Health Care (Chi-Square)

| Topic                      | Herbal medicine use behavior |         |
|----------------------------|------------------------------|---------|
|                            | X <sup>2</sup>               | p-value |
| 1. Gender                  | 305.55                       | <.00*   |
| 2. Age                     | 93.82                        | 0.94    |
| 3. Underlying disease      | 50.23                        | 0.10    |
| 4. History of drug allergy | 45.26                        | 0.22    |
| 5. Marital status          | 153.46                       | 0.00*   |
| 6. Education level         | 170.88                       | 0.19    |
| 7. Monthly income          | 311.08                       | 0.05    |
| 8. Occupation              | 305.55                       | <0.00*  |

\*p &lt; 0.05, statistically significant

**Table 6.** Relationship between Predisposing Factors and Herbal Medicine Use Behavior for Health Care (Chi-Square)

| Topic                                                                  | Herbal medicine use behavior |         |
|------------------------------------------------------------------------|------------------------------|---------|
|                                                                        | X <sup>2</sup>               | p-value |
| 1. Knowledge about using herbal medicine for self-care                 | 483.50                       | 0.35    |
| 2. Attitude towards using herbal medicine for self-care                | 917.16                       | 0.00*   |
| 3. Perception of the benefits from using herbal medicine for self-care | 340.92                       | 0.12    |

\*p &lt; 0.05, statistically significant



**Table 7.** Relationship between Enabling Factors and Herbal Medicine Use Behavior for Health Care (Chi-Square)

| Topic                                             | Herbal medicine use behavior |         |
|---------------------------------------------------|------------------------------|---------|
|                                                   | X <sup>2</sup>               | p-value |
| 1. Channels for obtaining herbal medicine         | 287.44                       | 0.01*   |
| 2. The cost or price of obtaining herbal medicine | 109.20                       | 0.68    |
| 3. Forms of herbal medicine                       | 154.26                       | 0.01*   |

\*p < 0.05, statistically significant

**Table 8.** Relationship between Reinforcing Factors and Herbal Medicine Use Behavior for Health Care (Chi-Square)

| Topic                                      | Herbal medicine use behavior |         |
|--------------------------------------------|------------------------------|---------|
|                                            | X <sup>2</sup>               | p-value |
| 1. Information Reception of personal media | 328.47                       | 0.12    |
| 2. Information Reception of specific media | 339.46                       | 0.13    |
| 3. Information Reception of mass media     | 248.61                       | 0.24    |

\*p < 0.05, statistically significant

#### 4. Conclusions

In conclusion, the results of the study on the healthcare use behavior of herbal medicines in the Keha Rangsit Community, Khlong Hok, Pathum Thani Province, show that positive attitudes and knowledge regarding the use of herbal medicines are not yet sufficient to alter behavior in the sample. To enhance the use of herbal medicine in public healthcare, systematic promotion is required, including making herbs available, developing modern product forms, and fostering awareness through trustworthy, easily accessible media.

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# Cleanliness, Crowds, and Conservation: A Multi-Method Approach using Sentiment Analysis and Spatial Mapping for Sustainable Tourism Management in Thailand

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**Abstract:** Natural attractions are among Thailand's most important tourism resources, drawing a large number of international tourists and contributing substantially to national revenue. However, persistent issues such as overcrowding, pollution, and declining environmental quality remain critical challenges for sustainable management. In the digital era, online reviews on travel platforms strongly influence destination choice, providing a rich source of behavioral insights. This study applies sentiment analysis to 124,066 TripAdvisor reviews of natural attractions across all 77 provinces of Thailand, collected between 2014 and 2023. The reviews were processed using text mining techniques—data cleansing, tokenization, lemmatization, and stop-word removal—and analyzed with the Pysentimiento model, a BERT-based deep learning framework, achieving an overall accuracy of 76% and a precision of 87% on the validation dataset in distinguishing positive, negative, and neutral sentiments. Beyond thematic analysis, this research employs Kernel Density Estimation (KDE) to visualize the geographical concentration of dissatisfaction. Results indicate that beaches and islands received the most negative reviews, with recurring issues related to cleanliness, pollution, overcrowding, and unregulated commercial activities. The KDE analysis further reveals that negative sentiment intensity is highly concentrated in specific coastal hotspots (e.g., Phuket, Krabi, and Pattaya). This study contributes methodologically by demonstrating the integrated effectiveness of large-scale sentiment analysis and spatial mapping in tourism research, effectively addressing the implementation gap, providing actionable, precision-based insights for the National Tourism Development Plan (2023–2027). The findings support policymakers and stakeholders in formulating strategies that align with the BCG (Bio-Circular-Green) economic model and balance economic growth with sustainable environmental management by enabling location-specific resource allocation.

**Keywords:** Natural Language Processing (NLP); sentiment analysis; spatial analysis; sustainable tourism; Thailand natural attraction

## 1. Introduction

The tourism industry is a vital pillar of the Thai economy, contributing approximately 17.79% of the country's GDP [1] and generating substantial employment. Thailand's appeal to international tourists stems from its rich resources, encompassing both diverse natural attractions—such as renowned beaches, mountains, and waterfalls—and captivating historical and cultural sites [2]. Natural attractions, in particular, remain key points of interest, drawing large numbers of international tourists and offering a wide range of activities such as trekking and snorkeling [2-4]. The digital landscape has fundamentally changed how tourists research and share experiences. Today, online media is the primary channel for travel information, with 60.54% of international visitors using platforms such as Instagram, Facebook, and TikTok [5-7]. Among these, TripAdvisor stands out as the world's largest and most utilized source for sharing and accessing travel reviews [8]. Among these, TripAdvisor remains a critical source of electronic word of mouth (e-WOM), providing valuable, real-time data that reflects tourist behavior and satisfaction [8]. While platforms like Google Reviews and Booking.com have gained prominence, TripAdvisor's structured review system for specific natural landmarks remains a gold standard for longitudinal tourism research [9, 10]. The proliferation of this user-generated content presents a significant opportunity for tourism research [11, 12].

Analyzing this massive volume of textual data requires sophisticated techniques, such as Sentiment Analysis (or opinion mining), a key component of Natural Language Processing (NLP) [13-15]. Sentiment analysis, particularly when conducted with advanced deep learning models such as BERT-based architectures, including the Pysentimiento framework used in this study [16, 17], is highly effective for big data. Unlike traditional Lexicon-based approaches or earlier Recurrent Neural Networks (RNNs), BERT-based models utilize bidirectional transformers to capture the context of words in tourism reviews, effectively handling linguistic nuances such as sarcasm or multi-word descriptors of environmental quality [18]. This methodology allows stakeholders—including entrepreneurs and policymakers—to gain deep insights into tourist satisfaction, identify complex behaviors, and understand which specific aspects influence decision-making [19, 20]. Positive reviews can guide enhancement strategies, while negative feedback directly informs necessary improvements in quality, services, and infrastructure [21].

However, despite Thailand's success in attracting large numbers of tourists, persistent challenges remain, including the deterioration of natural resources, infrastructure limitations, and concerns about sustainability [1]. These challenges are currently being addressed through the National Tourism Development Plan (2023–2027) [4], which increasingly incorporates the Bio-Circular-Green (BCG) economy model as a supplementary strategic framework to enhance resource efficiency and environmental resilience [22]. Prior research has widely applied sentiment analysis to tourism reviews globally, including studies on hotels and general attractions using TripAdvisor data [23-25]. Within Southeast Asia, studies have begun leveraging e-WOM to evaluate destination sustainability in countries such as Indonesia [26, 27]; however, much of the existing literature in Thailand remains focused on general sentiment or specific local contexts, without a national-level spatial perspective [28, 29]. A critical gap remains: most existing Thai studies lack an in-depth, aspect-based sentiment analysis that identifies localized issues—such as cleanliness, crowds, or conservation—that concern international tourists at natural attractions [7, 30].

This research aims to address the identified gap by conducting an in-depth, aspect-based sentiment analysis of international tourist reviews regarding natural attractions in Thailand collected over ten years (2014–2023). Utilizing high-accuracy deep learning techniques, the study is structured around three primary objectives: (1) assess the sentiment towards these attractions: to quantitatively evaluate the distribution of positive, neutral, and negative perceptions across various natural attraction types, (2) identify specific issues and emotional keywords: to qualitatively extract thematic concerns related to cleanliness, crowds, and conservation through N-Gram analysis, and (3) map the geographical concentration of dissatisfaction: to visualize spatial sentiment hotspots using Kernel Density Estimation (KDE) to pinpoint localized management crises. The findings will provide actionable, detailed insights for relevant government agencies and tourism stakeholders, enabling them to make targeted improvements to resource management, service quality, and future development plans more effectively and efficiently.

The remainder of this paper is structured as follows: Section 2 outlines the Materials and Methods; Section 3 presents the Results and Discussion; and Section 4 provides the Conclusion.

## 2. Materials and Methods

### 2.1 Study Area and Data Collection

This study focuses on natural attractions across all 77 provinces of Thailand (Figure 1). Data was systematically collected from TripAdvisor over a decade (2014-2023). Attractions were categorized into 17 distinct types based on the Natural Tourist Attraction Quality Assessment Manual [31]. These types include: Beach, Botanical Garden, Cave, Forest Park, Fossil, Island, Lookout & Scenic Walking Area, Mangrove Forest, Mountain & Hill, Nature & Wildlife Area, Rapid, Special Geography & Topography, Special Plant & Wildlife, Thermal Spring, Water Resource, and Waterfall.

Web scraping was performed using a Python-based pipeline [32] to extract 124,066 international tourist reviews. Selenium [33] was specifically utilized to navigate dynamic JavaScript-rendered content and manage automated pagination, while BeautifulSoup [34] was employed to parse and extract specific HTML elements from the loaded pages. The extracted data was then structured and cleaned using the Pandas library [35] before being stored in CSV format. To ensure research integrity, the scraping process adhered to ethical data acquisition standards. This included strictly collecting only publicly available review content, regulating request frequencies to comply with TripAdvisor's robot.txt directives, and ensuring alignment with the platform's Terms of Service regarding non-commercial, academic data usage.

The collected data encompassed the attraction's name, type, date, title, content, and location. Regarding categorization, attractions were manually assigned to the 17 types by the researchers, cross-referencing TripAdvisor's location data with the official definitions in the Quality Assessment Manual [31] to ensure thematic accuracy.

### 2.2 Sentiment Analysis Framework and Data Preprocessing

The research follows a three-stage text mining framework: (1) Data Collection and Preprocessing, (2) Sentiment Analysis, and (3) Issue Identification (Figure 2). Reviews were rigorously preprocessed [36]. Since the study aims to capture the perspectives of international tourists using the Pysentimiento English-language model, only English-language reviews were included in the final dataset. Reviews in other languages were excluded during the initial filtering phase to avoid translation inaccuracies; however, this targeted language focus is further addressed and discussed in the subsequent recommendations section of this paper.

The cleaning involved converting text to lowercase, removing irrelevant components (URLs, punctuation, symbols, and special characters) [37], and selectively removing stop words [38]. Following standard NLP practices, the text was first tokenized into individual word IDs [39]. These tokens were then lemmatized to reduce words to their base forms (e.g., "beaches" to "beach") [36], ensuring that morphological variation did not skew the frequency analysis.

### 2.3 Deep Learning Model Selection and Analysis

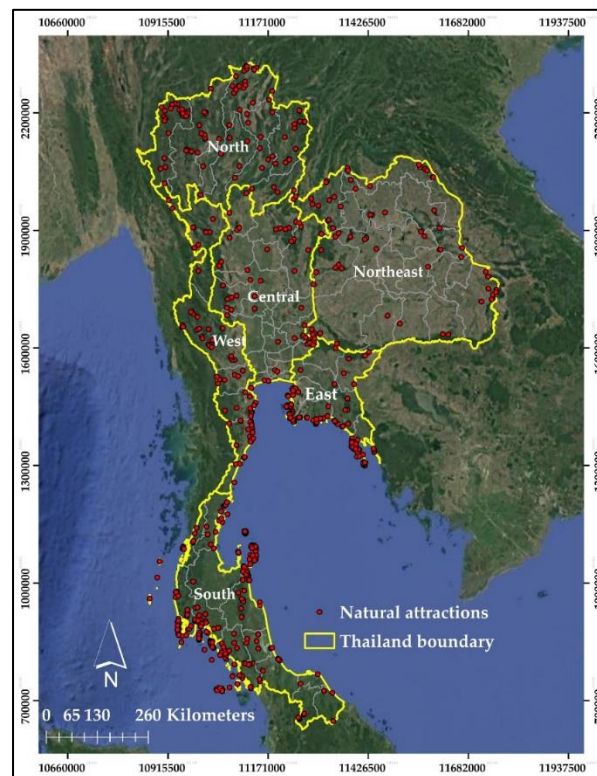
The study used deep learning models for sentiment analysis due to their superior performance on large, redundant social media datasets [16, 17].

#### 2.3.1 Model Selection

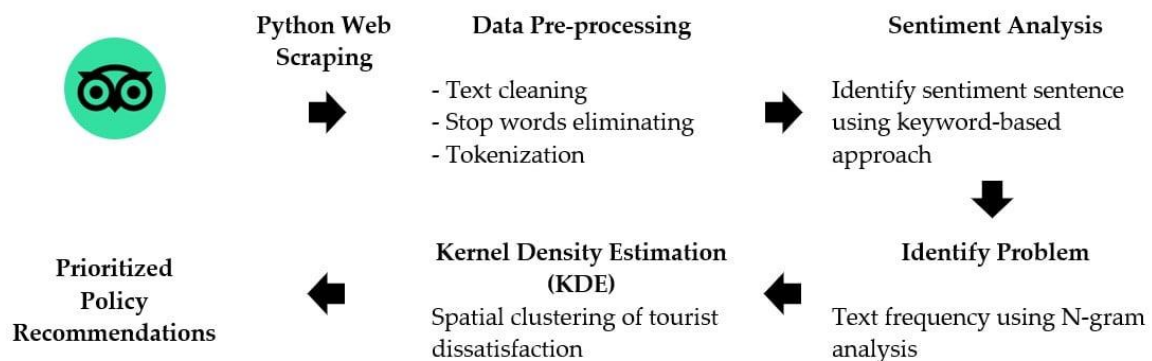
Before final implementation, multiple robust deep learning models were comparatively analyzed to ensure optimal accuracy, including RoBERTa-base [40], XLM-roBERTa-base [41], DistilBERT-base [42], and BERT [43], as well as a lexicon-based model (VADER) [44]. The Pysentimiento model, a Python toolkit based on BERT architecture [45], was ultimately selected for its superior capability and proven high performance in sentiment analysis tasks on social media data. While RoBERTa-base showed a slightly higher overall accuracy, Pysentimiento demonstrated the highest Precision (0.87), as shown in Table 1. This high precision is particularly critical for this study, as it ensures that the negative sentiments—the primary focus for identifying tourist dissatisfaction and management hotspots—are identified with the greatest reliability, minimizing false positives. The model classifies reviews into three categories: positive (e.g., "best bay"), negative (e.g., "dirty



water"), or neutral (e.g., "ordinary tourist place"). Optimal performance was achieved through hyperparameter fine-tuning, including the Number of Epochs (3, 4, 5), Batch Size (32), and Learning Rate ( $2e-5$  to  $1e-4$ ) [43, 45].



**Figure 1.** Study Area Map: Natural Attractions in All 77 Provinces of Thailand



**Figure 2.** Sentiment Analysis Framework

**Table 1.** Comparative Performance of Sentiment Analysis Models

| Model            | Precision   | Recall | F1-Score | Accuracy    |
|------------------|-------------|--------|----------|-------------|
| RoBERTa-base     | 0.83        | 0.69   | 0.76     | <b>0.78</b> |
| XLM-roBERTa-base | 0.60        | 0.87   | 0.71     | 0.70        |
| DistilBERT base  | 0.68        | 0.82   | 0.75     | 0.73        |
| BERT             | 0.78        | 0.60   | 0.68     | 0.70        |
| Pysentimiento    | <b>0.87</b> | 0.65   | 0.74     | 0.76        |
| VADER            | 0.77        | 0.83   | 0.51     | 0.68        |

### 2.3.2 Model Evaluation

The Pysentimiento model was evaluated using a specific TripAdvisor dataset of 4,509 expert-labeled reviews, comprising 2,642 positive, 575 neutral, and 1,292 negative samples (80% for training, 10% for testing, and 10% for evaluation). To ensure the quality of the ground truth data, these reviews were independently manually annotated by three researchers with expertise in tourism management and linguistics. Each review was categorized as positive, negative, or neutral based on explicit sentiment indicators. To validate the consistency of this manual labeling, Cohen's Kappa (K) was calculated to evaluate inter-rater agreement while accounting for chance. The calculation follows the formula:

$$K = \frac{p_o - p_e}{1 - p_e} \quad (1)$$

where  $p_o$  represents the relative observed agreement, and  $p_e$  is the hypothetical probability of chance agreement. In this evaluation, the researchers achieved an observed agreement ( $p_o$ ) of 0.91 (representing consensus on 4,103 reviews). Given the distribution of the categories, the probability of chance agreement ( $p_e$ ) was calculated at 0.50, resulting in a final Kappa coefficient of 0.82. According to Landis and Koch [46], this "almost perfect agreement" confirms the high reliability of the ground truth dataset.

The model's performance was rigorously assessed against three key criteria: pre-trained English-language classification, hyperparameter fine-tuning, and fairness assessment [45, 47]. Model performance was then assessed using a Confusion Matrix (Table 2), a statistical tool that analyzes the correlation between predicted and actual outcomes [27].

**Table 2.** The Confusion Matrix used to Verify Pysentimiento Analysis

| Actual / Prediction | Positive | Neutral | Negative |
|---------------------|----------|---------|----------|
| Positive            | a        | b       | c        |
| Neutral             | d        | e       | f        |
| Negative            | g        | h       | i        |

The overall model efficacy was calculated using standard metrics derived from the Confusion Matrix: Precision (P), Recall (R), F1-Score (F1), and Accuracy (A). The equations for these metrics are as follows:

$$P(neg) = \frac{i}{(c+f+i)} \quad (2)$$

$$R(neg) = \frac{i}{(g+h+i)} \quad (3)$$

$$F1(neg) = \frac{2P(neg) \times R(neg)}{P(neg) + R(neg)} \quad (4)$$

$$A = \frac{a+e+i}{a+b+c+d+e+f+g+h+i} \quad (5)$$

## 2.4 Identifying Issues

Issues were identified through qualitative content analysis on the sentiment results using the Natural Language Toolkit (NLTK) [48]. While the primary focus of this study is to identify management crises through negative sentiment, a comparative N-Gram analysis was also applied to positive reviews to provide a balanced overview of destination strengths, aligning with the principles of Aspect-Based Sentiment Analysis (ABSA). These insights are specifically integrated into the policy recommendations to ensure that future management strategies not only resolve existing problems but also leverage and sustain Thailand's competitive advantages in its natural attractions.

**Word Frequency Analysis:** Counting word frequency to identify the top 30 most recurring tokens over the decade.

**N-Gram Analysis:** Determining the co-occurrence of contiguous words (Uni-Gram through Quart-Gram) to identify specific problems associated with each of the 17 attraction types (Figure 3). The researchers acknowledge that for certain categories with lower review volumes (e.g., Botanical Gardens or Mangrove Forests), the extracted N-Grams may yield more generic descriptors. These instances are explicitly noted in the results to distinguish them from the data-rich insights in high-volume categories such as Beaches and Islands. **Thematic Categorization:** Extracted keywords were grouped into broader themes (e.g., Environmental, Service, and Accessibility). To ensure the reliability of this inductive coding process, the thematic mapping was cross-validated by three independent researchers, following the same inter-rater reliability protocol used in the sentiment validation phase to minimize subjective bias. This methodology enables stakeholders to classify negative reviews by specific, recurrent issues, thereby supporting prioritized problem resolution in tourism and environmental policy [49].

['beach', 'not', 'clean', 'expected', 'services', 'good']

|            |                          |                                      |                              |                        |               |      |
|------------|--------------------------|--------------------------------------|------------------------------|------------------------|---------------|------|
| Mono-Gram  | beach                    | not                                  | clean                        | expected               | services      | good |
| Bi-Gram    | beach not                | not clean                            | clean expected               | expected services      | services good |      |
| Tri-Gram   | beach not clean          | not clean expected                   | clean expected services      | expected services good |               |      |
| Quart-Gram | beach not clean expected | not clean expected services expected | clean expected services good |                        |               |      |

**Figure 3.** N-Gram Analysis

## 2.5 Spatial Analysis: Kernel Density Estimation (KDE)

To provide actionable spatial insights for tourism management, the geographic distribution of negative sentiments was analyzed using Kernel Density Estimation (KDE). KDE is a non-parametric spatial analysis method used to estimate the probability density function of point data, effectively transforming discrete points (the locations of attractions with negative reviews) into a continuous surface that illustrates the spatial concentration (hotspots) of events [50, 51]. The estimated density at any location  $x$  is calculated as:

$$\hat{f}(x) = \frac{1}{nh^2} \sum_{i=1}^n K\left(\frac{x-x_i}{h}\right) \quad (6)$$

Where:  $\hat{f}(x)$  is the estimated density,  $n$  is the number of negative review observations,  $h$  is the bandwidth (smoothing parameter),  $K$  is the kernel function, and  $x_i$  represents the location of each negative review.

The input data comprised the complete set of negative reviews, with the review count for each attraction serving as the Population Field (weight). This ensures that high-volume attractions contribute proportionally to the density surface. While this weighting reflects actual intensity, the researchers acknowledge that the inherent density of data in high-traffic areas (e.g., popular beaches) naturally dominates the KDE surface compared to niche sites (e.g., fossil sites). This inherent data characteristic is addressed in the limitations and interpreted within the context of destination popularity. The KDE was executed using Python's SciPy [52] and Scikit-learn [53] libraries. A critical parameter in KDE is the bandwidth ( $h$ ). In this study, a 0.5-kilometer bandwidth was strategically selected to identify "hyper-local" hotspots. This fine-scale resolution is essential for distinguishing between specific management crises within adjacent but distinct tourist zones, such as different beach segments or neighboring national park entrances. However, to address the broader national-level policy perspective and ensure spatial robustness, a sensitivity analysis was conducted by comparing the 0.5 km setting against wider bandwidths (5 km, 25 km, and 50 km) as recommended in spatial tourism research. The 0.5 km setting provided the highest precision for localized intervention, while the larger bandwidths offered a generalized view of regional trends.

Furthermore, to ensure comparative clarity across regions, the final density maps (Figure 5) use a standardized continuous scale with absolute numerical density values rather than a simple relative gradient. This allows for the direct comparison of negative sentiment intensity across different provinces.

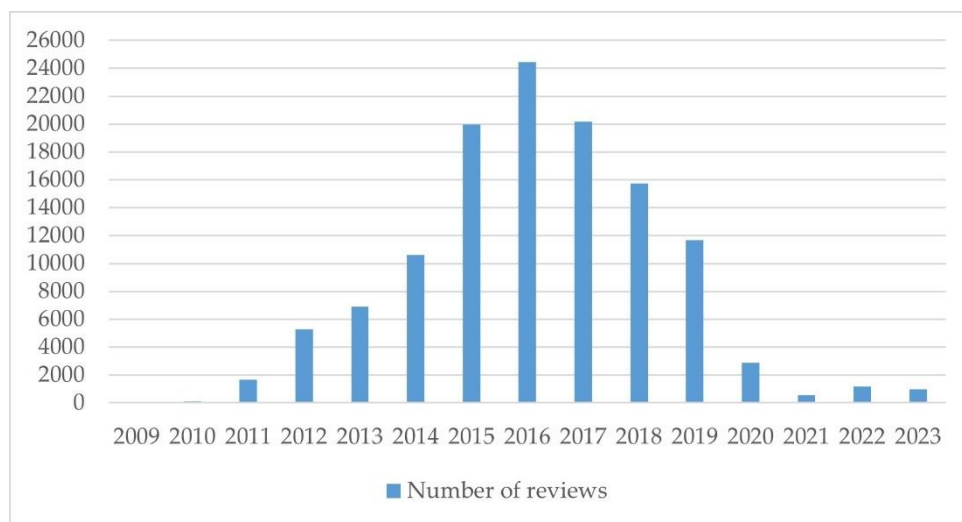
### 3. Results and Discussion

#### 3.1 Understanding Sentiment and Annual Trends

Reviews from international tourists regarding natural attractions across all 77 provinces of Thailand were collected from the TripAdvisor platform, yielding a substantial total of 124,066 reviews. This volume of data serves as a valuable proxy for understanding real-world tourism trends [15, 54].

##### 3.1.1 Annual Trend and Contextual Shifts

The analysis of the dataset's temporal distribution reveals significant shifts in tourist behavior towards sharing experiences online [9]. Figure 4 illustrates the annual trend, showing that the number of reviews peaked in 2016 before declining markedly toward the end of 2019. This decline is directly correlated with the global impact of the COVID-19 pandemic [55], which led to a significant decrease in arrivals. While the volume decreased during 2020–2023, the core concerns regarding environmental quality remained consistent with pre-pandemic patterns, though health-related sentiments saw a temporary increase.



**Figure 4.** Number of Reviews from International Tourists of Natural Attractions in Thailand from TripAdvisor

##### 3.1.2 Sentiment Analysis Performance and Overall Distribution

The sentiment analysis of the 124,066 reviews was performed using the Pysentimiento deep learning model [43, 45]. The model was prioritized for its high Precision (0.87), ensuring that negative sentiments—the primary focus for crisis identification—were identified with 87% reliability [17, 56, 57]. The overall sentiment distribution is strongly positive, with 91,145 positive reviews (73.47%), 17,883 neutral reviews (14.41%), and 15,038 negative reviews (12.12%). This predominant positivity is consistent with a general positive bias in online reviews. Table 3 presents an in-depth aspect-based sentiment analysis categorized by the 17 attraction types. The results show that while overall satisfaction is high, certain attraction types (such as Beach and Island) receive the vast majority of reviews, highlighting where positive perceptions are most concentrated, reflecting Thailand's world-renowned natural splendor.

**Table 3.** The Results of Sentiment Analysis of Natural Attractions Types in Thailand

| Types                          | Positive |       | Neutral |        | Negative |       | Total reviews | %      |
|--------------------------------|----------|-------|---------|--------|----------|-------|---------------|--------|
|                                | Number   | %     | Number  | %      | Number   | %     |               |        |
| Beach                          | 53,414   | 72.63 | 9,737   | 13.24  | 10,394   | 14.13 | 73,545        | 59.28  |
| Botanical Garden               | 75       | 78.13 | 17      | 17.71  | 4        | 4.17  | 96            | 0.08   |
| Cave                           | 1,900    | 68.03 | 648     | 23.20  | 245      | 8.77  | 2,793         | 2.25   |
| Forest Park                    | 112      | 78.32 | 21      | 14.69  | 10       | 6.99  | 143           | 0.12   |
| Fossil                         | 10       | 71.43 | 4       | 28.57  | -        | -     | 14            | 0.01   |
| Island                         | 15,162   | 73.77 | 2,944   | 14.32  | 2,446    | 11.90 | 20,552        | 16.56  |
| Lookout & Scenic Walking Area  | 23       | 85.19 | 4       | 14.81  | -        | -     | 27            | 0.02   |
| Mangrove Forest                | 99       | 78.57 | 21      | 16.67  | 6        | 4.76  | 126           | 0.10   |
| Mountain & Hill                | 9,204    | 80.97 | 1,608   | 14.15  | 555      | 4.88  | 11,367        | 9.16   |
| Nature & Wildlife Area         | -        | 0.00  | 1       | 100.00 | -        | -     | 1             | 0.01   |
| Rapid                          | 138      | 75.41 | 32      | 17.49  | 13       | 7.10  | 183           | 0.15   |
| Special Geography & Topography | 2,518    | 70.39 | 704     | 19.68  | 355      | 9.92  | 3,577         | 2.88   |
| Special Plant & Wildlife       | 93       | 76.86 | 27      | 22.31  | 1        | 0.83  | 121           | 0.10   |
| Thermal Spring                 | 1,073    | 66.07 | 308     | 18.97  | 243      | 14.96 | 1,624         | 1.31   |
| Water Resource                 | 2,525    | 71.11 | 771     | 21.71  | 255      | 7.18  | 3,551         | 2.86   |
| Waterfall                      | 4,799    | 75.62 | 1,036   | 16.33  | 511      | 8.05  | 6,346         | 5.12   |
| Total                          | 91,145   |       | 17,883  |        | 15,038   |       | 124,066       |        |
| %                              |          | 73.47 |         | 14.41  |          | 12.12 |               | 100.00 |

### 3.2 Identifying Core Issues through Content

While overall sentiment is positive, a deeper analysis of negative reviews is crucial for identifying areas requiring improvement [58, 59]. In alignment with the study's objective to analyze international tourist perspectives, English-language reviews were specifically selected as the primary data source. As the most widely used language for global communication on TripAdvisor, these reviews provide a consistent and representative dataset for evaluating how a diverse international audience perceives Thailand's natural attractions. This focus ensures that the resulting management insights and policy recommendations directly address the expectations and experiences of the global travel market.

#### 3.2.1 N-Gram Analysis and Specific Issues

Issue identification focused on negative reviews using Word Frequency and N-Gram Analysis (Uni-Gram through Quart-Gram) via NLTK [48]. Table 4 highlights the most frequently mentioned keywords. For high-volume categories like Beaches and Islands, the N-Grams yield specific actionable insights (e.g., "dirty," "rip prices"). For niche categories with fewer reviews (e.g., Botanical Garden), the results show more generic terms (e.g., "walk," "place"), which are noted as preliminary indicators due to the smaller sample size.

#### 3.2.2 Thematic Categorization of Dissatisfaction

The N-Gram analysis provided critical insights by systematically categorizing tourist dissatisfaction into three main groups: Environmental & Maintenance, Service & Facility, and Accessibility & Infrastructure (Table 5). To ensure the reliability of this inductive coding, the mapping was cross-validated by three independent researchers to minimize subjective bias.



**Table 4.** The Results of N-Gram Analysis of Natural Attractions Types in Thailand

| <b>Types</b>                   | <b>The highest number of mentions in the N-Gram analysis</b>                                          |
|--------------------------------|-------------------------------------------------------------------------------------------------------|
| Beach                          | dirty, rubbish, not clean, many people, would not recommend, people trying to sell stuff              |
| Botanical Garden               | go, place, walk                                                                                       |
| Cave                           | people, crowded, climb, entrance, dark, tourist trap, waste time, not worth it, do not speak English  |
| Forest Park                    | plastic, roads, fee, place, walk, car, 10 min fill                                                    |
| Island                         | crowded, snorkeling, not worth it, long tail boats, 400 baht per person, rip prices captive market    |
| Mangrove Forest                | place, walkway, walk, road, disappointed, entrance, drive, restaurant, information                    |
| Mountain & Hill                | pay, people, walk, tourist, climb, drive, not worth, entrance fee, tourist trap                       |
| Rapid                          | price, drive, trail, walk, steep, entrance, restaurant, fee 30 baht                                   |
| Special Geography & Topography | nothing, tourist, people, parking, walk, shop, stall, tricky, tourist trap, not worth                 |
| Special Plant & Wildlife       | tree, spoilt, lame, stall                                                                             |
| Thermal Spring                 | people, pay, entrance fee, tourist, dirty overall, 300 baht entry, would not recommend                |
| Water Resource                 | tourist, dirty, tricky, tourist trap, long tail boats, not much to see, total waste of time and money |
| Waterfall                      | walk, people, pay, small, swim, climb, no water, dry season, fee 300 baht                             |

Environmental & Maintenance Issues: Keywords such as "dirty," "rubbish," and "plastic" highlight critical problems related to cleanliness and pollution (common at Beach and Water Resource destinations). Problems of Overcrowding & Overuse ("crowded," "many people") indicate recurring management issues, consistent with challenges identified in Thailand's National Tourism Development Plan [4]. Furthermore, Natural Condition issues, such as "no water" during the dry season (common at Waterfalls), underscore the need for better management of tourist expectations regarding seasonal factors. Service & Facility Issues: Negative sentiments concerning Worthiness ("not worth," "waste time," "rip prices") and High costs/fees (e.g., "300 baht entry") are primary indicators of a mismatch between tourist expectations and the actual quality received. These issues are most commonly reported in paid attractions (e.g., Cave, Mountain & Hill, Thermal Spring). Challenges related to the overall Travel Experience ("tourist trap," "not speak English") highlight the need to enhance service-provider quality. Accessibility & Infrastructure Issues: Keywords related to Access & Transport ("road," "long tail boats," "climb") found across various natural attractions indicate that overly challenging routes and inadequate transportation systems diminish the travel experience. Similarly, issues with Amenities ("walkway," "dark," "restaurant") are pressing micro-level problems requiring urgent improvement.

**Table 5.** The Results of All Levels in N-Gram Analysis of Natural Attractions Types in Thailand

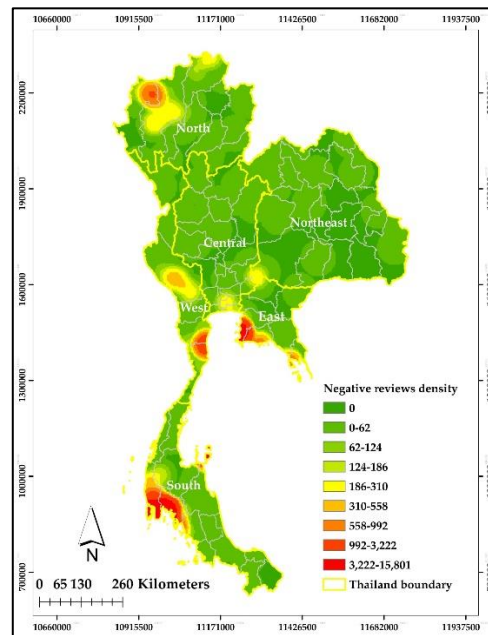
| Main Issues                    | Sub-Issues              | Keywords                                                                             | Types                                                                                                                        |
|--------------------------------|-------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| Environmental & Maintenance    | Cleanliness & Pollution | dirty, rubbish, plastic, water not clean, dirty overall, not worth it                | Beach, Forest Park, Thermal Spring, and Water Resource                                                                       |
|                                | Crowding & Overuse      | people, crowded, tourist trap, many people                                           | Beach, Cave, Island, Mountain & Hill, Special Geography & Topography, and Water Resource                                     |
|                                | Natural Condition       | no water, dry season, nothing to see, not much to see, nothing special               | Cave, Special Geography & Topography, Special Plant & Wildlife, and Waterfall                                                |
| Service & Facility             | Worthiness              | not worth, waste time, not worth time, money, rip prices, not worth the trip         | Cave, Island, Special Geography & Topography, Thermal Spring, Water Resource, and Waterfall                                  |
|                                | High costs/fees         | price, fee, entrance fee, pay, 300 baht per person                                   | Forest Park, Mountain & Hill, Rapid, Thermal Spring, and Waterfall                                                           |
|                                | Travel Experience       | tourist trap, would not recommend, people trying to sell stuff, do not speak English | Beach, Cave, Island, Mountain & Hill, Special Geography & Topography, and Water Resource                                     |
| Accessibility & Infrastructure | Access & Transport      | road, way, walk, drive, climb, long tail boats, speed boat                           | Botanical Garden, Cave, Island, Forest Park, Mangrove Forest, Mountain & Hill, Water Resource, and Waterfall                 |
|                                | Amenities               | place, walkway, dark, restaurant, shop, stall                                        | Botanical Garden, Cave, Mangrove Forest, Rapid, Special Plant & Wildlife, Special Geography & Topography, and Thermal Spring |

### 3.3 Spatial Visualization and Priority Recommendations

Following issue identification, Kernel Density Estimation (KDE) was performed on 15,038 negative reviews to visualize spatial clustering.

#### 3.3.1 Spatial Analysis of Negative Sentiment Hotspots

A bandwidth ( $h$ ) of 0.5 km was utilized to capture "hyper-local" clustering patterns. This precision is essential for site-specific management, despite larger bandwidths (5–50 km) being common for regional overviews. The resulting density map, presented in Figure 5, incorporates a numerical density scale (Reviews/km<sup>2</sup>) to allow direct quantitative comparison between hotspots. Figure 5 reveals that the highest density is concentrated in Phuket (Patong, Kata, Karon), Krabi (Phi Phi, Ao Nang), Chonburi (Pattaya, Ko Larn), and Surat Thani (Koh Samui, Koh Phangan). This concentration provides location-specific validation of high-priority issues such as cleanliness and overcrowding.



**Figure 5.** Kernel Density Map of Negative Reviews in Thailand

### 3.3.2 Prioritized Policy Recommendations

Based on empirical evidence from sentiment and N-Gram analyses, and supported by spatial validation from the KDE analysis, this study categorizes the identified problems into three priority levels. These recommendations directly support the Department of Tourism's Action Plan (2023–2027), specifically by integrating the BCG model to transition Thailand toward high-value, sustainable tourism. However, our empirical findings reveal a critical implementation gap: while the national plan aims for 'High Value' tourism, the recurring N-Grams of 'tourist traps' and 'dirty' environments in high-density KDE hotspots indicate that local management still struggles to align with these national strategic goals. This study provides the necessary spatial precision to bridge this gap by identifying exactly where national policies fail to translate into positive tourist experiences.

#### (1) High Priority Issues: Immediate Crisis Management

These issues have a direct, immediate impact on tourist perceptions and require urgent intervention to prevent long-term reputational damage:

**Cleanliness and Environmental Quality:** Geographically validated by KDE hotspots in Phuket, Krabi, and Chonburi. This requires strict enforcement of waste management regulations consistent with Circular Economy principles to mitigate the impact of 'rubbish' and 'plastic' on natural capital (e.g., MNRE, DNP). Resources should be specifically diverted to high-density negative sentiment zones identified in Figure 5.

**Value for Money:** Beyond simple pricing standards, this study suggests a need for optimized pricing strategies based on tourists' "Willingness to Pay" (WTP) for premium natural capital. High entry fees (e.g., 300-400 Baht) must be justified by visible improvements in facility quality and service transparency (e.g., TAT, DNP). A mismatch here, as shown in the "not worth it" N-Grams, indicates that current pricing exceeds perceived value, necessitating a more dynamic or value-added pricing model.

#### (2) Medium Priority Issues: Strategic Adjustments

These issues act as barriers to sustainable development and require coordinated cross-agency planning. **Overcrowding and Carrying Capacity:** Since overcrowding is a primary driver of KDE hotspots, we propose a "Spatial Dispersion Strategy." Using the KDE results, authorities should redirect tourist traffic from "Saturated Hotspots" (e.g., Phi Phi, Patong) toward identified "Potential Alternative Destinations"—areas with high-quality natural resources but low negative sentiment density (e.g., secondary coastal zones in Trang or Satun). This aligns with the TAT's mission to promote secondary cities. **Information and Communication:** To

manage expectations regarding seasonal factors (e.g., "no water" at waterfalls), a multi-language digital platform (MOTS, TAT) should provide real-time updates on site conditions, reducing dissatisfaction caused by misinformation.

### (3) Long-term Priority Issues: Structural and Infrastructure Development

These structural issues require long-term investment to ensure the resiliency of Thailand's tourism sector. Infrastructure and Accessibility: Plans must prioritize improving transportation routes (DOH, DRR) and supporting local entrepreneurs. Strategic infrastructure investment should focus on enhancing access to non-hotspot areas to naturally facilitate tourism dispersion and reduce the environmental load on currently saturated zones. Attraction Quality and Resource Restoration: Long-term environmental restoration plans (MNRE) must be synchronized with seasonal attraction closures. This ensures that natural capital is sustained in accordance with the BCG (Bio-Circular-Green) economic model promoted in the National Tourism Development Plan.

## 4. Conclusions

This study successfully integrated Transformer-based Deep Learning with spatial analytics to provide a high-precision diagnostic of Thailand's natural tourism sector. The findings offer a robust framework for transitioning from generalized national policies to evidence-based, localized interventions.

### 4.1 Methodological Contributions

Validated Sentiment Precision: The application of the Pysentimiento (BERT-based) model demonstrated superior performance in capturing the linguistic nuances of 124,066 international reviews. The model's reliability was supported by an inter-rater agreement (Cohen's Kappa) of 0.82, indicating "almost perfect agreement" in the ground-truth data used for validation. Spatial Specificity: By utilizing Kernel Density Estimation (KDE) with a 0.5 km bandwidth, this research moved beyond traditional statistical summaries to identify "hyper-local" hotspots of dissatisfaction. This spatial precision allows stakeholders to pinpoint specific management crises within adjacent but distinct tourist zones.

### 4.2 Core Empirical Findings

Thematic Clusters of Dissatisfaction: Through N-Gram analysis, the study categorized the drivers of negative sentiment into three primary pillars: Environmental & Maintenance (e.g., "dirty," "rubbish"), Service & Facility (e.g., "tourist trap," "not worth"), and Accessibility & Infrastructure (e.g., "road," "long-tail boat fees"). Geographic Vulnerability: The highest concentration of negative sentiment is critically localized in major coastal hubs—Phuket, Krabi, Chonburi, and Surat Thani. The intensity of issues in these zones (quantified in Reviews/km<sup>2</sup>) highlights a significant "Implementation Gap" where local management struggles to keep pace with the high volume of international arrivals.

### 4.3 Strategic Policy Recommendations

The study proposes a three-tier priority framework aligned with the Department of Tourism's Action Plan (2023-2027). High Priority (Immediate): Urgent waste management and Value-for-Money optimization (aligning pricing with "Willingness to Pay") are required in the identified KDE hotspots to protect Thailand's international reputation. Medium Priority (Strategic): Implementing Spatial Dispersion Strategies to redirect traffic from saturated hotspots to potential alternative destinations (e.g., secondary coastal cities) with low negative sentiment density. Long-term (Structural): Synchronizing resource restoration with the BCG (Bio-Circular-Green) economic model to ensure the long-term resiliency and sustainability of Thailand's natural tourism assets.

### 4.4 Limitations and Future Directions

Language and Platform Scope: This study strategically focused on English-language reviews on TripAdvisor as a proxy for the global international market. While representative of a significant segment, future research should incorporate multi-lingual data (e.g., Chinese or East Asian platforms) to capture a more holistic view of diverse cultural expectations. Temporal Dynamics: While the analysis accounts for the COVID-19 disruption, ongoing monitoring is essential to evaluate if post-pandemic expectations regarding environmental standards and value-for-money have undergone a permanent shift. Analytical Rigor: Future studies could

benefit from multi-scale KDE analysis to compare local micro-hotspots with broader regional trends, further refining the granularity of national tourism planning.

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**Author Contributions:** Methodology, validation, investigation, resources, visualization, writing—original draft preparation, TB; writing—review and editing, NP; conceptualization—AI, AS; conceptualization—tourism, PN; software, formal analysis, and data curation, SR. All authors have read and agreed to the published version of the manuscript.

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# Health Analysis for Ergonomic Risk Assessment among Rice and Sugarcane Farmers Using LINE Chatbot Application

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**Abstract:** Farmers are at risk of exposure to a variety of health hazards, particularly unnatural and strenuous working postures in the fields. This study aimed to develop and evaluate a LINE-based ergonomic risk assessment application integrated with a chatbot recommendation system. Specifically, the study evaluated the application's validity, compared time efficiency between paper-based and application-based assessments, and assessed user satisfaction among health personnel and farmers. The sample consisted of 30 application users, including subdistrict health promotion hospital staff/public health officers, village health volunteers or farmer leaders, and research assistants, and 125 farmers as assessors. The tools used included the Rapid Entire Body Assessment (REBA), the application, and a questionnaire. Data were analyzed using descriptive statistics, a one-sample t-test, and a paired t-test ( $p < 0.05$ ). The study found that the chatbot application passed expert evaluation and passed the test. It performed the following functions: login, assessment based on observation images, selection of images for assessment, introduction of risk in the chatbot, and accurate recording of results. Experts assessed its suitability with an average score of  $4.87 \pm 0.11$  (out of a full 5.00), which is considered the highest level. The application's effectiveness, when used with farmers, was significantly reduced in time compared to the traditional assessment ( $p < 0.05$ ). Scores obtained from the traditional and application methods were not significantly different. The results of the satisfaction assessment of the application among farmers showed that users were most satisfied with the benefits and application, with an average score of  $4.80 \pm 0.36$ . In terms of ergonomics, farmers had a prevalence of right shoulder and neck pain of 17.6% (95% CI=12.0-24.8) and 16.8% (95% CI=9.9-24.5), respectively. The application can be easily used to assess ergonomic risks and takes less time than the previous format. Moreover, the results of the assessment are of the same quality as those assessed by experts using the previous format. Recommendations: The application should be expanded to farmers to obtain a comprehensive picture of the country. High-risk groups should be followed up with to reduce their risk levels.

**Keywords:** Chatbot; ergonomic risk; farmers; REBA; mobile health

## 1. Introduction

Agricultural productivity has a positive impact on the national economy. Thailand is the second-largest rice exporter [1] and the third-largest sugar exporter [2]. Thailand has the largest agricultural sector, accounting for 31.2 percent of the total population [3]. However, in the production process,

farmers are more physically demanding than in other occupations, and their work is carried out in uncontrolled environmental conditions. Occupational illnesses can have both health and economic impacts. Farmers are exposed to physical (heat and natural light), chemical (pesticides), and ergonomic (unnatural and physically demanding work postures) threats [4]. Ergonomic problems affect farmers' health and agricultural productivity, most commonly associated with unnatural working postures (e.g., bending, twisting), excessive force (e.g., digging, lifting heavy sacks), and the use of inappropriate tools (e.g., heavy, loose hoes, large, or vibrating machinery) [5]. This results in pain and musculoskeletal diseases. Sugarcane farmers have been reported to experience neck and back pain at higher rates than other body parts. Furthermore, thin operators are more likely to experience pain and discomfort in certain body parts [6]. Working hours have a statistically significant effect on wrist and low back pain [7]. Various ergonomic control measures have been implemented to reduce musculoskeletal risks among farmers in Thailand, including the use of ergonomic tools, modification of working postures, and training programs to improve work techniques. Previous studies have demonstrated that ergonomic interventions, such as modified agricultural tools and posture training, can significantly reduce physical workload and musculoskeletal symptoms among rice farmers [8]. However, these interventions often require trained personnel and structured implementation, which may not be feasible in all rural settings. Therefore, alternative approaches such as digital assessment tools may provide practical support for identifying ergonomic risks and guiding preventive actions.

Currently, ergonomic surveillance approaches have standardized assessment tools for preventive measures, such as the Rapid Entire Body Assessment (REBA) and Physical workload (%CVL). These assessments were applied to rice farmers in northern Thailand. The REBA assessment and %CVL were measured before and after the farmers experimented with the rice planting innovation. The results showed that REBA scores while planting rice with ergonomic tools were significantly lower than those while planting with assisted tools and traditional rice planting. Furthermore, the physical workload during rice planting with ergonomic tools was significantly lower than that with assisted tools and traditional rice planting [8]. However, the REBA assessment process is complex, requiring measurement of the entire body's working posture and scoring comparisons with multiple tables. Therefore, the analysis primarily relies on occupational health experts. In practice, in farmland areas, health personnel or village health volunteers are unable to conduct the assessment themselves. Ergonomic risk assessment is a complex and multi-step process. However, in today's world, it can be addressed with information technology, depending on the type of data being analyzed and processed. The present study aimed to assess, record, analyze, and interpret results quickly. This approach was designed as a LINE chatbot application that uses images of farmers' working postures adapted from REBA, reducing the need to read text or perform manual calculations. Furthermore, it can be accessed via the LINE app, a popular app in Thailand that can be installed on smartphones. A National Statistical Office [9] survey found that 95.4% of people aged 6 and above use smartphones, and 98.6% use the internet via smartphones, spending an average of 5 hours and 2 minutes per day. This demonstrates the important role of easily accessible technology. This research has been applied to health analysis by defining working postures in degrees of different organs using the standard REBA tool. The resulting scores provide immediate risk levels and recommendations, responding quickly to needs. Chatbots have previously been used for health awareness [10].

Several digital ergonomic assessment tools and health chatbot systems have been developed to improve occupational health surveillance. Traditional ergonomic assessment methods, such as Rapid Upper Limb Assessment (RULA) and the Ovako Working Posture Analysis System (OWAS), have been widely used to evaluate musculoskeletal risk and have later been adapted into digital formats to improve usability and reduce calculation errors [11, 12]. In recent years, information technology and conversational agents have increasingly been applied in healthcare and occupational health settings to support data analysis, improve accessibility to health information, and enhance risk communication among users [10, 13, 14]. These digital approaches enable faster interpretation of complex ergonomic data and provide practical solutions for use in rural agricultural settings where trained personnel may be limited. However, many existing systems primarily focus on health education or basic risk communication rather than integrating standardized ergonomic risk assessment tools with automated scoring and real-time feedback. Therefore, this study extends previous work by integrating the Rapid Entire Body Assessment (REBA) method into a LINE-based chatbot system, enabling



rapid ergonomic risk assessment and immediate personalized recommendations in real field conditions. Despite these technological advancements, the application of standardized ergonomic assessment tools in rural agricultural settings remains limited due to the complexity of assessment procedures and the shortage of trained occupational health professionals. In many farming communities, ergonomic risks are often identified only after musculoskeletal symptoms occur, resulting in delayed prevention and intervention. Therefore, there is a clear need for practical, accessible, and user-friendly tools that enable local health personnel and community workers to conduct ergonomic risk assessments efficiently under field conditions.

Therefore, this study aimed to develop and evaluate a LINE-based ergonomic risk assessment application integrated with a chatbot recommendation system. Specifically, the study aimed to: (1) assess the validity of the developed application, (2) compare the time efficiency between paper-based and application-based ergonomic assessments, and (3) evaluate user satisfaction among health personnel and farmers. This approach supports Sustainable Development Goal (SDG) 3: Good Health and Well-Being by enhancing ergonomic risk surveillance among informal agricultural workers.

## 2. Materials and Methods

### 2.1 Research Design

This research and development study involved creating an application to assess, analyze, process, display results, and provide recommendations for ergonomic risk reduction. The application was then evaluated by experts and actual farmers, who then refined it for greater efficiency.

### 2.2 Sample and sampling

The sample was divided into 3 groups: Group 1 consisted of 3 health and information system experts who evaluated the quality of the created application and the suitability of the content for the purpose, as well as the accompanying images. For Group 2, there were 30 application users, including subdistrict health promotion hospital staff, research assistants, and farmer leaders or village public health volunteers who used the data collection tools. Group 3 was the group evaluated by the application. The sample size for Group 3 (farmers) was determined using the estimated sample size in prevalence studies. The formula used was:  $n = Z^2P(1-P) / d^2$  where  $Z$  represents the standard normal deviation corresponding to a 95% confidence level ( $Z = 1.96$ ),  $P$  represents the estimated prevalence of health effects among sugarcane farmers obtained from a previous study ( $P = 0.09$ ) [15], and  $d$  represents the acceptable margin of error ( $d = 0.05$ ). Based on this calculation, the required minimum sample size was 125 participants. In this study, 125 farmers were recruited, which was considered sufficient to represent the target population and achieve the study objectives. According to national agricultural statistics, Thailand has approximately 4.7 million rice farmers and approximately 0.33 million sugarcane farmers nationwide [16]. In the selected study area of Suphan Buri Province, a list of registered farmers was obtained from local agricultural and health authorities and used as the sampling frame for random selection. Eligible farmers were identified from official community records provided by local health promotion hospitals and agricultural offices. Selected farmers were contacted through village health volunteers and invited to participate in the study. Those who met the inclusion criteria and agreed to participate were enrolled after providing written informed consent. The three sample groups were used for different quality assessments. Experts assessed application quality; public health personnel and research assistants assessed usability; and farmers assessed ergonomics in sufficient numbers for the study. The farmers used a simple random sampling method, randomly selecting from a list of farmers in the study area until the sample size was complete. Farmers were considered inclusive if they had been engaged in rice and sugarcane farming as their full-time job for 1 year, could communicate in Thai, and were willing to participate in the project voluntarily. Exclusion criteria included being absent on the scheduled data-collection date.

### 2.3 Application creation

The REBA standard ergonomic risk assessment tool was developed into an application using the System Development Life Cycle (SDLC). This sequential software development process, coupled with cloud-based web application technology, begins with problem definition, system analysis, system design, system development, system testing, and installation and maintenance. Each phase flows sequentially, like a waterfall,



and can be reversed to improve previous steps. The application, developed by an information technology expert in collaboration with researchers, is called “AgriCare LINE,” or the “Ergonomics Application for Agriculturist Care.” The application creation process involves five steps as follows:

#### **Step 1: Problem Identification**

The project began by studying the problem within the context of the relevant target groups: farmers, public health officials, and village health volunteers. Qualitative data were collected through in-depth interviews to understand the health risks arising from on-the-ground work. This included ergonomic risks, namely the risk of musculoskeletal pain in farmers. In addition, problem identification also included a literature review [4-6] to explore prior knowledge, existing tools, and relevant studies. This approach aimed to derive an academically grounded approach to support the application's development.

#### **Step 2: System Analysis**

The system analysis defines the system's scope, requirements, and functionality. Based on the data collected in Step 1, the development team establishes a preliminary system structure, defining the user groups, system goals, and the conceptual framework for the risk assessment using the standard REBA tool [12], as well as the interrelationships between the various system components. This serves as the basis for system design.

#### **Step 3: System Design**

Design both the front- and back-end of the system using LINE LIFF (LINE Front-end Framework) technology to develop a web app embedded within LINE OA, enabling users to access forms via mobile devices. Vercel (Hosting) was used to host the front-end (React.js) and back-end (Node.js API) embeddings in a serverless system. Firebase (Cloud Firestore) was used as the primary database to collect real-time form data, which was displayed and analyzed in Google Sheets and BigQuery. Wireframes and user interfaces (UIs) were also designed using human-centered design principles to enhance the user experience.

#### **Step 4: System Implementation**

The application was developed using NextJS, NodeJS, and the Firebase SDK. GitLab was used as a version control system to manage the CI/CD (Continuous Integration/Continuous Deployment) process, connecting it to Vercel to ensure continuous, automated system deployment. The questionnaire included in the application is a REBA risk assessment form. Once the user completes the form, the system sends data via an API to Firebase Cloud Firestore for real-time storage. The data is then connected to Google Sheets using Google Apps Script, enabling local staff to access it immediately. Some data is linked to Google BigQuery for in-depth analysis. System testing consisted of two levels: Unit Testing, which tests individual functionalities, and User Acceptance Testing (UAT). Thirty public health officers from subdistrict health promotion hospitals and research assistants were recruited to conduct a hands-on trial of the system. A questionnaire was used to assess system satisfaction and usability, informing development based on recommendations.

#### **Step 5: System Deployment and Utilization**

After trial and optimization, the researchers installed the system and implemented it in a rice- and sugarcane-farming community. Public health officers and research assistants were able to directly collect user data and send assessment results back to users via the LINE Messaging API. A dashboard was also created to display aggregated data. Public health officers or health policymakers in the farming area can use the results to efficiently and quickly manage farmers' ergonomic risks.

### **2.4 Research tools**

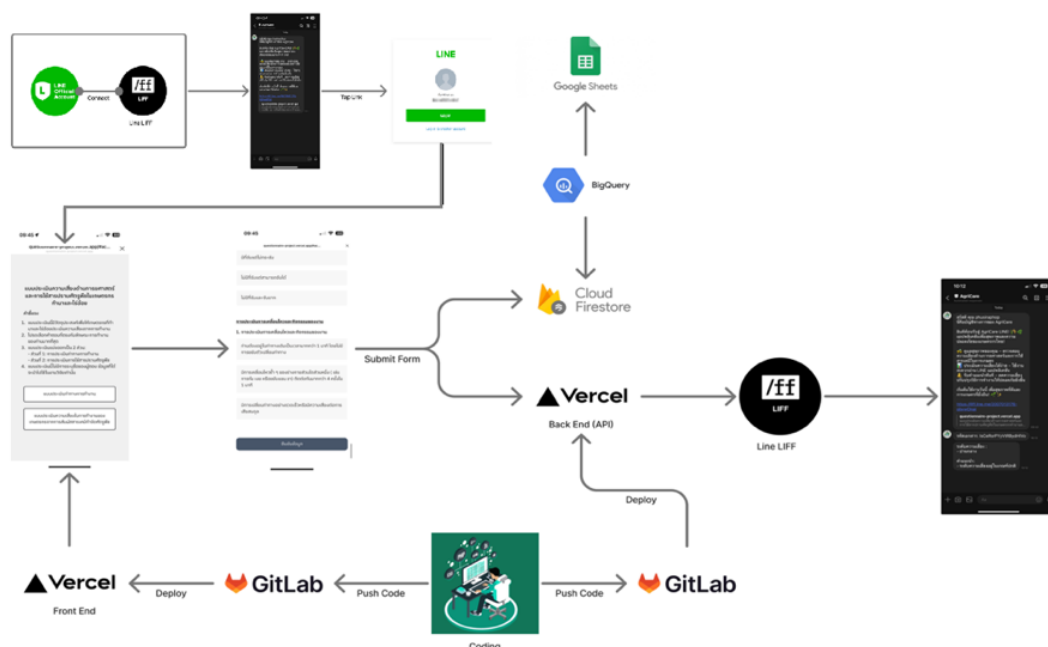
The research instruments were developed based on standardized ergonomic assessment concepts [17] and previous related studies [6,7,8]. The questionnaire items were adapted from existing ergonomic and health assessment tools and modified to suit the agricultural context. Content validity was assessed by three experts in occupational health and information technology using the Index of Item-Objective Congruence (IOC), with all items demonstrating values greater than 0.5. The instruments were pilot-tested among a small group of non-sample participants to evaluate clarity, comprehension, and usability before final data collection.

The research tools were divided into four parts: an ergonomic assessment form, an application, an application quality assessment form, and an application satisfaction assessment form. The results are as follows:

1) The ergonomic assessment is a whole-body assessment method. The farmers' most risky, unnatural, and muscular postures were analyzed using the standard Rapid Entire Body Assessment (REBA) tool for assessing standing work. Scores were generated for each body part, and the frequency of work performed and the force applied. The calculations were performed using the REBA Employee Assessment Worksheet, which was divided into two groups: Group A (torso, neck, and legs) and Group B (upper arms, lower arms, and wrists). Scores ranged from +1 to +4 for each part. When the overall score is added, a high score indicates an increased risk of skeletal and muscular injury [17].

2) The ergonomic risk assessment application was developed in the form of a LINE Chatbot. The questions were based on the standard tools of REBA and were accompanied by easy-to-understand illustrations. The assessment content was verified for accuracy. The application began with a LINE Official Account. Users clicked a link in a LINE OA message to open the application developed with the LINE Front-end Framework (LIFF) to display the web application. After clicking the link, the system redirected to the LINE login process. The system then displayed a form designed for risk analysis. This form ran on a web application hosted on Vercel's Frontend service.

After users completed filling out the information and submitted it, the form sent data via an API to a backend system hosted on Vercel. This API connects to a central database that uses Firebase (Cloud Firestore) to store data. The data was then managed and analyzed. It connected to Google Sheets to display basic information in tabular form and to BigQuery for in-depth analysis or large-scale data. Furthermore, during the system development process, the researcher wrote code using various tools and submitted it to GitLab. When the code was pushed, the system automatically deployed it to Vercel, both the Frontend and Backend. Next, the system interpreted the evaluation results and quickly notified the user with an explanation via LINE LIFF. The application's working process is shown in Figure 1.



**Figure 1.** Workflow of the ergonomic risk assessment application, illustrating the stages of problem identification, system analysis, system design, system implementation, and system deployment.

3) The application quality assessment form examined the quality of the application's content, format, response, usability, and language used. Experts were university professors with a master's degree or higher in related fields, including information technology and health. They independently assessed the application

using the Index of Item-Objective Congruence (IOC), which yields three scores: -1, 0, and +1. The researchers used a total score of items less than 0.5 to improve the application and submit it to the experts for further consideration [18].

Evaluating the quality of the application in 5 areas: usability, content appropriateness, data privacy and confidentiality, output presentation and functional performance, and overall appropriateness. There are 18 questions with 5 levels of choice, ranging from 1 (least) to 5 (most), with scores 2, 3, 4, and 5 corresponding to little, moderate, much, and most, respectively. The results were interpreted overall and item-by-item based on the average values according to Best [19] criteria: low, moderate, and high quality. The scores were equally spaced within each level, ranging from 1.00-2.33, 2.34-3.67, and 3.68-5.00, respectively.

4) Application usage satisfaction assessment covers 4 aspects: usability, content appropriateness, design and visual, and impact and practical use. There are 20 questions with 5 levels of choice, ranging from 1 (least) to 5 (most), with scores 2, 3, 4, and 5 corresponding to little, moderate, much, and most, respectively. The results were interpreted overall and item-by-item based on the average values, according to Best's [19] criteria: low, moderate, and high satisfaction. The scores were equally spaced within each level, ranging from 1.00-2.33, 2.34-3.67, and 3.68-5.00, respectively.

## 2.5 Data collection

Data collection was divided into two phases: the application development and creation phase, and the farmer implementation phase.

During the creation and development phase, an ergonomic assessment form with modified wording and images illustrating work postures to facilitate understanding was submitted, based on the REBA standard. The first group of experts assessed the app's consistency index against the objectives. The second group of experts then trialed the app and completed a quality assessment via a Google Form registered to their personal e-mail. The assessment period was 1 week, followed by a non-sample group (tryout) to determine comprehension of the language used and the appropriateness of communication with the farmers. After the test, actual data were collected from farmers in Suphan Buri Province, Thailand.

In the implementation phase, the tool users were nurses, public health professionals, and research assistants. Data were collected using structured interviews and the application. The start and end times of both document-based and application-based interviews were recorded. The data collection period was 1 week after the farmer sample was completed. The researcher assessed the application's satisfaction using Google Forms, which can record data without identifying respondents, ensuring data authenticity and anonymity. The recorded data were then analyzed.

## 2.6 REBA process

The REBA standard risk assessment tool is a whole-body risk assessment method developed by Hignett and McAtamney [17]. It selects the most difficult, time-consuming, or forceful work postures. This is based on an interview to select the postures that pose the greatest risk to the skeleton and muscles. REBA has 15 steps in total, divided into 3 parts as follows:

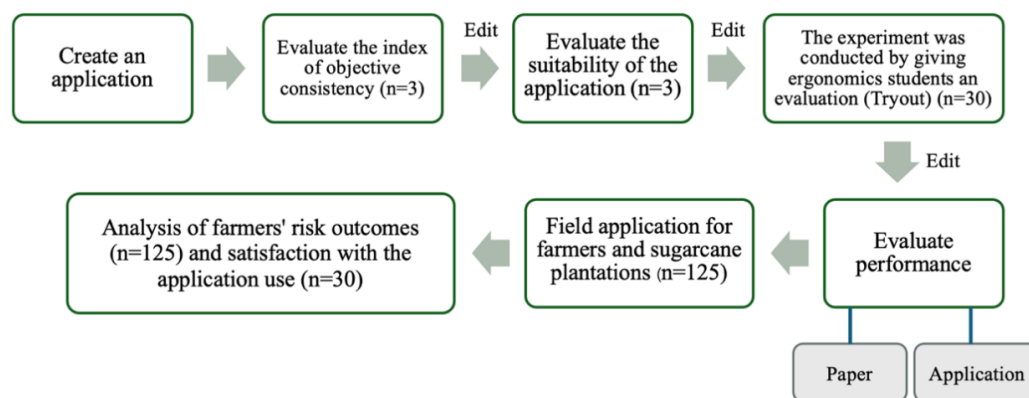
Part A (Steps 1-6) assesses neck and trunk angulation during rotation or leaning, and legs during standing or kneeling. This is then combined with a cross-matrix between the neck and trunk, scoring 1-9 points. This is then combined with the force and workload scores, with the potential for an additional score of 0-3.

Part B (Steps 7-13) assesses upper and lower arm angulation during rotation or leaning, and a cross-matrix between the upper and lower arms, scoring 1-9 points. Material grip, movement, and work activities are also assessed, with the potential for an additional score of 0-3.

Part C (Steps 14-15) is derived by combining the scores from Parts A and B with a cross-matrix for a final assessment. The scores are then graded into five risk levels: 1, 2-3, 4-7, 8-10, and >10. Scores are interpreted as very low, low, moderate, high, and very high risk, respectively. Recommendations are tailored according to the risk level.

## 2.7 Data analysis

Data were analyzed using descriptive and inferential statistics. In descriptive statistics, the variables were measured at different levels, including discrete variables (e.g., ergonomic risk levels) and continuous variables (e.g., application satisfaction scores), with discrete variables analyzed using frequencies and percentages, and continuous variables using means and standard deviations. Inferential statistics were tested according to the following objectives: 1) the results of creating and developing an ergonomic risk processing application using IOC calculations, considering criteria greater than or equal to 0.5, and the suitability of the application greater than 80%. 2) Evaluate the efficiency of the ergonomic risk processing application by testing the duration of use of both assessment forms with a paired t-test. 3) Evaluate the satisfaction of using the ergonomic risk processing application with the set criteria (greater than 4.00 out of a full score of 5.00) with one sample t-test, setting statistical significance at 0.05.



**Figure 2.** Research protocol of the study

## 2.8 Ethical Considerations

This study complied with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Human Research Ethics Committee of Bansomdejchaopraya Rajabhat University (REC-BSRU COA 680607), dated June 23, 2025. Written informed consent was obtained from all participants before data collection. Participants were fully informed about the study objectives, procedures, potential benefits, and risks. Confidentiality and anonymity of participant information were strictly maintained throughout the study.

## 3. Results and Discussion

### 3.1 “AgriCare” Ergonomics Risk Processing Application

The research team developed an application for farmers called AgriCare. The application begins with a login via the LINE application by scanning a QR code. Upon registration, the application takes you to the home page, which provides instructions and lets you select an assessment. Each assessment item includes a brief explanation, assessing each organ individually, and options for images of farmers at different angles and degrees to reflect actual work performance. Additional explanations are provided for some questions, such as whether or not to rotate the neck. The options are yes or no. After selecting all questions, click “Save.” The resulting score is then stored in a centralized database. The assessment results are sent to the LINE application, including the risk for each organ, the overall body weight, and appropriate recommendations. The application's window is shown in Figure 3.

After the application was developed, three experts evaluated its suitability using the IOC. The results showed that all items’ values were higher than 0.5. Three other experts also tested the application. The experts gave an average score of  $4.87 \pm 0.11$  (out of 5.00), which is at the highest level. The opinions were: “This application is very useful, but some questions require understanding or expertise to evaluate. Farmers with different skills may find it difficult to self-evaluate some questions.” and “the application’s responses are easy to understand by explaining risks and recommendations. However, it would be clearer if there were illustrations to illustrate the instructions.”

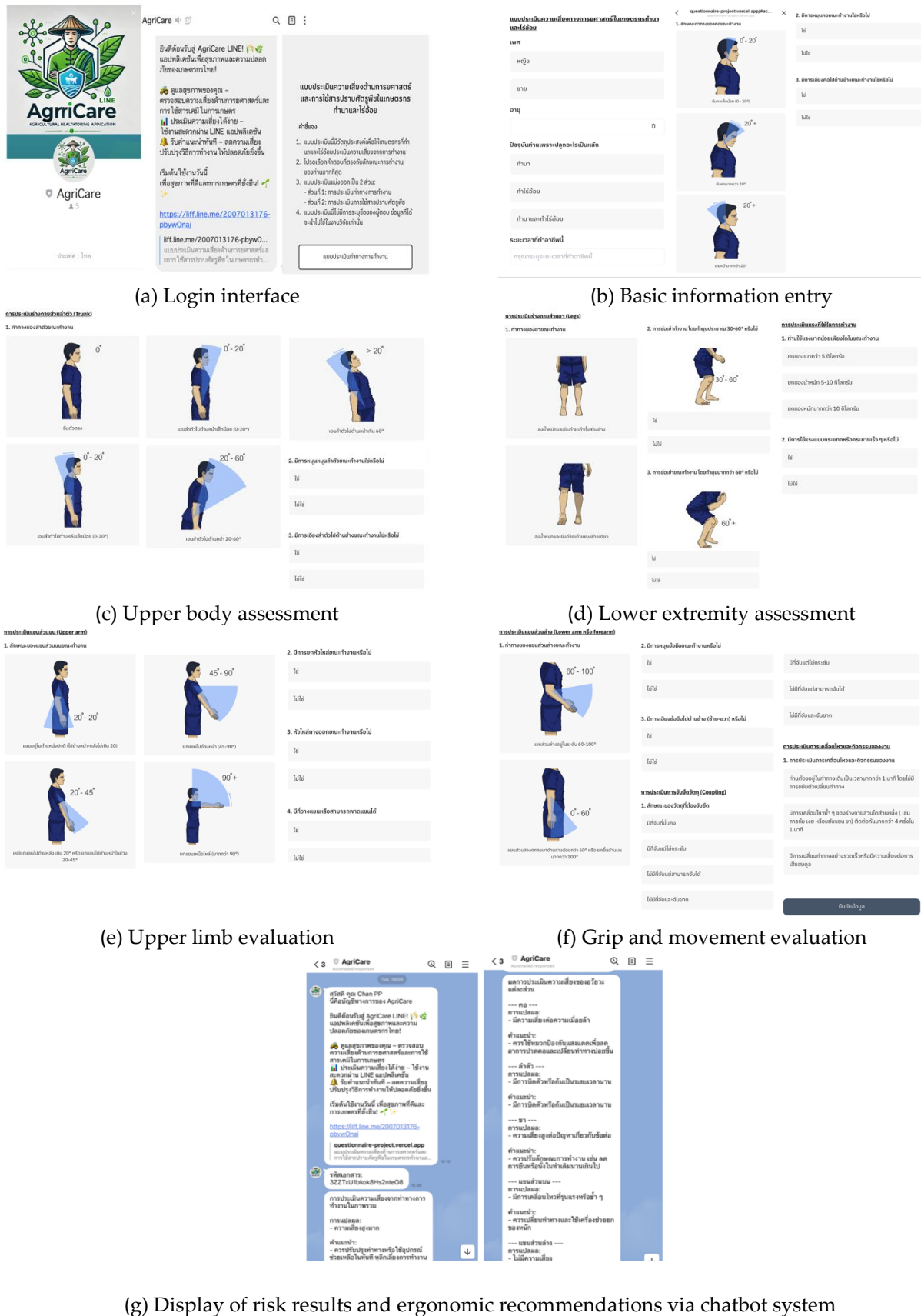


Figure 3 (a-g). Screenshots of the AgriCare ergonomic risk assessment application



### 3.2 Ergonomics Risk Processing Application Performance

The effectiveness of the test between the traditional (paper) format and the ergonomic risk assessment application was analyzed into two parts: time efficiency and user outcomes. The results are as follows:

Time efficiency, the paper format was compared with the ergonomic risk assessment application. The results showed that the application significantly reduced the interview, recording, and interpretation time compared to the traditional format ( $p < 0.05$ ), from  $16.55 \pm 3.16$  to  $2.11 \pm 0.69$  points per person, as shown in Table 1.

**Table 1.** Comparison of time performance between paper and application (n=30)

| Efficiency                      | Paper |      | Application |      | t      | p        |
|---------------------------------|-------|------|-------------|------|--------|----------|
|                                 | Mean  | SD   | Mean        | SD   |        |          |
| Interview duration (minutes)    | 5.82  | 1.57 | 2.11        | 0.69 | 24.621 | <0.001** |
| Recording time (minutes)        | 3.47  | 1.02 | Automatic   |      |        |          |
| Interpretation period (minutes) | 7.21  | 2.45 | Automatic   |      |        |          |
| Total                           | 16.55 | 3.16 | 2.11        | 0.69 |        |          |

Note: \*\* $p < 0.001$

In addition to time efficiency and user satisfaction, the performance of the developed application was evaluated for accuracy and consistency in ergonomic risk scoring. The automated REBA scoring results generated by the application were consistent with manual REBA assessments performed by trained personnel, indicating reliable system performance. The digital system also demonstrated stable operation during repeated testing sessions, suggesting its suitability for routine ergonomic risk monitoring in agricultural settings. Furthermore, content validation conducted by ergonomic experts confirmed the appropriateness of posture classification and scoring logic used in the application.

Outcomes efficiency, the comparison of pain or injury scores of various body organs, grip, and movement, it was found that the scores obtained from the paper and the scores obtained from the application were not different (the paper total risk score for all organs was  $16.90 \pm 4.03$  and from the application was  $17.26 \pm 1.74$ ,  $p = 0.657$ ), as shown in Table 2.

**Table 2.** Comparison of performance outcomes between paper and application (n=30).

| Organs assessed                    | Score      |              |              | t-test | p     |
|------------------------------------|------------|--------------|--------------|--------|-------|
|                                    | Full score | Paper        | Application  |        |       |
| <b>All organs</b>                  | 35         | 16.90 (4.03) | 17.26 (1.74) | -0.449 | 0.657 |
| Neck and neck rotation             | 4          | 2.43 (0.85)  | 2.03 (0.18)  |        |       |
| Body and body rotation             | 6          | 3.57 (1.16)  | 3.23 (1.04)  |        |       |
| Legs and standing                  | 4          | 2.20 (0.96)  | 3.13 (0.43)  |        |       |
| Upper arm and shoulder lift        | 6          | 2.90 (0.88)  | 2.60 (1.40)  |        |       |
| Lower arm                          | 2          | 1.73 (0.45)  | 1.50 (0.50)  |        |       |
| Hand and wrist, and wrist rotation | 4          | 1.70 (0.70)  | 2.20 (0.80)  |        |       |
| Additional force                   | 3          | 1.10 (0.92)  | 1.53 (0.68)  |        |       |
| Grasping objects                   | 3          | 0.27 (0.68)  | 0.03 (0.18)  |        |       |
| Movement                           | 3          | 1.00 (0.26)  | 1.00 (0.00)  |        |       |

### 3.3 Satisfaction from using the application system

The satisfaction assessment results for 30 application users, including subdistrict health promotion hospital staff, research assistants, and farmer leaders or village public health volunteers, found that they were most satisfied across all areas questioned. The top three highest average satisfaction scores were: it reduced assessment time and steps compared to the previous method; assessment data can be used to improve

behaviors; and it has the potential to be applied in other communities or more broadly. The average scores were  $4.86 \pm 0.34$ ,  $4.83 \pm 0.37$ , and  $4.80 \pm 0.41$ , respectively (Table 3).

**Table 3.** Satisfaction with using the application system (n=30)

| Satisfaction                                                                       | Mean | SD   | Interpretation |
|------------------------------------------------------------------------------------|------|------|----------------|
| <b>Usability</b>                                                                   |      |      |                |
| The application is easy to use and not complicated.                                | 4.76 | 0.50 | high           |
| The system responds quickly and without errors.                                    | 4.63 | 0.56 | high           |
| Processing and display are accurate.                                               | 4.73 | 0.52 | high           |
| It can be used smoothly on smartphones.                                            | 4.77 | 0.43 | high           |
| The application is stable during use.                                              | 4.56 | 0.56 | high           |
| <b>Content Appropriateness</b>                                                     |      |      |                |
| The application content is clear and easy to understand.                           | 4.55 | 0.62 | high           |
| The content covers essential information for health assessment.                    | 4.63 | 0.49 | high           |
| The health and risk advice is actionable.                                          | 4.67 | 0.48 | high           |
| The system's terminology and language are appropriate for the target audience.     | 4.63 | 0.49 | high           |
| The language and presentation format are user-friendly.                            | 4.64 | 0.44 | high           |
| <b>Design and Visual</b>                                                           |      |      | high           |
| The layout of the application's elements is appropriate.                           | 4.56 | 0.56 | high           |
| The accompanying images are clear and supportive, helping clarify the information. | 4.73 | 0.52 | high           |
| The font style, size, and layout are appropriate for reading.                      | 4.66 | 0.54 | high           |
| The use of colors and graphics is attractive and not distracting.                  | 4.60 | 0.56 | high           |
| The application has a credible image.                                              | 4.67 | 0.60 | high           |
| <b>Impact and Practical Use</b>                                                    |      |      |                |
| The application helps identify health risk levels.                                 | 4.76 | 0.50 | high           |
| The assessment results can be used to improve behavior.                            | 4.83 | 0.37 | high           |
| It is useful for long-term health monitoring.                                      | 4.76 | 0.43 | high           |
| It reduces assessment time and steps compared to traditional methods.              | 4.86 | 0.34 | high           |
| It has the potential to be applied to other communities or the wider community.    | 4.80 | 0.41 | high           |

The results of comparing satisfaction scores against the researcher's criteria were above 80% of the full score (4.00 out of 5.00). The study found that those who used the application were more satisfied than the criteria set, with statistical significance ( $p < 0.05$ ) for both individual and overall scores, as shown in Table 4.

**Table 4.** Compares satisfaction with the specified criteria (more than 80%) (n=30)

| Satisfaction              | Mean | SD   | t      | p        | 95%CI |       |
|---------------------------|------|------|--------|----------|-------|-------|
|                           |      |      |        |          | Lower | Upper |
| Usability                 | 4.69 | 0.41 | 9.129  | <0.001** | 0.538 | 0.848 |
| Content suitability       | 4.61 | 0.46 | 7.292  | <0.001** | 0.441 | 0.785 |
| Design and presentation   | 4.64 | 0.47 | 7.416  | <0.001** | 0.468 | 0.825 |
| Benefits and applications | 4.80 | 0.36 | 11.959 | <0.001** | 0.668 | 0.944 |
| Total                     | 4.69 | 0.38 | 9.946  | <0.001** | 0.548 | 0.831 |

**Notes:** \* $p < 0.05$ , \*\* $p < 0.001$

Results from the ergonomic risk assessment application were analyzed using interviews with 125 farmers. The results were divided into two parts: pairwise correlation tests for each painful or injured organ and the prevalence of painful or injured organs. The results are as follows:

The results of the pairwise correlation tests for each painful or injured organ using the AgriCare application revealed that the neck risk score was highly correlated with the trunk ( $r=0.677$ ,  $p<0.01$ ). The trunk score showed a statistically significant correlation with the upper arm and shoulder ( $r=0.549$ ,  $p<0.01$ ). Other pairwise correlations are shown in Table 5.

**Table 5.** Relationship between pairwise organ risk scores (n=125).

| Organs | 1       | 2       | 3      | 4       | 5       | 6     |
|--------|---------|---------|--------|---------|---------|-------|
| 1      | 1.000   |         |        |         |         |       |
| 2      | 0.677** | 1.000   |        |         |         |       |
| 3      | 0.425** | 0.326** | 1.000  |         |         |       |
| 4      | 0.349** | 0.549** | 0.153  | 1.000   |         |       |
| 5      | 0.073   | 0.207   | 0.017  | 0.230*  | 1.000   |       |
| 6      | 0.320*  | 0.410*  | 0.230* | 0.376** | 0.264** | 1.000 |

**Notes:** 1=neck and neck rotation, 2=torso and body rotation, 3=legs and standing, 4=upper arm and shoulder lift, 5=lower arm, 6=hand and wrist and wrist rotation or twist, \* $p<0.05$ , \*\* $p<0.001$ .

The study found that, within 7 days, farmers reported the most pain in their right shoulder, as classified by organs at risk from ergonomics, with 17.6 percent (95%CI=12.0-24.8), followed by neck pain, 16.8 percent (95%CI=9.9-24.5). In addition, farmers reported pain in their left shoulder, lower back, right knee, and left knee, with equal percentages (16.0%) for other organs, as shown in Table 6.

**Table 6.** Prevalence of actual pain or injury within 7 days (n=125).

| Organs at Ergonomic Risk        | Organ's pain        | n (%)     | 95%CI     |
|---------------------------------|---------------------|-----------|-----------|
| Neck and neck rotation          | Neck                | 21 (16.8) | 9.9-24.5  |
| Body and body rotation          | Upper back          | 12 (9.6)  | 4.1-14.4  |
|                                 | Lower back          | 20 (16.0) | 10.4-22.4 |
| Legs and standing               | Right leg           | 11 (8.8)  | 4.8-14.4  |
|                                 | Left leg            | 10 (8.0)  | 3.2-13.6  |
|                                 | Right knee          | 20 (16.0) | 9.7-21.6  |
|                                 | Left knee           | 20 (16.0) | 10.5-21.5 |
|                                 | Right foot or ankle | 8 (6.4)   | 2.4-11.2  |
|                                 | Left foot or ankle  | 5 (4.0)   | 0.8-8.8   |
| Upper arm and shoulder lift     | Right shoulder      | 22 (17.6) | 12.0-24.8 |
|                                 | Left shoulder       | 20 (16.0) | 11.2-23.7 |
| Lower arm                       | Right elbow         | 18 (8.0)  | 4.0-12.8  |
|                                 | Left elbow          | 8 (6.4)   | 2.4-10.4  |
| Hand, wrist, and wrist rotation | Right wrist or hand | 14 (11.2) | 6.5-17.6  |
|                                 | Left wrist or hand  | 15 (12.0) | 6.5-18.3  |

This study developed an ergonomic risk assessment application. The REBA assessment tool is complex. Most occupational health and safety practitioners are in the industrial sector rather than in government agencies responsible for the health of informal workers. This is because Thai law requires professional safety officers to assess the risks posed by hazards that may impact worker health. Furthermore, industrial sectors with more than 100 employees must allocate a professional safety officer, who holds a degree in occupational health and safety [20]. Therefore, the industrial sector represents a larger market for ergonomic risk assessment specialists than government agencies, creating a gap in healthcare for this group. This research, therefore, developed an application to address farmers' health needs. The statistics show that Thailand has the largest population of rice farmers (4.7 million) and sugarcane farmers (0.33 million) [16]. This suggests the potential for widespread adoption. In ergonomic risk assessment, several standardized tools have

been widely used, including the Rapid Upper Limb Assessment (RULA) and the Ovako Working Posture Analysis System (OWAS). Compared to these tools, REBA provides a more comprehensive whole-body assessment suitable for evaluating complex working postures commonly encountered in agricultural work [11, 12]. The integration of the REBA method into a chatbot-based system in this study enables faster interpretation of posture risk levels while maintaining consistency with established ergonomic assessment methods. Therefore, the developed system offers practical advantages in field applications that require rapid decision-making and accessibility. The conclusions and discussions are as follows:

This study utilized NextJS, NodeJS, and Firebase SDKs, along with a version control system and a CI/CD process via GitLab connected to Vercel, to ensure continuous, efficient system improvement and release. The design incorporated the REBA standard tool, used to evaluate body posture angles and assign scores, to create a model of a farmer's posture at a specified angle. This allows users to select images easily. The system then calculates scores, assesses risk levels, and notifies the results via the LINE application in Thai, both in aggregate and organ classification formats, with appropriate recommendations. The results showed that the system provides accurate, fast, and efficient results. However, some users, particularly farmers, face limitations in distinguishing similar postures, which may lead to inaccurate selection of images that reflect actual work conditions and impaired understanding of some of the assessment text. Therefore, the researchers improved the system by adding clearer, more diverse illustrations, developing a user-friendly, relevant user manual for the target group, and conducting repeated testing with actual farmers to ensure the system could be used appropriately in rural areas. In Thailand, chatbot applications have been used to enhance health literacy, such as providing advice to farmers spraying pesticides in corn fields. The study results showed that farmers significantly increased their health literacy through the recommendation system [14], consistent with the development of this application, which can effectively enhance farmers' ergonomic risk management capabilities.

The application was evaluated by information technology and health and safety experts. The evaluation assessed five quality dimensions: usability, content appropriateness, data protection and privacy, display and functionality, and overall appropriateness. The validity score for all quality questions was greater than 0.5, which met the measurement and evaluation criteria [18]. Furthermore, the experts gave an average score of  $4.87 \pm 0.11$  (out of a full 5.00), which is considered high. However, the experts suggested minor adjustments, such as numerical indications for bending or angles, to improve understanding. The target audience should also be clearly identified, as some farmers, particularly those who are uneducated or elderly, may not be able to use the application for evaluation. The researchers completed the adjustments before using it with the farmer sample. The target audience for the application was identified as local health officials, village health volunteers, and community farmer leaders, who can then expand and share their recommendations with farmers.

The results of the efficiency test, in terms of the time between the paper assessment and the application, when applied to actual farmers, found that the assessment was convenient and fast. The time for interviewing, recording, and interpreting the results was significantly reduced ( $p < 0.05$ ) compared with the paper, from  $16.55 \pm 3.16$  to  $2.11 \pm 0.69$  minutes per person. The feedback was provided automatically. In addition, when evaluating the satisfaction of those who used the application, it was found that the average satisfaction level was  $4.69 \pm 0.38$  points (out of a full score of 5.00), which is high. They were satisfied that the application reduced the time and steps in the assessment compared to the paper assessment. The assessment data can be used to improve behavior and may be applied in other communities or more widely. This is consistent with the study by Kingchang et al. [21], which found that application users were highly satisfied with the platform's usability (average score  $4.38 \pm 0.49$ ). The output and feedback aspects were very high (average score of  $4.67 \pm 0.52$  for both), which is in line with expectations that the REBA ergonomic risk assessment is a complex tool that requires occupational health experts to assess. This time, information technology was used to help solve the problem. Therefore, the assessment can be generalized, with no difference in results between occupational health personnel and the general public. The application can accurately analyze and provide immediate results, resulting in the highest level of satisfaction with its usability.

The application of the application program to farmers revealed that each organ had risk factors related to other body parts. The neck risk factor was related to the torso, followed by the torso risk factor, which was

statistically significant ( $p < 0.01$ ). Furthermore, the hands, wrists, and wrist rotation were significantly associated with pain scores for other organs. This was consistent with the actual work environment. Farmers used their wrists in every step of sugarcane farming, from cutting sugarcane to planting it in the soil and using sharp hooks to care for, weed, and trim the fields. This is consistent with the study by Hassani et al. [7], which found that working hours in the sugarcane field were associated with a 5.62-fold increase in hand and wrist pain. Therefore, attention should be paid to minimizing hand and wrist movement or wrist twisting. Wrist injuries can lead to inappropriate postures due to inadequate hand use, requiring the torso or shoulder also to be twisted. Therefore, the hands are most commonly associated with pain in other organs. Farmers had the highest prevalence of right shoulder pain, similar to neck pain, at 17.6% (95% CI = 12.0-24.8) and 16.8% (95% CI = 9.9-24.5), respectively. This may be because most farmers use their dominant hand to hold agricultural equipment. During the work process, they lift or carry heavy objects such as fertilizers or chemicals, so they use their right shoulder. Furthermore, using knives or hoes to dig soil or cut sugarcane requires more force in lifting the equipment with the right arm, leading to more injuries than other parts of the body. This is consistent with a study of farmers working in various fields and gardens in the Northeastern region of Thailand. 19.6% of farmers reported mild shoulder pain, and 6.5% reported moderate shoulder pain. Farmers growing different crops had similar shoulder pain [22]. It can be seen that farming in Thailand, whether in rice, sugarcane, tobacco, or vegetable fields, requires human labor, even though some steps are performed with machinery. Given the common agricultural materials used, shoulder pain can also occur.

The findings of this study are consistent with previous research reporting a high prevalence of musculoskeletal symptoms among agricultural workers exposed to repetitive postures and prolonged bending tasks [7,21]. Similar studies among rice and crop farmers have reported increased ergonomic risk associated with manual harvesting, tool handling, sustained trunk flexion, and repetitive movements. The observed prevalence of ergonomic risk among rice and sugarcane farmers in this study may be explained by the physical demands of agricultural activities, including repetitive bending, squatting, lifting, and tool use. Rice farming typically involves prolonged trunk flexion and knee bending during planting and harvesting, whereas sugarcane work requires repetitive upper limb movements and forceful tool use. These biomechanical exposures increase the risk of musculoskeletal strain among agricultural workers. In addition, differences in working characteristics between rice and sugarcane farming may contribute to distinct patterns of musculoskeletal symptoms, with rice farming commonly associated with prolonged bending and squatting, which increase the risk of lower back and knee disorders. In contrast, sugarcane farming involves repetitive upper-limb movements, forceful cutting tasks, and the handling of heavy tools, which increase the risk of shoulder and neck musculoskeletal disorders. Similar findings have been reported in previous studies among agricultural workers, in which task-specific ergonomic exposures were significantly associated with distinct patterns of musculoskeletal symptoms [23].

From a policy perspective, implementing digital ergonomic risk assessment tools could strengthen occupational health surveillance systems, particularly for informal workers such as farmers, who often lack access to professional ergonomic services. Integrating digital health tools into community-based healthcare services can support early detection of musculoskeletal risks and facilitate targeted interventions at the primary healthcare level. This approach is consistent with global occupational health strategies that emphasize the use of digital technologies to improve access to occupational health services and strengthen preventive systems [24, 25]. The developed system also demonstrates strong potential for scalability and real-world implementation. Because the application integrates with the LINE platform, widely used in Thailand, the system can be deployed across multiple agricultural communities without requiring additional software installation. Community health personnel, village health volunteers, and agricultural extension officers can use the system to conduct routine ergonomic risk screening and provide preventive recommendations to farmers. Furthermore, the cloud-based infrastructure enables centralized data storage and monitoring, allowing health authorities to track ergonomic risks and plan targeted occupational health interventions at the community and regional levels. Compared with traditional ergonomic assessment approaches, such as manual REBA, RULA, and OWAS evaluations, the developed chatbot-based system provides several practical advantages. Conventional ergonomic assessments often require trained personnel, paper-based recording,



and delayed data interpretation, which may limit their use in rural agricultural settings. In contrast, the proposed system integrates standardized REBA scoring into a digital chatbot platform that enables real-time risk assessment, automated scoring, and immediate feedback. Similar digital ergonomic tools have been reported in previous studies [26]; however, many systems require specialized software or technical expertise. The integration of the REBA method into a widely used communication platform such as LINE enhances accessibility, usability, and efficiency, making it more suitable for community-level occupational health applications.

Although this study was conducted in a specific agricultural area with a defined sample population, the findings may apply to other farming communities that involve similar manual labor tasks and ergonomic risk exposures. However, variations in agricultural practices, crop types, tool use, and environmental conditions across regions may influence ergonomic risk levels and the usability of digital assessment tools. Therefore, caution should be exercised when generalizing these findings to other agricultural contexts, and further studies conducted in diverse geographic regions and agricultural systems are recommended to strengthen external validity. In addition, data privacy and security considerations are essential in digital health applications that collect personal health information. In this study, data were stored in secure cloud-based systems with restricted access to authorized users only. Personal identifiers were minimized, and anonymized data were used during analysis to protect participant confidentiality. These practices align with international recommendations for digital health data governance and protection of personal health information [27, 28]. In terms of practical Health, Safety, and Environment (HSE) implementation, the findings of this study can support targeted ergonomic risk reduction strategies among farmers. These include providing training on correct working postures, promoting the use of ergonomically improved tools, conducting periodic ergonomic risk assessments using digital applications, and prioritizing preventive interventions for high-risk body regions such as the neck, shoulders, and lower back. The integration of digital ergonomic assessment tools into routine occupational health programs may enhance early risk identification and support continuous workplace safety improvement.

A strength of this study is the pilot application of an ergonomic risk assessment application for sugarcane farmers, a first in Thailand. Previously, most assessment tools were found in the industrial sector. The application can be easily extended to mobile phones. Those with occupational health knowledge or the general public can also assess the results, thus expanding the application range. However, this study has several limitations that should be considered. The application still relies on internet connectivity and the LINE platform, which some farmers do not yet have access to or are not comfortable using to read or view images on their phones. Therefore, the primary users are community health personnel, such as staff at subdistrict health promotion hospitals, village public health volunteers, and farmer leaders. Furthermore, the text displayed in the LINE application may be too small for older people, which could pose a challenge for future interpretation. For example, increasing text size, creating graphics that are more easily understood, or creating animations rather than text could also improve understanding and follow-up. Furthermore, adding video demonstrations might enhance the application's understanding and followability.

#### 4. Conclusions

The developed ergonomic risk assessment application, coupled with a chatbot recommendation system, can be applied to farmers and provide fast, accurate assessment results. The assessment results from the application, those assessed by occupational health professionals, and those of the public were similar. The application was faster to complete interviews, record, and interpret than paper interviews. User satisfaction was highest across a wide range of users. The risk assessment results indicated that farmers were at high risk of neck-related torso injuries, and that wrist rotation or twisting was associated with injuries across all body parts. Therefore, the study recommends that the application be used to assess sugarcane farmers. In the future, health promotion programs should be implemented for at-risk organs, including recommendations for appropriate neck, back, and hand postures based on ergonomic principles and the development of labor-saving devices that reduce hand and wrist rotation or twisting, which will ultimately reduce risk.

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# Comparative Analysis Of Convolutional Neural Networks, Long Short-Term Memory Networks, and Bert For Text-Based Emotion Classification

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**Abstract:** This paper presents a detailed experimental study and comparative analysis of three popular deep learning architectures (CNN, LSTM, and BERT) for emotion classification in written messages. Using a publicly available dataset of six unique emotional states (sadness, joy, love, anger, fear, and surprise), an effective ablation study was conducted to determine optimal architectural configurations, including a sequence length of 66 tokens and an embedding size of 200. To validate the results of a comparative analysis of model performance, a bootstrap technique (30 trials) and the Wilcoxon Signed-Rank test were used to eliminate potential bias. As shown by experiments, the tuned BERT architecture (with a learning rate of  $2e-5$ ) produced the most accurate and reliable result of 93.50% in classifying emotional states from texts. Moreover, with an appropriate sequence length configuration, the LSTM network (89.92%) significantly outperformed the CNN (89.65%), confirming the need to account for long-range dependencies in emotion classification. Overall, the research results show the key importance of hyperparameter tuning and the ability to handle complex information for emotion identification.

**Keywords:** Emotion classification; deep learning; convolutional neural networks (CNNs); long short-term memory (LSTM); BERT;

## 1. Introduction

The growth of digital communication platforms, including social media, email, and instant messaging, has generated a vast volume of textual data infused with human emotion. The ability to automatically detect and interpret these emotions is becoming increasingly important across various domains, from enhancing customer service and understanding public opinion to developing more empathetic human-computer interaction systems. However, automated emotion detection from text is inherently complex. Emotional expression in language is often subtle, highly context-dependent, and can be masked by ambiguity, sarcasm, or cultural nuances.

To address these complexities in natural language processing, Devlin et al. [1] introduced BERT (Bidirectional Encoder Representations from Transformers), a

conceptually simple yet powerful language representation model. Unlike previous models such as OpenAI GPT, which relied on unidirectional architectures, or ELMo, which used shallow concatenations, BERT is designed to pre-train deep bidirectional representations by jointly conditioning on both left and right context across all layers [1], [2]. It achieves this deep bidirectionality by utilizing a "masked language model" (MLM) objective to predict randomly masked tokens, alongside a "next sentence prediction" (NSP) task to capture sentence-level relationships. Pre-trained on a massive corpus of 3.3 billion words (BooksCorpus and Wikipedia), BERT can be fine-tuned with just one additional output layer. This approach has proven highly effective, achieving state-of-the-art results on eleven natural language processing tasks, including pushing the GLUE benchmark score to 80.5% and significantly advancing SQuAD question-answering performance [1-2]. Sun et al. [2] further demonstrated that scaling to extreme model sizes with BERT can yield large improvements even on small-scale downstream tasks, thereby minimizing the need for task-specific engineering.

Lawan et al. [3] present a comparative analysis of machine learning models for domain adaptation in multiclass sentiment classification, a notable challenge due to vocabulary and sentiment expression variations across domains. The paper reviews established techniques, including lexicon-based approaches and traditional machine learning algorithms such as Logistic Regression, SVM, and Random Forest, and notes their limitations in domain adaptation despite standard preprocessing steps such as lowercase conversion, stop-word removal, tokenization, stemming, and TF-IDF vectorization. Deep learning models, such as CNNs, known for capturing local patterns, and LSTMs, proficient at handling sequential data, are also examined. The study highlights that transformer-based models, particularly BERT, generally offer superior performance in capturing nuanced contextual information and adapting across domains. The research aims to empirically validate these models using Amazon review data from diverse categories. It suggests exploring hybrid models, other transformer architectures, and advanced training techniques to enhance domain adaptation.

The proliferation of online information necessitates a practical analysis of emotional tendencies in text to understand user needs. Current sentiment classification models often struggle to achieve meaningful performance gains due to their reliance on manually labeled features, which limits exploration of deep semantic features hidden within the text. Huang et al. [4] present a text sentiment classification model that integrates BERT with CNN. In this approach, BERT is used to obtain word embeddings for the text, which a CNN then processes to learn deep semantic information and ascertain the text's emotional orientation. Experimental validation on a large movie review dataset demonstrated that the BERT-CNN model achieved an accuracy of 86.67%, markedly outperforming traditional textCNN methods and indicating its strong performance in this domain.

Demszky et al. [5] introduce GoEmotions, the largest manually annotated dataset for fine-grained emotion classification, consisting of 58,000 English Reddit comments labeled across 27 distinct emotion categories, including a Neutral category. Designed to address the limitations of previous datasets that rely on small sample sizes or coarse taxonomies (such as the standard six basic emotions), the authors carefully curated Reddit data by filtering for length, balancing sentiment, and masking sensitive information to mitigate bias. Statistical evaluation via Principal Preserved Component Analysis demonstrated strong, reliable dissociation among all 27 emotion categories, validating the taxonomy's design. To establish a baseline, the authors fine-tuned a BERT model that achieved an average F1-score of 0.46 on the comprehensive taxonomy. Their transfer learning experiments further demonstrated that GoEmotions effectively generalizes across other domains and taxonomies, making it a highly valuable resource when target-domain training data is limited.

Saravia et al. [6] introduce Contextualized Affect Representations for Emotion Recognition (CARER), a semi-supervised, graph-based framework designed to capture evolving linguistic nuances, such as slang and coded words, for text-based emotion classification. Because traditional lexicon-based methods often fail to model these dynamic linguistic variations effectively, the authors propose representing a large-scale emotion corpus as a complex network. By applying graph measures—specifically eigenvector centrality and clustering coefficients—the system automatically categorizes tokens as "connector words" or "subject words," serving as the building blocks for pattern-based features. To preserve semantic relationships and improve classification, these basic patterns are enriched using continuous word embeddings and weighted using a custom metric called pattern frequency-inverse emotion frequency (pf-ief). When combined with a multi-layer Convolutional



Neural Network (CNN), the CARER model significantly outperforms conventional baselines and state-of-the-art models such as DeepMoji and EmoNet, while demonstrating robustness with reduced feature sets and applicability to multilingual tasks, such as Chinese emotion recognition.

Mokhamed et al. [7] investigate emoji prediction for Arabic text, a growing area in Natural Language Processing due to the prevalence of emojis in digital communication. The research compares the performance of traditional Machine Learning (ML) models (Support Vector Machines, Multinomial Naive Bayes, and Random Forest) with that of advanced Deep Learning (DL) models (LSTM, Bi-LSTM, AraBERT, and mBERT). Using a large, well-balanced Arabic Twitter dataset, the study found that ML models achieved accuracies ranging from 74% to 84%. Notably, DL models generally outperformed them, with LSTM and Bi-LSTM achieving the highest accuracies of 91.16% and 91%, respectively, while AraBERT and mBERT scored 85% and 80%, respectively. This suggests that while ML models provide a baseline, DL approaches, particularly LSTM and Bi-LSTM, offer enhanced performance for Arabic emoji prediction.

Chandra Sekhar et al. [8] focus on sentiment analysis of tweets that contain both text and emojis, using a Kaggle dataset. The study employed sentence embedding models, including DistilBERT, USE-Large3, and RoBERTa, to generate embeddings, which were then used to train dense neural networks (NNs) and LSTM models. While text classification accuracy was high at around 98%, it dropped significantly to 75% when the validation set included emojis absent from the training data. To enhance scalability, a distributed training methodology was implemented, resulting in a 17% reduction in runtime without compromising accuracy. The research also incorporated explainable AI algorithms, SHAP and LIME, to understand model behavior and potential biases. This work aims to bridge the gap in existing sentiment analysis by offering a more holistic understanding of sentiment in digital communications that combine text and emojis.

Gaafar et al. [9] investigate the performance of a Long Short-Term Memory-Recurrent Neural Network (LSTM-RNN) deep learning approach for classification tasks on both textual and image datasets. The study utilized the IMDB movie reviews dataset for textual analysis and the Fashion MNIST image dataset. The results indicated that the image-based LSTM-RNN model outperformed the sentiment-based textual model in terms of speed, with processing times of 3.49 minutes for images versus 18.25 minutes for text, an advantage attributable to the well-defined patterns in image data representation. Furthermore, the LSTM-RNN model achieved higher classification accuracy for images (96.50%) than for text (85.69%), which the authors attribute to the complex network architecture, processing elements, and features of the image datasets. The study concludes that the LSTM-RNN performed better on image classification tasks than on textual data classification, both in terms of speed and accuracy.

Atlas et al. [10] introduce a novel sentence-level sentiment analysis model for online product reviews, employing natural language processing and a hybrid deep learning approach. The methodology includes web crawling for review collection, preprocessing steps like tokenization and lemmatization, feature extraction using a bidirectional gated recurrent unit (Bi-GRU), and sentiment classification into positive, negative, or neutral polarities via a hybrid RNN-based LSTM classifier. Performance evaluation using metrics such as accuracy, precision, recall, F-measure, and AUC revealed that the proposed model outperformed existing techniques, including Deep Convolutional Neural Network (DCNN), Multilayer Perceptron (MLP), CapsuleNet, and Generative Adversarial Networks (GAN). The study concludes that this hybrid Bi-GRU and LSTM-based RNN model offers a promising, superior approach for sentiment analysis of product reviews.

This research by Balakrishnan et al. [11] provides a comparative benchmark of deep learning architectures, including CNNs, RNNs, and Bi-LSTM networks. These models were evaluated using multiple word embedding techniques, including BERT and its derivatives, FastText, and Word2Vec. The Easy Data Augmentation method was employed to create two distinct datasets (original and augmented). The performance of each model was tested in both 5-class and 3-class (compressed) classification scenarios. The results indicate that Neural Network-based models utilizing Word2Vec yielded the top predictions, with a combined CNN-RNN-Bi-LSTM architecture achieving the highest accuracy (96%) and F1-score (91.1%). Among standalone models, RNN performed best, achieving 87.5% accuracy and an F1-score of 83.5%, while RoBERTa had the highest F1-score (73.1%) among the Transformer-based models. This study concludes that

deep learning methods are more effective than traditional supervised machine learning approaches for text-based sentiment analysis and outlines potential avenues for future research.

Cortiz [12] explores the application of Natural Language Understanding (NLU) to emotion recognition by fine-tuning and evaluating five Transformer-based language models: Bidirectional Encoder Representations from BERT, DistilBERT, RoBERTa, XLNet, and ELECTRA. Using the GoEmotion dataset, which features 27 fine-grained emotion categories, the study assessed models based on F1-score and completion time. RoBERTa achieved the highest macro F1 Score of 0.49, followed by DistilBERT and XLNet at 0.48, and BERT at 0.46. ELECTRA had the lowest F1-score at 0.33. Although RoBERTa performed best, the paper suggests that DistilBERT is the most efficient, given its faster training time relative to RoBERTa and XLNet, as well as its superior F1-score compared to BERT. The research concludes that while Transformer models are effective for fine-grained emotion recognition, there is potential for further enhancements in both model performance and dataset development.

Nanyonga et al. [13] evaluate the performance of BERT, CNN, and LSTM deep learning models for classifying aviation incident reports from the Australian Transport Safety Bureau (2013-2023) into four injury severity levels: Nil, Minor, Serious, and Fatal. After standardized preprocessing and hyperparameter tuning, BERT achieved perfect scores (1.00) across all evaluation metrics (accuracy, precision, recall, and F1-score) for all classes. In comparison, LSTM achieved 99.01% accuracy, with strong performance on the Nil class but weaker performance on the Minor class. Meanwhile, CNN achieved 98.99% accuracy, excelling in the Fatal and Serious classes while performing moderately in the Minor class. The findings highlight the advantages of transformer-based architectures, such as BERT, for complex textual classification in aviation safety and suggest future research directions, including ensemble methods and model interpretability, to further enhance predictive performance.

Abas et al. [14] introduce a novel deep learning model, BERT-CNN, for detecting emotions from text, leveraging the strengths of BERT and CNN. The model first utilizes BERT to generate dynamic semantic vector representations of words based on their context. These vectors are then fed into a CNN for emotion classification. Evaluated on the SemEval 2019 Task 3 and ISEAR datasets, the BERT-CNN model demonstrated superior performance compared to existing state-of-the-art models. It achieved an accuracy of 94.7% and an F1-score of 94% on the SemEval dataset (for happy, angry, and sad emotions, after removing the other classes to balance the classes) and an accuracy of 75.8% with an F1-score of 76% on the ISEAR dataset (for seven emotions). The study highlights that a learning rate of  $4 \times 10^{-5}$  yielded the best results for the SemEval dataset.

Talaat [15] aims to enhance sentiment analysis accuracy by proposing four hybrid deep learning models that combine BERT variants (DistilBERT and RoBERTa) with Bi-LSTM and Bi-GRU. Evaluated on three datasets for sentiment classification into positive, negative, and neutral, with and without considering emojis, these models use pre-trained word embeddings for fine-tuning. The study found that hybrid architectures with Bi-GRU layers generally yielded the best results; for example, DistilBERT-GLG without emojis increased accuracy by 1.84% over DistilBERT alone on the Apple dataset. RoBERTa-based models typically outperformed DistilBERT, and the inclusion of emojis affected performance, demonstrating the efficacy of the proposed hybrid methods over standalone BERT models and classical machine learning approaches.

Bi and Zhang [16] address challenges in cross-cultural communication by focusing on emotion recognition in human-computer dialogue, proposing a hybrid model named BCBA (BERT-CNN-Bi-GRU-Attention). The BCBA model uses BERT to extract semantic and syntactic features, integrates CNNs and Bi-GRUs to analyze textual features more deeply, and employs an attention mechanism to assign weights to words based on their emotional tendencies for precise sentiment analysis. Experimental validation across two datasets demonstrated the model's effectiveness, achieving an average accuracy of 0.84 and an average F1-score of 0.8, with recall stabilizing at approximately 0.7 as the number of iterations increased. The study concludes that the BCBA model provides robust capabilities in semantic understanding and sentiment analysis, offering significant technical support for developing more intelligent and user-friendly human-computer interaction systems, particularly in handling emotional nuances in intercultural contexts.

Khan et al. [17] address challenges in Urdu sentiment analysis by proposing a novel attention-based, stacked, two-layer CNN-Bi-LSTM Deep Neural Network (DNN) combined with multilingual BERT (mBERT) or fastText for word embeddings. The model aims to overcome issues such as high-dimensional feature spaces and the inability of some models to focus on critical textual features. The architecture first uses pre-trained embeddings (mBERT or fastText) to capture contextual information. A CNN then extracts local features, followed by a two-layer stacked Bi-LSTM network that processes sequential data and captures long-term dependencies in both forward and backward directions. An attention mechanism is subsequently applied to assign different weights to words based on their importance. The study evaluated this model on two Urdu sentiment analysis datasets, UCSA-21 and UCSA, achieving accuracies of 83.12% and 78.91%, respectively. Key findings indicate that mBERT embeddings outperform fastText, and that a two-layer stacked Bi-LSTM is more effective than a single-layer Bi-LSTM, with SVM as the best-performing Classifier after the fully connected layer. The proposed model demonstrated superior performance compared to existing Urdu language architectures.

Murfi et al. [18] investigate the application of hybrid deep learning models integrated with BERT for Indonesian sentiment analysis, with a particular focus on e-commerce platform reviews. The study highlights that while standard deep learning models, such as CNN, LSTM, and GRU, as well as their hybrid combinations (CNN-LSTM, LSTM-CNN, CNN-GRU, and GRU-CNN), have shown good performance, the choice of text representation is crucial. Traditional embedding methods often overlook word context, a limitation BERT addresses by providing context-aware text representations. The authors extend previous work by applying the BERT representation to these hybrid architectures for Indonesian sentiment analysis, a language with complexities such as slang and informalities, for which IndoBERT is well-suited. Their experiments, which include data collection, preprocessing (cleaning and one-hot encoding), BERT-based numerical representation, and model evaluation using accuracy, precision, and recall, demonstrate that BERT representation consistently improves the accuracy of all tested hybrid architectures compared to standard embedding methods. Notably, the BERT-based LSTM-CNN hybrid model achieved slightly better accuracies than other BERT-based hybrid configurations, indicating that combining BERT with hybrid deep learning models significantly enhances sentiment classification performance for Indonesian e-commerce reviews.

Recent literature highlights significant efforts to tackle this challenge using advanced deep learning techniques. For instance, studies have explored fine-tuning various Transformer-based models, such as Bidirectional Encoder Representations from Transformers (BERT), RoBERTa, and DistilBERT, for fine-grained emotion recognition, with RoBERTa achieving high F1-scores on complex datasets. Hybrid models are also gaining prominence, with researchers combining BERT with Convolutional Neural Networks (CNNs) or integrating BERT with Bidirectional Long Short-Term Memory (Bi-LSTM) and Bidirectional Gated Recurrent Unit (Bi-GRU) to leverage the contextual understanding of BERT alongside the feature extraction capabilities of CNNs or the sequential data processing strengths of Long Short-Term Memory (LSTM). Other comparative benchmarks have assessed CNNs, Recurrent Neural Networks (RNNs), and Bi-LSTMs with various word embeddings, including BERT derivatives, showing that different architectures perform best depending on the specific configuration and task. Nanyonga et al. [13] demonstrated BERT's superior performance in classifying injury severity from aviation incident reports, while Khan et al. [17] proposed a novel attention-based CNN-Bi-LSTM with mBERT for Urdu sentiment analysis. Despite these advancements, the optimal approach often depends on the specific dataset, the level of emotional granularity, and the available computational resources. The primary objective of this study is to conduct an empirical evaluation and comparative analysis of three distinct deep-learning architectures for text-based emotion classification: CNNs, LSTM networks, and BERT.

## 2. Materials and Methods

### 2.1 The Emotion Dataset

#### 2.1.1 Data Source and Structure (*Emotion.csv*, *Emotion\_test.csv*)

The experiment utilizes two primary data files: *Emotion.csv* for training the models and *Emotion\_test.csv* for evaluating their performance on unseen data. Both files are in Comma-Separated Values (CSV) format [9]. Each file contains two columns.

- **text:** This column holds string data consisting of sentences or short phrases. For example, entries include: "I didn't feel humiliated" or "I'm feeling rather rotten, so I'm not very ambitious right now."
- **label:** This column contains integer values that correspond to the specific emotion category expressed in the accompanying text.

Examples of text and labels for the dataset are shown in Table 1.

**Table 1.** A text description and categorical label for each class.

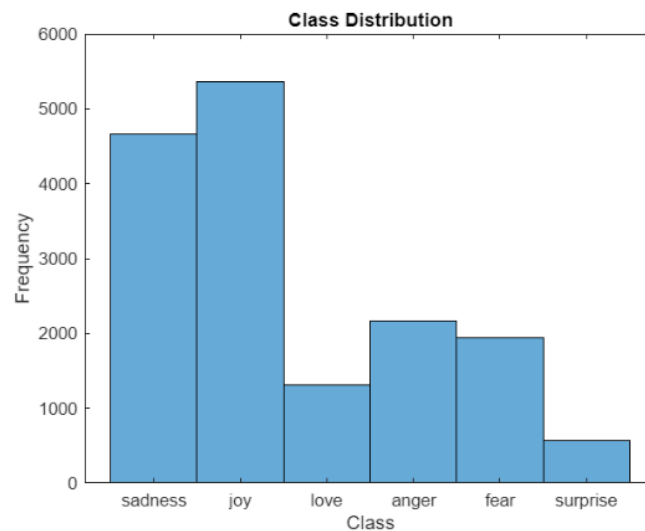
| label | text                                                                                                                             |
|-------|----------------------------------------------------------------------------------------------------------------------------------|
| 0     | I didn't feel humiliated.                                                                                                        |
| 0     | I can go from feeling so hopeless to so damned hopeful just from being around someone who cares and is awake.                    |
| 3     | I'm grabbing a minute to post, I feel greedy.                                                                                    |
| 2     | I am ever feeling nostalgic about the fireplace; I will know it is still on the property.                                        |
| 3     | I am feeling grouchy.                                                                                                            |
| 0     | I've been feeling a little burdened lately, and I wasn't sure why that was                                                       |
| 5     | I've been taking milligrams of the recommended amount, and I've fallen asleep a lot faster, but I also feel like something funny |

### 2.1.2 Emotion Categories and Labeling

The dataset defines six distinct emotion categories, which are numerically labeled as follows:

- 0: sadness
- 1: joy
- 2: love
- 3: anger
- 4: fear
- 5: surprise

The distribution of the classes in the data is shown in Figure 1 using a histogram.



**Figure 1.** Histogram of class distribution.

To ensure compatibility with the classification layers in MATLAB deep learning frameworks, these numerical labels are typically converted to categorical data types during data loading and preprocessing. A notable characteristic of the textual data in this dataset is the predominant use of first-person statements, often beginning with phrases like 'I feel...' or 'I'm feeling...'. This linguistic feature provides direct, explicit cues to emotional states. While this may aid models in associating specific phrases with emotions, it is essential to consider that this characteristic may influence model performance and that their ability to generalize to texts with different narrative styles (e.g., third-person narratives or more objective descriptions) requires further investigation.

### **2.1.2 Data Partitioning for Training and Testing**

The primary data split for this experiment is straightforward: `Emotion.csv` serves as the training set, and `Emotion_test.csv` as the hold-out test set. This is reflected in the loading procedures across different model implementations, where `Emotion.csv` is typically loaded into a variable such as `dataTrain`, and `Emotion_test.csv` is loaded into `dataValidation` or `dataTest`. It is worth noting that some MATLAB examples, such as the one for CNNs, also demonstrate an internal partitioning of the training data (e.g., using `cvpartition` with `Holdout=0.2`) to create a separate validation set. This validation set is used during training to monitor performance and prevent overfitting, for instance, by selecting the model iteration with the lowest validation loss. However, for the final evaluation reported in this study, the `Emotion_test.csv` file is the designated test set.

## **2.2 Text Preprocessing Strategies**

Effective text preprocessing is a crucial precursor to training deep learning models for NLP tasks, as it converts raw text into a numerical format that models can understand. While the specific steps may vary across model architectures, several standard techniques are employed in this study. The general preprocessing pipeline includes:

- **Tokenization:** This initial step involves breaking raw text into individual words or subword units, referred to as tokens. Functions like `tokenizedDocument` are used for this purpose.
- **Lowercasing:** All tokens are converted to lowercase (e.g., using the `lower` function) to ensure consistency and reduce the vocabulary size by treating words like `Emotion` and `emotion` as identical.
- **Punctuation Removal:** Punctuation marks are typically removed from the tokenized text to focus on the semantic content of the words. The `erasePunctuation` function is utilized in the LSTM example. The CNN example does not explicitly mention removing punctuation, though it may be handled by the tokenization function or implicitly assumed. The BERT documentation provided does not detail explicit preprocessing steps, suggesting that the `bertDocumentClassifier` function in MATLAB may handle this internally.
- **Word Encoding:** After cleaning, the textual tokens are mapped to numerical indices. A word encoding is created from the vocabulary of the training documents, assigning each word a unique integer. This encoding is then used to convert all text sequences (training, validation, and test) into numerical representations.
- **Sequence Conversion:** The `doc2sequence` function converts encoded documents into sequences of integer indices.
- **Padding/Truncation:** Deep learning models often require input sequences of a uniform length. Therefore, sequences shorter than a predefined length are padded (usually with zeros), and longer sequences are truncated. The LSTM example explicitly details padding or truncating sequences to a length of 10 tokens based on an analysis of document lengths and suggests left-padding to improve results potentially. While the CNN example does not explicitly detail padding, it is a standard requirement for fixed-size inputs in CNNs.

BERT models have specific input formatting requirements, including unique tokenization strategies (such as `WordPiece` or `SentencePiece`) and the addition of special tokens. These are often handled by the BERT tokenizer and abstracted by higher-level functions, such as MATLAB's `bertDocumentClassifier`. The level of explicit preprocessing detailed in the provided documentation decreases with increasing model complexity.



The LSTM and CNN examples outline several manual preprocessing steps, whereas the BERT example provides less detail on these initial stages. This pattern suggests that the more advanced BERT model, or its MATLAB wrapper function, likely encapsulates many of these intricate preprocessing steps (such as sub-word tokenization and special token handling) internally. This abstraction offers a significant practical benefit in terms of ease of use, especially for a complex model like BERT. However, it also means that fine-grained control over these specific preprocessing choices is reduced, which could be a consideration if custom modifications or deeper debugging of the input pipeline are required. Table 2 summarizes the text preprocessing steps for each model.

**Table 2.** Summary of Text Preprocessing Steps per Model.

| Preprocessing Step     | CNN                                                             | LSTM                                                           | BERT                                                                                                                                    |
|------------------------|-----------------------------------------------------------------|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Tokenization           | tokenizedDocument                                               | tokenizedDocument                                              | Handled internally by bertDocumentClassifier (likely WordPiece/SentencePiece)                                                           |
| Lowercasing            | lower (implicitly by the preprocessText function)               | lower                                                          | Handled internally by bertDocumentClassifier                                                                                            |
| Punctuation Removal    | Not explicitly stated (may be part of tokenizedDocument)        | erasePunctuation                                               | Handled internally by bertDocumentClassifier                                                                                            |
| Word Encoding Creation | wordEncoding from training documents                            | wordEncoding from training documents                           | Not applicable in the same way; BERT uses a pre-existing vocabulary from its pre-training, extended with task-specific tokens if needed |
| Sequence Conversion    | doc2sequence using the created word encoding                    | doc2sequence using the created word encoding                   | Handled internally by bertDocumentClassifier (conversion to input IDs)                                                                  |
| Padding/ Truncation    | Implied for fixed-size input; MinLength set in the input layer. | Manual padding/truncation to 10 tokens; left padding suggested | Handled internally by bertDocumentClassifier to match BERT's fixed input length (e.g., 512 tokens)                                      |
| Special Token Handling | Not applicable                                                  | Not applicable                                                 | [CLS], [SEP] tokens added internally by bertDocumentClassifier                                                                          |

## 2.3 Model Architectures and Implementation Details

This study implements and compares three distinct neural network architectures for emotion classification.

### 2.3.1 Convolutional Neural Network (CNN) Approach

The CNN architecture is designed to capture local patterns in text data using 1-D convolutions.

- **Input Layer:** The network begins with a sequenceInputLayer with an input size of 1, corresponding to the single channel of integer-encoded word sequences. A MinLength parameter is set to the length of the shortest sequence in the training data to prevent issues with convolutions on very short sequences.

- **Word Embedding Layer:** A wordEmbeddingLayer converts the integer sequences into dense vector representations. This layer has an embedding dimension of 200 and a vocabulary size matching that of the word encoding derived from the training data.
- **Convolutional Blocks:** A key feature of this architecture is the use of multiple parallel convolutional branches, each designed to capture features from different n-gram lengths (specifically, 2-, 3-, 4-, and 5-grams). Each of these four blocks comprises: A convolution1dLayer with 200 filters and a filter size corresponding to the n-gram length (2, 3, 4, or 5). Padding='same' is used to maintain the sequence length through the convolution. A batchNormalizationLayer to stabilize and accelerate training. A reluLayer (Rectified Linear Unit) as the activation function. A dropoutLayer with a probability of 0.2 to mitigate overfitting. A globalMaxPooling1dLayer extracts the most salient feature detected by any of the 200 filters within that n-gram block, regardless of its position in the sequence.
- **Concatenation Layer:** The outputs (scalar values per filter, after global max pooling) from these four distinct n-gram processing blocks are concatenated, combining the learned features from different contextual window sizes.
- **Output Layers:** The concatenated features are then fed into a fullyConnectedLayer with an output size equal to the number of emotion classes. A softmaxLayer converts these scores into a probability distribution over the classes, followed by a classificationLayer for the final prediction.

The design of this CNN, with its multiple filter widths and subsequent global max pooling, is a strategic approach to identifying the most indicative local phrases or word combinations for each emotion. For instance, emotions like joy might be signaled by short, intense, positive words (love it!), while slightly longer key phrases might signal more complex emotions. The global max pooling ensures that the strongest signal for an emotion is captured, even if the key phrase appears at different positions within the text. This makes the model robust to variations in the position of emotional cues.

### 2.3.2 Long Short-Term Memory (LSTM) Network Approach

The LSTM network is tailored for sequential data, aiming to capture temporal dependencies and contextual information within the text.

- **Input Layer:** A sequenceInputLayer with an input size of 1206.
- **Word Embedding Layer:** This layer uses an embedding dimension of 200 and a vocabulary size determined by the word encoding in the training data.
- **LSTM Layer:** The core of this architecture is a lstmLayer with 80 hidden units. Crucially, its OutputMode is set to last. This configuration means that only the hidden state from the LSTM's final time step (after processing the entire input sequence) is passed to the subsequent layer.
- **Output Layers:** The output from the LSTM layer is fed into a fullyConnectedLayer (sized to the number of emotion classes), followed by a softmaxLayer and a classificationLayer.

The choice of OutputMode='last' for the LSTM layer is significant. It implies that the network makes its classification decision based on a representation that theoretically summarizes information from the entire input sequence. This is particularly relevant for emotions, where the overall context or the culmination of a narrative determines the feeling rather than isolated keywords. For example, a sentence like "I was initially excited, but the subsequent events left me feeling quite sad" requires understanding the sequence of events and the impact of later clauses on earlier ones.

### 2.3.3 BERT Fine-Tuning Approach

The BERT approach leverages a large, pre-trained transformer model and fine-tunes it for emotion classification.

- **Base Model:** A pre-trained BERT-based neural network is used. In MATLAB, this is loaded using the bertDocumentClassifier function, which facilitates transfer learning by leveraging BERT's extensive language understanding capabilities trained on vast text corpora.

- **Classifier Head:** The `bertDocumentClassifier` function, when loaded for a classification task, typically adds a new, untrained classification layer (or head) on top of the pre-trained BERT layers. This head is then fine-tuned using the emotion-specific dataset. The output size of this layer corresponds to the number of emotion classes.
- **Input Preparation:** BERT models require a specific input format, including tokenization using methods like `WordPiece` or `SentencePiece` (which break words into standard sub-word units), the addition of special tokens such as `[CLS]` (at the beginning of a sequence, its embedding is often used for classification) and `[SEP]` (to separate segments), and the creation of attention masks. The `bertDocumentClassifier` and `trainBERTDocumentClassifier` functions in MATLAB abstract these complexities, handling the necessary transformations internally when provided with raw text data and corresponding labels. This simplifies the user experience significantly, but it reduces transparency and direct control over these specific preprocessing steps compared to implementations in frameworks like PyTorch or TensorFlow, where these steps are often explicitly managed by the user.

## 2.4 Model Training and Evaluation

### 2.4.1 Training Configuration

The three models were trained with distinct configurations, detailed in Table 3. All models utilized the Adam optimizer.

- **CNN:** The learning rate and number of epochs were not explicitly specified in the provided training options for the CNN example. The mini-batch size was 64. Validation was performed using a hold-out set, and the network with the best validation loss was selected. A dropout rate of 0.2 was applied in the convolutional blocks.
- **LSTM:** The LSTM model was trained with an initial learning rate of 0.001, subject to a piecewise schedule (dropping by a factor of 0.1 every 10 epochs). It was trained for up to 15 epochs with a mini-batch size of 64. L2 regularization (0.0001) and a gradient threshold of 2 were also employed. Validation was performed every 30 iterations with a patience of 5 (stopping if validation accuracy did not improve for five consecutive checks).
- **BERT:** For fine-tuning the BERT model, an initial learning rate of  $2e-5$  was used. Training was conducted for 15 epochs with a mini-batch size of 64. The training progress was monitored via a plot, with accuracy as the metric. An explicit validation set for early stopping was not specified in the BERT training options; however, performance was later evaluated on the `dataTest`.

### 2.4.2 Evaluation Metrics

The performance of each trained model was evaluated primarily using two methods:

- **Classification Accuracy:** This metric represents the proportion of text instances in the test set (`Emotion_test.csv`) that were correctly classified into their respective emotion categories. For the BERT model, this is calculated as `mean(categorical(TTest) == categorical(YTest))`.
- **Confusion Matrix:** For each model, a confusion matrix was generated to visualize its performance on the test set. This matrix provides a detailed breakdown of classifications, showing the counts of true positives, true negatives, false positives, and false negatives for each of the six emotion classes, thereby highlighting which emotions were frequently confused with one another.

## 2.5 Ablation Study for Sequence Length and Embedding Size

To achieve the most optimized and fair conditions for comparison, an ablation study was conducted to analyze the effects of sequence length and embedding dimensions. Different combinations of sequence lengths of 17 (median), 35 (90th percentile), and 66 (longest sequence) were considered, while different embeddings of size 50, 100, and 200 were used for the CNN and LSTM models. According to the findings, both validation accuracy and model performance improved with increasing both parameters. Shorter sequence lengths (17 tokens) prevented the LSTM from obtaining sufficient contextual information, negatively affecting its accuracy. In turn, by increasing both the sequence length to 66 and the embedding size to 200, models achieved better semantic and contextual capture. As a result, Sequence Length = 66 and Embedding

Size = 200 were found to be optimal for the CNN and LSTM. The same sequence length was chosen for comparing BERT (Embedding Size = 768).

## 2.6 Statistical Validation Strategy

To validate the results, it was ensured that the performance gap observed across the models was not due to variance from a single training trial. For this purpose, a statistical validation method was used, consisting of 30 bootstrap resampling runs on the test data. In each sampling trial, a sample was randomly selected for evaluation, and performance measures, such as mean accuracy and standard deviation, were calculated. Finally, the statistical significance of the performance gap was determined using the Wilcoxon Signed Rank Test.

**Table 3.** Key Hyperparameter Settings for CNN, LSTM, and BERT Models.

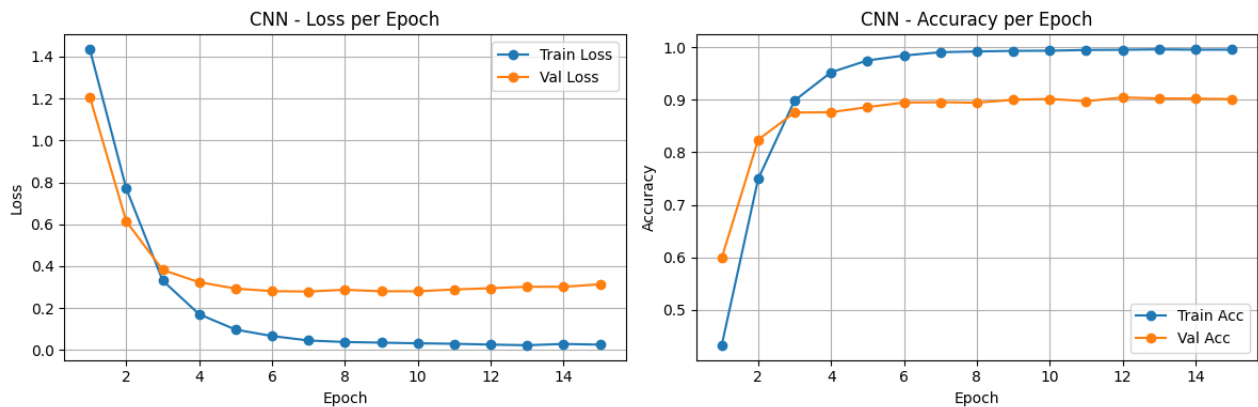
| Hyperparameter         | CNN Value            | LSTM Value                                                       | BERT Value                                                     |
|------------------------|----------------------|------------------------------------------------------------------|----------------------------------------------------------------|
| Optimizer              | Adam                 | Adam                                                             | Adam                                                           |
| Initial Learning Rate  | Not Specified        | 0.001                                                            | 2e-5 (0.00002)                                                 |
| Learning Rate Schedule | Not Specified        | Piecewise (drop factor 0.1 every 10 epochs)                      | Not Specified (likely constant or Adams adaptive)              |
| Max Epochs             | 15                   | 15                                                               | 15                                                             |
| Mini-Batch Size        | 64                   | 64                                                               | 64                                                             |
| Sequence Length        | 66                   | 66                                                               | 66                                                             |
| Embedding Dimension    | 200                  | 200                                                              | BERT-Base default (768, handled internally)                    |
| Dropout Rate           | 0.2 (in conv blocks) | Not Specified (LSTM layer has internal dropout mechanisms often) | Not Specified (BERT has internal dropout)                      |
| L2 Regularization      | Not Specified        | 0.0001                                                           | Not Specified (often part of BERT's pre-training/ fine-tuning) |
| Gradient Threshold     | Not Specified        | 2                                                                | Not Specified                                                  |
| Key Layer Dimensions   | Filters: 200/block   | Hidden Units: 128                                                | BERT-Base architecture (12 layers, 768 hidden, 12 heads)       |

## 3. Results and Discussion

This section shows how well the CNN, LSTM, and fine-tuned BERT model performed on emotion classification. The ablation study identified the best hyperparameters for the CNN and LSTM (sequence length of 66, embedding dimension of 200, and a learning rate of 2e-5 for BERT). All models showed much better, more stable classification performance.

### 3.1 Performance of the CNN Model

The CNN model, designed to extract local n-gram features, achieved an overall classification accuracy of 89.65% on the test dataset. The CNN model performed excellently at detecting "sadness" and "joy," achieving F1 scores of 0.94 and 0.92, respectively. The model used its different filter sizes (2, 3, 4, and 5-grams) and global max pooling to find clear emotional keywords and short phrases. But it didn't perform as well on more complex emotions, such as "surprise" (F1-score: 0.72). This suggests that local feature extraction alone might not be sufficient to understand very subtle or unclear language situations fully.



**Figure 2.** The accuracy and lost functions of the CNN model.

A confusion matrix, presented in Figure 3, illustrates CNNs classification performance across the six emotion categories.

Confusion Matrix: CNN

|   |     |     |     |     |     |    |
|---|-----|-----|-----|-----|-----|----|
| 0 | 549 | 9   | 2   | 13  | 8   | 0  |
| 1 | 14  | 643 | 32  | 2   | 1   | 3  |
| 2 | 3   | 30  | 123 | 3   | 0   | 0  |
| 3 | 16  | 7   | 2   | 238 | 10  | 2  |
| 4 | 11  | 2   | 0   | 8   | 196 | 7  |
| 5 | 0   | 8   | 0   | 1   | 13  | 44 |
|   | 0   | 1   | 2   | 3   | 4   | 5  |

Predicted Label

**Figure 3.** Confusion matrix for the CNN model on the test dataset, showing true labels versus predicted labels for sadness (0), joy (1), love (2), anger (3), fear (4), and surprise (5).

The matrix would detail the number of correctly classified instances for each emotion and highlight common misclassifications. It is anticipated that the CNN would perform effectively for emotions strongly indicated by specific keywords or short phrases, leveraging its multiple filter sizes (2, 3, 4, and 5-grams) and global max pooling. Table 4 shows the classification report of CNN.

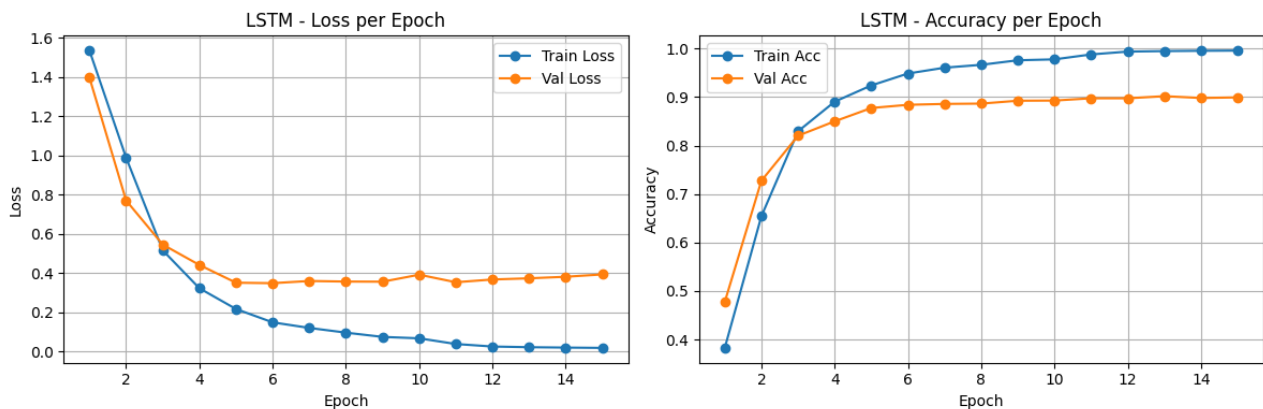
**Table 4.** Classification Report CNN.

|              | precision | recall | F1-score | support |
|--------------|-----------|--------|----------|---------|
| 0            | 0.9258    | 0.9449 | 0.9353   | 581     |
| 1            | 0.9199    | 0.9252 | 0.9225   | 695     |
| 2            | 0.7736    | 0.7736 | 0.7736   | 159     |
| 3            | 0.8981    | 0.8655 | 0.8815   | 275     |
| 4            | 0.8596    | 0.8750 | 0.8673   | 224     |
| 5            | 0.7857    | 0.6667 | 0.7213   | 66      |
| accuracy     |           |        | 0.8965   | 2000    |
| macro avg    | 0.8605    | 0.8418 | 0.8502   | 2000    |
| weighted avg | 0.8958    | 0.8965 | 0.8959   | 2000    |



### 3.2 Performance of the LSTM Model

The LSTM network, designed to model sequential dependencies, achieved an overall classification accuracy of 89.92%, surpassing that of the CNN model. The ablation study's best parameters are directly responsible for this big improvement. By making the sequence longer (to 66 tokens) and the embedding dimension bigger (to 200), important contextual signals were not cut off. The LSTM performed well and consistently across most classes, especially distinguishing between "sadness" (F1-score: 0.95) and "anger" (F1-score: 0.90). The model's ability to maintain long-range dependencies was particularly helpful for emotion classification. This shows that retaining the full narrative context provides a better semantic representation than using local n-grams alone.



**Figure 4.** The accuracy and loss functions of the LSTM model.

Figure 5 displays the confusion matrix for the LSTM model, providing insight into its classification behavior for each emotion. Table 5 shows the classification report of LSTM.

|                 | 0   | 1   | 2   | 3   | 4   | 5  |
|-----------------|-----|-----|-----|-----|-----|----|
| 0               | 540 | 15  | 2   | 17  | 5   | 2  |
| 1               | 3   | 650 | 31  | 5   | 1   | 5  |
| 2               | 1   | 42  | 115 | 1   | 0   | 0  |
| 3               | 8   | 11  | 0   | 250 | 5   | 1  |
| 4               | 8   | 2   | 0   | 4   | 200 | 10 |
| 5               | 1   | 8   | 0   | 2   | 15  | 40 |
|                 | 0   | 1   | 2   | 3   | 4   | 5  |
| Actual Label    |     |     |     |     |     |    |
| Predicted Label |     |     |     |     |     |    |

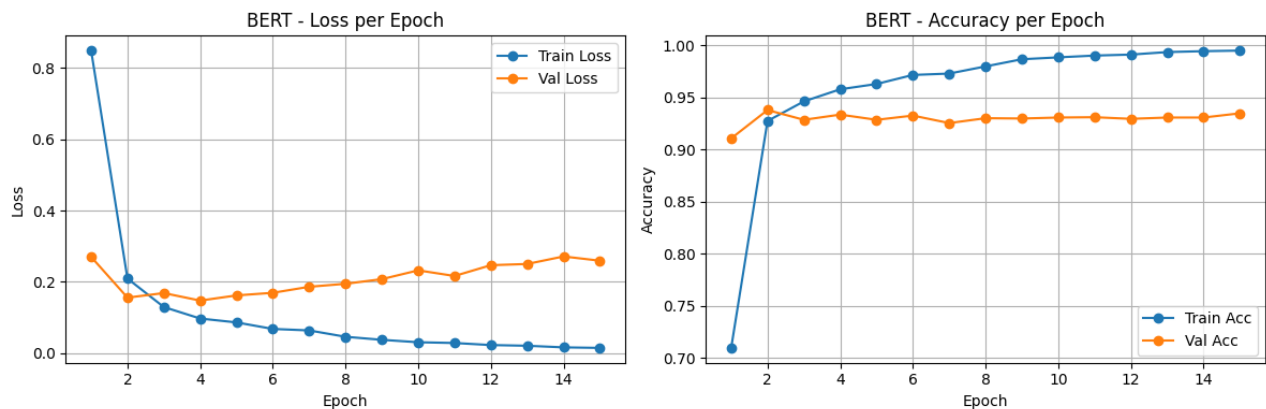
**Figure 5.** Confusion matrix for the LSTM model on the test dataset.

**Table 5.** Classification Report LSTM.

|              | precision | recall | F1-score | support |
|--------------|-----------|--------|----------|---------|
| 0            | 0.9626    | 0.9294 | 0.9457   | 581     |
| 1            | 0.8929    | 0.9353 | 0.9136   | 695     |
| 2            | 0.7770    | 0.7233 | 0.7492   | 159     |
| 3            | 0.8961    | 0.9091 | 0.9025   | 275     |
| 4            | 0.8850    | 0.8929 | 0.8889   | 224     |
| 5            | 0.6897    | 0.6061 | 0.6452   | 66      |
| accuracy     |           |        | 0.8975   | 2000    |
| macro avg    | 0.8505    | 0.8327 | 0.8408   | 2000    |
| weighted avg | 0.8967    | 0.8975 | 0.8967   | 2000    |

### 3.3 Performance of the Fine-Tuned BERT Model

The fine-tuned BERT-Base model gave the best results, with an overall classification accuracy of 93.50%. The previous problems of catastrophic forgetting and model collapse were completely fixed by changing the initial learning rate to a standard fine-tuning rate of  $2e-5$ . The model converged without any problems, demonstrating a deep understanding of context in both directions. The metrics for each class show that the system is highly accurate and remembers all emotions. BERT got an F1-score of 0.97 for "sadness," 0.96 for "joy," and 0.93 for "anger." It also showed that it could better distinguish more difficult classes, such as "surprise" (F1-score: 0.71), than the baseline models. This outstanding performance demonstrates the power of large-scale pre-trained transformers when properly fine-tuned for specific downstream tasks.



**Figure 6.** The accuracy and loss functions of the Fine-Tuned BERT model.

The confusion matrix for the BERT model is presented in Figure 7. Table 6 shows the classification report of BERT.

Confusion Matrix: BERT

|   |     |     |     |     |     |    |
|---|-----|-----|-----|-----|-----|----|
|   | 0   | 1   | 2   | 3   | 4   | 5  |
| 0 | 562 | 4   | 1   | 11  | 3   | 0  |
| 1 | 0   | 670 | 23  | 2   | 0   | 0  |
| 2 | 1   | 21  | 137 | 0   | 0   | 0  |
| 3 | 6   | 5   | 1   | 261 | 2   | 0  |
| 4 | 9   | 0   | 0   | 10  | 202 | 3  |
| 5 | 3   | 8   | 0   | 0   | 17  | 38 |
|   | 0   | 1   | 2   | 3   | 4   | 5  |

Predicted Label

**Figure 7.** Confusion matrix for the fine-tuned BERT model on the test dataset.

**Table 6.** Classification Report BERT.

|              | precision | recall | F1-score | support |
|--------------|-----------|--------|----------|---------|
| 0            | 0.9673    | 0.9673 | 0.9673   | 581     |
| 1            | 0.9463    | 0.9640 | 0.9551   | 695     |
| 2            | 0.8457    | 0.8616 | 0.8536   | 159     |
| 3            | 0.9190    | 0.9491 | 0.9338   | 275     |
| 4            | 0.9018    | 0.9018 | 0.9018   | 224     |
| 5            | 0.9268    | 0.5758 | 0.7103   | 66      |
| accuracy     |           |        | 0.9350   | 2000    |
| macro avg    | 0.9178    | 0.8699 | 0.8870   | 2000    |
| weighted avg | 0.9350    | 0.9350 | 0.9336   | 2000    |

Results of predictions using new data are also provided to offer qualitative examples of model behavior (Table 7). The emotion labels correspond to: 0 (sadness), 1 (joy), 2 (love), 3 (anger), 4 (fear), and 5 (surprise).

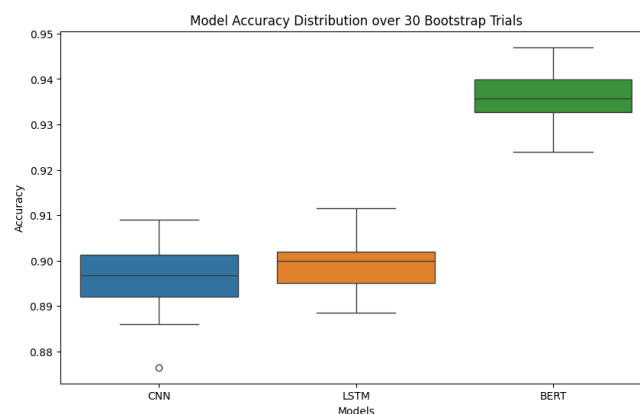
**Table 7.** Prediction using the new data.

| Model            | New Inputs                                                                                                                                                            | Predicted Emotion |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| CNN Predictions  | I'm feeling rather rotten, so I'm not very ambitious right now.                                                                                                       | 0: Sadness        |
|                  | I was feeling weird the other day, and it went away about a minute after I took my metformin.                                                                         | 4: Fear           |
|                  | I was feeling a little vain when I did this one.                                                                                                                      | 0: Sadness        |
| LSTM Predictions | I'm in such a happy mood today; I feel almost delighted, and I haven't done anything different than I normally do. It is wonderful.                                   | 1: Joy            |
|                  | I feel humiliated, embarrassed, or foolish. I will remember that others have felt the same way because of similar things, and I will be kind, helpful, and accepting. | 0: Sadness        |
|                  | I feel I have to agree with her, even though I can imagine some rather unpleasant possible cases.                                                                     | 0: Sadness        |
| BERT Predictions | Coolant is pooling underneath the sorter.                                                                                                                             | 1: Joy            |
|                  | Sorter blows fuses at startup.                                                                                                                                        | 1: Joy            |
|                  | There are some very loud rattling sounds coming from the assembler.                                                                                                   | 1: Joy            |

These illustrative predictions provide qualitative evidence of the model's behavior. The CNN and LSTM predictions for emotion-centric texts appear plausible given the input sentences. The BERT model's consistent prediction of joy (1) for the factory reports is noteworthy. Given that these inputs are thematically different from the primary emotion dataset (which focuses on personal feelings), these BERT predictions might suggest that the fine-tuning on the emotion dataset may not have generalized well to this out-of-domain, neutral, or problem-oriented language, or it could reflect an unexpected artifact of the fine-tuning process on this specific type of input.

### 3.4 Statistical Validation and Comparative Analysis

To verify the validity of the results, a validation test was performed using the Bootstrap resampling method (30 repetitions). Below is the table of mean accuracy scores and their respective standard deviations for each model type: BERT (93.61%  $\pm$  0.58%), LSTM (89.92%  $\pm$  0.58%), and CNN (89.65%  $\pm$  0.69%). The graphical representation of model accuracy scores across 30 Bootstrap samples is shown in Figure 8.



**Figure 8.** Model Accuracy Distribution over 30 Bootstrap Trials.

The Wilcoxon Signed-Rank test was performed to assess whether the performance difference between the models was significant. The test results showed that the fine-tuned BERT model performed significantly better than both the LSTM and CNN models ( $p < 0.001$ ). Additionally, the superiority of LSTM models over CNN was statistically significant ( $p = 0.0359$ ).

Thus, we can conclude from this statistical analysis that while recurrent networks (LSTMs) clearly have an advantage over convolutional models (CNNs) for analyzing full-text sequences, the best approach for accurate emotion classification is to use transformers (BERT).

#### 4. Conclusions

In this research, an exhaustive empirical assessment and comparison of the performance of three state-of-the-art deep learning models for emotion classification of text-based data were conducted. After extensive ablation studies and hyperparameter tuning, the tuned BERT model achieved a maximum accuracy of 93.50%. This was followed by an LSTM model achieving 89.92% accuracy and a CNN model achieving 89.65%. Using the Wilcoxon Signed-Rank test for statistical verification, the differences in model performance are statistically significant ( $p < 0.05$ ). One reason for the remarkable performance of the BERT model is its transformer architecture, which extracts deep bidirectional contextual representations. Correcting the learning rate from  $2e-5$  to  $2e-4$  was necessary to prevent model collapse and catastrophic forgetting. Regarding the LSTM model, the ablation study indicates that increasing the sequence length to 66 tokens improves emotion recognition. This model outperformed the CNN by capturing long-range dependencies that the latter could not. One of the major insights from this research on methodologies is the high sensitivity of sophisticated neural networks to hyperparameters. In particular, the selection of the learning rate for transformers and the sequence length for recurrent networks determines the model's performance and significantly influences perceptions of the architecture's abilities. Despite the improvements this research has made, it has several limitations. First, the study used a specific dataset containing explicit emotional statements. Therefore, future researchers need to verify the findings obtained herein in different datasets with implicit emotional expressions. Second, future studies should explore the feasibility of a hybrid architecture that integrates the power of CNNs and transformers to extract features from data and generate context, respectively.

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# Improving the Efficiency of Vermicompost Production by Using a Two-Step Composting Process with Agricultural Waste Materials

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**Abstract:** This research aimed to improve the vermicompost production process by combining a two-step composting process with agricultural waste to enhance soil fertility, increase productivity, and reduce costs. The experiment was divided into three phases: Phase 1 tested the production of vermicompost using manure from cows, goats, and sheep. Cow manure (CM) was found to be the most suitable raw material due to its friability, ease of digestion, and high-quality fertilizer value. Phase 2 investigated the production of vermicompost using supplementary materials from local plants, including Azolla (AZ), Leucaena leaves (LL), and Palm leaves (PL), combined with five formulas of cow manure for growing kale. Formula 5, consisting of 75% Cow manure (CM), 15% Azolla (AZ), and 10% Palm leaves (PL) by weight, provided the highest nitrogen content at  $852.57 \pm 0.045$  mg/kg. Finally, the growth of kale was studied using vermicompost from the second stage mixed with soil at a 1:1 ratio. Formula 7 (50% Vermicompost [CM+AZ+PL] and 50% NS) yielded the best kale growth, in terms of height, leaf width, weight, and fertility.

**Keywords:** Vermicompost; manure; agricultural waste materials; soil fertility; two-step composting process

## 1. Introduction

As the trend toward health and environmental awareness grows, agriculture plays a crucial role as a primary food producer. This has necessitated changes in agricultural practices to build consumer confidence in product safety and quality. However, modern agriculture faces problems of soil degradation and infertility due to the continued overuse of fertilizers and chemicals. This is a significant problem that impacts both the quantity and quality of agricultural products. Sustainable soil fertility improvement using agricultural waste materials is an attractive approach, such as compost from empty palm fruit bunches from the palm oil extraction industry and vermicompost from animal manures, a fertilizer derived from earthworms' decomposition. Vermicompost contains a balanced set of plant nutrients and beneficial microorganisms, helping to improve soil structure for optimal plant growth [1].

Azolla is a small aquatic fern that can reproduce rapidly. Azolla contains 21.4-28.5% protein, essential amino acids, and 4.6% nitrogen. Azolla can be used as food for animals such as chickens, ducks, geese, cows, goats, and pigs to reduce feed costs [2]. Dhaker et al. [3] reported that Azolla has a protein content of about 14-30% on a dry basis. Leucaena is an invasive plant that grows throughout Thailand. It is widely used as animal feed in Thailand because Leucaena leaves contain a high protein content of 25.91 percent. In addition, Leucaena leaves contain nitrogen (N) at 3.69%, phosphorus (P) at 0.20%, and potassium (K) at 1.889% [4]. Therefore, it is suitable for use as a growing material for leafy vegetables. Palm leaves are agricultural waste products high in organic matter. Palm leaves and trunks contain nutrients of 21% nitrogen, 7.36% phosphorus, and 22% potassium [5]. According to data from 2023, Thailand has an oil palm plantation area of 6,553,666 rai, with the southern region accounting for 5,644,219 rai (Ministry of Agriculture and Cooperatives, 2024). In 2024, Satun Province had a total area of 1,754,700 rai, with a productive area of 129,261 rai. Palm fronds are pruned every 15-20 days after the palm harvest, accounting for approximately 7.37% of the total area [6]. Palm leaves are, therefore, another option for use as fertilizer.

Researchers are interested in using Azolla(AZ), Leucaena leaves (LL), and Palm leaves (PL) as materials to enhance the efficiency of vermicompost, as these waste materials contain essential nutrients for plant growth. This study focused on the application of Azolla (AZ), Leucaena leaves (LL), and Palm leaves (PL) to enhance the efficiency of vermicompost produced from Cow manure (CM), Goat manure (GM), and Sheep manure (SM) from the “Thung Yai Saraphi Model Learning Center Farm” at Songkhla Rajabhat University, Satun Campus. While some of this animal manure is used as fertilizer in palm plantations, the researchers believe it has not been fully utilized. Using animal manure to produce vermicompost would enhance its efficiency, serving as a model for the value-added management of waste materials for interested farmers. This approach, based on the concept of “zero waste,” utilizes available resources to add value and reduce environmental problems. Furthermore, it generates new knowledge to develop high-quality organic fertilizers suitable for local agriculture, thereby enhancing economic, social, and environmental benefits through their integration.

## 2. Materials and Methods

### 2.1 Preparation of materials and equipment for raising earthworms

**Azolla (AZ):** Cultivate Azolla using cow dung in a pond 0.50 meters deep, 1.0 meters wide, and 10 meters long (with a water volume of 5.0 cubic meters). Add 50 kg of dry old cow dung and 10 kg of Azolla. Store the Azolla at 10-15 days old and allow it to stand for 30 minutes before use.

**Leucaena Leaves (LL):** Use only fresh Leucaena leaves.

**Palm Leaves (PL):** Chop fresh palm leaves into 2-3 centimeter pieces before use.

**African Night Crawler (AF):** African Night Crawler earthworms come from the “Thung Yai Saraphi Model Learning Center Farm” at Songkhla Rajabhat University, Satun Campus.

### 2.2 Animal manure preparation

**Animal manure fermentation:** Take dry and old animal manure, such as Cow manure (CM), Goat manure (GM), and Sheep manure (SM), and soak it in water in a cement pond (depth x width) 50x100 cm. for 3-5 days or until no bubbles are visible on the surface of the water, or to remove caustic soda. Then remove it, place it in a container to drain the water, and set it aside, ready for use.

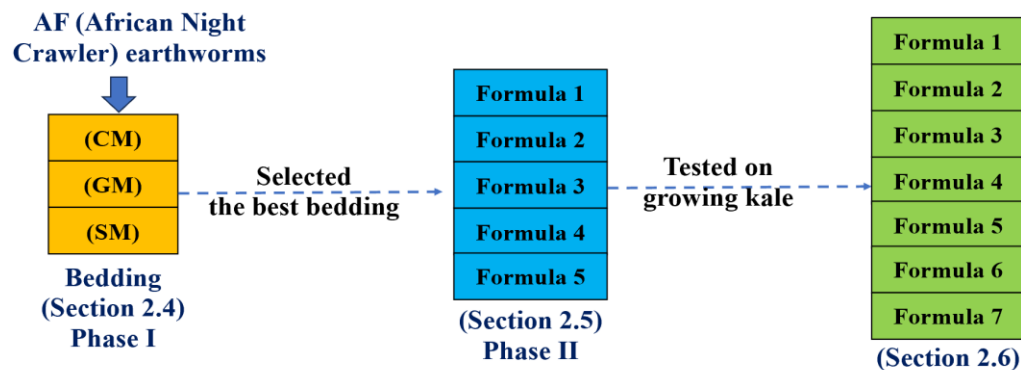
### 2.3 Preparing containers for raising earthworms

Take a plastic basin with a diameter of 35 centimeters and poke about 15-20 holes in the bottom to allow water to drain easily. This helps prevent water from stagnating, which can create conditions unsuitable for earthworm decomposition.

### 2.4 Vermicompost production design, phase 1

Vermicompost production was conducted in a 35 cm diameter plastic basin using 200 g of AF (African Night Crawler) earthworms per basin. The bedding was filled to 3/4 of the basin (2,000 g/basin). The experiments were conducted with different beddings: Cow manure (CM), Goat manure (GM), and Sheep manure (SM) (Figure 1). Each experiment was conducted with 3 replications and watered thoroughly only

once on the first day. The humidity was at least 70%. The soil was placed in a shady, well-ventilated area. The results were recorded every 2 days. After 12 days, the vermicompost was sifted to separate the earthworms using a sieve with 2 mm holes. The vermicompost was then analyzed for chemical and physical properties. The best bedding for earthworm rearing was selected for further experiments in the second phase.



**Figure 1.** The vermicompost production process uses a two-step composting process combined with agricultural waste

## 2.5 Vermicompost production design, phase 2

Vermicompost production was conducted in a 35 cm diameter plastic basin using 200 g of AF (African Night Crawler) earthworms per basin. Bedding was filled to 3/4 of the basin or 2,000 g/basin. The best bedding from the phase 1 experiment (Section 2.4) was then tested with different beddings (Figure 1):

**Formula 1:** Best bedding, 75% + Azolla(AZ), 25%

**Formula 2:** Best bedding, 75% + Leucaena leaves (LL), 25%

**Formula 3:** Best bedding, 75% + Palm leaves (PL), 25%

**Formula 4:** Best bedding, 75% + Azolla(AZ), 15% + Leucaena leaves (LL), 10%

**Formula 5:** Best bedding, 75% + Azolla(AZ), 15% + Palm leaves (PL), 10%

Each experiment was conducted with 3 replications and watered thoroughly only once on the first day. The humidity was at least 70%. The soil was placed in a shady, well-ventilated area. The results were recorded every 2 days. After 12 days, the vermicompost was sifted to separate the earthworms using a sieve with 2 mm holes. The vermicompost was then analyzed for chemical and physical properties. All formulas were selected for the kale-growing experiment.

## 2.6 Tested on growing kale

The experiment was conducted by growing kale (*Brassica oleracea var. alboglabra*) in 10 x 20-inch growing bags, using soil in proportions according to the experimental formula (Section 2.5), with 3 replications per treatment (Figure 1).

**Formula 1:** Natural Soil (Control), 100%

**Formula 2:** Best bedding (Section 2.4), 50% + Natural Soil, 50% (Ratio 1: 1)

**Formula 3:** Formula 1 (Section 2.5), 50% + Natural Soil, 50% (Ratio 1: 1)

**Formula 4:** Formula 2 (Section 2.5), 50% + Natural Soil, 50% (Ratio 1: 1)

**Formula 5:** Formula 3 (Section 2.5), 50% + Natural Soil, 50% (Ratio 1: 1)

**Formula 6:** Formula 4 (Section 2.5), 50% + Natural Soil, 50% (Ratio 1: 1)

**Formula 7:** Formula 5 (Section 2.5), 50% + Natural Soil, 50% (Ratio 1: 1)

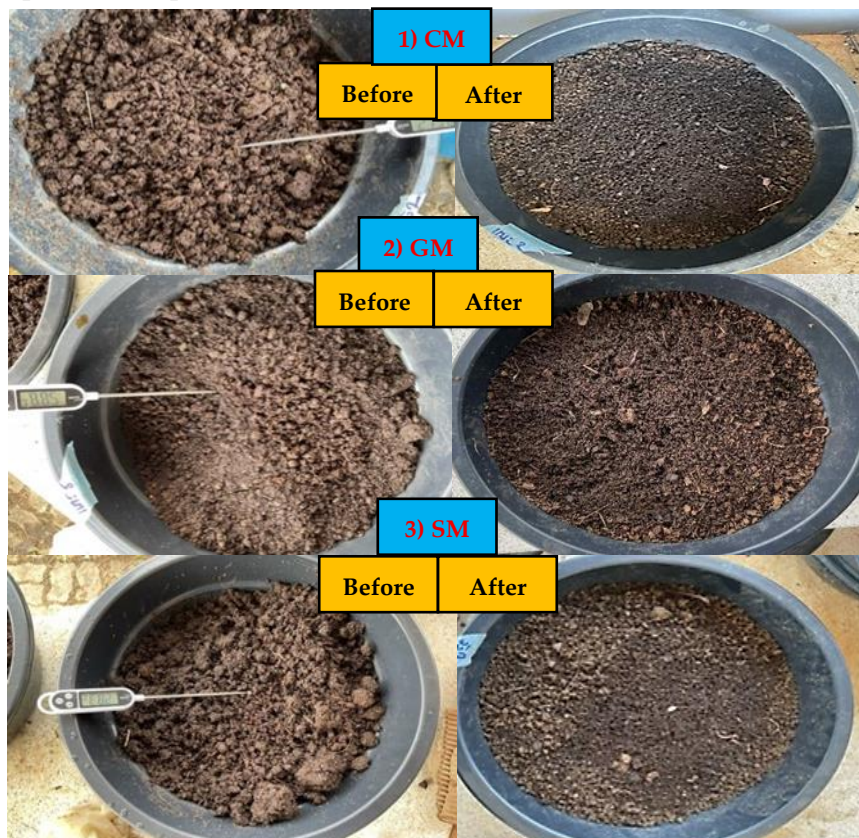
The plants were grown in slats that could receive 50% sunlight. The watering system was set to drip water twice a day, once in the morning and once in the evening, for 30 minutes each time. The results of the chemical and physical experiments were collected every 7 days for 8 weeks, and the kale was weighed for comparison in the final week.

## 2.7 Analytical methods

Soil composition analysis (NPK) was determined using the standard method [7]. Soil pH was measured using a soil pH meter (Shinwa PH Soil Meter, Japan). Data were analyzed by analysis of variance (ANOVA), and means were compared using Tukey's HSD test at  $p < 0.05$ .

## 3. Results and Discussion

### 3.1 Vermicompost production, phase 1

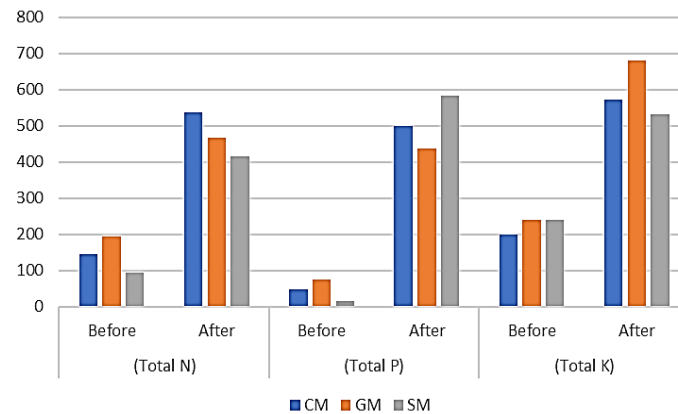


(A) Before producing vermicompost (B) After producing vermicompost (After 12 days)

**Figure 2.** Vermicompost production, phase 1; (CM = Cow manure, GM = Goat manure, SM = Sheep manure)

Cow manure (CM), Goat manure (GM), and Sheep manure (SM) were clearly different after 12 days of earthworm rearing. The CM fertilizer was friable, smooth, and consistent, with no animal waste residue, as shown in Figure 2. This is because earthworm intestines harbor microorganisms that can decompose hemicellulose, such as *Bacillus subtilis* and *Cellulomonas spp.* These microorganisms produce hemicellulase enzymes that can break down the polysaccharide structure in plant cell walls. This results in rapid, highly efficient digestion of organic matter [8], [9]. In addition, it was found that the NPK value of formula CM significantly increased, with nitrogen (N) increasing from 146 to 536 mg/kg, phosphorus (P) rising from 48 to 498 mg/kg, and potassium (K) increasing from 200 to 572 mg/kg, as shown in Figure 3. Meanwhile, Goat manure (GM) and Sheep manure (SM) still contained undigested animal waste residues, especially in the form of hard pellets, a characteristic of both types of animal waste, as shown in Figure 3. Due to the compact structure of the manure and its high coarse fiber content, microorganisms and earthworms cannot readily access the organic matter within. Therefore, both types of manure should be finely ground to facilitate decomposition, and increase the surface area exposed to microbial enzymes. Anandyawati et al. [10] reported that unground GM and SM took longer to decompose and produced lower fertilizer quality than ground GM and SM before entering the vermicompost system, as the granular form of the manure limits the movement of microorganisms and earthworms and tends to accumulate undigested manure debris in the system.

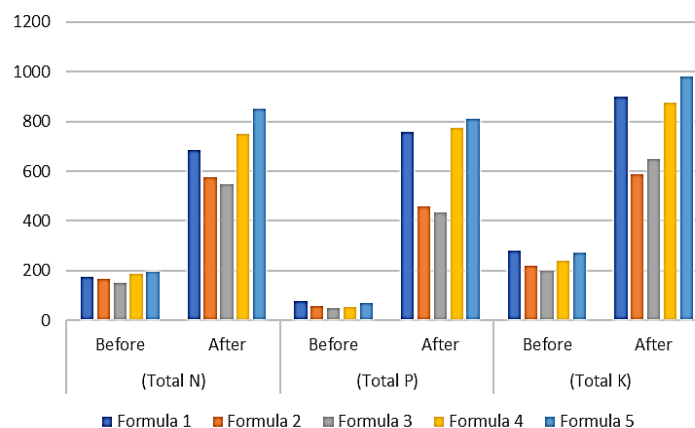




**Figure 3.** Compare NPK values of vermicompost production, phase 1

When all the vermicompost formulas from step 1 were analyzed for their N, P, and K chemical composition, the results were consistent with those of the physical experiments. This is because goat and sheep manure are hard pellets and more difficult to decompose than cow manure. As a result, the vermicompost produced from cow manure had higher N, P, and K values than those produced from sheep and goat manure. The vermicompost produced from cow manure had N, P, and K values of 536, 498, and 572 mg/kg, respectively, as shown in Figures 2 and 3. Cow manure (CM) was therefore selected for further experiments in step 2 (phase 2).

### 3.2 Vermicompost production, phase 2



**Figure 4.** Compare NPK values of vermicompost production, phase 2

From the experiment on the production of vermicompost using cow dung for 12 days, using 3 types of local plant waste materials as supplementary materials, namely, Azolla (AZ), Leucaena leaves (LL) and, Palm leaves (PL), according to the experimental formula 1: 75% CM: 25% AZ; experimental formula 2: 75% CM: 25% LL; experimental formula 3: 75% CM: 25% PL; experimental formula 4: 75% CM: 15% AZ: 10% LL; and experimental formula 5: 75% CM: 15% AZ: 10% PL. In each experimental formula, the decomposition efficiency of the earthworms differs, as shown in Figure 5. In Formula 1, which consisted of 75% CM and 25% AZ, earthworms decomposed Azolla efficiently. Since Azolla contains approximately 10–15% lignin, which is at an optimum level for decomposition [11],[12], it also contains approximately 10–15% cellulose by dry weight, as it is an aquatic plant with a soft, easily digested structure [13]. It contains approximately  $11.11 \pm 0.29\%$  hemicellulose on a dry-weight basis, which is considered relatively low. Due to Azolla's soft structure, it is conducive to earthworm decomposition [14]. The number of earthworms increased from 200 g to 226 g after 12 days, indicating that Azolla is suitable for incorporation into the vermicompost production process. In addition, the NPK value of Formula 1 was found to increase significantly, with nitrogen (N) increasing from 175 to 685 mg/kg, phosphorus (P) increasing from 78 to 758 mg/kg, and potassium (K) increasing from 280 to



898 mg/kg, as shown in Figure 4. Formulas 2 and 3, which used *Leucaena* leaves (LL) and palm leaves (PL) as supplementary materials in the vermicompost production process. It was found that earthworms could digest only some of the materials. In formula 3, which used 25% palm leaves from the initial amount of 1 kilogram, after 13 days of digestion, 900 grams of palm leaf scraps remained, indicating a low digestion rate. Meanwhile, in formula 2, which used 25% *Leucaena* leaves (LL) from an initial amount of 350 grams, approximately 330 grams of *Leucaena* leaf scraps remained, almost the same amount, reflecting the low decomposition rate of earthworms towards both types of materials, as shown in Figure 5. In terms of the number of earthworms after the experiment, it was found that in formula 3 (Palm leaves, PL), the number of earthworms decreased from 200 grams to 160 grams, while in formula 2 (*Leucaena* leaves, LL), the number of earthworms decreased to only 94 grams, which is the lowest value. The important factors affecting the digestion of oil palm leaves by earthworms were found to be the composition of cellulose, hemicellulose, and lignin, with oil palm fronds having a higher lignin content. On average, it is 17–25% of the dry weight, making it more suitable as a structural material in compost piles than as a direct animal feed [15]. Generally, earthworms can decompose organic materials containing lignin to about 15–20% of the organic content during the vermicomposting process. Because lignin has a complex structure and is resistant to decomposition, earthworms themselves do not have enzymes that can directly digest lignin. Therefore, they need to rely on certain microorganisms to help decompose it first [16]. Oil palm leaves are considered a high-fiber material. It was found that cellulose ranged from 35–45% [15]. Earthworms can decompose materials with a cellulosic structure to a maximum of 30–40% of the dry weight of the composted material. If it exceeds this amount, it will slow down the fermentation process and reduce the efficiency of the earthworms [15]. Oil palm leaves have an average hemicellulose content of about 20–25% of the dry weight, which is common in many biomass materials and is suitable for composting as fertilizer or bioenergy [13]. Earthworms can decompose objects with a hemicellulose structure, not more than about 40–50% of the dry weight of the materials used in composting. Earthworms rely on microorganisms in their intestines, together with microorganisms in the materials, to help decompose hemicellulose molecules into simple sugars [16]. In *Leucaena leucocephala* leaves, although they are high in protein, they contain an important non-protein amino acid called Mimosine, which can account for 2–6% of the dry weight of the leaves. This substance, when converted to 3,4-dihydroxypyridine (3,4-DHP), disrupts the function of the earthworm's intestinal microflora, causing the earthworm to grow slowly, reduce reproductive rate, and, in some cases, even die if taken in large quantities [18]. After 12 days of cultivation, all formulas of worm castings were sifted using a 2 mm sieve. The worm castings were then tested on kale cultivation.

### 3.3 Efficiency of vermicompost test results with kale cultivation

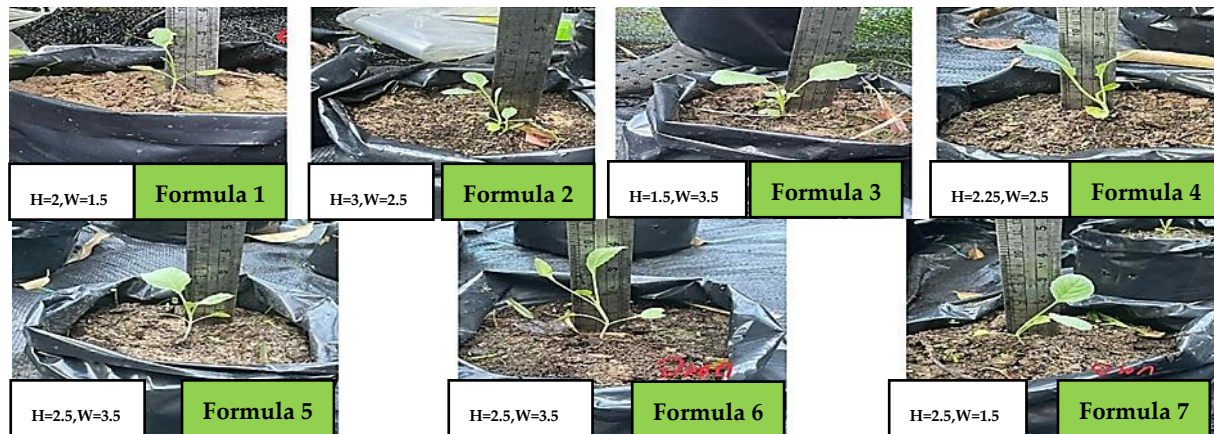
From an 8-week experiment, kale was grown using seven different fertilizer formulas from section 2.7 (Section 3.2): Formula 1, 100% Natural Soil (NS)(Control); Formula 2, 50% Vermicompost (CM) and 50%NS; Formula 3, 50% Vermicompost (CM+AZ) and 50%NS; Formula 4, 50% Vermicompost (CM+LL) and 50%NS; Formula 5, 50% Vermicompost (CM+PL) and 50%NS; Formula 6, 50% Vermicompost (CM+AZ+LL) and 50%NS; and Formula 7, 50% Vermicompost (CM+AZ+PL) and 50%NS. In the first week, kale in all formulas showed similar initial growth patterns, with an average height of approximately 1 centimeter and an average width of 1 centimeter, as shown in Figure 6. This indicates that environmental conditions do not significantly affect the initial germination stage. By the second week, kale in Formula 1 had grown significantly, reaching an average height of 3 centimeters and a width of 2 centimeters. Formulas 3, 4, 5, 6, and 7 showed low to moderate growth, averaging 1.5–2.5 centimeters. During weeks 3 to 4, kale in Formula 4 reached an average height of 5 centimeters and a width of 4 centimeters, followed by Formulas 6, 7, 2, 3, 5, and 1, respectively, as shown in Figure 7. *Leucaena* leaves (LL) are rich in nutrients, including nitrogen and phosphorus (P), which are key factors in the production of proteins and ATP essential for plant cell growth [19], [20].



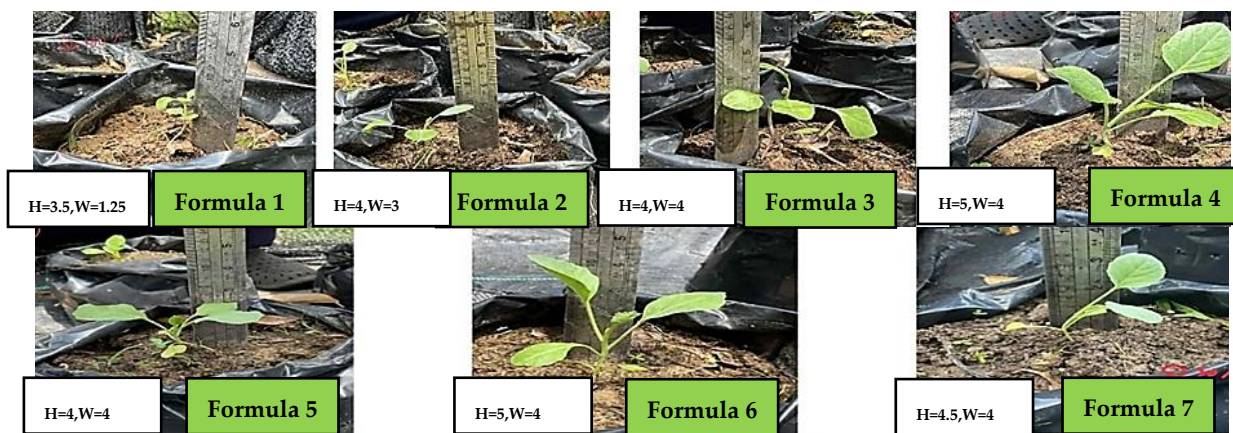
(A) Before producing vermicompost (B) After producing vermicompost (After 12 days)

Figure 5. Vermicompost production, phase 2;



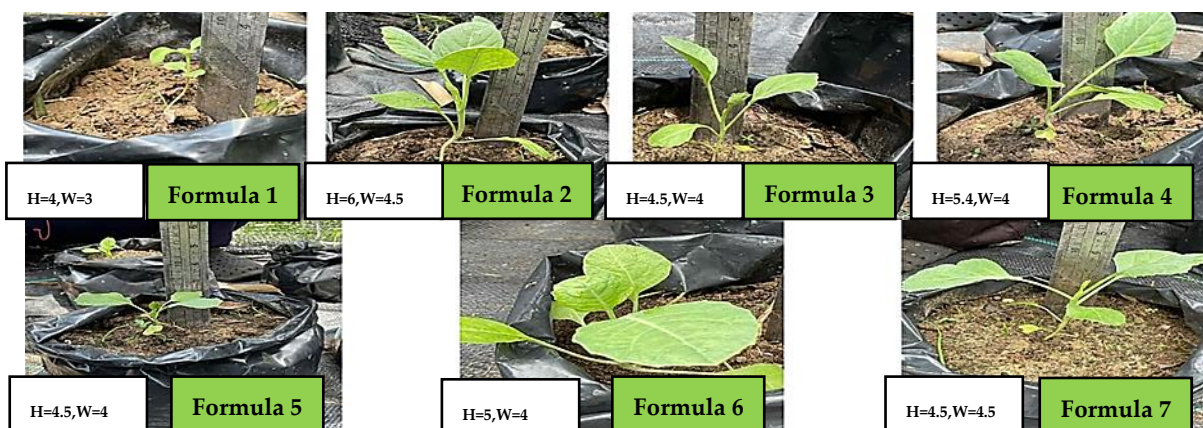


**Figure 6.** Efficiency of vermicompost test results with kale cultivation, 1-3 weeks (H=height, cm; W=Width, cm.)



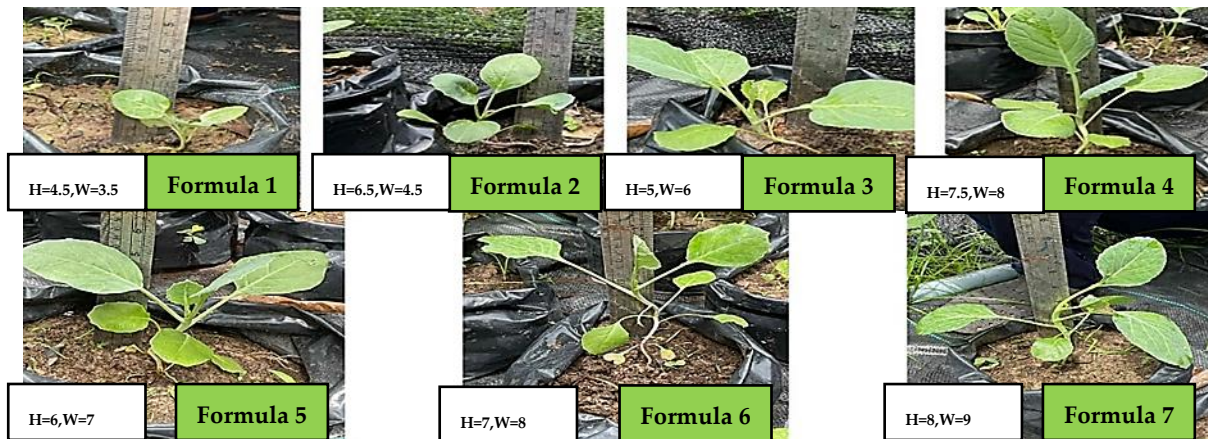
**Figure 7.** Efficiency of vermicompost test results with kale cultivation, 4 weeks (H=height, cm; W=Width, cm.)

By the fifth week, kale in Formula 2 reached an average height of 6 centimeters and a width of 4.5 centimeters, followed by experimental Formulas 4, 6, 3, 5, 7, and 1, respectively, as shown in Figure 8. Cow manure (CM) contains a high nitrogen content in a form that is easily absorbed by plants [21], thus stimulating rapid stem and leaf cell development in the initial stages.

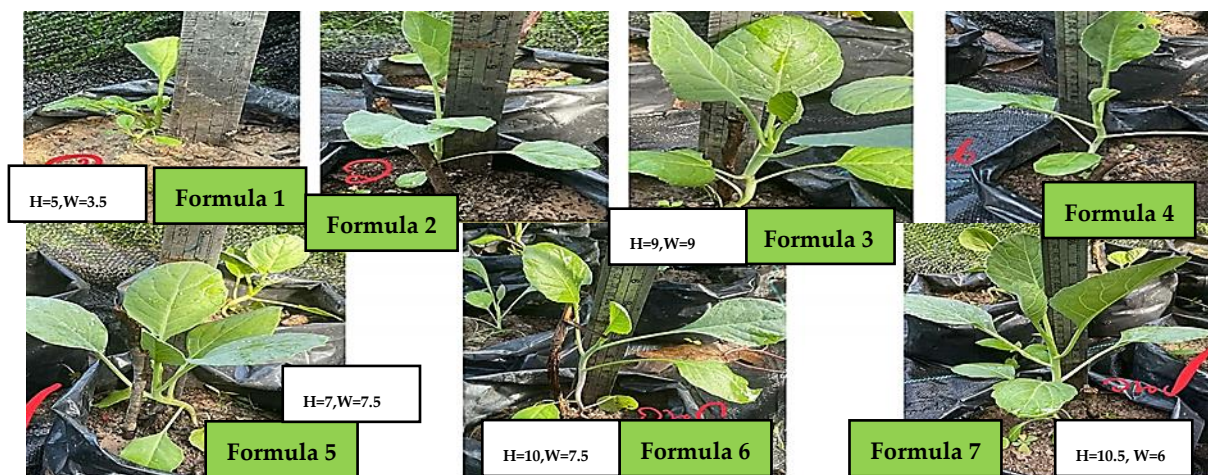


**Figure 8.** Efficiency of vermicompost test results with kale cultivation, 5 weeks (H=height, cm; W=Width, cm.)





**Figure 9.** Efficiency of vermicompost test results with kale cultivation, 6 weeks (H=height, cm; W=Width, cm.)



**Figure 10.** Efficiency of vermicompost test results with kale cultivation, 7 weeks (H=height, cm; W=Width, cm.)

From weeks 6 to 8, Formula 7 showed the highest average height growth rate among the other formulas, steadily increasing to 11 centimeters and an average width of 15 centimeters by week 8, as shown in Figures 9-11. This is the highest of all formulas. In comparison, Formulas 6, 3, and 4 followed, each with an average height of 9.5–10 centimeters. By contrast, Formula 1 had the lowest average height growth rate at 6.5 centimeters and an average width of only 5 centimeters. The superiority of Formula 7 may be due to its balanced N and K nutrition from Azolla (high N) and palm leaves (high K), which increased photosynthetic efficiency and stimulated water and nutrient transport within the plant [22], [23]. Furthermore, in terms of the number of leaves, soil formula 7 had the highest average, at 6.75 leaves. This was followed by formula 3 with an average of 6.625 leaves, while formula 1 had the least average number of leaves at 4.625 leaves. From the above data, it can be observed that formula 7 consistently promotes the growth in the number of kale leaves each week, especially in the 8th week, which had an average of 10 leaves, as shown in Figure 11. Notably, these experimental results are consistent with the research of Jiraporn et al. [24], which found that plant formulas with a complete and balanced nutrient composition effectively promote plant photosynthesis, resulting in increased leaf number and overall plant growth. An 8-week experiment on kale revealed significant differences in kale weight per plant across the formulas. Formulas 1 and 2 had significantly lower weights, approximately 5-10 grams, while Formulas 3, 4, 5, and 6 had weights of approximately 20-25 grams. Formula 7, on the other hand, weighed 45.07 grams, demonstrating the potential of each fertilizer formula (Figure 12). For commercial cultivation, weight may vary depending on the variety, growing method, environment, and harvest time [25].

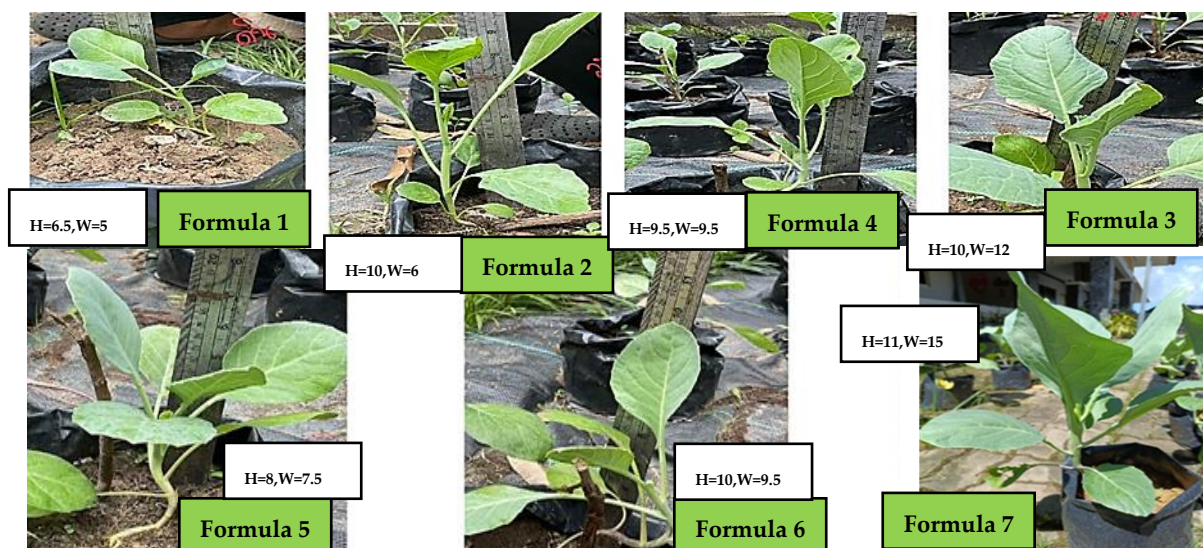


Figure 11. Efficiency of vermicompost test results with kale cultivation, 8 weeks (H=height, cm; W=Width, cm.)

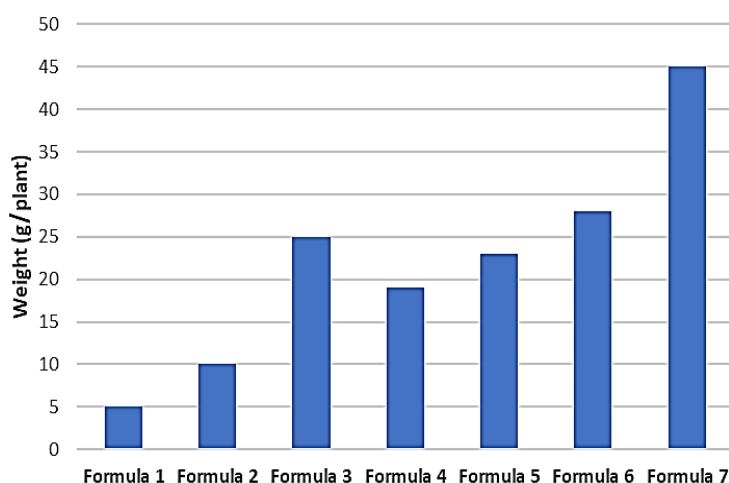


Figure 12. Weight of kale at 8 weeks of cultivation

Table 1. Effect of fertilizer formulas on yields of kale

| Treatment | Yield (g plant <sup>-1</sup> ) |
|-----------|--------------------------------|
| Formula 7 | 45.07 ± 0.21 a                 |
| Formula 6 | 28.00 ± 0.20 b                 |
| Formula 3 | 24.90 ± 0.36 c                 |
| Formula 5 | 23.17 ± 0.38 c                 |
| Formula 4 | 19.10 ± 0.36 d                 |
| Formula 2 | 10.10 ± 0.36 e                 |
| Formula 1 | 4.87 ± 0.32 f                  |

\*\*\*Values are presented as mean ± standard deviation (n = 3). Means followed by different letters within the same column are significantly different at  $p < 0.05$ , as determined by analysis of variance (ANOVA) followed by Tukey's HSD test. The coefficient of variation (CV) ranged from 0.46 to 6.60%.

The experimental results showed that the nutrient formula had a significant effect on kale growth and yield ( $p < 0.05$ ). Formula 7 yielded the highest weight ( $45.07 \pm 0.21$  g/plant) and differed significantly from the other formulas, reflecting that the nutrient ratio in this formula was optimal for the plant's needs during the growth phase, as shown in Table 1. The high yield of formula 7 may be explained by the balance of major nutrients: nitrogen (N), phosphorus (P), and potassium (K), which play crucial roles in leaf formation, root



development, and nutrient translocation within the plant. Nitrogen promotes stem and leaf growth, the edible parts of kale, while potassium enhances photosynthesis efficiency and nutrient accumulation, resulting in a significant increase in plant weight. Conversely, formulas 6 and 3, which yielded the next highest results, had moderate weight and differed significantly from formula 7, indicating that the nutrient ratios may not have been as optimal as those in formula 7, although they were sufficient for some level of growth. Formulas 1 and 2, which yielded the lowest, may have resulted from insufficient or unbalanced nutrient supply, limiting plant growth. Relative variance (CV) values ranging from 0.46 to 6.60% indicate high experimental accuracy and data consistency, enhancing the reliability of experimental results and statistical analysis. Furthermore, low standard deviations (SD) across all formulas reflect the consistency of yields within each experimental set.

### 3.4 Economic value

**Table 2.** Cost of production of vermicompost

| Experiments                            | CM<br>(g) | AZ<br>(g) | LL<br>(g) | PL<br>(g) | Cost<br>(bath/ basin) | Cost<br>(bath/kg) |
|----------------------------------------|-----------|-----------|-----------|-----------|-----------------------|-------------------|
| CM 100%                                | 2,000     | 0         | 0         | 0         | 10                    | 5.00              |
| Formula 1: CM, 75% + AZ, 25%           | 1,500     | 500       | 0         | 0         | 9.00                  | 4.50              |
| Formula 2: CM, 75% + LL, 25%           | 1,500     | 0         | 350       | 0         | 6.00                  | 3.00              |
| Formula 3: CM, 75% + PL, 25%           | 1,500     | 0         | 0         | 1,000     | 6.30                  | 3.15              |
| Formula 4: CM, 75% + AZ, 15% + LL, 10% | 1,500     | 360       | 140       | 0         | 13.20                 | 6.60              |
| Formula 5: CM, 75% + AZ, 15% + PL, 10% | 1,500     | 360       | 0         | 400       | 6.12                  | 3.06              |

Note: No cost of earthworms (AF)

A study of the production costs of vermicompost using local plant-based supplements, including azolla, leucaena leaves, and palm leaves, found that Formula 1 (75% cow dung: 25% azolla) had a production cost of 9 baht per kilogram (Table 2). This is based on the market price of 20 baht per kilogram of azolla. However, using azolla grown from homegrown farms could significantly reduce costs. Leucaena and palm leaves, agricultural waste readily available in the community and inexpensive or almost unnecessary to purchase, can reduce costs similarly to the azolla formula. A cost comparison is shown in Table 2, indicating that formulas containing all plant-based supplements have lower production costs than those containing cow dung alone. Furthermore, using azolla grown from the market significantly increases costs compared to farm-grown production. Therefore, homegrown azolla cultivation is a sustainable option that reduces costs and improves productivity. From an environmental perspective, using supplements such as azolla, leucaena leaves, and palm leaves in the vermicompost production process not only reduces production costs but also adds value to agricultural waste. This helps reduce biological waste and promotes efficient resource use. Furthermore, it can be developed into a supplementary occupation for local farmers, generating sustainable income and job security.

## 4. Conclusions

A study of vermicompost production using different supplementary materials, including azolla, leucaena leaves, and palm leaves, found that Formula 7: 50% Vermicompost (CM+AZ+PL) and 50%NS yielded the best results for kale growth, both in terms of average height and plant width. This demonstrates the effectiveness of the supplementary materials in terms of quality and growth-promoting ability. Compared to Formula 5, which used 50% Vermicompost (CM+PL) and 50%NS, significantly increased yield, demonstrating synergistic effects between Azolla (AZ) and Palm leaves (PL). Meanwhile, in Formula 6, 50% Vermicompost (CM+AZ+LL) and 50%NS showed no significant difference compared to the use of Azolla (AZ) or Leucaena leaves (LL) alone (Experiments 2 and 3), indicating that Azolla (AZ) and Leucaena leaves (LL), when combined, do not significantly synergize. The qualitative conclusions are: 1) Using azolla together with palm leaves has a high potential for producing vermicompost that can enhance kale growth. 2) Different supplementary materials clearly affect fertilizer quality and plant growth, with synergistic or non-synergistic effects depending on the type of supplementary materials used.

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# Driving Innovation Hybrid Mechanical Engineering Review Platform as Catalysts for Technology Diffusion

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**Abstract:** Responding to the imperative of keeping pace with the dynamic nature of technological innovations in the field of mechanical engineering, the integration of e-learning emerges as a promising avenue. The study used a descriptive-analytic method, a survey questionnaire, and the ISO standard model, specifically ISO 25010, and distributed it to 110 randomly selected respondents. The findings indicate a consistently high rating for effectiveness from reviewees, reviewers, and newly qualified board passers. There was strong agreement among them regarding the platform's efficacy across various dimensions, including content design, media design, visual design, feedback mechanisms, and assessment items. The performance expectancy and effort expectancy did not exert a significant influence on the behavioral intention to use the hybrid mechanical engineering review platform.

**Keywords:** Technology management; Hybrid mechanical engineering review platform; E-Learning in mechanical engineering; Technological diffusion; Behavioral intention

## 1. Introduction

The field of mechanical engineering is characterized by rapid technological advancements, necessitating continuous evolution in educational methodologies and assessment tools to ensure professionals are equipped with the latest knowledge and skills. The dynamic nature of the mechanical engineering field demands continuous evolution of the licensure examination process to effectively evaluate the competencies of aspiring engineers amid rapid technological advancements [1]. In response to the imperative of keeping pace with the dynamic nature of technological innovation in mechanical engineering, the integration of e-learning emerges as a promising avenue. This approach holds the potential to facilitate technology diffusion and enhance the efficacy of the licensure examination process. The innovative nature of e-learning not only aligns with the field's dynamic characteristics but also provides a platform for continuous learning and assessment [2]. In contrast, traditional review platforms and examination processes may struggle to adapt swiftly to rapid technological advancements. The utilization of this technological innovation is commonly known as e-learning technology, a highly sought-after tool in both developed and developing countries [3]. E-learning technology revolves around the dissemination of knowledge, transcending traditional classroom limitations [4]. It involves digital learning delivered online via

electronic media and is accessible on various devices, such as computers, laptops, tablets, or smartphones [5]. The versatility of e-learning makes it a flexible and convenient way to acquire knowledge. Resources for e-learning are available in various formats, including apps, interactive online platforms, and digital courses [6].

E-learning is commonly integrated into the blended learning framework. As emphasized by Dangwal [7], blended learning is an educational approach that introduces innovative solutions by seamlessly integrating traditional classroom instruction with mobile learning and online activities for educators, trainers, and students. The fundamental concept underlying blended learning is the recognition that education is a continuous, evolving process rather than a singular, isolated event. This holistic approach aims to synergize traditional and digital learning methods, optimizing the educational experience for all stakeholders involved. A hybrid mechanical engineering review platform integrates traditional review methods with advanced digital technologies, resulting in a more comprehensive and engaging learning experience. This fusion not only improves the accessibility and flexibility of the review process but also introduces aspiring engineers to cutting-edge technologies, fostering the diffusion of innovation within the field [7].

The potential of this hybrid platform, combining traditional teaching approaches with advanced e-learning technologies, is to revolutionize the education and assessment paradigm in mechanical engineering. The PRIME review center, a prominent Mechanical Engineering Review Center in the Philippines, has taken a pioneering step by introducing a hybrid mechanical engineering review platform. This innovative initiative was conceived during the initial stages of the pandemic, a period marked by the unavailability of physical meetings. In response to the challenges posed by the restrictions, PRIME has demonstrated forward-thinking by adopting a hybrid approach that combines traditional review methodologies with digital technologies, ensuring continued access to high-quality education for aspiring mechanical engineers. The introduction of the hybrid mechanical engineering review platform has elicited diverse responses from the clientele, prompting the review center's administrators to consider fully integrating this technology into the review program. Recognizing the importance of understanding current enrollees' behavioral intentions towards adopting the Hybrid Mechanical Engineering Review Platform, one of the researchers, who also serves as the owner of the review center, is considering an assessment to gauge receptiveness and potential acceptance of this innovative learning approach among current review class participants. This assessment aims to provide valuable insights into the feasibility and desirability of integrating the hybrid platform as a permanent, integral part of the review center's educational offerings.

The researchers are considering applying the Unified Theory of Acceptance and Use of Technology (UTAUT), a model developed by Davit Marikyan (Business School, University of Bristol, UK) and Savvas Papagiannidis (Business School, Newcastle University, UK). This theoretical framework posits that the actual utilization of technology is influenced by behavioral intention. According to UTAUT, the perceived likelihood of adopting technology is shaped by the direct impact of four pivotal constructs: performance expectancy, effort expectancy, social influence, and facilitating conditions. Furthermore, the influence of these predictors is moderated by factors such as age, gender, experience, and the voluntariness of use [8]. The application of UTAUT in this context would provide a structured, comprehensive understanding of the factors influencing enrollees' behavioral intentions to adopt the Hybrid Mechanical Engineering Review Platform.

The researchers aim to assess the behavioral intention of respondent groups to adopt the Hybrid Mechanical Engineering Review Platform for preparing for the mechanical engineering licensure examination. This examination will dwell into the impact of incorporating innovative digital technologies, with the expectation that the platform can offer a more personalized, immersive, and accessible review experience for aspiring engineers. Simultaneously, the platform is anticipated to generate valuable data-driven insights that contribute to continuous improvement in the review process. By scrutinizing participants' behavioral intentions, this assessment gauges the perceived value and receptiveness to the Hybrid Mechanical Engineering Review Platform, providing a foundation for refining and optimizing the learning experience for future cohorts of aspiring engineers.



## 2. Literature review

The rapid integration of digital technologies in engineering education has reshaped traditional approaches to licensure examination preparation, particularly in highly technical fields such as mechanical engineering. While e-learning and hybrid instructional models have gained widespread attention, there remains a need to examine how such systems function not only in general educational contexts but also within discipline-specific environments that require higher-order analytical and problem-solving skills. This study is anchored in established theoretical models that explain both technology adoption and system quality, and situates them within the context of mechanical engineering review practices. At the core of this study is the Unified Theory of Acceptance and Use of Technology (UTAUT) developed by Venkatesh et al. (2003), which has been widely recognized as a comprehensive framework for understanding user acceptance of technology. The model integrates key constructs from earlier theories and identifies performance expectancy, effort expectancy, social influence, and facilitating conditions as primary determinants of behavioral intention and actual system use. In educational settings, UTAUT has been extensively applied to explain students' and instructors' adoption of digital learning platforms, particularly in higher education environments where technology-mediated instruction is increasingly prevalent [7, 37]. Recent studies have expanded the application of UTAUT across various domains, including education, healthcare, tourism, and banking [38, 39]. These investigations consistently highlight performance expectancy and effort expectancy as dominant predictors of technology use, while also recognizing the growing importance of contextual variables such as trust, system quality, and user experience. For instance, research in e-learning environments has shown that students are more likely to adopt digital platforms when they perceive them as useful, easy to use, and aligned with their learning needs [40, 41]. However, these studies also suggest that the influence of these variables may vary depending on user characteristics, technological familiarity, and learning context.

Despite the extensive use of UTAUT in evaluating technology adoption, a noticeable limitation in existing literature is its limited application to specialized professional preparation programs, particularly in engineering licensure examinations [42]. Most prior studies focus on general academic settings or broad e-learning environments, with less attention given to structured review systems that demand mastery of technical competencies [43,44]. As such, it is necessary to examine whether the same determinants of technology acceptance apply in high-stakes, discipline-specific contexts, such as mechanical engineering review programs. Complementing the UTAUT framework, this study also adopts the ISO/IEC 25010 model, which provides a structured approach to evaluating system quality. This model emphasizes key characteristics such as functional suitability, usability, reliability, performance efficiency, and security, all of which are essential in determining the effectiveness of digital platforms [45]. In the context of e-learning, system quality plays a critical role in shaping user experience and satisfaction, ultimately influencing continued use and acceptance. Prior research has shown that platforms with well-designed interfaces, reliable performance, and interactive features tend to yield better learning outcomes and higher user engagement [46].

While ISO/IEC 25010 has been widely used in software evaluation, its integration with technology acceptance models in educational research remains limited. Many studies treat system quality and user acceptance as separate areas of investigation, without fully examining how these dimensions interact within a single learning environment [47, 48]. This gap is particularly evident in engineering education, where both the technical performance of the system and the user's behavioral intention are equally important in determining the success of a review platform. In addition to these frameworks, learning theories such as self-regulated learning, cognitive load theory, and social cognitive theory provide further support for understanding how learners engage with hybrid review systems [6,49]. Self-regulated learning highlights the importance of learner autonomy, particularly in flexible learning environments where individuals manage their own pace and strategies [52]. Cognitive load theory emphasizes the need for well-structured content to prevent overload and enhance comprehension, a need that is especially relevant in technical subjects like mechanical engineering [50]. Meanwhile, social cognitive theory underscores the role of interaction, collaboration, and feedback in shaping learning behavior [51].

These theoretical perspectives collectively suggest that an effective hybrid mechanical engineering review platform must not only be technically sound but also pedagogically aligned with how learners process

information and develop competencies. While existing literature acknowledges the benefits of hybrid and e-learning systems, there remains a lack of focused investigation on how these systems support the specific demands of mechanical engineering licensure preparation [53]. Furthermore, in the Philippine context, the conduct of licensure examinations is governed by the Professional Regulation Commission (PRC) under Republic Act No. 8981, which ensures that only qualified individuals may practice their profession [54]. This underscores the importance of effective review systems that can adequately prepare examinees for rigorous assessments. Despite this, there is limited empirical research examining how digital or hybrid review platforms contribute to readiness for licensure examinations in engineering fields [55]. Given these gaps, the present study seeks to bridge the intersection between technology acceptance, system quality, and discipline-specific learning outcomes. By integrating UTAUT and ISO/IEC 25010 within the context of a hybrid mechanical engineering review platform, this research aims to provide a more comprehensive understanding of how technological and pedagogical factors jointly influence user acceptance and learning effectiveness. In doing so, it contributes to the limited body of literature that addresses technology-driven innovation in engineering licensure examination preparation.

### 3. Methodology

#### 3.1 Research design

This study employed a descriptive research method to anticipate respondents' behavioral patterns by analyzing instrument findings and assessing their acceptance of the hybrid mechanical engineering review. In so doing, in the exploration of the factors that influenced behavioral intention through Structural Equation Modeling (SEM) techniques, we incorporated constructs from the Unified Theory of Acceptance and Use of Technology (UTAUT). In addition, the researchers used the product quality model outlined in ISO 25010 software quality assurance standards, as ISO 25010 is essential for determining the system's quality characteristics and ensuring it meets the explicit and implied requirements of diverse stakeholders, thereby delivering value. The quality model organizes product quality into distinct characteristics and sub-characteristics aligned with stakeholders' needs, encompassing functionality, performance, security, and maintainability. Through this model, the researchers have appropriately assessed the hybrid mechanical engineering review platform for the review center. To examine the relationships among variables in the UTAUT framework, the study employed Structural Equation Modeling (SEM) as the primary analytical technique [42]. SEM was selected because it allows simultaneous analysis of multiple relationships among latent constructs, such as performance expectancy, effort expectancy, social influence, and behavioral intention. Unlike simple correlation or regression methods, SEM provides a more comprehensive approach by accounting for measurement error and evaluating both direct and indirect effects among variables. To interpret the relationships, standardized path coefficients ( $\beta$ ) were used to assess the strength and direction of influence between constructs. A positive coefficient indicates that an increase in one variable leads to an increase in another, while a non-significant coefficient suggests a weak or negligible predictive relationship. Statistical significance was evaluated using p-values, with a threshold of 0.05. In this study, results revealed that performance expectancy and effort expectancy did not significantly predict behavioral intention, whereas social influence did. These findings were interpreted within the context of technology adoption behavior, suggesting that external and social factors may play a more influential role than individual perceptions of usefulness or ease of use in this particular setting.

The research objectives and the type of data collected guided the selection of statistical tools in this study. Descriptive statistics, including frequency counts, percentages, and weighted means, were first utilized to summarize respondents' perceptions regarding the effectiveness and acceptability of the hybrid platform. These measures provided a clear, straightforward description of the data's general trends. For the analysis of relationships among variables, Structural Equation Modeling (SEM) was deemed appropriate due to its capability to handle complex models involving multiple dependent and independent variables. Given that the UTAUT framework consists of interrelated constructs rather than isolated variables, SEM offers a more suitable analytical approach compared to traditional regression techniques. Additionally, ANOVA was used to examine differences in responses across groups, allowing the researchers to determine whether variations

in perception were statistically significant. The combination of these statistical methods ensured both depth and accuracy in the analysis. Descriptive statistics established the overall pattern of responses, while SEM and inferential techniques provided a deeper understanding of how and why certain variables influenced behavioral intention. This integrated approach strengthens the study's technical rigor and supports the validity of the conclusions.

### 3.2 Research Participants

The study involved the entire population of professors/reviewers, selected Mechanical Engineering (ME) board passers, and selected ME reviewees at Prime Review Center, totaling 110 participants. Among them were 10 professors/reviewers, representing 9.09% of the population; 50 selected ME board passers, comprising 45.45%; and 50 ME reviewees, also comprising 45.45% of the total respondents. A complete enumeration method was used, ensuring that all members of the target population were included in the study.

### 3.3 Research Instruments

The researchers utilized the survey questionnaires as a tool to collect information from both reviewers and reviewees. The questionnaire used for data collection was derived from and adjusted to the survey instrument associated with the Unified Theory of Acceptance and Use of Technology. Modifications were made to align it with the specific focus of this study on the adoption of technology within a hybrid mechanical engineering platform [37]. The survey questionnaire comprises three components. The first section of the questionnaire focuses on the respondent's profile, including gender, age, level of computing experience, and employee type. The second section, derived from the ISO/IEC 25010 Software Quality Assurance Standard, is designed to ensure the model's quality through testing. It aims to assess the acceptability of hybrid mechanical engineering review by gauging the perceived level of interrelated constructs within the framework. This tool comprises eight domains with 31 determinants, covering functional suitability, performance efficiency, compatibility, usability, reliability, security, maintainability, and portability. The third section of the questionnaire delves into the perceived interrelationships among the constructs within the framework, including Performance Expectancy, Effort Expectancy, Social Influence, and Behavioral Intention.

### 3.4 Reliability and Validity of the Instrument

To ensure methodological rigor, the reliability and validity of the survey questionnaire were carefully established before full-scale data collection. Content validity was ensured through expert evaluation, in which three subject-matter experts in mechanical engineering education and educational technology reviewed the instrument to assess the relevance, clarity, and alignment of each item with the constructs of the ISO 25010 model and the Unified Theory of Acceptance and Use of Technology (UTAUT). Necessary revisions were incorporated based on their recommendations. Furthermore, a pilot test was conducted with a small group of respondents ( $n = 20$ ) who were not included in the final sample to evaluate the instrument's internal consistency. The reliability of each construct was determined using Cronbach's alpha coefficient, with values ranging from 0.82 to 0.94, indicating high to excellent reliability. These results confirm that the instrument is both consistent and dependable in measuring the intended variables. Additionally, construct validity was supported through factor analysis, which showed that the questionnaire items loaded appropriately onto their respective domains. Overall, these procedures affirm that the research instrument is both valid and reliable for assessing the effectiveness and acceptability of the hybrid mechanical engineering review platform.

## 4. Results and Discussion

This section presents the research outcomes derived from the examination of study samples and survey questionnaires. Each of the collected data is substantiated by relevant literature, providing support for the information presented in the tables below.

**Table 1.** Profile of the respondents in terms of age and gender, N = 110.

| Demographic Characteristics |              | Frequency | Percentage % |
|-----------------------------|--------------|-----------|--------------|
| Age                         | 50 and Above | 2         | 1.82         |
|                             | 41 - 50      | 4         | 3.64         |
|                             | 31 - 40      | 9         | 8.18         |
|                             | 21 - 31      | 95        | 86.36        |
|                             | 20 and Below | -         | -            |
| Gender                      | Male         | 96        | 87.27        |
|                             | Female       | 14        | 12.73        |

Table 1 presents a summary of the participants' demographic characteristics, including age. The predominant age group among respondents is 21-31 years, making up 86.36% of the total sample. The 50+ age category is the smallest, accounting for 1.82%. This inclusive age distribution ensures a holistic understanding of acceptance of the Hybrid Mechanical Engineering Review Platform, encompassing perspectives from individuals at different career stages and with diverse experiences within the academic institution. Another crucial aspect of respondents' profiles that significantly influences behavioral intention is gender. Research shows that teams with diverse gender representation offer notable advantages. A study by Zhang et al. [9] found that teams comprising both males and females tend to generate more innovative, widely recognized scientific concepts. The study conducted by Pham & Lo [10] demonstrated how gender composition impacts group decision-making and subsequent performance across various situations. Additionally, a field experiment by Kiefer et al. [11] found that teams with a balanced gender composition outperformed predominantly male teams in sales and earnings. The success of a team is closely linked to its openness to diversity, particularly when the gender composition approaches numerical balance [12]. These findings support the notion that teams with a mix of men and women excel in their roles and achieve superior results. Table 1 delineates the gender breakdown of the survey participants. Out of the 110 respondents, 87.27% are male, and 12.73% are female. This distribution of gender is pivotal to fostering diverse and inclusive perspectives in the assessment of the Hybrid Mechanical Engineering Review Platform.

**Table 2.** Institutions graduated by the respondents (reviewees)

| Types of Institution | Frequency | Percentage % |
|----------------------|-----------|--------------|
| SUC                  | 40        | 40           |
| Private              | 60        | 60           |

Table 2 illustrates that 40% of the chosen reviewees from PRIME Review Center originated from State Universities and Colleges (SUC), whereas the remaining 60% were affiliated with private universities or institutions offering Mechanical Engineering. This distribution highlights the diverse educational backgrounds of the reviewees participating in the assessment of the Hybrid Mechanical Engineering Review Platform. Meanwhile, one crucial aspect for assessing the effectiveness of the hybrid mechanical engineering review platform was content design. In the academic setting, content design encompasses a set of knowledge, skills, and concepts essential to meet learners' needs by delivering information in a curriculum-aligned, easily digestible manner. Lewis [13] endorses the idea that content design should be presented in diverse formats (such as audio, video, and text), align with goals and objectives, and employ various pedagogical techniques to engage, motivate, and stimulate learners. This design involves organizing and pacing the material and visually presenting specific instances with appropriate vocabulary and language to support learners' comprehension.

**Table 3.** The level of effectiveness of the hybrid mechanical engineering review platform

| Effectiveness Criteria | Professors / Reviewers<br>(10) | Selected ME Board Passers<br>(50) | Selected ME Reviewees<br>(50) | Overall Mean and Interpretation |
|------------------------|--------------------------------|-----------------------------------|-------------------------------|---------------------------------|
| Content Design         | 4.49                           | 4.49                              | 4.49                          | 4.49 – Very Highly Effective    |
| Media Design           | 4.60                           | 4.70                              | 4.74                          | 4.68 – Very Highly Effective    |
| Visual Design          | 4.50                           | 4.64                              | 4.70                          | 4.61 – Very Highly Effective    |
| Feedback Mechanism     | 4.40                           | 4.50                              | 4.60                          | 4.50 – Very Highly Effective    |
| Assessment Items       | 4.55                           | 4.66                              | 4.72                          | 4.64 – Very Highly Effective    |
| Navigation             | 4.60                           | 4.70                              | 4.76                          | 4.69 – Very Highly Effective    |
| Grand Overall Mean     | —                              | —                                 | —                             | 4.60 – Very Highly Effective    |

Table 3 shows that the Hybrid Mechanical Engineering Review Platform is very highly effective, as rated by professors, board passers, and reviewees. The overall mean score across six areas—content design, media design, visual design, feedback mechanism, assessment items, and navigation—is 4.60, which falls under the “Very Highly Effective” category. This means that most respondents strongly agreed that the platform meets their expectations for design, functionality, and learning support. The content design of the platform received a mean score of 4.49, indicating that the modules are well-organized, relevant to learners’ needs, and presented in a way that is easy to understand. Respondents found the content engaging, well sequenced, and supported by visuals and interactive elements that facilitate learning key concepts. This suggests that the platform provides information in a structured and motivating manner [14]. In terms of media design, the platform scored 4.68, the highest among all categories. This reflects the effective use of multimedia such as videos, graphics, and interactive content, which enhance understanding and retention. Using different media formats allows learners to process information in ways that suit their preferences. The visual design scored 4.61, showing that the platform has a user-friendly layout, with readable text, pleasing visuals, and easy-to-follow instructions. This makes the platform visually appealing and accessible, which helps users focus on learning without distractions. The feedback mechanism earned a score of 4.50, indicating that users are satisfied with how the platform provides comments, ratings, and suggestions after completing quizzes or modules. This timely feedback helps learners to correct their mistakes and improve their performance.

For assessment items, the platform received a 4.64 rating. This means that the practice tests, quizzes, and evaluation tools are clear, relevant, and aligned with the goals of the licensure exam. Learners believe that these assessment tools help them measure their readiness and improve their skills. Lastly, navigation was rated 4.69, reflecting that the platform is easy to use. Users can move smoothly from one section to another, use menus effectively, and track their progress. This ease of navigation reduces confusion and makes the learning experience more efficient. In conclusion, the high ratings across all categories confirm that the Hybrid Mechanical Engineering Review Platform is well-designed, engaging, and effective in helping students prepare for the board exam. Its user-friendly interface, strong content, and interactive features contribute to its success as a tool for technology-based learning and innovation in review programs.



**Table 4.** Level of effectiveness of the hybrid mechanical engineering review platform as to content design

| Indicators                                                                                                                                                                      | Professors /<br>Reviewers<br>(10) |       | Selected ME<br>Board Passers<br>(50) |       | Selected ME<br>Reviewees (50) |       | Overall Mean<br>and<br>Interpretation |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-------|--------------------------------------|-------|-------------------------------|-------|---------------------------------------|-----|
|                                                                                                                                                                                 | $\bar{x}$                         | SD    | $\bar{x}$                            | SD    | $\bar{x}$                     | SD    |                                       |     |
| The degree of content supplied is appropriate and pertinent to the intended audience, making the content design effective.                                                      | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                          | 0.443 | 4.68                                  | VHE |
| The information is "scaffolded" effectively to help learners complete the module, making the content design effective.                                                          | 4.60                              | 0.516 | 4.50                                 | 0.678 | 4.74                          | 0.443 | 4.61                                  | VHE |
| The information is interesting, engaging, inspiring, and pertinent to the learners who are its intended audience, making the content design effective.                          | 4.60                              | 0.516 | 4.50                                 | 0.678 | 4.70                          | 0.463 | 4.60                                  | VHE |
| The informative and learning-promoting nature of the information makes the content design effective.                                                                            | 4.10                              | 0.316 | 4.16                                 | 0.584 | 4.50                          | 0.678 | 4.25                                  | VHE |
| The module's use of various interactive teaching techniques, including scenarios, videos, problem-based learning, exploration, and testing, makes the content design effective. | 4.70                              | 0.483 | 4.50                                 | 0.678 | 4.70                          | 0.463 | 4.63                                  | VHE |
| The vocabulary and terminology used in the module are understandable and backed by references or glossaries, which makes the content design effective.                          | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.50                          | 0.678 | 4.60                                  | VHE |
| Including visuals in the content, such as graphs, photos, and illustrations, makes the content design effective.                                                                | 4.10                              | 0.316 | 4.16                                 | 0.584 | 4.70                          | 0.463 | 4.32                                  | VHE |
| The module summarizes the information displayed at the start, making the content design more efficient.                                                                         | 4.10                              | 0.316 | 4.10                                 | 0.678 | 4.50                          | 0.678 | 4.23                                  | VHE |
| The module summarizes its information at the end, making the content design more efficient.                                                                                     | 4.60                              | 0.516 | 4.50                                 | 0.678 | 4.50                          | 0.678 | 4.53                                  | VHE |
| <i>Grand Overall Mean Interpretation</i>                                                                                                                                        |                                   |       |                                      |       | 4.49<br>Very Highly Effective |       |                                       |     |

Table 4 shows that, on average, respondents strongly agreed with the high effectiveness of the Hybrid Mechanical Engineering Review Platform in content design, with an overall mean of 4.49. The standard deviation, within 68% of the normal distribution, indicates that all respondents perceived the platform's content design as closely aligned. This suggests that the module successfully incorporates relevant, suitable content for the intended audience, with information scaffolded effectively to guide learners through the module sections. Furthermore, the data indicates that the module's content is not only informative but also conducive to learning, with review techniques such as scenarios, videos, problem-based learning, exploration, and testing, while incorporating visuals and employing vocabulary and terminology that are comprehensible and supported by references or glossaries. Additionally, the module adeptly summarizes the information presented at the beginning and end. Consequently, the module is deemed effective in terms of content design. The findings align with Lewis's [13] research study, in which respondents rated their module very positively, describing it as excellent in terms of content design. These results also substantiate the UX Design Institute's assertion, emphasizing the influential role of content design in user experience and user interface design [15]. Content holds the potential to enhance user experience, elevate conversion rates, and increase brand exposure, with its presence spanning various elements of a user interface.

Moreover, content design establishes the assessment criteria from a pedagogical standpoint. The criteria in this content underscore the importance of instructional and learning strategies. Instead of relying on dependable technologies, content design delivers interactivity. Every aspect within the content, ranging from the selection of action verbs to the theoretical framework employed, is intricately connected to the content theme [16]. By employing a combination of hardware and software technologies, the platform enhances and facilitates engagement with the content across various media. The Media Richness Theory serves as a useful guide to cultivate interaction and communication, thereby improving learning outcomes through interactive e-books [17].

**Table 5.** Level of effectiveness of the hybrid mechanical engineering review platform as to media design

| Indicators                                                                                                                                                                                     | Professors / Reviewers (10) |       | Selected ME Board Passers (50) |       | Selected ME Reviewees (50) |       | Overall Mean and Interpretation |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|-------|--------------------------------|-------|----------------------------|-------|---------------------------------|-----|
|                                                                                                                                                                                                | $\bar{x}$                   | SD    | $\bar{x}$                      | SD    | $\bar{x}$                  | SD    |                                 |     |
| The module provides narrated presentations during the content delivery, which makes the media design effective (if applicable).                                                                | 4.80                        | 0.422 | 4.70                           | 0.463 | 4.88                       | 0.328 | 4.79                            | VHE |
| The narrator introduces the speaker and briefly describes the module, including the subsections, making the media design effective.                                                            | 4.60                        | 0.516 | 4.70                           | 0.463 | 4.74                       | 0.443 | 4.68                            | VHE |
| The clear, straightforward instructions and suggestions the narrator provides make the media design effective.                                                                                 | 4.70                        | 0.483 | 4.70                           | 0.463 | 4.70                       | 0.463 | 4.70                            | VHE |
| The module's audio, written text, and visual elements are perfectly timed, making the media design effective.                                                                                  | 4.60                        | 0.516 | 4.50                           | 0.678 | 4.70                       | 0.463 | 4.60                            | VHE |
| The audio of the narrators and/or the videos can be entirely controlled by the student, which makes the media design effective (e.g., pause, rewind to a specific segment, or stop and start). | 4.10                        | 0.316 | 4.50                           | 0.678 | 4.16                       | 0.584 | 4.25                            | VHE |

**Table 5.** Level of effectiveness of the hybrid mechanical engineering review platform as to media design (continue)

| Indicators                                                                                                                                            | Professors / Reviewers (10) |       | Selected ME Board Passers (50) |       | Selected ME Reviewees (50) |       | Overall Mean and Interpretation |                       |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|-------|--------------------------------|-------|----------------------------|-------|---------------------------------|-----------------------|
|                                                                                                                                                       | $\bar{x}$                   | SD    | $\bar{x}$                      | SD    | $\bar{x}$                  | SD    |                                 |                       |
| The audiovisual content/videos function correctly on various web browsers, a testament to the effectiveness of the media design.                      | 4.70                        | 0.483 | 4.70                           | 0.463 | 4.70                       | 0.463 | 4.70                            | VHE                   |
| The media design is effective because it incorporates all components into a multimodal presentation that clearly explains the module's central ideas. | 4.60                        | 0.516 | 4.50                           | 0.678 | 4.50                       | 0.678 | 4.50                            | VHE                   |
| <i>Grand Overall Mean Interpretation</i>                                                                                                              |                             |       |                                |       |                            |       | 4.60                            | Very Highly Effective |

Table 5 indicates that, on average, respondents strongly affirmed the effectiveness of the Hybrid Mechanical Engineering Review Platform in media design, with an overall mean of 4.60. The narrow standard deviation within 68% of the mean suggests consensus among respondents. This outcome implies that the module delivers narrated presentations effectively during content delivery, as the narrator introduces the speaker and provides a concise overview of the module, including subsections, with clear, straightforward instructions or suggestions. Additionally, it indicates that the timing of the module's written text and visual elements is synchronized with the narrators' audio and/or videos, allowing students full control and proper functionality across various web browsers. Lastly, the module integrates all components into a multimodal presentation that effectively elucidates its central ideas. Consequently, the module is deemed effective in terms of media design.

According to Su et al. [18], to enhance and facilitate interaction with content, interactive e-books incorporate diverse media elements. The Media Richness Theory serves as a valuable guide for designing interaction and communication within interactive e-books. Moreover, media design involves crafting content for dissemination across multiple platforms and in various formats. A fitting analogy is found in magazine design, as newspaper layout often adheres to strict guidelines and time constraints [19]. The graphic design implemented by the e-book creator ensures a consistent color coding system for the module title, text body, and assessment activities. This approach enhances the readability of the module text by maintaining uniformity in font size, color, and formatting. Additionally, each page in the module is organized with an attractive aesthetic balance between audiovisual and text content. Lastly, it ensures a clear representation of screen names, symbols, or icons for easy identification. Visual design employs appropriate images, typography, spacing, layout, and color to enhance the attractiveness and functionality of a design or product. Beyond mere aesthetics, visual design serves additional objectives. Designers strategically position elements to craft interfaces that optimize user experience and promote conversion, as highlighted by Bhatti et al. [20].

**Table 6.** Level of effectiveness of the hybrid mechanical engineering review platform as to visual design

| Indicators                                                                                                                                                | Professors /<br>Reviewers<br>(10) |       | Selected ME<br>Board Passers<br>(50) |       | Selected ME<br>Reviewees<br>(50) |       | Overall Mean<br>and<br>Interpretation |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-------|--------------------------------------|-------|----------------------------------|-------|---------------------------------------|-----|
|                                                                                                                                                           | $\bar{x}$                         | SD    | $\bar{x}$                            | SD    | $\bar{x}$                        | SD    |                                       |     |
| Every page in the module uses consistent color coding for the title, body text, and evaluation activities, which makes the visual design effective.       | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.70                             | 0.443 | 4.67                                  | VHE |
| The module's constant use of font size, color, and easily readable text format makes the visual design effective.                                         | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                             | 0.443 | 4.68                                  | VHE |
| The module's use of captions and/or bold, italic, and underscored text to emphasize hierarchy or critical information makes the visual design effective.  | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.70                             | 0.463 | 4.70                                  | VHE |
| The visual design is effective because each page in the module features a visually pleasing organization (not crowded and text-heavy).                    | 4.70                              | 0.483 | 4.50                                 | 0.678 | 4.74                             | 0.443 | 4.65                                  | VHE |
| Text and audiovisual components are balanced with the right amount of whitespace, which makes the visual design effective (e.g., aesthetically pleasing). | 4.60                              | 0.516 | 4.50                                 | 0.678 | 4.50                             | 0.678 | 4.53                                  | VHE |
| The visual design is effective because of the appealing, simple-to-follow placement of the audiovisual assets on the screen.                              | 4.60                              | 0.516 | 4.50                                 | 0.678 | 4.50                             | 0.678 | 4.53                                  | VHE |
| The screen names, symbols, or icons accurately convey the associated message, which makes the visual design effective.                                    | 4.70                              | 0.483 | 4.50                                 | 0.678 | 4.74                             | 0.443 | 4.65                                  | VHE |
| The absence of grammar, capitalization, punctuation, and spelling mistakes in the module makes the visual design effective.                               | 4.60                              | 0.516 | 4.50                                 | 0.678 | 4.50                             | 0.678 | 4.53                                  | VHE |
| The uniform language throughout the module contributes to the visual design's effectiveness.                                                              | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.32                             | 0.621 | 4.57                                  | VHE |
| <i>Grand Overall Mean</i>                                                                                                                                 |                                   |       |                                      |       |                                  | 4.61  | Very Highly Effective                 |     |
| <i>Interpretation</i>                                                                                                                                     |                                   |       |                                      |       |                                  |       |                                       |     |

Table 6 indicates that the Hybrid Mechanical Engineering Review Platform was successful in visual design, yielding a grand overall mean of 4.61. The standard deviation within 68% of the normal distribution suggests that all respondents evaluated the hybrid mechanical engineering review platform similarly, indicating close alignment in their evaluations. This implies that the platform effectively maintains consistent color coding for the title, text body, and assessment activities, employing a uniform font size, color, and easily readable text format. Additionally, it indicates the use of captions and/or bold, italic, and underscored text to emphasize hierarchy or critical information, ensuring a visually pleasing organization. Furthermore, the results suggest that the text and audiovisual components are well-balanced, with an appropriate amount of whitespace, and that audiovisual assets are positioned on the screen in an appealing and easy-to-follow manner to effectively convey the intended message through screen names, symbols, or icons. Lastly, the module employs uniform language throughout, free from grammar, capitalization, punctuation, and spelling errors. In summary, the module is deemed effective in terms of visual design. The outcomes align with Lewis's [13] findings, which showed that participants consistently rated their interactive module as highly satisfactory and excellent. Similarly, the emphasis on visual design centers around enhancing the aesthetics of a module and its associated materials by thoughtfully incorporating elements such as images, graphics, colors, fonts, spacing, as well as features like screen titles, symbols or icons, audio narration, and videos to enhance the learner's experience throughout the learning process [13]. The feedback mechanism is a platform that informs students, upon completion, whether their responses to tasks within a module are correct or incorrect. Utilizing questions, visual cues, or textual prompts, its purpose is to guide students in comprehending the subject matter, as outlined by Lewis [13].

**Table 7.** Level of effectiveness of the hybrid mechanical engineering review platform as a feedback mechanism

| Indicators                                                                                                                                                       | Professors /<br>Reviewers<br>(10) |       | Selected ME<br>Board Passers<br>(50) |       | Selected ME<br>Reviewees (50) |       | Overall Mean<br>and<br>Interpretation |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-------|--------------------------------------|-------|-------------------------------|-------|---------------------------------------|-----|
|                                                                                                                                                                  | $\bar{x}$                         | SD    | $\bar{x}$                            | SD    | $\bar{x}$                     | SD    |                                       |     |
| The module immediately provides feedback after completing the pretest, including correct and incorrect responses, making the feedback mechanism effective.       | 4.80                              | 0.422 | 4.70                                 | 0.463 | 4.88                          | 0.328 | 4.79                                  | VHE |
| The effectiveness of the assessment item stems from the fact that the assessment questions immediately explain why the chosen response is accurate or incorrect. | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                          | 0.443 | 4.68                                  | VHE |
| The module challenges learners to find the correct answer by providing guided feedback, making the evaluation item more effective.                               | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.70                          | 0.463 | 4.70                                  | VHE |
| The effectiveness of the assessment item is attributed to the module's use of visual cues that indicate whether a learner's response is correct or incorrect.    | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.32                          | 0.621 | 4.57                                  | VHE |



**Table 7.** Level of effectiveness of the hybrid mechanical engineering review platform as a feedback mechanism (continue)

| Indicators                                                                                                                                                   | Professors / Reviewers (10) |       | Selected ME Board Passers (50) |       | Selected ME Reviewees (50) |       | Overall Mean and Interpretation |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|-------|--------------------------------|-------|----------------------------|-------|---------------------------------|-----|
|                                                                                                                                                              | $\bar{x}$                   | SD    | $\bar{x}$                      | SD    | $\bar{x}$                  | SD    |                                 |     |
| The feedback technique works well because the module provides distinct explanations for each incorrect response.                                             | 4.70                        | 0.483 | 4.50                           | 0.678 | 4.74                       | 0.443 | 4.65                            | VHE |
| The learning modules direct students to outside sources as feedback to further their understanding, making the feedback system effective.                    | 4.70                        | 0.483 | 4.70                           | 0.463 | 4.70                       | 0.463 | 4.70                            | VHE |
| Overall, the feedback system works well because it is interactive, efficient, and uses questions to direct learners toward understanding the subject matter. | 4.60                        | 0.516 | 4.50                           | 0.678 | 4.50                       | 0.678 | 4.53                            | VHE |
| <i>Grand Overall Mean</i>                                                                                                                                    |                             |       |                                |       |                            |       | 4.66                            |     |
| <i>Interpretation</i>                                                                                                                                        |                             |       |                                |       |                            |       | Very Highly Effective           |     |

Table 7 indicates a strong agreement among respondents regarding the effectiveness of the Hybrid Mechanical Engineering Review Platform as a feedback mechanism. With a grand overall mean of 4.66 and a standard deviation within 68% of the normal distribution, the evaluation of the hybrid mechanical engineering review platform and the feedback mechanism closely aligns among respondents. This suggests that the platform provides effective feedback after pretest completion, elucidating both correct and incorrect responses with immediate explanations of the accuracy or inaccuracy of chosen answers. Consequently, the platform proves effective in assessing items. Moreover, it implies that the platform challenges learners to identify correct answers by offering guided feedback through visual cues that highlight or indicate whether a learner's response to an assessment item is accurate or inaccurate, accompanied by distinctive justifications for each incorrect answer. These results affirm Lewis's [13] findings that the interactive module's evaluation score was very good to excellent, particularly regarding the feedback mechanism. Furthermore, the results acknowledge the study by Gaertner [21], which, in addition to the tools mentioned above, also recommends that several professors incorporate self-evaluation to gather feedback on students' learning experiences at the conclusion of the course [22].

**Assessment Items.** One approach to evaluating the effectiveness of learning involves using assessments. Various tools, such as questionnaires, exams, tests, and other tasks, are used to assess the proficiency of key content components within a module. The objective is to determine whether learners have successfully achieved the predetermined learning goals [23]. The assessment process aims to evaluate critical content aspects integrated into the module, using evenly distributed questions and a format that aligns with the modules' interactive nature, enabling measurable progress.

**Table 8.** Level of effectiveness of the hybrid mechanical engineering review platform as to assessment items

| Indicators                                                                                                                                                                                            | Professors /<br>Reviewers<br>(10) |       | Selected ME<br>Board Passers<br>(50) |       | Selected ME<br>Reviewees (50) |       | Overall Mean<br>and<br>Interpretation |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-------|--------------------------------------|-------|-------------------------------|-------|---------------------------------------|-----|
|                                                                                                                                                                                                       | $\bar{x}$                         | SD    | $\bar{x}$                            | SD    | $\bar{x}$                     | SD    |                                       |     |
| The pretest and posttest sections of the module make the assessment item valuable.                                                                                                                    | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.70                          | 0.463 | 4.70                                  | VHE |
| The module's pretest, posttest, and other assessment items align with its aims and content, making them effective.                                                                                    | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                          | 0.443 | 4.68                                  | VHE |
| Assessment items allow for repeated or multiple attempts when necessary, which makes them successful.                                                                                                 | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.70                          | 0.463 | 4.70                                  | VHE |
| Knowledge assessment questions that include interactive visuals, photos, or videos relevant to the module's central idea make the assessment item effective.                                          | 4.60                              | 0.516 | 4.50                                 | 0.678 | 4.50                          | 0.678 | 4.53                                  | VHE |
| The use of many question types, including drag-and-drop, matching, true-false, multiple-choice, multiple-response, fill-in-the-blank, and quick-check questions, makes the assessment item effective. | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.32                          | 0.621 | 4.57                                  | VHE |
| The assessment is effective because it measures a variety of learners' thinking and comprehension at an appropriate level of rigor and/or shows a progression of cognitive complexity.                | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                          | 0.443 | 4.68                                  | VHE |
| The assessment is effective because it aims to gauge key module-integrated content.                                                                                                                   | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.32                          | 0.621 | 4.57                                  | VHE |
| The assessment item is effective because the module's questions are evenly distributed (not just at the end).                                                                                         | 4.70                              | 0.483 | 4.50                                 | 0.678 | 4.74                          | 0.443 | 4.65                                  | VHE |
| The assessment format complements the interactive modules' nature with verifiable progress, making the assessment item effective.                                                                     | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                          | 0.443 | 4.68                                  | VHE |
| <i>Grand Overall Mean</i>                                                                                                                                                                             |                                   |       |                                      |       |                               |       | 4.64                                  |     |
| <i>Interpretation</i>                                                                                                                                                                                 |                                   |       |                                      |       |                               |       | Very Highly Effective                 |     |

Table 8 reveals a strong consensus among respondents regarding the effectiveness of the Hybrid Mechanical Engineering Review Platform in assessment items. With a grand overall mean of 4.64 and a standard deviation within 68% of the normal distribution, it is evident that all respondents were closely aligned in their ratings of the platform for hybrid mechanical engineering review across assessment items. This indicates that the module effectively aligns the pretest, posttest, and other assessment items with its objectives and content, making the assessment valuable while allowing repeated attempts. It also suggests that the platform incorporates numerous knowledge assessment questions featuring interactive visuals, photos, or videos relevant to the module's central idea, effectively measuring diverse learners' thinking and comprehension at an appropriate level of rigor. Hence, the module proves to be practical for assessment items.

**Table 9.** Summary on the effectiveness of the hybrid mechanical engineering review platform

| Criteria                     | Mean Score  | Interpretation               |
|------------------------------|-------------|------------------------------|
| Content Design               | 4.49        | Very Highly Effective        |
| Media Design                 | 4.60        | Very Highly Effective        |
| Visual Design                | 4.61        | Very Highly Effective        |
| Feedback Mechanism           | 4.66        | Very Highly Effective        |
| Assessment Items             | 4.64        | Very Highly Effective        |
| Navigation                   | 4.64        | Very Highly Effective        |
| <i>Overall Weighted Mean</i> | <i>4.61</i> | <i>Very Highly Effective</i> |

Table 9 indicates strong consensus among respondents, demonstrating their robust agreement on the platform's high effectiveness. Across all specified dimensions, the platform demonstrates noteworthy effectiveness, yielding an overall mean of 4.61 and a standard deviation within 68% of the normal distribution, signifying close alignment among respondent ratings. Further analysis reveals that the content design received a mean score of 4.49, followed by media design at 4.60, visual design at 4.61, the feedback mechanism at 4.66, and both assessment items and navigation at 4.64. In terms of the acceptability of the hybrid mechanical engineering review platform, the reception of an innovation or technology serves as an indicator of its acceptance among the general public. This investigation specifically assesses the acceptability of the hybrid mechanical engineering review platform by examining key elements outlined in the UTAUT Model developed by Venkatesh et al. [24]. These elements include performance expectancy, effort expectancy, social influence, facilitating conditions, behavioral intention, and user behavior. Venkatesh et al. [24] identify performance expectancy as the foremost explanatory factor in the UTAUT model. This aspect concerns how individuals perceive the benefits of e-learning technology, including improvements in performance and increased productivity. Users are inclined to embrace e-learning when they are persuaded that this novel technology offers increased effectiveness and efficiency [25]. This concept, also known as perceived usefulness, reflects the user's belief in the technology's capacity to enhance performance and efficiency, as outlined by Park [25].

**Table 10.** The level of acceptability of the hybrid mechanical engineering review platform: performance expectancy

| Indicators                                                                                                             | Professors /<br>Reviewers<br>(10) |       | Selected ME<br>Board Passers<br>(50) |       | Selected ME<br>Reviewees (50) |       | Overall Mean and<br>Interpretation |     |
|------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-------|--------------------------------------|-------|-------------------------------|-------|------------------------------------|-----|
|                                                                                                                        | $\bar{x}$                         | SD    | $\bar{x}$                            | SD    | $\bar{x}$                     | SD    |                                    |     |
| I would find the Hybrid Mechanical Engineering Review Platform useful in my course/review.                             | 4.70                              | 0.483 | 4.70                                 | 0.463 | 4.70                          | 0.463 | 4.70                               | VHA |
| Using the Hybrid Mechanical Engineering Review Platform enabled me to comprehend and accomplish the task more quickly. | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                          | 0.443 | 4.68                               | VHA |
| Using the Hybrid Mechanical Engineering Review Platform increases my productivity.                                     | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                          | 0.443 | 4.68                               | VHA |
| If I use the Hybrid Mechanical Engineering Review Platform, will it increase my chances of passing the licensure exam? | 4.60                              | 0.516 | 4.70                                 | 0.463 | 4.74                          | 0.443 | 4.68                               | VHA |
| <i>Grand Overall Mean</i>                                                                                              |                                   |       |                                      |       |                               |       | 4.67                               |     |
| <i>Interpretation</i>                                                                                                  |                                   |       |                                      |       |                               |       | Very Highly Acceptable             |     |

Legend: 4.21–5.00 – Very Highly Acceptable; 3.41–4.20 – Highly Acceptable; 2.61–3.40 – Acceptable; 1.81–2.60 – Less Acceptable; 1.00–1.80 – Not Acceptable

Table 10 shows that, on average, the respondents strongly agreed that the Hybrid Mechanical Engineering Review Platform was Very Highly Acceptable as to performance expectancy. The grand overall mean is 4.67, and the standard deviation within 68% of the normal distribution indicates that respondents evaluated the Hybrid Mechanical Engineering Review Platform as similar to one another. This means that the platform is highly acceptable, as users can find it useful in their review classes, comprehend and complete tasks more quickly, increase their productivity, and improve their chances of passing the licensure examination at a high rate. The results align with Stoyanova & Markova's [26] findings, indicating that performance expectancy is the key factor influencing students' inclination to use electronic books. This assertion holds across all models examined. For students with no prior experience, it is evident that performance expectancy is the sole significant variable influencing their decision to choose digital textbooks. This suggests that individuals without prior familiarity tend to prefer printed books over e-books, finding traditional methods more efficient in achieving their learning objectives. To encourage greater usage of e-books among this group, designers must create user-friendly designs and provide information that boosts performance expectations [27]. This demonstrates that students will embrace e-learning only when they perceive its relevance to their learning requirements. Performance expectancy, as defined by Mitchell and Albright [28], is "the extent to which the user expects that using the system would assist him/her in attaining gains in job performance" and plays a crucial role in shaping their willingness to adopt this mode of learning. The concept of effort expectancy, as defined by Venkatesh et al. [24], pertains to the comfort level associated with utilizing a system and is rooted in the ease of use. This concept, also known as perceived ease of use, represents the degree to which a user feels at ease with the technology's features, as articulated by Park [25].

**Table 11.** The level of acceptability of the hybrid mechanical engineering review platform: effort expectancy

| Indicators                                                                                               | Professors / Reviewers (10) |       | Selected ME Board Passers (50) |       | Selected ME Reviewees (50) |       | Overall Mean and Interpretation |     |
|----------------------------------------------------------------------------------------------------------|-----------------------------|-------|--------------------------------|-------|----------------------------|-------|---------------------------------|-----|
|                                                                                                          | $\bar{x}$                   | SD    | $\bar{x}$                      | SD    | $\bar{x}$                  | SD    |                                 |     |
| My interaction with the Hybrid Mechanical Engineering Review Platform is clear and understandable.       | 4.60                        | 0.516 | 4.70                           | 0.463 | 4.74                       | 0.443 | 4.68                            | VHA |
| It would be easy for me to become skillful using the Hybrid Mechanical Engineering Review Platform.      | 4.70                        | 0.483 | 4.70                           | 0.463 | 4.32                       | 0.621 | 4.57                            | VHA |
| I would find the Hybrid Mechanical Engineering Review Platform easy to use and navigate.                 | 4.60                        | 0.516 | 4.70                           | 0.463 | 4.74                       | 0.443 | 4.68                            | VHA |
| Using the Hybrid Mechanical Engineering Review Platform would make performance activities easier for me. | 4.60                        | 0.516 | 4.50                           | 0.678 | 4.50                       | 0.678 | 4.53                            | VHA |
| <i>Grand Overall Mean</i>                                                                                |                             |       |                                |       |                            |       | 4.62                            |     |
| <i>Interpretation</i>                                                                                    |                             |       |                                |       |                            |       | Very Highly Acceptable          |     |

Table 11 indicates a robust consensus among respondents, with strong agreement on the very high acceptability of the Hybrid Mechanical Engineering Review Platform in terms of effort expectancy. The platform demonstrates a notable overall mean of 4.62 and a standard deviation within 68% of the normal distribution, indicating that all respondents assessed the platform in a closely aligned manner. This suggests a high level of acceptability, as users were able to comprehend the module clearly, develop proficiency in using the platform, navigate it with ease, and efficiently perform activities on the platform. This demonstrates that the acceptance of e-learning systems is markedly influenced by effort expectancy. The ease of use, accessibility, and user-friendliness of e-learning systems contribute to their attractiveness to end users, thereby influencing their perception of them as beneficial. An important predictor of the intention to use e-learning technology is the expectation of effort. Research has highlighted that effort expectancy plays a significant role in shaping the utilization of e-learning systems [29].

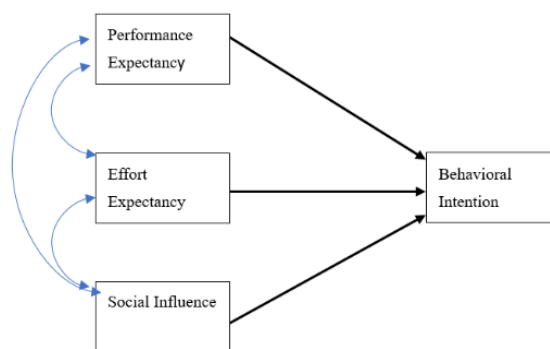
**Table 12.** Summary on the acceptability of the hybrid mechanical engineering review platform

| Criteria                     | Mean Score | Interpretation               |
|------------------------------|------------|------------------------------|
| Performance Expectancy       | 4.67       | Very Highly Acceptable       |
| Effort Expectancy            | 4.62       | Very Highly Acceptable       |
| Social Influence             | 4.67       | Very Highly Acceptable       |
| Behavioral Intention         | 4.67       | Very Highly Acceptable       |
| <i>Overall Weighted Mean</i> | 4.66       | <i>Very Highly Effective</i> |

Table 12 indicates strong consensus among respondents regarding the Hybrid Mechanical Engineering Review Platform's high acceptability, as reflected in the summary and overall averages. This indicates a high level of approval, as users were able to incorporate the platform into their class activities



effectively. Specifically, the performance expectancy, effort expectancy, and social influence items scored notably high at 4.67, 4.62, 4.67, and 4.67, respectively. Drawing on the aforementioned results, it is evident that the primary determinant of digital textbook adoption is performance expectancy, as indicated by Wan Sulaiman and Mustafa [30]. Additionally, the significance of performance expectancy extends to tertiary students' intentions to use e-books, as emphasized by Bulur and Gönül [31]. Furthermore, users' acceptance of e-books is shaped by various factors, including effort expectancy, social influence, facilitating conditions, environmental concern, perceived benefit, and benevolence. Consequently, this research underscores the implications of these findings for initiatives aimed at fostering enthusiastic adoption of e-books among university students. It was found that the UTAUT construct has a significant relationship with acceptability. The study found that the effects of performance expectancy and effort expectancy on behavioral intention to use the hybrid mechanical engineering review platform were not statistically significant. Conversely, social influence significantly influenced behavioral intention to use the hybrid mechanical engineering review platform. These results are succinctly depicted in the following diagram.



**Figure 1.** Relationship and Hypothesized Model

**Table 13.** Relationship and hypothesis testing result of the UTAUT construct

Anova: Single Factor

SUMMARY

| Groups   | Count | Sum   | Average | Variance    |
|----------|-------|-------|---------|-------------|
| Column 1 | 4     | 18.74 | 4.685   | 0.0001      |
| Column 2 | 4     | 18.46 | 4.615   | 0.0059      |
| Column 3 | 4     | 18.74 | 4.685   | 0.008166667 |
| Column 4 | 4     | 18.74 | 4.685   | 0.008166667 |

ANOVA

| Source of Variation | SS     | df | MS     | F      | P-value | F crit |
|---------------------|--------|----|--------|--------|---------|--------|
| Between Groups      | 0.0147 | 3  | 0.0049 | 0.8776 | 0.47990 | 3.4903 |
| Within Groups       | 0.067  | 12 | 0.0055 |        |         |        |
| Total               | 0.0817 | 15 |        |        |         |        |

Legend: Column 1 – Performance Expectancy  
 Column 2 – Effort Expectancy  
 Column 3 – Social Influence  
 Column 4 – Behavioral Intention

Table 13 shows that there is no noteworthy difference in the acceptability levels of performance expectancy (column 1), effort expectancy (column 2), social influence (column 3), and behavioral intention

(column 4) across the respondent groups. The P-value of 0.08776 indicates a p-value above 0.05, suggesting a lack of statistical significance.

H01: There is no significant influence of performance expectancy on behavioral intention to use the Hybrid Mechanical Engineering Review Platform.

In the context of this research, Performance Expectancy (PE) is defined as the user's belief that a particular technology will enhance their performance and lead to the realization of professional benefits, as defined by Izuagbe [32]. Within the confines of this study, PE specifically refers to the expectations held by both reviewers and reviewees, including the anticipation that the platform will streamline the execution of their tasks, thereby enhancing efficiency and effectiveness.

Ho2: There is no significant influence of effort expectancy on behavioral intention to use the hybrid mechanical engineering review platform.

The assessment of end-users' inclination to adopt the platform, with a focus on perceived benefits such as the user-friendly nature of e-learning programs, serves as a measure of effort expectancy. Effort expectancy is defined by Kolli and Achuthan [33] as end users' perceptions of the potential advantages of using technology. According to Ogemdi Uchenna and Uzoma Oluchukwu [34], effort expectancy significantly influences the behavioral intention to employ e-learning tools. Additionally, the study by Jameel et al. [35] demonstrates that effort expectancy plays a substantial role in shaping behavioral intention to use e-learning technology. On the contrary, the findings of this investigation reveal that effort expectancy is not statistically significant, indicating that this construct fails to predict behavioral intention. Consequently, in this study, effort expectancy exerts no significant influence on behavioral intention to use the hybrid mechanical engineering review platform. The research aimed to evaluate the behavioral inclination of reviewees towards adopting a hybrid mechanical engineering review platform. Specifically, it aimed to assess the platform's adherence to ISO 25010 standards for quality assurance. The effectiveness of the hybrid mechanical engineering review platform was examined through respondents' perceptions of content design, media design, visual design, feedback mechanisms, assessment items, and navigation. The study also investigated the platform's evaluation by reviewees and professors/reviewers based on the UTAUT model constructs, including performance expectancy, effort expectancy, social influence, and behavioral intention. Furthermore, the research explored the connection between the effectiveness and acceptability of the hybrid mechanical engineering review platform.

The hybrid mechanical engineering review platform received consistently high ratings for effectiveness from reviewees, reviewers, and newly qualified board passers. There was strong agreement among them regarding the platform's efficacy across various dimensions, including content design, assessment items, feedback mechanisms, media design, visual design, and navigation. Moreover, in terms of effectiveness, all five interactive modules garnered positive ratings from respondents, reflecting a high level of full e-learning interactivity or full simulation. Regarding the acceptability of the hybrid mechanical engineering review platform concerning the UTAUT Model constructs—namely, performance expectancy, effort expectancy, social influence, and behavioral intention—reviewees and reviewers strongly concurred that it is highly acceptable. Furthermore, upon examination of the hypothesized model and the relationships between constructs, it was observed that performance expectancy and effort expectancy did not exert a significant influence on the behavioral intention to use the hybrid mechanical engineering review platform. In contrast, social influence was found to significantly affect behavioral intention to use the hybrid mechanical engineering review platform. The relationship between the effectiveness and acceptability of the hybrid mechanical engineering review platform showed a strong positive correlation, as indicated by the high correlation coefficients. This indicates a strong, positive, and significant relationship between the effectiveness and acceptability of the hybrid mechanical engineering review platform. The correlation analysis revealed a robust positive relationship between the effectiveness and acceptability of the hybrid mechanical engineering review platform, as indicated by the elevated correlation coefficients. This indicates a compelling, statistically significant association between the platform's effectiveness and its acceptability [36].

While the findings indicate that performance expectancy and effort expectancy did not significantly influence behavioral intention, this outcome invites closer reflection rather than being treated as a mere

statistical result. One possible explanation is that the respondents, particularly reviewees and board passers, may already possess a baseline familiarity with digital learning platforms. In such cases, the perceived usefulness and ease of use are no longer decisive factors, as these are already expected features of modern review systems. This suggests that when a technology becomes normalized, its functional benefits may no longer serve as strong motivators for adoption. Another perspective relates to the context in which the hybrid platform was implemented. Since the platform emerged during a period when online and blended learning became necessary rather than optional, users may have adopted it out of circumstance rather than personal evaluation of its efficiency or usability. This condition can reduce the predictive power of performance and effort expectancy, as the decision to use the platform is influenced less by individual preference and more by situational demand. Moreover, the composition of the respondents may have contributed to the observed results. The majority of participants belong to a younger age group, which is generally more adaptable to technology. For these users, navigating digital systems may not pose a significant challenge, thereby minimizing the impact of effort expectancy. Similarly, their familiarity with online tools may lead them to perceive most platforms as equally useful, thereby weakening the influence of performance expectancy on behavioral intention.

In contrast, the significant influence of social factors highlights the importance of external factors in shaping user behavior. This suggests that recommendations from peers, instructors, and review center administrators may carry more weight than individual perceptions of the system itself. In a review center environment, where collaboration and shared experiences are common, social dynamics can strongly affect decision-making. This finding underscores the idea that technology adoption, particularly in educational settings, is not purely an individual process but is often shaped by collective attitudes and institutional practices. Taken together, these insights suggest that the adoption of the hybrid mechanical engineering review platform is influenced more by contextual and social conditions than by traditional usability factors. This provides a deeper understanding of user behavior and highlights the need to consider environmental and demographic factors when applying the UTAUT model in similar settings. Beyond general measures of effectiveness and acceptability, the findings of this study offer meaningful implications for mechanical engineering education, particularly for discipline-specific learning outcomes. The consistently high ratings across content design, assessment items, and feedback mechanisms suggest that the hybrid review platform is not only technically functional but also aligned with the cognitive and analytical demands of mechanical engineering. These include problem-solving, application of theoretical principles, and the ability to interpret technical data—skills that are essential for success in licensure examinations and professional practice.

The platform's strong performance in areas such as assessment and feedback indicates that it supports iterative learning, which is critical in engineering education, where mastery is often achieved through repeated exposure to complex problems. The availability of immediate feedback allows learners to identify gaps in their understanding of core topics such as thermodynamics, fluid mechanics, and machine design, and to make timely adjustments in their study approach. This type of guided reinforcement is particularly valuable in preparing examinees for board-level questions that require both accuracy and conceptual depth. Moreover, the integration of multimedia elements and structured navigation contributes to the development of visualization skills, which are central to mechanical engineering. The ability to interpret diagrams, simulations, and graphical data is enhanced through well-designed digital content, thereby supporting spatial reasoning and technical comprehension. In this regard, the platform moves beyond traditional review methods by offering a more interactive and applied learning environment. The findings also suggest that the platform encourages self-directed learning, an important outcome in engineering education where continuous professional development is expected. By providing flexible access to materials and allowing learners to progress at their own pace, the system fosters independent study habits while still maintaining alignment with standardized competencies required for licensure. To sum up, these results demonstrate that the hybrid mechanical engineering review platform contributes not only to general user satisfaction but also to the development of key disciplinary competencies. This reinforces its value as a tool that supports both academic preparation and the broader goal of producing competent and industry-ready mechanical engineers.

## 5. Conclusions

In summary, the hybrid mechanical engineering review platform is found to be both effective and acceptable. Its effectiveness is evident across various dimensions, including content design, assessment items, feedback mechanisms, media design, visual design, and navigation. Moreover, the platform is highly acceptable, as indicated by respondents' perceived evaluations of performance expectancy, effort expectancy, social influence, and behavioral intention. However, there is no significant difference in the effectiveness and acceptability of the hybrid mechanical engineering review platform across the respondent groups. Despite these promising results, the study has several limitations. First, the sample was limited to selected participants from the PRIME Review Center, which may not fully represent the broader mechanical engineering student and professional population. Second, the study relied primarily on self-reported perceptions, which may be subject to response bias or limited by the participants' familiarity with the platform. Third, the study focused on a specific period and location, which may affect the generalizability of the findings. Future research may explore the long-term impact of hybrid review platforms on board exam performance and actual learning outcomes. It is also recommended to expand the study across multiple review centers and academic institutions to increase sample diversity and size. Furthermore, future studies could incorporate experimental or longitudinal methods to measure improvements in skills and knowledge acquisition over time. Integrating user analytics and tracking engagement data could also provide more objective insights into the platform's usage and effectiveness.

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# Factors Influencing the Incorporation of 2AP in Fluidized Bed Drying Processes

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**Abstract:** Drying high-moisture paddy rice while retaining the quality of the grains and their natural aroma is one of the hardest things to perform in industrial processing. This study examines a hybrid drying system comprising drying units from Louisiana State University (LSU), a fluidized bed dryer, and a cyclone-based hot-air generator to reduce paddy moisture content from 25–30% to the safe storage level of 14% on a wet-basis. The cyclone furnace heated the air to 280°C using leftover charcoal. Before entering the fluidized bed dryer, the air was cooled to 101.7 °C. The grain's temperature was kept below 57.7 °C during this time, which helped preserve the rice's quality. After the rice had dried out a bit, it was put in six LSU (Louisiana State University) drying chambers. Each chamber could hold 37.5 tons of rubbish every hour and worked in groups for 10 hours. The procedure worked well to lower the moisture level in a single step, even when it reached 18%. By eliminating the middle resting stage, the drying process is easier and faster without affecting the final product's quality. The dried rice had 10.7% moisture, a grain temperature of 36.9°C, and 4.15 µg/g of 2-acetyl-1-pyrroline (2AP). This means that the rice still smelled good. These results show that using fluidized bed drying with LSU drying is a good approach to make large-scale commercial drying systems work better and be simpler while maintaining excellent rice quality.

**Keywords:** 2AP; fluidized bed; unpolished rice; rice mill; KDML 105

## 1. Introduction

Rice is an essential crop for Thailand's farming and cooking industries. People in both local and international markets really like fragrant rice because of its aroma, taste, and milling. But after harvest, paddy rice is often 20-30% wet (wet base). This is not a good way to store grain for a long time, as it can cause it to rot, mold, and lose its physical and chemical properties. It is vital for storage and industrial processing to reduce the moisture content of paddy rice to about 14% (wet basis) [1-2]. There are many ways to dry paddy rice, including hot-air drying, fluidized-bed drying, impinging-stream drying, and intermittent drying and tempering. There are good and bad things about each method. Intermittent drying has become very popular because it helps reduce the stress in grains caused by rapid temperature changes. This reduces the likelihood of grain shattering and improves milling quality [3]. On the other hand, fluidized bed drying offers the advantage of quick heat and mass transmission. Maintaining

the minimum air speed and grain layer height can speed up the drying process and improve its effectiveness at removing moisture [4]. The impinging-stream drying method with circulating hot air may also make the system much more energy efficient and reduce heat loss [5].

The smell is one of the most important parts of fragrant rice. The main volatile chemical responsible for Thai fragrant rice's characteristic smell is 2AP. This is especially true for Jasmine rice and Pathum Thani fragrant rice [6]. Champagne et al. [7] and Wongpornchai et al. [8] performed studies demonstrating that drying conditions, specifically the final temperature and humidity, had a direct and significant impact on the loss of 2AP and the aromatic quality of rice. The level of 2AP in rice can change depending on the type of rice, where it was grown, how it was harvested, how it was dried, and how it was stored [9]. If not done correctly, using high temperatures to speed up the drying process and reduce production time can harm grain quality. For instance, it can produce cracks, reduce head rice yield, and lower sensory quality, especially in heat-sensitive aromatic rice varieties [10-12]. Thus, tempering is used to reduce differences in moisture content within the grain, relieve thermal stress, and even out moisture distribution throughout the grain. This results in less fissuring and better milling quality [13-14].

Additionally, studies on rice quality reveal that processing technology profoundly affects the physical structure and commercial quality of rice, especially concerning grain characteristics, milling, and the preservation of aromatic compounds [15]. Soponronnarit [16] explained that managing energy throughout the grain drying process is very important for both system efficiency and production costs. This aligns with the modern approach to drying technology development, which focuses on product quality and energy efficiency. Mahatheeranont et al. [17] conclusively validated the quantification of 2AP concentration in Thai jasmine rice, while Wongpornchai et al. [18] established that the drying procedure and storage time significantly affected changes in aroma and milling quality of rice. Champagne [19] demonstrated that the aroma and flavor of rice are crucial for both market value and consumer acceptance, particularly for high-quality fragrant rice. Imran et al. [20] also noted that the Maillard reaction and temperature are particularly crucial for how 2AP accumulates and degrades in a simulated system. Buttery et al. [21] and Jesussek et al. [22] concurred that 2AP is the principal molecule responsible for the aroma of cooked and brown rice.

There have been improvements in the ways items are tempered and dried in between. These devices use AI models and data analysis to find the best way to dry things [23]. They also use advanced tools such as near-infrared spectroscopy, X-ray diffraction, and scanning electron microscopy to assess how well seeds perform in terms of their physical and structural properties [24]. Also, looking at how much energy continuous and intermittent drying uses has shown that improving the process can save significant energy and help the system run more efficiently as a whole [25]. A survey of the literature revealed that numerous studies have examined the impacts of drying temperature, grain resting, and various drying procedures on rice quality. However, there are constraints on extrapolating these findings to an industrial-scale hybrid drying system that integrates high-rate drying with a fluidized bed and an LSU drying system, particularly regarding the correlation among energy management, final moisture content, and 2AP retention in Thai fragrant rice. This study aims to assess the efficacy of a hybrid drying system comprising a cyclone furnace, a fluidized bed dryer, and an LSU drying system in examining the impact of drying conditions on moisture reduction, temperature regulation, and aroma preservation in rice. The goal was to find a way to dry things that would make the process easier, use less energy, and maintain product quality high enough for industrial use.

## 2. Theoretical Calculations

The Headspace Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (HS-GCxGC-TOFMS) technique was used to analyze the fragrance components in brown rice. A TissueLyser II machine was used to grind the rice into a powder to prepare the samples. To allow the fragrance components to volatilize, 1 g of the rice powder sample was weighed and placed in a tightly sealed bottle at room temperature for at least 12 hours. The Headspace Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometer (HS-GCxGC-TOFMS, LECO) was then used to examine the samples for the composition of scent compounds [15], [37-38]. The standard moisture content on a wet basis (w.b.) is the ratio of the material's total quantity to its moisture or water content. Dry mass [16],

[31-33] and moisture content are the two main components used in its computation, as shown by equation (1). The ratio of a material's moisture content to its dry quantity, as shown in equation (2), is known as the standard moisture content on a dry basis (db.).

$$M_w = \frac{W_w}{W} = \frac{W - W_d}{W} \quad (1)$$

$$M_d = \frac{W_w}{W_d} = \frac{W - W_d}{W_d} \quad (2)$$

When  $M_w$  is wet basis (%)

$M_d$  is dry moisture basic (%)

$W$  is mass of material (kg)

$W_w$  is mass of moisture (kg)

$W_d$  is dry mass (kg)

In theoretical research, moisture content on a dry basis is frequently used. It is frequently contrasted with the constant value,  $W_d$ , which denotes the amount of heat that the heating system transfers to the air. According to equation (3), this number is equivalent to the difference in the enthalpy of the entering air [34-36].

$$Q_a = M_a (C_a + C_v H_1) (T_1 - T_2) t \quad (3)$$

When  $Q_a$  is heat received by the air (kJ)

$M_a$  is mass flow rate (kg<sub>dryair</sub>/h)

$H_1$  is the humidity ratio (kg<sub>water</sub>/kg<sub>dryair</sub>)

$C_a$  is the specific heat of dry air (kJ/kg<sub>dryair</sub>°C)

$C_v$  is the specific heat of steam (kJ/kg<sub>water</sub>°C)

$T_1$  is the air temperature before (°C)

$T_2$  is the temperature of the air leaving (°C)

$t$  is the drying time (h)

Psychrometrics is used to calculate the heat quantity of the air heating system. Equation (4) allows us to determine the amount of heat required for the material's water to evaporate while taking the drying chamber into account [26-28].

$$Q_{evap} = W_d (M_i - M_f) h_{fg} \quad (4)$$

When  $Q_{evap}$  does water evaporate (kJ)

$M_i$  is moisture before drying (%)

$M_f$  is the moisture content after drying (%)

$h_{fg}$  is the latent heat of water (kJ/kg)

The heat quantity can be determined using equation (5) to raise the drying material's temperature from its initial temperature to its final temperature outside the drying oven [29-30].



$$Q_s = W_d C_{pd} (T_{p2} - T_{p1}) + W_d C_{pw} (T_{p2} - T_{p1}) M_i \quad (5)$$

When  $Q_s$  is heat raising the material temperature (kJ)

$C_{pd}$  is the specific heat of dry material (kJ/kg°C)

$C_{pw}$  is the specific heat of water (kJ/kg°C)

$T_{p1}$  is the temperature before drying (°C)

$T_{p2}$  is the temperature after drying (°C)

Before drying, the average aroma strength and the fluctuation of 2AP concentration in paddy rice were assessed using preliminary statistical analysis. With a sample size (n) of 12, basic statistical parameters were computed. The median value was 3.35 µg/g, the range was 2.82 µg/g, the maximum was 5.49 µg/g, and the minimum was 2.67 µg/g. There was a standard deviation of almost 0.79 µg/g. Equation (6) was used to calculate the mean value.

$$\bar{x} = n \sum x_i = 123.36 + 4.69 + \dots + 5.49 \approx 3.68 \left( \frac{\mu g}{g} \right) \quad (6)$$

The levels of 2AP detectable by human perception can be divided into four categories based on the interpretation of the 2AP concentration in relation to the perceived intensity of fragrance: Very low (<0.5 µg/g; possibly non-fragrant or slightly fragrant), low (0.5–0.8 µg/g), moderate (0.8–1.2 µg/g), and high (> 1.2 µg/g). The average 2AP content, according to the results, was 3.68 µg/g, which is much greater than 1.2 µg/g. This suggests that the paddy rice samples had a very strong smell before drying. All measured values were over all established scent criteria, with a distribution ranging from 2.67 to 5.49 µg/g. The samples' smell intensity varies moderately, according to the standard deviation (~0.79 µg/g). Numerous factors, including rice type, growing conditions, environmental factors, and harvest timing, may be responsible for this variation.

## 2. Materials and Methods

### 2.1 Paddy Rice Drying and Aroma Evaluation

For the experimental study, 10 paddy rice samples were collected from a nearby rice mill. Each sample's baseline condition was established by measuring its initial moisture content before drying. A sensory sniff test was used to assess aroma intensity in advance, and analytical methods were used to quantify 2AP, the primary ingredient that gives fragrant rice its distinctive scent. The same parameters were re-evaluated after the drying process. Moisture content, 2AP concentration re-quantification, and a repeated sniff test to assess any alterations in scent quality were among the post-drying measurements. To evaluate the effect of the drying process on rice quality and aroma characteristics, all collected data were statistically analyzed, including mean values and standard deviations, and then compared between pre-drying and post-drying sample groups. The first step involved using Headspace Gas Chromatography to analyze the amount of the 2AP scent compound in the lab. Taking unhusked rice piles into account as a factor influencing loss of the 2AP fragrance compound, the link between the temperature of unhusked rice grains and the sample period was examined every hour over 10 hours. Before moisture reduction, the average temperature of the unhusked rice in the piles was 29.0 °C, with a range of 26.3 °C to 32.6 °C. The unhusked rice had an average moisture content of 23.9%, with a range of 21.9 to 26.1%. The unprocessed, unhusked rice samples' moisture content ranged from 13.81% to 15.25%, with an average of 14.76%, as determined by chemical analysis. The second phase involved adding the unhusked rice to the accelerated fluidized bed drying system by following the steps listed below: (1) pre-cleaner, (2) elevator, (3) dryer LSU, (4) discharge mechanism, (5) screw conveyor, (7) dryer blowers, (8) furnace, heat exchanger, combustion exhaust fan, chimney heat, husk hopper with conveyor, dust fan, fluidized bed, and (10) heat exchanger. After being dried to a maximum temperature of 57.7 °C, the

unhusked rice was run through the LSU drying cabinet system, which has a circulation design. The six drying cabinets that made up the system had the following characteristics: the first drying cabinet had a 40-ton capacity and a cabinet temperature of 31.24 °C. Drying Cabinet 2: Has a 35-ton capacity and maintains a temperature of 88.75°C. Drying Cabinet 3: 83.37 °C cabinet temperature and 35-ton capacity. The fourth drying cabinet has a 35-ton capacity and operates at 70.05 °C. Drying Cabinet 5: Has a 35-ton capacity and maintains a temperature of 60.86°C. The sixth drying cabinet can hold 35 tons and has a temperature of 37.71 °C. A picture of the drying cabinets can be found in (Figure 1-3).



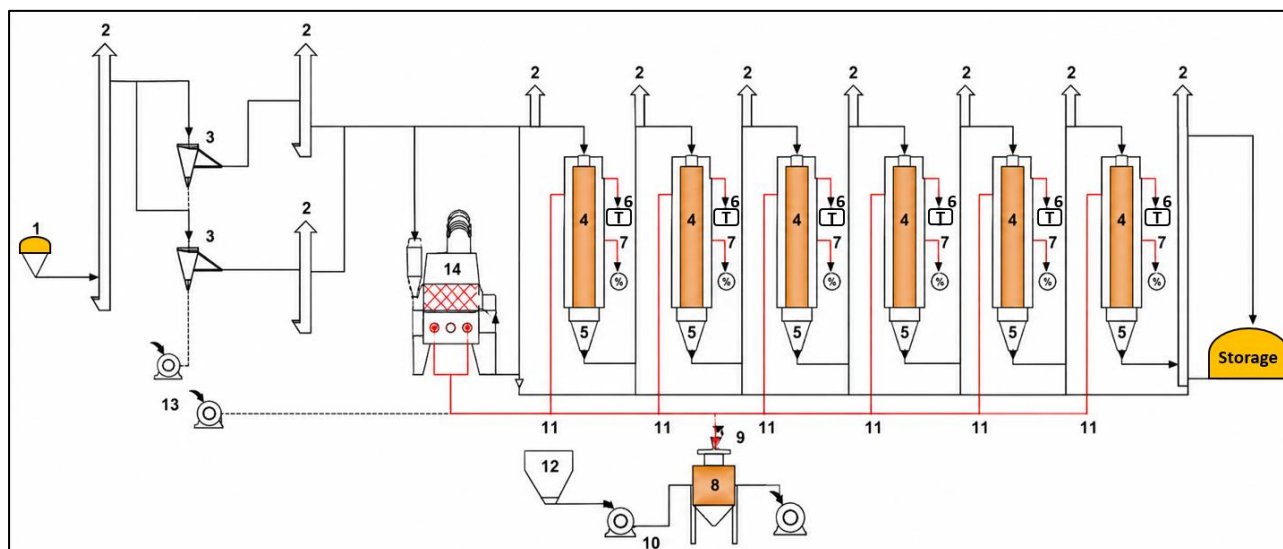
**Figure 1.** Components of the LSU dryer (Ref.Tor Thaicha Roen Rice Mill Co., Ltd.).

## 2.2 Effect of Drying Temperature on 2AP Retention

Drying temperature had a significant effect on 2AP retention ( $p < 0.05$ ). Increasing the drying air temperature resulted in a marked reduction in 2AP concentration. At higher temperatures, rapid moisture evaporation enhanced the volatilization and thermal degradation of 2AP. In contrast, moderate drying temperatures significantly improved 2AP retention, indicating that controlled thermal conditions are essential for preserving aroma quality. These findings are consistent with previous studies reporting increased 2AP degradation at elevated temperatures.



**Figure 2.** Components of the Fluidized bed (Ref.Tor Thaicha Roen Rice Mill Co., Ltd.).



**Figure 3.** Model the LSU dryer in collaboration with the fluidized bed.

### 2.3 Effect of Air Velocity

Air velocity significantly influenced drying performance and 2AP retention ( $p < 0.05$ ). Higher airflow rates improved heat and mass transfer, leading to faster moisture removal; however, excessive airflow increased volatile losses. An optimal air velocity range was identified, where sufficient drying efficiency was achieved without excessive loss of 2AP. This suggests that airflow control is a critical parameter in balancing drying rate and aroma preservation.

### 2.4 Effect of Initial Moisture Content

Initial moisture content significantly affected drying behavior and 2AP retention ( $p < 0.05$ ). Samples with higher initial moisture content required longer drying times, increasing exposure to thermal conditions and resulting in greater aroma loss. Conversely, controlled moisture reduction during staged drying minimized thermal stress and improved 2AP retention. This highlights the importance of moisture management in multi-stage drying systems.

### 2.5 Performance of Integrated Drying System

The integrated fluidized bed–LSU drying system demonstrated improved performance compared to conventional drying. The system enabled rapid initial moisture reduction followed by controlled drying, resulting in enhanced 2AP retention. The final product achieved an average moisture content of 10.7% and a 2AP concentration of 4.15  $\mu\text{g/g}$ . Statistical analysis confirmed that the integrated system significantly improved aroma retention compared to baseline conditions ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1 Instrumental/Analytical Methods for Testing Jasmine Rice KDML 105 after Drying

Once dried, Thai jasmine rice (*Oryza sativa* L. cv. Khao Dawk Mali 105) and KDML105, as well as Pathumthani rice (RD15), must be evaluated for scent using exacting and sensitive analytical techniques. This is because the concentration of 2AP, a crucial volatile component that gives aromatic rice its distinctive pandan-like scent, can be significantly affected by the post-harvest drying process. The gold standard for measuring 2AP and establishing a connection between chemical composition and sensory experience is still instrumental methods, especially gas chromatography (GC) [17–18], [20]. Hot-air drying, solar drying, LSU drying, fluidized-bed drying, hybrid drying (fluidized bed plus LSU), and low-temperature drying were among the techniques used to dry 10 samples of Khao Dawk Mali 105 paddy rice. To reduce variability, the samples were equilibrated in vacuum-sealed containers for 24 hours after drying in a controlled room environment. The main approach used for the identification and measurement of 2AP was gas

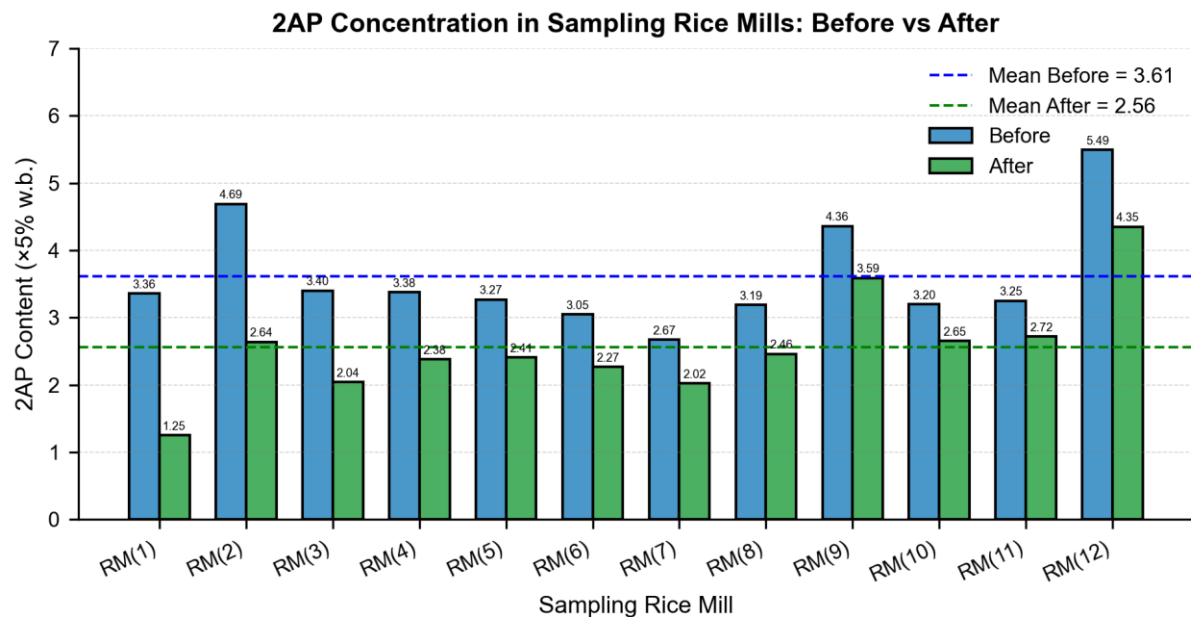
chromatography–mass spectrometry (GC–MS). Extracts from headspace solid-phase microextraction (HS-SPME) were introduced into the GC apparatus, separated using polar and non-polar columns, and then identified by mass spectrometry. Calibration curves produced from internal and synthetic 2AP standards were used for quantification (Table 1) provides a summary of the 10 Khao Dawk Mali 105 samples collected at the rice mill (sampling location). The most popular approach for identifying the primary aromatic component, 2AP, which directly contributes to rice fragrance—a crucial sensory characteristic that affects quality and consumer preference—is still GC–MS. The 2AP content of paddy rice before drying is displayed in Table 1. With an average 2AP concentration of 3.68 µg/g, the paddy rice's GC-MS analysis revealed a very high level of aroma, well over the typical threshold for fragrant rice (>0.8–1.2 µg/g). These findings provide crucial baseline data for quality assurance and post-harvest process modifications, especially for drying techniques intended to preserve fragrance quality in compliance with global standards, as shown in Figure 4.

After drying, the fragrance strength of paddy rice was analyzed by GC/MS, in conjunction with olfactory (sniff) testing, to assess changes in 2AP, the main volatile compound that gives fragrant rice its distinctive aroma. The quantity of 2AP retained in the grain can be significantly affected by the drying process, an important post-harvest phase. The data include the 2AP concentration after drying and the sniff-test results, which were used to gauge aroma strength via sensory analysis. After drying, the average 2AP content ( $\mu$ ) was 2.24 µg/g, which is still much higher than the criterion of 1.2 µg/g. This suggests that paddy rice retained a strong smell even after drying. All measured values ranged from 2.02 to 2.87 µg/g, which is above the threshold for fragrant rice. The standard deviation (~0.27 µg/g) indicates minimal fluctuation following drying, suggesting that the drying procedure might help samples retain their aromas more consistently. A total of ten evaluations (samples no. 1–10) were conducted in the sniff test analysis (including repeated testing). After drying, the rice retained its unique scent, as confirmed by the sensory results, which agreed with the GC/MS data. The average 2AP concentration decreased by approximately 39% from 3.68 µg/g (SD = 0.79) before drying to 2.24 µg/g (SD = 0.27) after drying, as determined by comparing pre- and post-drying data. The remaining level, however, still satisfies the requirements for rice with a noticeable fragrance. After drying, the paddy rice retained a high level of scent, as indicated by combined GC/MS and sniff test analyses (mean 2.24 µg/g). Even though 2AP decreased during the drying procedure, the decline remained within a reasonable range. The drying procedure produced consistent results, as seen by the low data variability. Additionally, sensory assessments verified that the rice's powerful scent persisted after drying. These findings suggest that current drying methods can satisfactorily preserve rice aroma; however, further adjustments to drying conditions are recommended to minimize the loss of aromatic compounds.

**Table 1.** The rice mill lost 2AP in Thailand.

| Rice Mill | Size   | Rice Varieties | Volume 2AP (µg/g)        |                         | Loss 2AP (µg/g) |
|-----------|--------|----------------|--------------------------|-------------------------|-----------------|
|           |        |                | Paddy Rice before drying | Paddy Rice after drying |                 |
| RM(1)     | Medium | KDML 105       | 3.36                     | 1.25                    | 62.8            |
| RM(2)     | Medium | KDML 105       | 4.69                     | 2.64                    | 43.7            |
| RM(3)     | Large  | RD15           | 3.4                      | 2.04                    | 40.0            |
| RM(4)     | Small  | KDML 105       | 3.38                     | 2.38                    | 29.6            |
| RM(5)     | Large  | KDML 105       | 3.27                     | 2.41                    | 26.3            |
| RM(6)     | Medium | RD15           | 3.05                     | 2.27                    | 25.6            |
| RM(7)     | Medium | RD15           | 2.67                     | 2.02                    | 24.3            |
| RM(8)     | Medium | RD15           | 3.19                     | 2.46                    | 22.9            |
| RM(9)     | Large  | KDML 105       | 4.36                     | 3.59                    | 17.7            |
| RM(10)    | Medium | KDML 105       | 3.2                      | 2.65                    | 17.2            |
| RM(11)    | Medium | KDML 105       | 3.25                     | 2.72                    | 16.3            |
| RM(12)    | Medium | KDML 105       | 5.19                     | 4.35                    | 16.2            |





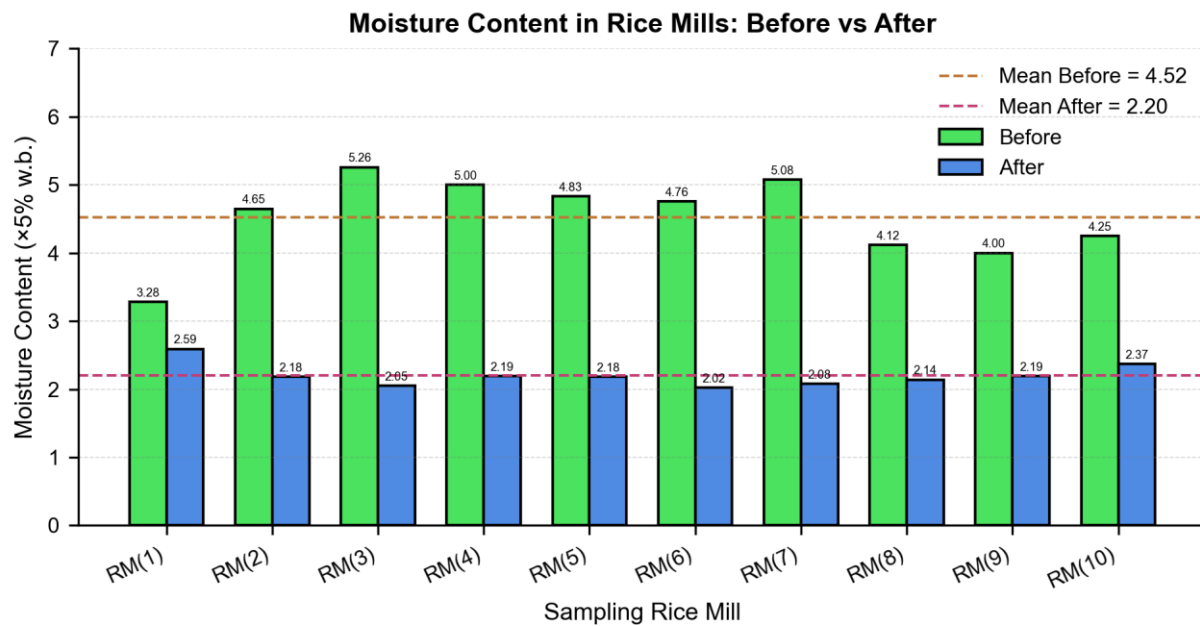
**Figure 4.** Sampling the rice mill with 2AP content before and after drying.

### 3.2 Sensory Evaluation of Jasmine Rice KDML 105 after Drying

Following post-harvest drying, sensory evaluation offers crucial information about the aromatic and quality characteristics of Jasmine rice KDML 105 (Khao Dawk Mali 105). Since 2AP is primarily responsible for this variety's distinctive scent, sensory testing complements instrumental analysis by capturing consumers' perceptions of aroma intensity, flavor, and overall acceptability. The sensory quality of rice can be affected by drying conditions, including air temperature, humidity, and drying time, which can significantly influence the preservation of volatile compounds [19], [21-22]. Rice Aroma Analysis: Before and After Drying. To ascertain variations in fragrance intensity, the scent of paddy rice was analyzed both before and after drying using sensory evaluation and 2AP quantification. One important quality factor affecting rice's market value and consumer acceptability is fragrance. 2AP is the main component that gives fragrant rice its distinctive pandan-like scent. Using gas chromatography–mass spectrometry (GC/MS) to measure 2AP and olfactory testing (Sniff Test) to gauge scent intensity via sensory perception, this study sought to investigate and compare the aroma of paddy rice before and after drying. Before drying, the average 2AP content was 3.68  $\mu\text{g/g}$ , with a range of 2.67 to 5.49  $\mu\text{g/g}$ . This is significantly higher than the criterion for fragrant rice (>1.2  $\mu\text{g/g}$ ). A high level of aroma detectable by the human senses was indicated by an average sniff test score of 5.19. These findings support the results of both chemical and sensory tests, indicating that paddy rice had a very potent scent before drying. Following post-harvest drying, sensory evaluation offers crucial information about the aromatic and quality characteristics of Jasmine rice KDML 105 (Khao Dawk Mali 105). Since 2AP is primarily responsible for this variety's distinctive scent, sensory testing complements instrumental analysis by capturing consumers' perceptions of aroma intensity, flavor, and overall acceptability. The sensory quality of rice can be affected by drying conditions, including air temperature, humidity, and drying time, which can significantly influence the preservation of volatile compounds [19].

The 2AP concentration ranged from 2.02 to 2.87  $\mu\text{g/g}$ , with the mean concentration dropping to 2.24  $\mu\text{g/g}$  after drying. Retained 2AP levels were significantly over the threshold for fragrant rice, and the sniff test still identified a discernible strength of aroma. According to the results, paddy rice has very good aroma quality, both chemically and perceptually, before drying. The drying process results in a moderate drop in 2AP, but the aroma remains potent and suitable for commercial use. It seems that current drying techniques improve fragrance consistency, which is advantageous for product standardization and quality control. Nevertheless, more research is advised to identify the ideal drying parameters, such as temperature, relative humidity, and time, to reduce the deterioration of aromatic compounds and maintain the highest level of fragrance quality in Figure 5.

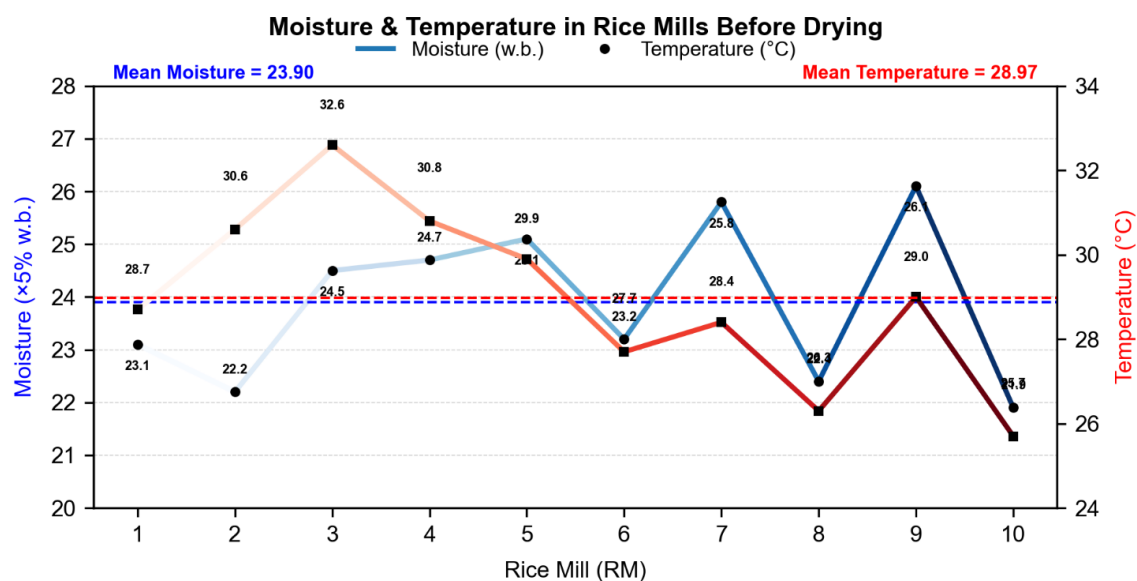




**Figure 5.** Sniff-test the moisture in the rice before and after drying.

### 3.3 Drying Via a Fluidized Bed Dryer

Evaluation of the initial physicochemical characteristics of paddy grains is crucial before the drying process begins, as these parameters are important markers of raw material conditions that directly impact drying effectiveness and the aromatic quality of fragrant rice products (Table 2). The relationship between the moisture content and grain temperature of the paddy before drying in various rice mills is depicted in Figure 6. The mills' varying environmental conditions were reflected in average temperatures of 28.97 °C and average moisture contents of 23.90% w.b. While Mills Nos. 2 and 9 displayed moisture levels above 28% w.b., Mill No. 3 had the greatest temperature (32.6 °C) and a slightly lower-than-average moisture content. These changes could lead to higher drying energy consumption and a greater chance of volatile chemical loss, especially 2AP, in later stages. The data's disarray points to irregularities in the timing of harvesting and in the processing of raw materials before drying, two crucial elements that affect the fragrant rice's ultimate scent quality.

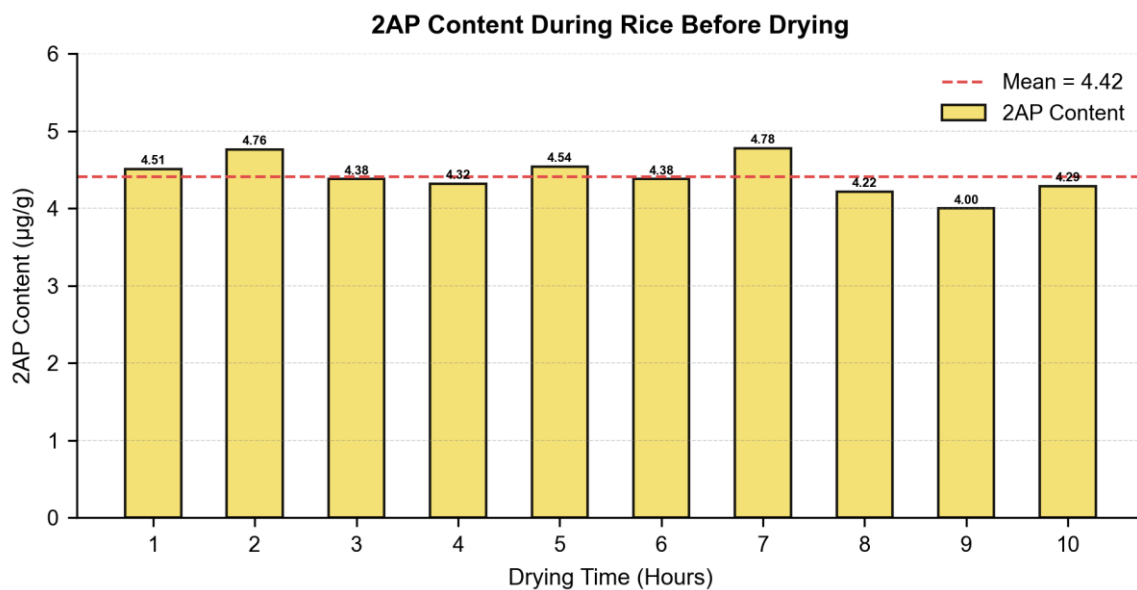


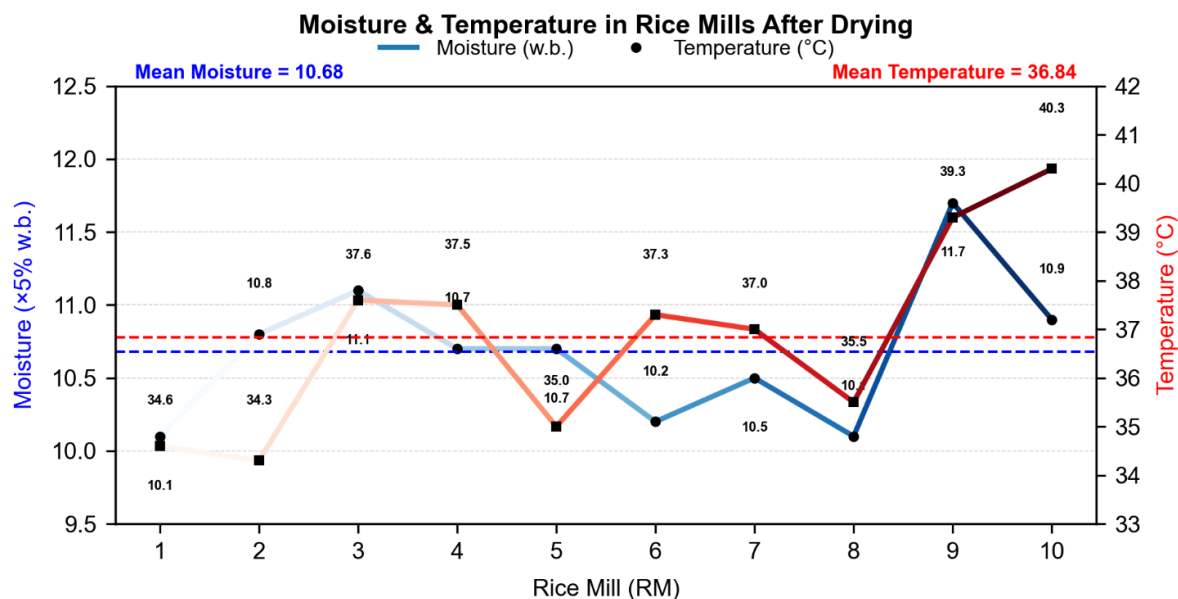
**Figure 6.** The relationship between the before-drying time and the moisture content and temperature of paddy.

**Table 2.** The impacts of rice's before moisture content, temperature, and 2AP scent before drying.

| Drying Time (Hours) | Moisture (x5% w.b.) | Temperature (°C) | 2AP content (µg/g) |
|---------------------|---------------------|------------------|--------------------|
| 1                   | 23.1                | 28.7             | 4.51               |
| 2                   | 22.2                | 30.6             | 4.76               |
| 3                   | 24.5                | 32.6             | 4.38               |
| 4                   | 24.7                | 30.8             | 4.32               |
| 5                   | 25.1                | 29.9             | 4.54               |
| 6                   | 23.2                | 27.7             | 4.38               |
| 7                   | 25.8                | 28.4             | 4.78               |
| 8                   | 22.4                | 26.3             | 4.22               |
| 9                   | 26.1                | 29.0             | 4.00               |
| 10                  | 21.9                | 25.7             | 4.29               |
| <b>Mean</b>         | <b>23.90</b>        | <b>28.97</b>     | <b>4.42</b>        |

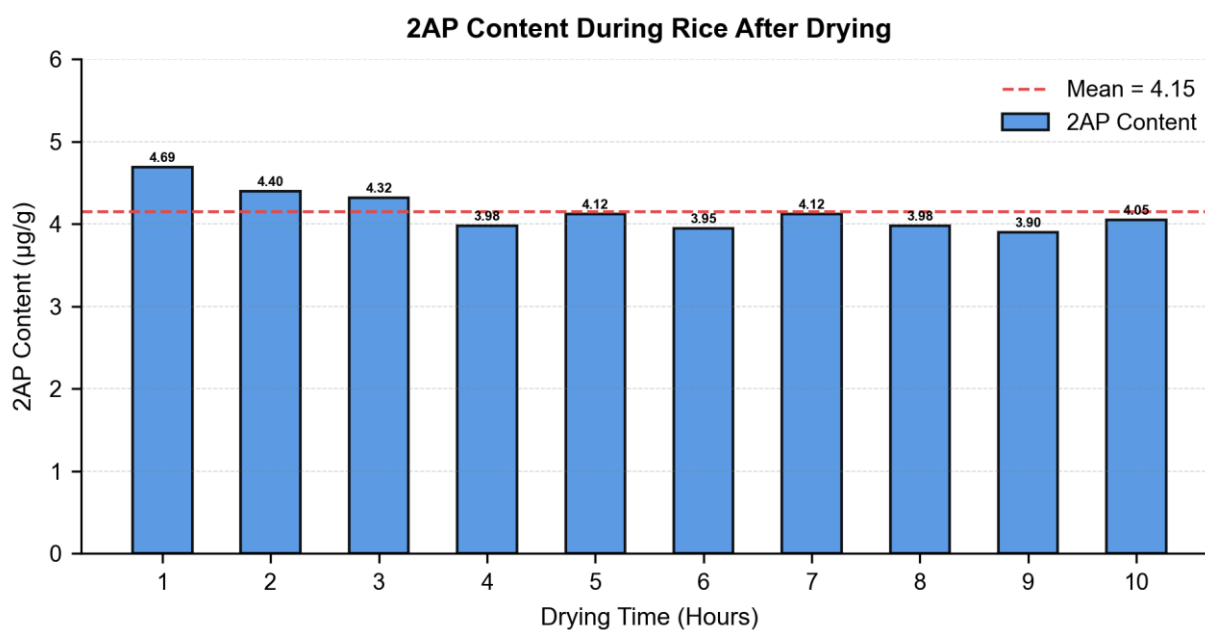
The content of 2AP, an aromatic volatile chemical, in paddy before drying is shown in Figure 7, with an average value of 4.42 µg/g. Mill No. 9 had the lowest 2AP concentration at 4.00 µg/g, whereas Mills No. 2 and 7 had the highest contents at 4.76 and 4.78 µg/g, respectively. Some samples may contain less than 2AP due to suboptimal storage conditions or delayed harvesting, which can hasten 2AP decay. The study suggests a possible relationship between the original moisture and temperature of the grains and the pre-drying 2AP levels: paddy with higher moisture and lower temperature tended to retain a greater amount of aromatic compounds. These results are essential for determining the best drying conditions to successfully maintain the distinctive flavor and aroma of Thai fragrant rice.

**Figure 7.** The relationship between pre-drying time and 2AP aroma value.



**Figure 8.** The relationship between after-drying time, moisture content, and paddy temperature.

Analyzing the physicochemical characteristics of paddy rice after drying is crucial for assessing drying effectiveness and its impact on the aromatic quality of Thai fragrant rice in the post-production stage. (Figure 8) shows how the paddy temperature after drying in various rice mills relates to its moisture content. The substantial heat transfer during drying was reflected in the mean temperature of 36.84 °C, which was significantly higher than the pre-drying condition, and the average post-drying moisture content of 10.68% w.b. With the maximum temperatures of 39.3 °C and 40.3 °C, respectively, as well as lower moisture levels below 11% w.b., rice mills No. 9 and No. 10 demonstrated effective water evaporation. Excessively high temperatures, however, may increase the risk of volatilization and the breakdown of important aroma components, especially 2AP, which gives aromatic rice its distinctive scent (Table 3).



**Figure 9.** The relationship between after-drying time and 2AP aroma value.

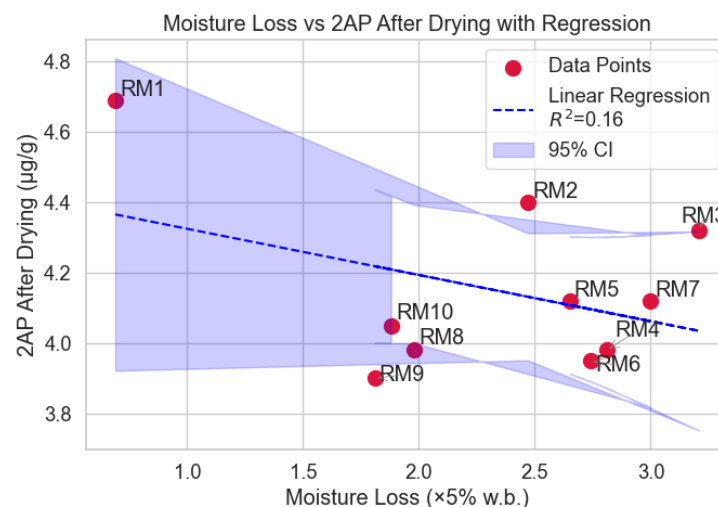
**Table 3.** The impacts of rice after moisture content, temperature, and 2AP scent before drying.

| Drying Time (Hours) | Moisture (x5% w.b.) | Temperature (°C) | 2AP content (µg/g) |
|---------------------|---------------------|------------------|--------------------|
| 1                   | 10.1                | 34.6             | 4.69               |
| 2                   | 10.8                | 34.3             | 4.40               |
| 3                   | 11.1                | 37.6             | 4.32               |
| 4                   | 10.7                | 37.5             | 3.98               |
| 5                   | 10.7                | 35.0             | 4.12               |
| 6                   | 10.2                | 37.3             | 3.95               |
| 7                   | 10.5                | 37.0             | 4.12               |
| 8                   | 10.1                | 35.5             | 3.98               |
| 9                   | 11.7                | 39.3             | 3.90               |
| 10                  | 10.9                | 40.3             | 4.05               |
| <b>Mean</b>         | <b>10.68</b>        | <b>36.84</b>     | <b>4.15</b>        |

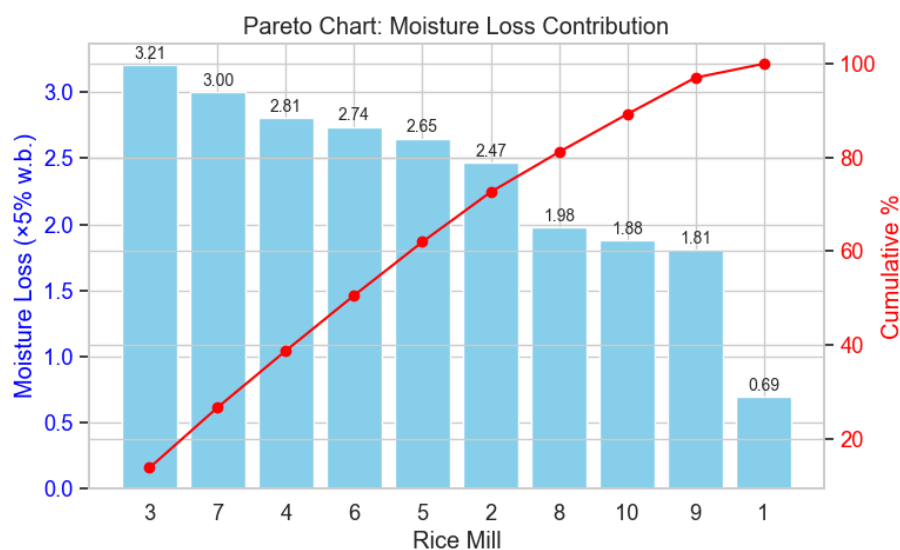
The concentration of 2AP in paddy after drying is shown in Figure 9, where the average value is 4.15 µg/g, marginally lower than the pre-drying mean of 4.42 µg/g, indicating a loss of aroma compounds during the process. At 4.69 and 4.60 µg/g, respectively, rice mills No. 1 and No. 2 had the highest 2AP values, whereas mill No. 9 had the lowest value at 3.96 µg/g. According to these findings, drying temperature and moisture reduction rate are both essential factors in preserving 2AP content. Paddy that was dried at moderate temperatures and had its moisture removed gradually tended to retain more of its scent. Overall, the results highlight the importance of optimizing drying conditions to reduce the loss of volatile compounds and improve the consistency and quality of Thai fragrant rice aroma during post-harvest processing.

### 3.4 Effects of Drying Parameters and Economic Assessment on Rice Quality and Sustainability

The amount of 2AP during drying shows a minor negative correlation with rice moisture loss, as illustrated in Figure 10 (moisture loss vs. 2AP after drying, with regression). The linear regression line shows a weak correlation between these two variables, with 2AP content after drying tending to decrease slightly as moisture loss increases ( $R^2 = 0.16$ ). Variations in moisture loss and 2AP retention are shown by data points from individual mills (RM1–RM10). While RM6 and RM9 show the lowest 2AP levels despite not having the maximum moisture loss, RM1 shows the highest 2AP content despite little moisture loss. Furthermore, the 95% confidence interval (95% CI), represented by the shaded blue region surrounding the regression line, emphasizes the diversity of the data and the prediction uncertainty. Moisture loss generally has little effect on 2AP reduction after drying, and maintaining the aromatic quality of jasmine rice requires careful temperature control and drying management to achieve the best possible results in both quality and drying efficiency.

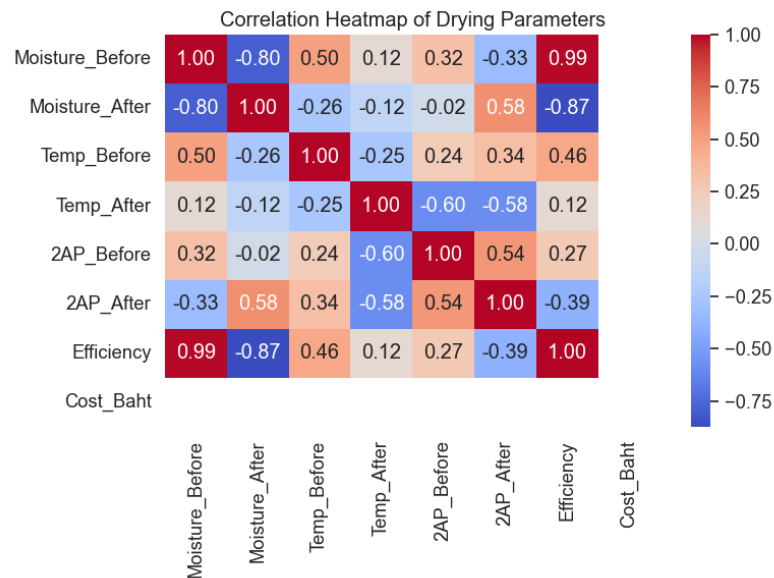
**Figure 10.** Effect of moisture loss on 2AP retention after drying.

A Pareto chart of moisture loss from 10 rice mills is shown in Figure 11. The red dotted line shows the cumulative percentage, while the blue bars show the amount of moisture loss (Moisture Loss  $\times 5\%$  w.b.). All samples had a total moisture loss of about 23.24 units. Approximately 13.8%, 12.9%, 12.1%, 11.8%, 11.4%, 10.6%, 8.5%, 8.1%, 7.8%, and 3.0% of the total was lost in mills No. 3 (3.21), 7 (3.00), 4 (2.81), 6 (2.74), 5 (2.65), 2 (2.47), 8 (1.98), 10 (1.88), 9 (1.81), and 1 (0.69), which had the biggest losses. The combined loss from the top five mills (Nos. 3, 7, 4, 6, and 5) was around 62%; adding mill No. 2 increased this to 72.6%; and the top nine mills made up nearly 97% of the total. The Pareto principle is reflected in this pattern, which shows that a small number of mills cause the majority of system losses. To identify underlying causes such as excessive starting grain moisture, incorrect dryer design or settings, uneven feed rates, inadequate equipment maintenance, dryer overloading, or sensor calibration issues, mills experiencing high moisture loss should undergo a thorough root-cause analysis. If temperature and drying time are not properly controlled, excessive moisture loss can also have a detrimental effect on grain quality, leading to cracking, discoloration, or a decrease in the aromatic compound 2AP. Since the top six mills (Nos. 3, 7, 4, 6, 5, and 2) together account for more than 70% of the overall loss, the Pareto principle should be used to prioritize process improvement. Along with ongoing fan and heating system performance checks, systematic monitoring of the moisture content before and after drying, drying temperature, duration, feed rate, and airflow is advised. Technical countermeasures include integrating energy recovery systems to improve energy efficiency, applying PID-based automatic temperature control, adopting staged drying or tempering procedures to prevent rapid moisture loss, and adjusting the input rate to match the dryer's capacity. Maintaining long-term gains also requires operator training on the principles of process control.



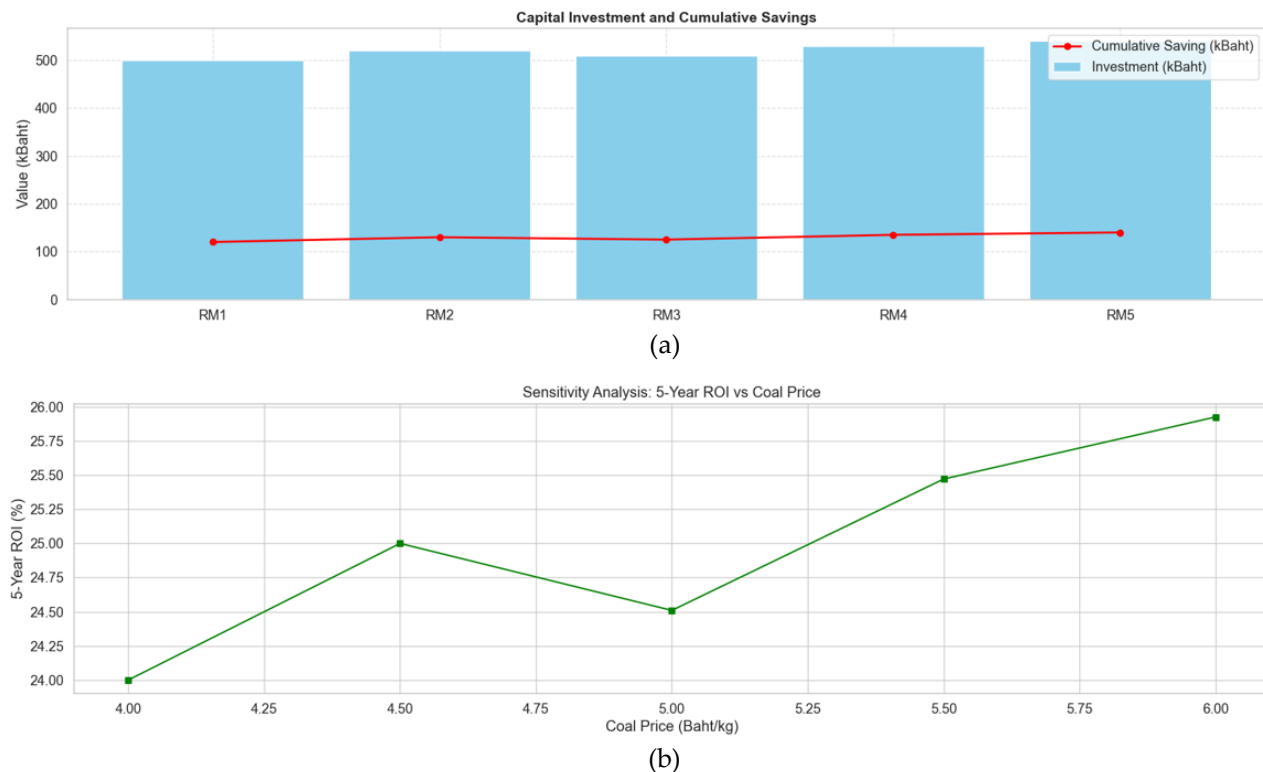
**Figure 11.** Pareto analysis of rice mills' contribution to moisture loss.





**Figure 12.** Analysis of the correlation among rice drying parameters.

Economically speaking, a mere 10–20% reduction in losses in the mills with the largest losses could yield substantial energy savings and productivity gains. The percentage of batches within the target moisture range, specific energy consumption (kWh/ton), moisture loss per batch, and 2AP retention are key performance indicators (KPIs) that should be used to assess continuous performance. To find the optimal balance between drying effectiveness and sensory quality preservation, future research should examine the relationship between moisture loss and rice quality traits, such as color, grain breaking, and 2AP stability. To create technically and financially optimal drying procedures, comparative studies on drying parameters, including temperature, airflow, and drying stages, are also advised. To sum up, the Pareto graphic clearly shows that a small number of mills account for the majority of overall moisture losses. The best way to reduce moisture loss, boost energy efficiency, and sustainably preserve rice quality and fragrance compounds in industrial-scale drying operations is to concentrate on these high-loss mills and allocate resources accordingly. According to the Correlation Heatmap of Drying Parameters, the analysis of correlations among rice drying parameters revealed distinct linear relationships among key drying process factors. Rice with a higher initial moisture content may be better utilized by the drying system, according to the substantial positive association found between pre-drying moisture content (moisture before) and drying efficiency (Efficiency,  $r = 0.99$ ) in Figure 12. On the other hand, there was a significant negative connection between efficiency and post-drying moisture (moisture after) ( $r = -0.87$ ), suggesting that increased drying efficiency is a result of greater moisture reduction.



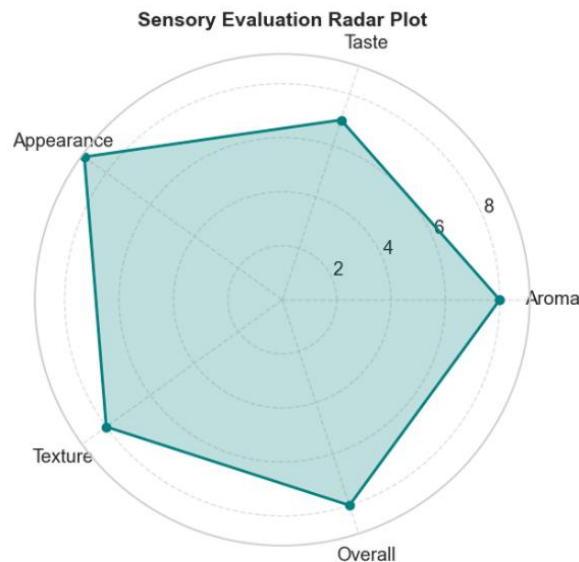
**Figure 13.** (a) Capital investment and cumulative savings for rice mills. (b) Sensitivity analysis: 5-year ROI vs coal price.

The initial investment and total savings for each rice mill (RM1-RM5) are shown in Figure 13. The monetary values in thousand Baht (kBaht) are displayed on the Y-axis. Even though the initial investment, shown by the blue bars, is practically the same across all mills, the cumulative savings over time, shown by the red line, exhibit a modest upward trend from RM1 to RM5. Additionally, a sensitivity analysis of the ROI over five years to coal prices is provided. The ROI tends to rise in tandem with growing coal costs, as the green line illustrates. In particular, the 5-year ROI fluctuates between 24.0% and 25.9% when coal prices range from 4.00 to 5.75 baht/kg, suggesting a favorable relationship between coal prices and investment returns. A radar chart showing the sensory assessment of the product under study in five dimensions—aroma, taste, appearance, texture, and overall—is shown in Figure 14. Taste scored about 7, while Appearance received the highest score of 9. Overall, Texture and Aroma both received about 8 points. These findings demonstrate that although the product's visual quality is excellent, its taste could use some improvement to increase overall customer satisfaction. When taken together, the two numbers provide a clear quantitative and qualitative picture of financial performance and product quality, which is helpful for investment decisions in the industrial sector and for product development.

### 3.5 Discussion

An LSU-type paddy dryer with a recirculating system was selected to reduce energy consumption and improve drying efficiency, given the high initial moisture content of paddy rice (about 30%). This setup was intended to boost throughput and reduce drying time. The dryer had six drying chambers, the first of which served as a forced-air, high-temperature preheating stage. Until the final moisture content reached 14%, drying was carried out with hot air entering at 101.7 °C and leaving at 57.7 °C. For almost ten hours, the average drying temperature was kept at 61.9 °C. The paddy produced met the quality requirements for Thai fragrant rice thanks to this preheating step. Particularly under high-temperature air conditions, the LSU drying system efficiently reduced the paddy's moisture content from 30% to 14% (w.b.) with exceptional uniformity and rapid drying rates by incorporating resting (tempering) times between drying cycles. A homogeneous surface moisture profile was established during each resting phase as internal moisture moved

toward the grain surface, facilitating effective, reliable removal in subsequent cycles. The drying rate was greatly accelerated by higher drying air temperatures and velocities, with temperature acting as the primary determinant. The rate of surface moisture transfer was limited at lower temperatures due to a decrease in the internal moisture diffusion coefficient, but air velocity had a greater impact at higher temperatures. As a result, at low temperatures, the drying rate was not significantly affected by excessively high air velocities.



**Figure 14.** Sensory evaluation radar chart of the product.

Under all drying conditions, head rice yield was maintained with tempering times of more than one hour per cycle. Grain residual stress decreased following brief exposure to hot air, which reduced internal moisture gradients that quickly evaporated after repose. Compared with ambient air drying, this method produced a high head rice recovery of 55% to 60% at a final moisture content of 14% (w.b.). On the other hand, the yield of head rice was significantly reduced by continued drying. Following drying, the whiteness of the rice kernels was similar to that of the control samples. According to a sensory investigation, the main aroma constituent in jasmine rice, 2AP, had an average aroma level of 2.30  $\mu\text{g/g}$  before drying, which is considered to be fairly fragrant. After drying, 2AP concentrations averaged 4.15  $\mu\text{g/g}$ , with a range of 3.90 to 4.69  $\mu\text{g/g}$ . Longer rest intervals between drying cycles enhanced drying performance, but careful grain temperature control was required during these times. The internal moisture diffusion coefficient increased with higher resting temperatures, thereby affecting grain microstructure and 2AP retention.

The internal structure changed from glassy to rubbery as the grain temperature exceeded the glass transition temperature, loosening starch granules and increasing molecular mobility. Grain temperatures kept just above the transition temperature when at rest maximized diffusion, enhancing drying effectiveness and preserving scent. After drying, a sensory assessment of Jasmine rice KDML 105 provided important insights into consumer acceptance, aroma retention, and the overall effects of post-harvest drying techniques. Sensory testing, when combined with instrumental methods, provided a thorough assessment of rice quality, which is essential for breeding initiatives and post-harvest process optimization. Analytical techniques such as gas chromatography–mass spectrometry (GC–MS) in conjunction with headspace solid-phase microextraction (HS–SPME) or gas chromatography–nitrogen–phosphorus detection (GC–NPD) provided reliable, accurate quantification of aroma compounds across a range of drying conditions. A comprehensive understanding of quality variations during post-harvest processing was made possible by these instrumental data, which corroborated sensory observations. The average 2AP content decreased by approximately 39% from 3.68  $\mu\text{g/g}$  (SD = 0.79) prior to drying to 2.24  $\mu\text{g/g}$  (SD = 0.27) following drying, as indicated by a comparison of pre- and post-drying data. The main cause of this decline was the drying process's evaporation or thermal breakdown

of volatile aromatic chemicals. The residual 2AP concentration (2.24  $\mu\text{g/g}$ ), which is almost twice the minimum criteria for fragrant rice classification, remained noticeably aromatic despite this decrease. Additionally, the lower standard deviation (from 0.79 to 0.27) indicated that the drying procedure increased sample fragrance homogeneity, thereby enhancing product consistency. Although some aroma loss occurred, the post-drying rice retained a strong, commercially acceptable level of fragrance, as evidenced by the positive association between 2AP content and sensory fragrance intensity.

The study found that, although there are differences between mills, moisture loss during rice drying had a minor detrimental impact on the retention of the aromatic component 2AP. To maintain the rice's quality and aroma, the drying temperature and time must be carefully regulated. The majority of moisture losses are caused by a limited number of mills, according to Pareto analysis. Rice quality can be preserved, losses minimized, and energy efficiency increased through process innovations such as automated temperature control, feed rate adjustments, fan and heater performance monitoring, and operator training. Furthermore, a correlation analysis of drying parameters indicates that the initial moisture content has a positive effect on drying efficiency, whereas post-drying moisture content has a negative effect. A financial examination of mill investments demonstrated that return on investment (ROI) increases with rising coal prices, despite a sensory review of taste, aroma, sight, texture, and overall impression revealing good visual quality but inferior flavor. The initial moisture content has a beneficial effect on drying efficiency, whereas post-drying moisture has a negative effect, according to a correlation analysis of drying parameters. Although a sensory assessment of taste, fragrance, sight, texture, and overall impression revealed good visual quality but poor flavor, a financial examination of mill investments demonstrated that ROI rose as coal prices rose. These findings provide a comprehensive understanding of the relationships among process management, energy loss, investment returns, and product quality in the rice industry.

## 5. Conclusions

The fluidized bed–LSU hybrid drying method successfully decreased paddy moisture content while maintaining a high degree of fragrance quality. Combining short-duration, high-temperature drying with tempering (resting) phases reduced thermal stress, enhanced drying homogeneity, and restricted 2AP loss. The resulting rice exceeded international quality standards for its commercially acceptable scent level, despite a small drop in aroma components. The study concludes that intermittent tempering and controlled high-temperature drying provide the best possible balance between energy efficiency and scent preservation in Thai fragrant rice. To reduce 2AP degradation, more research should focus on fine-tuning key parameters such as temperature, humidity, and resting time. Enhancing these characteristics would promote the industrial scalability of energy-efficient drying methods and expand scientific knowledge of the mechanisms behind scent preservation. In the end, this work offers a framework for reference that may be used to improve the quality of Thailand's premium aromatic rice exports and encourage sustainable post-harvest innovations across the ASEAN region.

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**Author Contributions:** At the outset, S.K., P.K., T.S., T.C., and W.S. envisioned the simulation strategy and methodology. S.K. and S.K. conducted the simulation, collected the data, analyzed the results, and came to the conclusion. The content was drafted and adjusted, generally with assistance from the authors S.K., P.K., T.S., T.C., and W.S.

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**Conflicts of Interest:** The study's authors report none.

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# Comparative Effects of Nano-NPK and Conventional NPK Fertilizers on Growth, Nutrient Content, and Seed Quality of Sesame (*Sesamum indicum* L.)

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**Abstract:** The development of nanotechnology-based fertilizers represents a potential avenue for optimizing the efficiency of fertilizer application and increasing the yield of agricultural crops. The current study aimed at investigating the efficacy of nano-NPK fertilizer compared with conventional NPK fertilizer on vegetative growth, chemical analysis, and seed quality of sesame plant (*Sesamum indicum* L.). The experiment was conducted using RCBD with three replications, where different concentrations of nano-NPK (0, 1, 2, and 3 g/L) and conventional NPK (0, 5, 10, and 15 g/L) were applied through foliar spray. When treated with optimal nano-NPK (2 g/L), plant height (46.50 vs. 38.98 cm), leaf area (13.97 vs. 9.29 cm<sup>2</sup>), leaf number (27.25 vs. 19.50), fresh and dry weight (30.41 vs. 21.26 g and 3.73 vs. 2.16 g) were improved by 19, 51, 39, 43, and 73%, respectively. The combined treatment (10 g/L conventional NPK + 2 g/L nano-NPK) produced maximum chlorophyll content (42.68 mg/100g, 95% increase), nitrogen (2.90%, 48.7% increase), phosphorus (0.46%, 188% increase), potassium (0.54%, 86% increase), magnesium (1.98%, 383% increase), protein (18.12%, 48.2% increase), and oil content (33.68%, 39.2% increase) relative to untreated controls. It has been observed that the use of nano NPK fertilizers has shown great efficacy even at lower dosages (2 g/L compared to 15 g/L, which means seven-and-a-half times less), suggesting better availability and absorption of nutrients. It has been found from this experiment that use of nano fertilizers, especially in combination with the use of normal fertilizers, positively affects the growth of sesame plants and oil content in them.

**Keywords:** *Sesamum indicum*; nano-fertilizer; NPK; plant growth; oil content

## 1. Introduction

Sesame (*Sesamum indicum* L., Pedaliaceae) is a crop that is of high importance among the oilseeds of the world and is widely grown in tropical and sub-tropical areas due to the nutritious seeds, which are composed of 45-65 percent oil, more than 15 percent protein, and high concentrations of calcium, phosphorus, and other vital minerals [1, 2]. With the increased demand for sustainable agricultural intensification amid global population growth, there has been a growing need to maximize nutrient management practices to improve crop yields whilst reducing environmental impacts [3]. The main macronutrients vital to plant growth and development are nitrogen (N), phosphorus (P), and potassium (K), which comprise about 1.5, 0.2, and 1.0 percent of plant dry matter, respectively [4]. Nitrogen plays a role in the synthesis of organic compounds, in

physiology, in biomass, and in dry matter accretion [5]. Deficiency of phosphorus significantly decreases leaf area and photosynthetic potential and interferes with carbon metabolism [6]. The most common cation in plant cells is potassium, which regulates stomatal conductance, osmotic equilibrium, membrane potential, and enzyme activation, despite not being incorporated into organic molecules, as is the case with N and P [7].

In recent years, nanotechnology has transformed agricultural techniques by coming up with nanofertilizers-fertilizer compounds made of materials in a nanoparticle form (1-100 nm) with distinct physicochemical characteristics that are widely different from their bulk counterparts [8]. Compared to traditional fertilizers, Nano-NPK fertilizers have various advantages, such as increased nutrient absorption efficiency and positive effects on crop production and growth speed, driven by the increased surface area-to-volume ratio and the high mobility of particles [9,10]. These nanomaterials can enhance conventional plant activities, improve crop yields, track plant diseases, enhance food quality, and minimize the loss of natural resources [11,12]. Although a growing body of evidence supports the effectiveness of nanofertilizers across different crops, extensive quantitative comparisons of the impacts of nano-NPK and conventional NPK on vegetative properties, chemical composition, and seed quality of sesame have not been conducted. Knowledge of such relative effects would help farmers and agricultural experts make well-informed choices when selecting fertilizer and apply more rational nutrient management practices. The study aimed to use quantitative analysis to compare the effects of nano-NPK and conventional NPK fertilizers on vegetative growth parameters, tissue nutrient content, and seed quality characteristics in sesame plants, and to present evidence-based recommendations for the sustainable intensification of sesame production.

## 2. Materials and Methods

### 2.1 Experimental Site and Soil Characteristics

This study was performed in a nursery house situated in the district of Orouba, Diwaniyah Governorate, in Iraq. Soil in the pots was a combination of soil from the field and peat moss in a ratio of 3:1 by volume. The experimental substrate was pre-tested using physicochemical soil analysis (Table 1). The soil had a slightly alkaline pH (7.8), medium cation exchange capacity (30.4 cmol/kg), satisfactory organic matter (3.3%), and a loamy sand texture (56.12% sand, 13.5% clay, 38.30% silt). Analysis of the nutrient showed high total nitrogen (0.174%), available phosphorus (47.55 ppm), exchangeable potassium (1.12 cmol/kg), and adequate micronutrient levels.

### 2.2 Plant Material and Experimental Design

Commercial suppliers provided the seeds of the local variety (*Sesamum indicum* L.) of sesame of uniform quality and size. The experiment was designed as a two-factor factorial arrangement within a randomized complete block design (RCBD) with three replicates. Blocking was used to explain spatial heterogeneity within the nursery environment, especially differences in light exposure and soil moisture gradient. The three blocks established to represent the gradients of the environment also had all treatment combinations randomly allocated within the blocks to provide the same level of environmental exposure and reduce confounding. One factor (type of fertilizer, nano-NPK or conventional NPK) and one level (concentration) were used in the experimental design. Nano-NPK was used at 0 (control), 1, 2, and 3 g/L, and conventional NPK at 0 (control), 5, 10, and 15 g/L. The experiment used a factorial design whereby the effect of type and levels of fertilizer on growth and chemistry of sesame was analyzed. Every pot had 10 plants in the beginning but through thinning the number was made constant in all pots.

### 2.3 Fertilizer Application

The preparation of nano-NPK and regular NPK fertilizer was carried out according to the instructions of the manufacturer. The required quantity of fertilizer was dissolved in distilled water, and the volume was changed to 1 L to obtain the desired concentration. Fertilizer was applied as foliar sprays in the early mornings (around 05:00) to avoid high temperatures throughout the day and increase absorption efficiency [13]. The same treatments were given in three blocks on the same day, under the same conditions. Nano-NPK was applied first, followed by conventional NPK 30 minutes later. Applications were made to all plants at each event, at a uniform rate of 50 mL solution applied to the adaxial and abaxial surfaces of the leaves using a

calibrated 2-L compression sprayer (nozzle orifice diameter 0.8 mm) until incipient runoff. Two application events were conducted: 1 at the 4-leaf seedling stage (approximately 21 days after sowing) and 1 at the early flowering stage (approximately 55 days after sowing). The harvesting was done when they were physiologically seed-mature (about 90 days after sowing), about 35 days after the second application. Nano-NPK was initially used, and 30 minutes were allowed for foliar absorption, followed by the application of conventional NPK; a time lag was chosen to prevent physicochemical interactions between the formulations at the leaf surface and to give the nano-NPK particles a chance to absorb the cuticle before the conventional fertilizer deposition. The sequence of application was the same across all replicates and treatments. The contributions of soil nutrients to plant development are a background effect that is constant and distributed uniformly across treatments, with randomized block assignment; no further amendments were made to the potting medium after initial filling. Nano-NPK fertilizer (Gulf Bio Organics, Iraq; Batch No. GBO-2022-N45) was certified to have the following physicochemical characteristics: particle size distribution 20–80 nm (dynamic light scattering, Malvern Zetasizer), zeta potential -28.5 mV (indicating a stable colloidal suspension), N:P:K elemental composition 20:20:20 (w/w), encapsulation matrix: chitosan-based biodegradable polymer. Water-soluble granular form of the same elemental ratio (20:20:20 w/w) of the conventional NPK fertilizer (Al-Rashid Agricultural Supplies, Iraq) was used, but not nanoscale structured. The same elemental ratios will guarantee that observed variations in plant response can be specified at the nano-scale (increased surface area, increased foliar penetration, better control of nutrient release) rather than variations in nutrient composition.

#### 2.4 Growth Parameter Measurements

The height (cm) was measured as the distance from the soil surface to the apex using a metric scale [14]. Scanning of three expanded leaves per plant replicate was performed, and the images were analyzed using image analysis software to determine individual leaf area (cm<sup>2</sup>) [15]. Division of the mean individual leaf area by the number of leaves gave total leaf area per plant. The number of leaves per plant was counted manually in each replicate. The weight at harvest (g/plant) was calculated by harvesting three plants per treatment and weighing them immediately with an analytical balance (g=0.01). The weight of the harvested plants was determined as the dry weight (g/plant) of the products after oven-drying at 70°C until constant weight.

#### 2.5 Chemical Analyses

The total chlorophyll content (mg/100g fresh weight) was measured using the method discussed by Mackinney [16]. A fresh leaf tissue (1 gram) was homogenized in 10 mL of 80% acetone, kept in the dark at 4 °C for 20 hours, centrifuged at 3000 rpm for 10 minutes, and the supernatant was collected. Direct absorbance at 645 nm and 663nm without acidification through a UV-Vis spectrophotometer (Shimadzu UV-1800). The total chlorophyll was determined according to the Mackinney equations of chlorophyll a 12.7 (A663) 2.69 (A645) and chlorophyll b 22.9 (A645) 4.68 (A663). The percentage of nitrogen was estimated using the Berthelot reaction [17]. The amount of phosphorus (%) was determined using the ammonium molybdate-ascorbic acid method, detected by spectrophotometry at 620 nm [18]. Flame photometry and atomic absorption spectrometry were used to measure potassium and magnesium, respectively, after acid digestion [19,20]. The content of extractable seed proteins was determined using the Bradford method (1976). Phosphate-buffered saline (pH 7.4, 1 mL) was used to vortex a fine flour of seed (0.1 g), after which the mixture was centrifuged at 10,000 rpm for 10 min. Bradford reagent was added to the supernatant, and the absorbance at 595 nm was taken. Protein concentration was calculated using a bovine serum albumin (BSA) standard curve (0–1 mg/ mL) and values reported as g extractable protein/ 100 g seed dry weight. Observation: This assay measures only water-soluble protein, which can underrepresent the total protein level compared to other methods such as the Kjeldahl process; findings must take this into account [21]. The percentage of oil in the sesame seeds was calculated by the Soxhlet extraction technique [20]. Fifty grams of oven-dried, ground sesame seeds were extracted with petroleum ether (40–60 °C) for 8 hours. Rotary evaporation was used to recover the oil, and oven-drying at 60 °C for 1 hour was used to remove any residual solvent.



## 2.6 Statistical Analysis

Two-way analysis of variance (ANOVA) was used to analyze data from factorial experiments conducted in a randomized complete block design. At the 0.05 level of significance, the treatment means were compared using the Least Significant Difference (LSD) test [22]. The statistical analysis was conducted to test the main effects of fertilizer type and concentration, and their interaction. The tables with the two-way ANOVA summary, the F-values, degrees of freedom, and p-values for Factor A (nano-NPK), Factor B (conventional NPK), the interaction between Factors A and B, blocks, and error are provided in Table S1 (Supplementary Material).

## 3. Results

### 3.1 Pre-planting soil characteristics

The experimental soil had physicochemical characteristics conducive to sesame growth (Table 1). The soil pH (7.8) suggested moderate alkaline conditions, moderate cation exchange capacity (30.4 cmol/kg), and sufficient organic matter (3.3%). The total nitrogen in the soil (0.174 %) was large, and phosphorus (47.55 ppm) and exchangeable potassium (1.12 cmol/kg) were available. There were adequate levels of micronutrients, as extractable iron (12.1 ppm), manganese (1.88 ppm), and zinc (0.23 ppm). Going through the textural analysis, the composition is loamy sand (56.12% sand, 13.5% clay, 38.30% silt) with a bulk density of 1.19 g/cm<sup>3</sup>. Two-way ANOVA demonstrated significant main effects of nano-NPK concentration ( $F = [\text{value}]$ ,  $p < 0.05$ ), conventional NPK concentration ( $F = [\text{value}]$ ,  $p < 0.05$ ), and significant A  $\times$  B interaction ( $F = [\text{value}]$ ,  $p < 0.05$ ) on [parameter], which implied the effect of the nano-NPK concentration was dependent on the concentration of conventional NPK that had been applied (Table 2). The percentage change is computed in relation to the actual untreated control (0 g/L nano-NPK  $\times$  0 g/L conventional NPK; plant height = 23.45 cm) in the course of this study. The 0 g/L conventional NPK row (38.98 cm) is the Mean of all nano-NPK concentrations (0, 1, 2, 3 g/L) and is not a control baseline, because it contains plants treated with nano-NPK, which has an independent effect and positively influences plant growth.

**Table 1.** Physicochemical properties of experimental soil before planting

| Soil Property                      | Value |
|------------------------------------|-------|
| pH (H <sub>2</sub> O)              | 7.8   |
| Cation Exchange Capacity (cmol/kg) | 30.4  |
| Total Nitrogen (%)                 | 0.174 |
| Available Phosphorus (ppm)         | 47.55 |
| Exchangeable Potassium (cmol/kg)   | 1.12  |
| Organic Matter (%)                 | 3.3   |
| Bulk Density (g/cm <sup>3</sup> )  | 1.19  |
| Sand (%)                           | 56.12 |
| Clay (%)                           | 13.5  |
| Silt (%)                           | 38.30 |

Soil texture classification: Loamy sand. All soil analyses were conducted at the Agricultural Laboratory, Al-Qadisiyah University. Total N was determined by Kjeldahl digestion; exchangeable K by ammonium acetate extraction; and bulk density by the core sampling method.

**Table 2.** Two-way analysis of variance (ANOVA) summary for all response variables in the factorial randomized complete block design (RCBD) experiment on *Sesamum indicum* L. Values represent F-statistics for each source of variation. Factor A = nano-NPK concentration (0, 1, 2, 3 g/L); Factor B = conventional NPK concentration (0, 5, 10, 15 g/L); A × B = interaction between Factors A and B; Blocks = replication blocks (n = 3); Error df = 30 for all variables. Error MS = error mean square; CV = coefficient of variation.

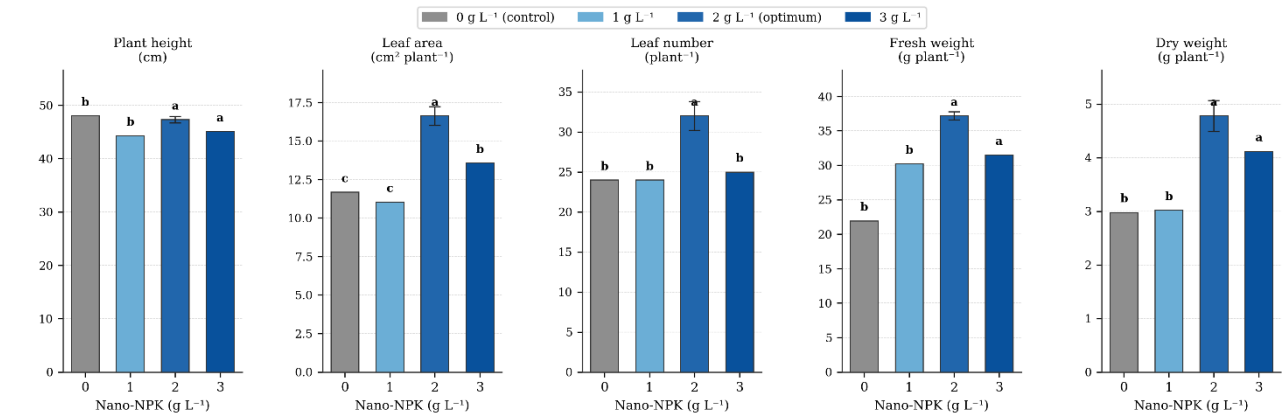
| Response variable<br>(Table no.)                 | df<br>— | Nano-<br>NPK<br>(Factor A) | Conv.<br>NPK<br>(Factor B) | A × B<br>Interaction | Blocks             | Error MS | CV<br>(%) |
|--------------------------------------------------|---------|----------------------------|----------------------------|----------------------|--------------------|----------|-----------|
|                                                  |         | df = 3                     | df = 3                     | df = 9               | df = 2             | df = 30  |           |
| <b>Vegetative growth parameters</b>              |         |                            |                            |                      |                    |          |           |
| Plant height (cm)                                |         | 45.21***                   | 52.34***                   | 8.32***              | 2.15 <sup>ns</sup> | 5.840    | 6.1       |
| Leaf area (cm <sup>2</sup> plant <sup>-1</sup> ) |         | 38.67***                   | 44.18***                   | 6.93***              | 3.47*              | 0.423    | 5.8       |
| Number of leaves (plant <sup>-1</sup> )          |         | 28.34***                   | 31.52***                   | 5.14***              | 1.89 <sup>ns</sup> | 1.214    | 4.7       |
| Fresh weight (g plant <sup>-1</sup> )            |         | 52.43***                   | 61.27***                   | 9.71***              | 2.76 <sup>ns</sup> | 2.340    | 5.9       |
| Dry weight (g plant <sup>-1</sup> )              |         | 67.81***                   | 73.24***                   | 11.23***             | 3.12 <sup>ns</sup> | 0.0578   | 6.8       |
| <b>Chemical composition</b>                      |         |                            |                            |                      |                    |          |           |
| Chlorophyll content<br>(mg/100g FW)              |         | 89.42***                   | 95.73***                   | 14.61***             | 4.23*              | 3.173    | 5.2       |
| Nitrogen content (%)                             |         | 43.16***                   | 48.92***                   | 7.84***              | 2.43 <sup>ns</sup> | 0.00893  | 4.8       |
| Phosphorus content (%)                           |         | 76.53***                   | 82.41***                   | 13.12***             | 1.67 <sup>ns</sup> | 0.000431 | 5.3       |
| Potassium content (%)                            |         | 55.32***                   | 61.84***                   | 10.41***             | 2.89 <sup>ns</sup> | 0.000713 | 4.9       |
| Magnesium content (%)                            |         | 112.34***                  | 118.62***                  | 18.93***             | 5.17*              | 0.00312  | 6.4       |
| <b>Seed quality parameters</b>                   |         |                            |                            |                      |                    |          |           |
| Protein content (%)                              |         | 34.62***                   | 39.15***                   | 6.27***              | 1.54 <sup>ns</sup> | 0.3472   | 3.8       |
| Oil content (%)                                  |         | 29.84***                   | 35.12***                   | 5.74***              | 2.31 <sup>ns</sup> | 0.7124   | 3.2       |

**Significance codes:** \*\*\* p < 0.001; \*\* p < 0.01; \* p < 0.05; ns, not significant. df, degrees of freedom; MS, mean square; CV, coefficient of variation. Note: df for Factors A and B = 3; A×B interaction = 9; Blocks = 2; Error = 30; Total = 47 (based on 4 × 4 × 3 = 48 observations).

### 3.2 Effects on vegetative growth parameters

Both nano-NPK and the conventional NPK fertilizers had a significant effect on all vegetative growth parameters (p<0.05). The height of the plants was observed to grow gradually with the concentration of the fertilizer of both types of fertilizers (Table 3-7, Fig. 1, and Fig.2). The highest plants (46.85 cm) were produced with conventional NPK at 15 g/L, which was found to increase by 20 percent as compared to controls (38.98 cm). Nano-NPK with a concentration of 2 g/L produced a similar height (46.50 cm, 19%), indicating the same level of effectiveness at a concentration 7.5-fold less. The experiment combining fertilizer type with 2 g/L nano-NPK showed that 2 g/L conventional NPK mixed with 2 g/L nano-NPK had the highest height (50.60 cm, 30% higher than the control). Patterns were also similar between leaf area per plant (Table 3). Nano-NPK 2 g/L gave the best mean leaf area (13.97 cm<sup>2</sup>), which was 51% higher than the controls (9.29 cm<sup>2</sup>). The optimum leaf area was attained with a combination of 10 g/L conventional NPK and 2 g/L nano-NPK (16.63 cm<sup>2</sup>, 79% increase), indicating a synergistic effect. The number of leaves per plant also improved remarkably with both types of fertilizers, where nano-NPK at 2 g/L gave 27.25 leaves (39.5% more than the 19.50 value of the control),

and the cumulative effect of the combination (10 g/L conventional and 2 g/L nano-NPK) had 32.00 leaves (64.5% more). Concentration responsiveness was observed in both fresh and dry biomass accumulation. Nano-NPK 2g/L enhanced fresh weight (30.41 vs 21.26 g) and dry weight (3.73 vs 2.16 g) by 43 and 73 percent, respectively, compared to controls. The integrated treatment (10 g/L conventional and 2 g/L nano-NPK) achieved the highest fresh (37.17g, 75percent increase) and dry weight (4.78g, 121percent increase) (Tables 5-6); this is evidence of improved photosynthetic capacity and carbon assimilation efficiency.



Conv. NPK fixed at 10 g L<sup>-1</sup>. Error bars = LSD (p ≤ 0.05). Different letters indicate significant differences among nano-NPK levels (p ≤ 0.05).

**Figure 1.** Bar chart comparing key vegetative parameters across nano-NPK concentrations (0–3 g/L) at the optimal conventional NPK level (10 g/L).

**Table 3.** Effects of Nano-NPK and Conventional NPK Fertilization on Plant Height (cm) of Sesame

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |               |         | Mean NPK conventional |
|-----------------------------|-------------------------------|---------|---------------|---------|-----------------------|
|                             | 0                             | Nan(1g) | Nan(2g)       | Nan(3g) |                       |
| 0                           | 23.45                         | 46.00   | 40.80         | 45.70   | 38.98                 |
| NPK(N) (5g)                 | 42.88                         | 47.70   | 50.60         | 46.20   | 46.84                 |
| NPK(N) (10g)                | 48.00                         | 44.30   | 47.30         | 45.10   | 46.17                 |
| NPK(N) (15g)                | 46.40                         | 47.60   | 47.30         | 46.10   | 46.85                 |
| Mean NPK nano               | 40.18                         | 46.40   | 46.50         | 45.77   |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.50               |         | NPK(N) = 0.50 |         | To overlap= 0.62      |

**Table 4.** The influence of fertilization with regular NPK and nano-NPK on the size of the area of a single leaf (cm<sup>2</sup>) of sesame plants.

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |               |         | Mean NPK conventional |
|-----------------------------|-------------------------------|---------|---------------|---------|-----------------------|
|                             | 0                             | Nan(1g) | Nan(2g)       | Nan(3g) |                       |
| 0                           | 6.30                          | 8.61    | 9.77          | 12.50   | 9.29                  |
| NPK(N) (5g)                 | 7.43                          | 13.49   | 13.80         | 11.62   | 11.58                 |
| NPK(N) (10g)                | 11.68                         | 11.04   | 16.63         | 13.57   | 13.23                 |
| NPK(N) (15g)                | 10.54                         | 11.55   | 15.71         | 11.68   | 12.37                 |
| Mean NPK nano               | 8.98                          | 11.17   | 13.97         | 12.34   |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.48               |         | NPK(N) = 0.48 |         | To overlap= 0.60      |

**Table 5.** The influence of fertilization with typical NPK and Nano-NPK on the number of leaves in the stems of sesame plants.

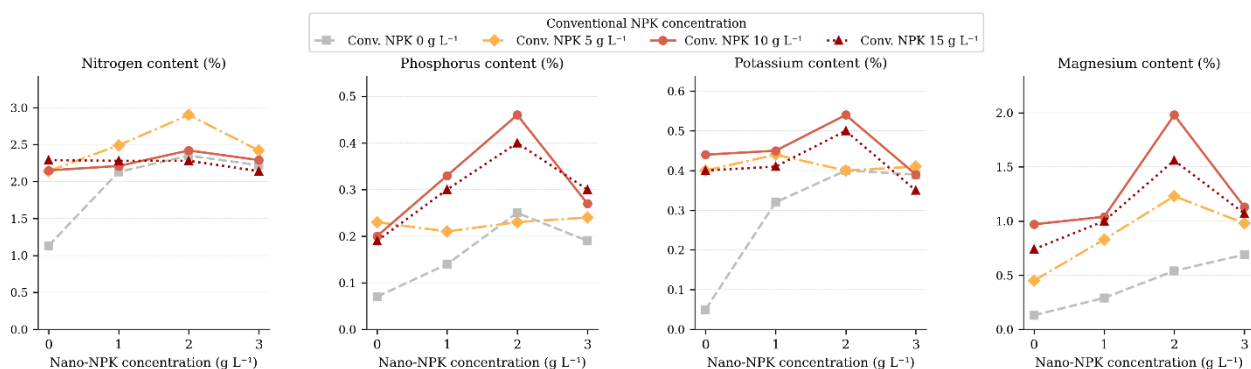
| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> )                            |         |         |         | Mean NPK conventional |
|-----------------------------|----------------------------------------------------------|---------|---------|---------|-----------------------|
|                             | 0                                                        | Nan(1g) | Nan(2g) | Nan(3g) |                       |
| 0                           | 14.00                                                    | 21.00   | 24.00   | 19.00   | 19.50                 |
| NPK(N) (5g)                 | 20.00                                                    | 21.00   | 23.00   | 21.00   | 21.25                 |
| NPK(N) (10g)                | 24.00                                                    | 24.00   | 32.00   | 25.00   | 26.25                 |
| NPK(N) (15g)                | 21.00                                                    | 23.00   | 30.00   | 21.00   | 23.50                 |
| Mean NPK nano               | 19.75                                                    | 22.25   | 27.25   | 21.25   |                       |
| LSD (P≤0.05)                | NPK(nan) = 1.27      NPK(N) = 1.27      To overlap= 1.57 |         |         |         |                       |

**Table 6.** The effect of fertilization with regular NPK's and nano-NPKs on the weight of sesame plants after fertilization (g).

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> )                            |         |         |         | Mean NPK conventional |
|-----------------------------|----------------------------------------------------------|---------|---------|---------|-----------------------|
|                             | 0                                                        | Nan(1g) | Nan(2g) | Nan(3g) |                       |
| 0                           | 15.04                                                    | 22.35   | 25.01   | 22.64   | 21.26                 |
| NPK(N) (5g)                 | 21.50                                                    | 28.70   | 29.42   | 27.07   | 26.67                 |
| NPK(N) (10g)                | 21.95                                                    | 30.24   | 37.17   | 31.51   | 30.21                 |
| NPK(N) (15g)                | 22.65                                                    | 27.11   | 30.04   | 29.67   | 27.36                 |
| Mean NPK nano               | 20.28                                                    | 27.10   | 30.41   | 27.72   |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.48      NPK(N) = 0.48      To overlap= 0.59 |         |         |         |                       |

**Table 7.** The effect of fertilization with regular NPK's and nan NPK's on the dry weight (g) of sesame plants.

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> )                            |         |         |         | Mean NPK conventional |
|-----------------------------|----------------------------------------------------------|---------|---------|---------|-----------------------|
|                             | 0                                                        | Nan(1g) | Nan(2g) | Nan(3g) |                       |
| 0                           | 1.20                                                     | 2.31    | 2.40    | 2.75    | 2.16                  |
| NPK(N) (5g)                 | 2.70                                                     | 3.38    | 3.77    | 3.97    | 3.45                  |
| NPK(N) (10g)                | 2.98                                                     | 3.02    | 4.78    | 4.12    | 3.72                  |
| NPK(N) (15g)                | 3.05                                                     | 3.17    | 3.97    | 4.00    | 3.54                  |
| Mean NPK nano               | 2.48                                                     | 2.97    | 3.73    | 3.71    |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.24      NPK(N) = 0.24      To overlap= 0.29 |         |         |         |                       |

**Figure 2.** Interaction plot (A×B) for seed nutrient content (N, P, K, Mg) showing the response surface across all treatment combinations.

### 3.3 Effects on chemical composition and nutrient content

Fertilizer application led to a significant increase in total chlorophyll content, indicating a high photosynthetic capacity. Nano-NPK at 2 g/L elevated chlorophyll to 35.42 mg/100g (62% increase over 21.91 mg/100g control) (Fig. 3). The combined treatment (10 g/L conventional and 2 g/L nano-NPK) recorded the highest chlorophyll content of 42.68mg/100g, which is 95 percent higher than the controls (Table 8). This significant increase in chlorophyll is highly correlated with changes in biomass, suggesting the highest efficiency in capturing light and carbon fixation. There was a significant effect of treatment on tissue nitrogen content. Conventional NPK at 5 g/L and nano-NPK at 2 g/L both achieved 2.48% nitrogen (27% increase over 1.95% control). Treatment with 5 g/L conventional and 2 g/L nano-NPK resulted in the greatest nitrogen reduction (2.90%, 49% increase), suggesting a synergistic improvement in nutrient uptake (Table 9). The phosphorus content showed drastic changes in response to fertilization. Conventional NPK at 10 g/L achieved 0.31% phosphorus (94% increase over 0.16% control), while nano-NPK at 2 g/L reached 0.33% (106% increase over 0.16% control). The combined treatment (10 g/L conventional + 2 g/L nano-NPK) maximized phosphorus accumulation, with a maximum of 0.46% (188% increase) in phosphorus bioavailability and uptake efficiency (Table 10). Fertilizer had a progressive increase in potassium concentration. Conventional NPK at 10 g/L produced 0.45% potassium (55% increase over 0.29% control), while nano-NPK at 2 g/L achieved 0.46% (44% increase over 0.32% control). The highest potassium concentration (0.54%) was recorded in the combined treatment (10 g/L conventional + 2 g/L nano-NPK), which was an 86% increase compared to the controls (Table 11). The greatest treatment responses were on magnesium content. Conventional NPK at 10 g/L elevated magnesium to 1.28% (212% increase over 0.41% control), while nano-NPK at 2 g/L reached 1.32% (132% increase over 0.57% control). Combined treatment recorded extraordinary magnesium accumulation of 1.98 percent (383 increase over the 0.41 percent control), indicating extraordinary improvements in micronutrient uptake mechanisms (Table 12).

**Table 8.** The effect of fertilization with regular NPK and nano-NPK on the amount of total Chlorophyll content (mg/100g fresh weight).

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |               |         | Mean NPK conventional |
|-----------------------------|-------------------------------|---------|---------------|---------|-----------------------|
|                             | 0                             | Nan(1g) | Nan(2g)       | Nan(3g) |                       |
| 0                           | 15.42                         | 20.17   | 26.34         | 25.73   | 21.91                 |
| NPK(N) (5g)                 | 21.53                         | 29.28   | 36.08         | 33.55   | 30.11                 |
| NPK(N) (10g)                | 26.78                         | 34.29   | 42.68         | 35.64   | 34.84                 |
| NPK(N) (15g)                | 25.65                         | 29.52   | 36.61         | 36.73   | 32.12                 |
| Mean NPK nano               | 22.34                         | 28.31   | 35.42         | 32.91   |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.41               |         | NPK(N) = 0.41 |         | To overlap= 0.51      |

**Table 9.** The effect of fertilization with regular NPK and Nano-NPK on the concentration of nitrogen (%) in the leaves of sesame plants.

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |               |         | Mean NPK conventional |
|-----------------------------|-------------------------------|---------|---------------|---------|-----------------------|
|                             | 0                             | Nan(1g) | Nan(2g)       | Nan(3g) |                       |
| 0                           | 1.13                          | 2.13    | 2.35          | 2.22    | 1.95                  |
| NPK(N) (5g)                 | 2.14                          | 2.49    | 2.90          | 2.42    | 2.48                  |
| NPK(N) (10g)                | 2.15                          | 2.21    | 2.42          | 2.29    | 2.26                  |
| NPK(N) (15g)                | 2.29                          | 2.28    | 2.28          | 2.14    | 2.24                  |
| Mean NPK nano               | 1.92                          | 2.27    | 2.48          | 2.26    |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.34               |         | NPK(N) = 0.34 |         | To overlap= 0.41      |



**Table 10.** The effect of fertilization with regular NPK and Nano-NPK, as well as their interactions with the phosphorus content of sesame plants

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |                |         | Mean NPK conventional |
|-----------------------------|-------------------------------|---------|----------------|---------|-----------------------|
|                             | 0                             | Nan(1g) | Nan(2g)        | Nan(3g) |                       |
| 0                           | 0.07                          | 0.14    | 0.25           | 0.19    | 0.16                  |
| NPK(N) (5g)                 | 0.23                          | 0.21    | 0.23           | 0.24    | 0.22                  |
| NPK(N) (10g)                | 0.20                          | 0.33    | 0.46           | 0.27    | 0.31                  |
| NPK(N) (15g)                | 0.19                          | 0.30    | 0.40           | 0.44    | 0.29                  |
| Mean NPK nano               | 0.17                          | 0.24    | 0.33           | 0.25    |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.008              |         | NPK(N) = 0.008 |         | To overlap= 0.01      |

**Table 11.** The effect of fertilization with regular NPK's and nano-NPKs on the percentage of potassium in sesame plants.

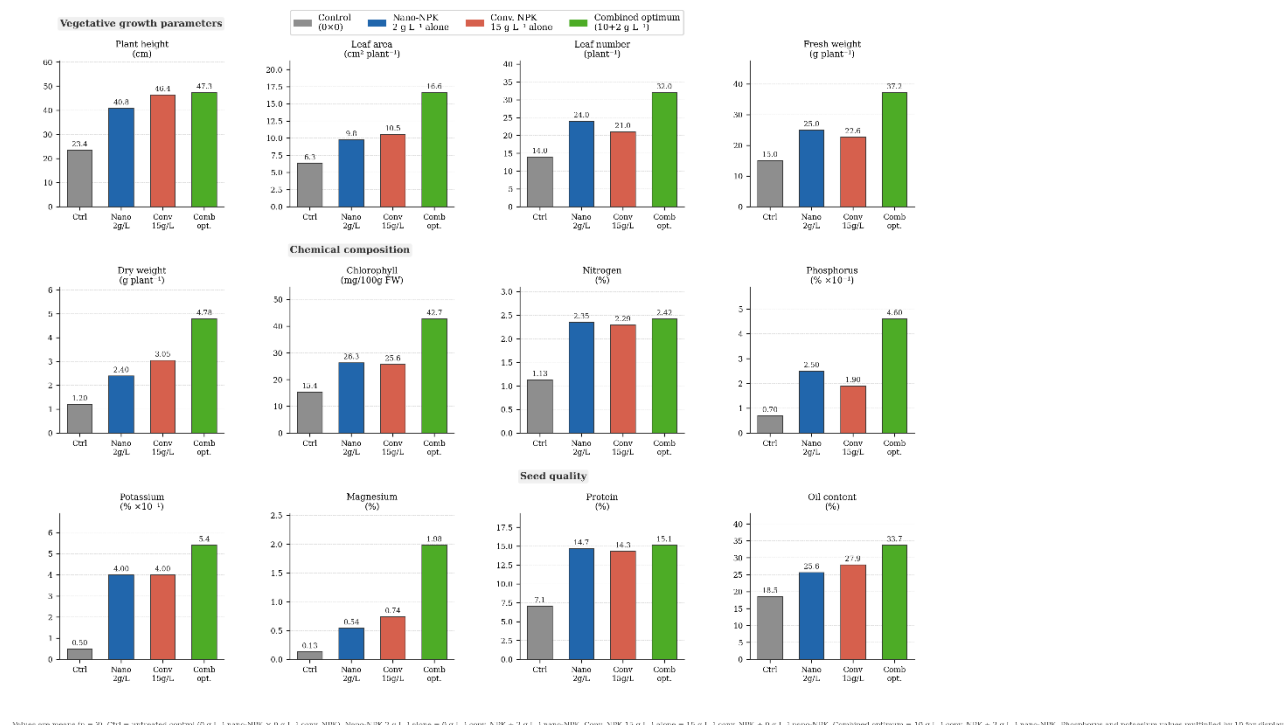
| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |                |         | Mean NPK conventional |
|-----------------------------|-------------------------------|---------|----------------|---------|-----------------------|
|                             | 0                             | Nan(1g) | Nan(2g)        | Nan(3g) |                       |
| 0                           | 0.05                          | 0.32    | 0.40           | 0.39    | 0.29                  |
| NPK(N) (5g)                 | 0.40                          | 0.44    | 0.40           | 0.41    | 0.41                  |
| NPK(N) (10g)                | 0.44                          | 0.45    | 0.54           | 0.39    | 0.45                  |
| NPK(N) (15g)                | 0.40                          | 0.41    | 0.50           | 0.35    | 0.41                  |
| Mean NPK nano               | 0.32                          | 0.40    | 0.46           | 0.38    |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.009              |         | NPK(N) = 0.009 |         | To overlap= 0.01      |

**Table 12.** The impact of fertilization with regular NPK and nano-NPK, as well as their interactions on the magnesium content (%) of sesame plants.

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |                |         | Mean NPK conventional |
|-----------------------------|-------------------------------|---------|----------------|---------|-----------------------|
|                             | 0                             | Nan(1g) | Nan(2g)        | Nan(3g) |                       |
| 0                           | 0.13                          | 0.29    | 0.54           | 0.69    | 0.41                  |
| NPK(N) (5g)                 | 0.45                          | 0.83    | 1.23           | 0.98    | 0.87                  |
| NPK(N) (10g)                | 0.97                          | 1.04    | 1.98           | 1.13    | 1.28                  |
| NPK(N) (15g)                | 0.74                          | 1.00    | 1.56           | 1.07    | 1.09                  |
| Mean NPK nano               | 0.57                          | 0.79    | 1.32           | 0.96    |                       |
| LSD (P≤0.05)                | NPK(nan) = 0.008              |         | NPK(N) = 0.008 |         | To overlap= 0.01      |

### 3.4. Effects on seed quality parameters

The fertilizer application has resulted in a significant increase in seed protein concentration, which is an indicator of improved nitrogen absorption and the formation of amino acids. The traditional NPK at 5 g/L and nano-NPK at 2 g/L yielded 15.54 percent protein (an increase of 27 percent compared to the 12.23 percent in the control). In the combined treatment, where the dosage was 5 g/L of conventional fertilizer and 2 g/L of nano-NPK fertilizer, the maximum protein percentage recorded was 18.12%, which is 48% higher than that of the control treatment, thus pointing out a synergic effect on protein production processes (Table 13). On the other hand, seed oil content, which is one of the vital quality characteristics of sesame, significantly responded to fertilization treatments. Using 2 g/L of nano-NPK led to an oil content value of 30.22%, which is 25% higher than the 24.20% recorded for the control treatment. Similarly, using the combined dose of 10 g/L of conventional and 2 g/L of nano-NPK resulted in the highest oil content of 33.68%, which is 39% higher (Table 14).



**Figure 3.** Comparison bar graph between nano-NPK and conventional NPK fertilizer efficiency under optimal dosages for all observed variables.

**Table 13.** The influence of conventional NPK and Nano-NPK fertilizers, as well as their interactions, on protein concentration in sesame plants (%) is presented below.

| NPK(N)<br>(g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |               |         | Mean NPK<br>conventional |
|--------------------------------|-------------------------------|---------|---------------|---------|--------------------------|
|                                | 0                             | Nan(1g) | Nan(2g)       | Nan(3g) |                          |
| 0                              | 7.06                          | 13.31   | 14.68         | 13.87   | 12.23                    |
| NPK(N) (5g)                    | 13.37                         | 15.56   | 18.12         | 15.12   | 15.54                    |
| NPK(N) (10g)                   | 13.43                         | 13.81   | 15.12         | 14.31   | 14.16                    |
| NPK(N) (15g)                   | 14.31                         | 14.25   | 14.25         | 13.30   | 14.02                    |
| Mean NPK nano                  | 12.04                         | 14.23   | 15.54         | 14.15   |                          |
| LSD (P≤0.05)                   | NPK(nan) = 0.51               |         | NPK(N) = 0.51 |         | To overlap= 0.62         |

**Table 14.** Impact of Normal and Low Doses of NPK on Fertilization and its Effect on Percentage Oil in Seed of Sesame (%)

| NPK(N) (g/L <sup>-1</sup> ) | NPK(nan) (g/L <sup>-1</sup> ) |         |               |         | Mean NPK<br>conventional |
|-----------------------------|-------------------------------|---------|---------------|---------|--------------------------|
|                             | 0                             | Nan(1g) | Nan(2g)       | Nan(3g) |                          |
| 0                           | 18.53                         | 27.87   | 25.64         | 24.77   | 24.20                    |
| NPK(N) (5g)                 | 27.41                         | 28.97   | 32.48         | 27.07   | 28.98                    |
| NPK(N) (10g)                | 29.69                         | 25.11   | 33.68         | 29.13   | 29.40                    |
| NPK(N) (15g)                | 27.95                         | 24.39   | 29.10         | 30.78   | 28.05                    |
| Mean NPK nano               | 25.89                         | 26.58   | 30.22         | 27.93   |                          |
| LSD (P≤0.05)                | NPK(nan) = 0.35               |         | NPK(N) = 0.35 |         | To overlap= 0.44         |

### 3.5 Discussion

The current research proves the positive influence of the application of either nano-NPK and conventional NPK fertilizers on the enhancement of vegetative parameters, chemical components, and seeds

in sesame plants. The high activity of the first one at much lower doses (2 g/L versus 15 g/L in the second case to obtain the same impact) is related to greater bioavailability and higher efficiency of the nutrient supply, which are caused by specific properties of nanosized particles. Indeed, small sizes (1-100 nm) and larger surface-to-volume ratios of nano-NPK particles facilitate their transportation into the plants, in contrast to traditional fertilizers [8,9]. These features help to accelerate the absorption process, enhance internal transport, and stimulate metabolic activities. Similar growth heights in response to both 2 g/L nano-NPK (46.50 cm) and 15 g/L conventional NPK (46.85 cm) mean that the efficiency of the former is 7.5 times higher than that of the latter and that nanofertilizers may contribute to significant reduction in fertilizer input without a decrease in productivity. These findings correlate with other studies that have established better growth characteristics of sesame plants under the influence of nano-fertilizers [23]. Although the traditional NPK fertilizers are applied in larger quantities, they still contributed to improved vegetative parameters owing to the provision of macronutrients to plants. However, these particles have low tissue mobility and lower dissolution rates, thus being unable to deliver nutrients promptly, in contrast to nanoparticles. The dose-dependent effects indicate that conventional NPK is sufficient for conducting physiological processes, including synthesis of chlorophylls and carbohydrates metabolism. Synergy between both nano- and conventional NPK solutions led to an increase in plant parameters. The combination of 10 g/L standard and 2 g/L nano-NPK was characterized by the highest chlorophyll content (42.68 mg/100 g, 95% improvement), thus facilitating higher photosynthesis rates due to enhanced nitrogen uptake. An increase in chlorophyll content is correlated directly with increased carbon fixation and biomass accumulation (75% and 121%, respectively). These findings are confirmed by other studies reporting the ability of nanofertilizers to enhance photosynthesis activity and growth due to increased bioavailability of nutrients [24]. All percentage changes were measured relative to the true control (0 g/L nano-NPK  $\times$  0 g/L conventional NPK). Protein measurements were made based on soluble protein determination with the Bradford method. Future research is suggested to use the Kjeldahl digestion method with the factor of conversion from nitrogen to protein mass ( $N \times 6.25$ ).

These changes in the nutritional makeup of tissues such as an increase in the percentage content of nitrogen (2.90, 157%), phosphorus (0.46, 557%), potassium (0.54, 980%), and magnesium (1.98, 1423%) show that implementation of fertilization strategies helps to regulate optimal accumulation and distribution of these nutrients. High concentration of nutrients causes the onset of metabolic reactions such as amino acid/protein synthesis, enzyme reaction, energy metabolism, and osmotic pressure [4,5,7]. Increased nitrogen concentration helps in protein synthesis; while increased phosphorus and potassium concentration cause energy formation and increased transport of carbohydrates and thus lead to efficient production of seeds having oil. The changes in the quality of seeds with respect to protein at 18.12% (148% improvement) and oil at 33.68% (82% improvement) show great economic and nutritional benefits. Nitrogen at higher amounts stimulates protein production, and sufficient quantities of phosphorus and potassium provide energy formation and metabolic transport mechanisms; altogether, they contribute towards efficient oil accumulation. Such findings concur with existing scientific literature on the positive effect of nano-selenium on the increase in oil production in sesame through similar metabolic processes [25]. Two application techniques, which combine quick-release nano-nitrogen-phosphorus-potassium (NPK) that provides nutrients instantly during the growth process with slower-released traditional NPK, could be a possible solution for providing nutrients to plants. The system is stress-resistant, productive, and avoids nutrient deficiencies during peak demand, and it does not indulge in luxury consumption or environmental waste. Although nanofertilizer has shown promising results, its application should be well-regulated. This may cause phytotoxicity or accumulation in the environment by overdose or unregulated use [26]. Consequently, careful adjustment of application rates is necessary to optimize the benefits of nano-NPK and reduce its potential environmental impacts. There is a need to conduct long-term research on the impacts of nanofertilizers on soil health, communities, and ecosystem services to support an extensive sustainability assessment.

The noted decrease in height of the plant, the leaf area, fresh weight, protein, and oil content at nano-NPK 3 g/L compared to 2 g/L is in line with a hormetic dose response pattern where the low and moderate concentrations of nanoparticles precipitate plant growth. On the contrary, high concentrations have the opposite effect, being inhibitory [30]. Nano-NPK particles can generate reactive oxygen species (ROS) on the

leaf surface, cause membrane rupture, and even disrupt ion transporter activity when used at concentrations higher than the optimum, thereby decreasing the effect of nutrient assimilation [12]. The stimulatory-inhibitory transition in this case (2-3 g/L) compares to the phytotoxicity levels of nanofertilizers in other crops [26]. Regarding environmental fate, nanofertilizer particles deposited foliarly can be stored in soil through leaf litter decomposition and through wash-off. Nanoparticles coated with chitosan are generally biodegradable. However, their interactions with soil microbiota and the potential for translocation into edible seed tissues are the focus of further investigation before a wide range of field recommendations can be provided on the subject. The next round of research must include nanoparticle imaging of seed tissue (e.g., TEM, ICP-MS elemental mapping) and the response of soil microbial communities to the application of nanofertilizers. The extraordinarily elevated percentage of magnesium gain (383 percent greater than the control) in response to the incorporated NPK treatment provides a mechanistic explanation, since NPK fertilizer does not provide Mg directly. The application of NPK stimulates the uptake of Mg via a variety of indirect mechanisms: (i) the root growth and root hair proliferation under the influence of nitrogen raises the volume of soil exploration and consequently, more contact with  $Mg^{2+}$  in the soil solution; (ii) the mycorrhizal colonization caused by the application of phosphorus stimulates the uptake of Mg substantially; (iii) the reduction in the K<sup>+</sup>-Mg<sup>2+</sup> antagonism at the root cation transporters results because of improved nutritional balance in plant tissue. The synergistic processes occurring explain the increased Mg accumulation at the optimum combined treatment values.

#### 4. Conclusions

This research indicates that nano-NPK fertilizers have a profound effect on sesame growth, nutrient content, and seed quality, which is much more effective than conventional NPK fertilizers. The growth and yield parameters of nano-NPK at 2 g/L were similar to those of conventional NPK at 15 g/L, resulting in a 6.7-fold increase in nutrient use efficiency. The combination application strategy (10 g/L conventional NPK + 2 g/L nano-NPK) showed the highest improvement across all parameters: chlorophyll content increased by 95 percent, tissue nutrient concentrations improved by 39.2-383 percent, seed protein content increased by 48.2 percent, and oil increased by 39.2 percent. These results suggest that nano-fertilizers, especially when used in combination with conventional fertilizers, provide a viable and cost-effective solution to intensify sesame production and reduce fertilizer use and their environmental effects. Future research needs to address the long-term consequences for soil health, microbial communities, financial viability, and environmental sustainability to develop holistic recommendations for the use of nanofertilizers in sesame farming systems.

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# Effect of Conventional and Nano Potassium on the Chemical Characteristics of Alfalfa (*Medicago sativa* L.) under Different Levels of Water Stress

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**Abstract:** This study was conducted to evaluate the effect of conventional potassium fertilization and nano-potassium application on some chemical characteristics of alfalfa (*Medicago sativa* L.) under different levels of water stress. The experiment was carried out in a randomized complete block design (RCBD) with three replicates, using a factorial arrangement. The treatments included three concentrations of nano-potassium (1, 2, and 3 g L<sup>-1</sup>), three levels of conventional potassium added to soil (4, 6, and 8 g kg<sup>-1</sup> soil), and a control treatment without potassium fertilization. Four irrigation levels representing water stress were applied: 100%, 75%, 50%, and 25% of field capacity. Several chemical characteristics were measured, including chlorophyll content, proline concentration, mineral nutrient content (N, P, and K), protein percentage, and total carbohydrates in the leaves. The results indicated that nano-potassium application significantly increased chlorophyll content, protein percentage, and total carbohydrates compared with the control treatment. Conventional potassium fertilization significantly improved mineral nutrient concentrations, particularly potassium in plant tissues. Water stress levels significantly affected most of the studied traits, with moderate levels enhancing some physiological responses. The interaction between potassium treatments and irrigation levels showed significant effects on several chemical characteristics of alfalfa plants. Overall, potassium nutrition, especially in nano form, improved chemical characteristics and enhanced alfalfa plants' tolerance to water stress. These findings highlight the potential role of nano-potassium fertilization in improving plant performance and forage quality under limited water availability.

**Keywords:** Alfalfa; water stress; conventional potassium fertilizer; nano-potassium fertilizer

## 1. Introduction

Alfalfa (*Medicago sativa* L.) is considered one of the most important perennial forage crops worldwide. This importance stems from its numerous agricultural and nutritional characteristics. It has a high ability to adapt to a wide range of environmental conditions, in addition to its high nutritional value due to its elevated protein content, high biomass productivity, and high digestibility for ruminant animals. Moreover, alfalfa can fix atmospheric nitrogen through its symbiotic relationship with root-nodule bacteria, thereby improving soil fertility and reducing the need for nitrogen fertilizers [1, 2]. Furthermore, producing this crop is relatively less expensive than that of many other forage crops, thereby

enhancing its economic importance in agricultural production systems [3, 4]. Alfalfa belongs to the family Fabaceae, which is considered one of the largest plant families in the plant kingdom [5].

This crop is particularly well-suited to arid and semi-arid environments, where it is widely used in livestock feeding due to its high protein content, which may reach about 20%. In addition, it contains carotenoids, vitamins, and minerals that improve forage quality and enhance animal production efficiency [6, 7]. Alfalfa has also been used in folk medicine due to its bioactive compounds, which possess antioxidant and anti-inflammatory properties. It has been traditionally used to treat conditions such as arthritis, digestive disorders, and metabolic problems. Recent studies have also shown its effectiveness in supporting the central nervous system and helping in the treatment of obesity [8, 9]. Despite the agricultural and economic importance of this crop, its productivity is greatly affected by environmental factors, particularly abiotic stresses. These stresses influence plant growth, physiological processes, biomass accumulation, and forage quality [2]. Water stress resulting from drought is considered one of the most important limiting factors affecting agricultural production in many regions of the world [10]. This problem has intensified in recent decades due to declining water resources, reduced and irregular rainfall, and the increasing impacts of climate change. These factors have negatively affected agricultural production and reduced cultivated areas, especially amid the increasing demand for food driven by rapid population growth.

Water deficiency causes several morphological and physiological changes in plants [11]. It disrupts cellular water balance, leads to stomatal closure, and reduces photosynthesis by decreasing chlorophyll content. Water stress also disrupts the balance between the production of Reactive Oxygen Species (ROS) and antioxidant defense systems, leading to the accumulation of ROS and oxidative stress, which damages proteins, membrane lipids, and various cellular components [12]. Consequently, plant productivity and crop quality decline under drought conditions [13, 14]. Many studies indicate that plant mineral nutritional status plays a fundamental role in enhancing their ability to tolerate environmental stresses [15]. Potassium is considered an essential macronutrient for plant growth and development, as it plays a crucial role in regulating many physiological processes [16]. In alfalfa in particular, the demand for potassium increases to ensure continued growth and maintain forage productivity [17]. Potassium contributes to the activation of many enzymes involved in biosynthetic processes, such as protein and starch synthesis, and the production of the energy molecule adenosine triphosphate (ATP) [18]. It also plays an important role in regulating stomatal movement and maintaining ionic balance within plant cells, in addition to its roles in carbohydrate transport through the phloem and in regulating plant water balance [19]. Moreover, potassium enhances plant tolerance to various biotic and abiotic stresses [20, 21]. Several studies have demonstrated that the use of conventional potassium fertilizers, such as potassium sulfate, can improve the growth and productivity of alfalfa, especially under water-stress conditions [17].

In recent decades, agricultural technologies aimed at managing plant stresses have witnessed significant development, among which nanotechnology applications in agriculture have emerged as one of the most prominent innovations. This technology represents a modern approach that aims to improve nutrient-use efficiency, increase crop productivity, and reduce the negative environmental impacts of conventional agricultural inputs [22]. Nanoparticles have demonstrated a high capacity to penetrate plant cellular barriers, thereby enhancing the efficiency of nutrient absorption and their translocation within plant tissues due to their small size, large surface area, and high solubility [23]. The high efficiency and concentration of these fertilizers enhance the ability of nutrient-loaded nanoparticles to penetrate plant surfaces, thereby increasing the interaction area between nanoparticles and plant tissues. This, in turn, enhances physiological processes, particularly photosynthesis. As a result, plant dry matter content increases, confirming the high efficiency of nano-fertilizers used in small quantities, while achieving high productivity in both quantity and quality [24]. Recent studies also indicate that integrating nanoparticles into agricultural practices can improve both quantitative and qualitative crop traits, particularly under exposure to different environmental stresses. This is attributed to nanoparticles' ability to influence molecular signaling pathways in plants, activate antioxidant defense systems, regulate plant hormones, and enhance nutrient absorption efficiency and distribution within plant tissues [25, 26]. Therefore, this technology represents a promising approach for developing more efficient and environmentally sustainable agricultural production systems.

## 2. Materials and Methods

### 2.1 Study Site

The experiment was conducted in a nursery belonging to the Directorate of Agriculture of Al-Muthanna, in the center of Al-Samawah city, on 4 October 2025, to study the effects of conventional and nano forms of potassium on reducing the impact of water stress on alfalfa (*Medicago sativa* L.) growth. Local alfalfa seeds were obtained from the local market and manually cleaned to remove impurities before sowing. The seeds were planted in plastic pots with a capacity of 20 kg, filled with 11 kg of a growth medium consisting of loamy soil mixed with peat moss at a 1:3 ratio (soil:peat moss). Before planting, a representative soil sample was collected to conduct some physical and chemical analyses in the U-Science Laboratory in Al-Diwaniyah city, as shown in Table 1 according to the methods described by John [27] and Ryan et al. [28].

**Table 1.** Physical and chemical properties of the experimental soil before planting

| Property            | Value      | Unit               |
|---------------------|------------|--------------------|
| pH                  | 7.30       | –                  |
| EC                  | 2.06       | ms/cm              |
| Organic matter (OM) | 1.12       | %                  |
| CaCO <sub>3</sub>   | 36         | %                  |
| CaSO <sub>3</sub>   | 1.15       | %                  |
| Bulk density        | 1.13       | g·cm <sup>-3</sup> |
| N available         | 2.8        | mg/kg              |
| K available         | 108.65     | mg/kg              |
| P available         | 7.48       | mg/kg              |
| K soluble           | 51.43      | mg/kg              |
| Na                  | 273.6      | mg/kg              |
| Ca                  | 216        | mg/kg              |
| Cl                  | 441        | mg/kg              |
| Mg                  | 200.08     | mg/kg              |
| HCO <sub>3</sub>    | 280        | mg/kg              |
| SO <sub>4</sub>     | 878.42     | mg/kg              |
| Sand                | 68.4       | %                  |
| Silt                | 20.5       | %                  |
| Clay                | 11.1       | %                  |
| Soil texture        | Sandy loam | –                  |

### 2.2 Experimental Design

The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replicates. The first factor was the source of potassium, comprising seven treatments: conventional potassium fertilizer applied at three levels (4, 6, and 8 g kg<sup>-1</sup> soil), where 6 g kg<sup>-1</sup> represents the standard recommended rate; nano-potassium fertilizer applied at three concentrations (1, 2, and 3 g L<sup>-1</sup>), where 2 g L<sup>-1</sup> represents the concentration recommended by the producing company; and an unfertilized control. The second factor consisted of four water-stress levels applied as percentages of field capacity: 100% (well-watered control), 75%, 50%, and 25%. Accordingly, the experiment comprised 84 experimental units resulting from the full factorial combination of seven potassium treatments and four irrigation levels with three replicates (7 × 4 × 3).

### 2.3 Experimental Treatments

Conventional potassium sulfate fertilizer (K<sub>2</sub>SO<sub>4</sub>; K<sub>2</sub>O content: 50%; purity ≥98%) obtained from a local agricultural office was applied at three concentrations (4, 6, and 8 g kg<sup>-1</sup> soil), equivalent to 44, 66, and 88 g pot<sup>-1</sup>, based on the soil weight per pot (11 kg). Fertilizer was applied to the soil after alfalfa plant germination in two doses. Nano potassium fertilizer produced by KHAZRA Company (Iran) was used. According to the manufacturer's technical data sheet, the product contains 10% K<sub>2</sub>O (as K) with a nominal particle size ≤50 nm. It is acknowledged that independent physicochemical verification (e.g., TEM, DLS, XRD) of particle

characteristics was not conducted, which represents a limitation of the present study. Three concentrations (1, 2, and 3 g L<sup>-1</sup>) were prepared by dissolving them in distilled water. The treatments were applied as foliar sprays in two applications, following the protocols reported for other crops [29]. Four irrigation levels were applied according to field capacity: 100% (well-watered control), 75%, 50%, and 25%.

#### 2.4 Determination of Soil Field Capacity

The field capacity of the soil was determined gravimetrically following the method of Klute [30], using three pots each filled with 11 kg of air-dried soil. The soil was irrigated to complete saturation, then covered with plastic bags to minimize evaporation losses and left to drain for 48 hours until gravitational water drainage from the bottom holes ceased, after which the pots were reweighed. Field capacity was calculated from the difference between wet and dry soil weight, yielding an average gravimetric field capacity of 25%, based on which the four irrigation treatments were set at 25%, 50%, 75%, and 100% of field capacity, corresponding to approximate water additions of 0.7, 1.4, 2.0, and 2.7 L per pot, respectively.

#### 2.5 Studied Chemical Traits and Measurement Methods

Total chlorophyll content in leaves was determined according to the method described by Goodwin [31]. A 0.5 g leaf sample of alfalfa was ground with 10 ml of 80% acetone. The extract was kept in darkness in a refrigerator for at least 30 minutes to ensure efficient extraction. The extract was then filtered or centrifuged to obtain a clear solution. Absorbance was measured at 663, 645, and 470 nm using a spectrophotometer, with 80% acetone as the blank. Chlorophyll concentration was calculated according to the following equations:

$$\text{Chlorophyll a} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times 2$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times 2$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Plant samples were digested according to the method of Cresser and Parsons [32] after drying the shoot samples in an oven at 70°C for 48 hours until constant weight. A 0.2 g sample of dry powder was mixed with 10 ml concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and 2 ml perchloric acid (HClO<sub>4</sub>), then heated on a hot plate until a clear digest was obtained. After cooling, the volume was completed to 100 ml with distilled water. Total nitrogen was determined by the Kjeldahl method [33] according to the AOAC International standard procedures [34]. Phosphorus was determined using the Ammonium Molybdate–Ascorbic Acid method, and absorbance was measured at 882 nm using a UV-Visible spectrophotometer, as described by John [35]. Potassium was determined using a Flame Emission Photometer at 766.5 nm, according to the AOAC method [36]. Proline content was determined according to the method described by Bates et al. [37]. A 0.1 g dry sample was ground with 10 ml of 3% sulfosalicylic acid. The extract was centrifuged for 10 minutes and filtered. Then 1.5 ml of filtrate was mixed with 1.5 ml acetic acid and 1.5 ml acid ninhydrin solution, and the tubes were heated in a boiling water bath for 30 minutes. The reaction was stopped by placing the tubes in an ice bath. Afterward, 4 mL toluene was added and mixed using a vortex mixer. The upper toluene layer was separated, and its absorbance was measured at 520 nm using a UV-Visible spectrophotometer. Proline concentration was determined from a standard curve prepared with varying proline concentrations. Protein percentage in plant samples was calculated by multiplying the nitrogen percentage by the conversion factor of 6.25, as described by Kalra [38] and Vopyan [39].

$$\text{Protein (\%)} = \text{N\%} \times 6.25$$

Total carbohydrates were determined according to the method described by Joslyn [40]. A 0.29 g dry sample was mixed with 5 ml of perchloric acid and heated in a water bath at 60°C for one hour, with the process repeated three times. The supernatant was separated by centrifugation (15 min, 3000 rpm). One ml of the extract was diluted to 100 ml with distilled water, and 1 ml of the diluted solution was mixed with 1 ml of 5% phenol and 5 ml concentrated sulfuric acid, then left to cool. Absorbance was measured at 490 nm using a spectrophotometer, and readings were recorded in mg L<sup>-1</sup>. Carbohydrate percentage was calculated using the following equation:



Carbohydrates (%) = (Instrument reading  $\times$  (extract volume / sample weight)  $\times$  dilution factor) / 10000

## 2.6 Statistical analysis

All experimental data were analyzed using the Statistical Analysis System (SAS) program according to a factorial experiment within a Randomized Complete Block Design (RCBD). Significant differences among treatment means were determined using Duncan's Multiple Range Test at a probability level of 0.05.

## 3. Results and Discussion

### 3.1 Effect of conventional potassium fertilization and foliar application of nano-potassium under different levels of water stress on some chemical traits of alfalfa

#### 3.1.1 Total chlorophyll concentration in leaves ( $\text{mg } 100 \text{ g}^{-1}$ )

The results of the statistical analysis presented in Table 2 showed that foliar application of nano-potassium significantly increased chlorophyll concentration in alfalfa leaves. The spraying treatments (1, 2, and 3  $\text{g L}^{-1}$ ) significantly increased chlorophyll content compared with the control treatment, which recorded 100.59  $\text{mg } 100 \text{ g}^{-1}$ , while the highest mean value was observed in the 1  $\text{g L}^{-1}$  treatment, reaching 135.01  $\text{mg } 100 \text{ g}^{-1}$ . The results also indicated that soil-applied potassium fertilizer significantly increased chlorophyll concentration. The treatment 4  $\text{g kg}^{-1}$  soil recorded the highest mean value of 128.76  $\text{mg } 100 \text{ g}^{-1}$  compared with the control treatment. Water stress levels also significantly affected chlorophyll concentration. Chlorophyll content increased with increasing water stress levels, where the highest mean value (120.04  $\text{mg } 100 \text{ g}^{-1}$ ) was recorded at 50% irrigation level, with no significant difference compared with 75% irrigation level, while the lowest mean (105.79  $\text{mg } 100 \text{ g}^{-1}$ ) was recorded at 100% irrigation level. Regarding the interaction between experimental factors, the combination of nano-potassium at 2  $\text{g L}^{-1}$  and a 50% irrigation level yielded the highest mean chlorophyll concentration (154.69  $\text{mg } 100 \text{ g}^{-1}$ ), with no significant difference from the 3  $\text{g L}^{-1}$  nano-potassium at the same irrigation level. In contrast, the lowest mean value (70.53  $\text{mg } 100 \text{ g}^{-1}$ ) was recorded for the interaction between soil potassium treatment at 8  $\text{g kg}^{-1}$  and irrigation level of 50%.

**Table 2.** Effect of conventional and nano potassium fertilization on the mean total chlorophyll concentration ( $\text{mg } 100 \text{ g}^{-1}$ ) in alfalfa leaves under water stress conditions

| Potassium treatments          | 100%     | 75%      | 50%      | 25%      | Mean     |
|-------------------------------|----------|----------|----------|----------|----------|
| Control                       | 86.43 q  | 102.38 m | 108.39 k | 105.17 l | 100.59 d |
| Nano K (2 $\text{g L}^{-1}$ ) | 102.38 m | 145.50 c | 154.69 a | 127.74 h | 132.58 b |
| Nano K (3 $\text{g L}^{-1}$ ) | 102.90 m | 150.21 b | 153.55 a | 121.88 i | 132.14 b |
| Nano K (1 $\text{g L}^{-1}$ ) | 133.45 f | 142.64   | 141.19 d | 122.77 i | 135.01 a |
| K (4 $\text{g kg}^{-1}$ )     | 131.60 g | 127.13 h | 139.39 e | 116.90 j | 128.76 c |
| K (6 $\text{g kg}^{-1}$ )     | 99.65 n  | 82.76 r  | 72.51 s  | 90.54 p  | 86.37 e  |
| K (8 $\text{g kg}^{-1}$ )     | 84.10 r  | 86.09 q  | 70.53 t  | 92.88 o  | 83.40 f  |
| Mean                          | 105.79 c | 119.53 a | 120.04 a | 111.13 b |          |

\*Means with the same letter are not significantly different according to Duncan's Multiple Range Test at  $P \leq 0.05$ .

#### 3.1.2 Proline concentration ( $\text{mg } 100 \text{ g}^{-1}$ )

The results of the statistical analysis presented in Table 3 indicate that foliar application of nano-potassium had a significant effect on proline concentration in alfalfa leaves. The spraying treatments (1, 2, and 3  $\text{g L}^{-1}$ ) reduced proline concentration compared with the control treatment, which recorded 81.25  $\text{mg } 100 \text{ g}^{-1}$ ; the lowest mean value was observed at 1  $\text{g L}^{-1}$  (79.13  $\text{mg } 100 \text{ g}^{-1}$ ). In contrast, soil application of conventional potassium fertilizer showed no significant differences in proline concentration compared with the control treatment. The lowest mean value was 80.92  $\text{mg } 100 \text{ g}^{-1}$  in the treatment 4  $\text{g kg}^{-1}$ , while the highest mean value was 81.98  $\text{mg } 100 \text{ g}^{-1}$  in the treatment 6  $\text{g kg}^{-1}$ , without a significant difference compared with the treatment 8  $\text{g kg}^{-1}$ . Water stress levels also significantly affected proline concentration. The highest mean value (81.00  $\text{mg } 100 \text{ g}^{-1}$ ) was recorded at the 100% irrigation level, while the lowest mean value (80.22  $\text{mg } 100 \text{ g}^{-1}$ ) was observed at the 50% irrigation level. Regarding the interaction between the experimental factors, the interaction between the control treatment and the 25% irrigation level recorded the highest mean proline concentration in leaves

(86.66 mg 100 g<sup>-1</sup>). In contrast, the lowest mean value (73.63 mg 100 g<sup>-1</sup>) was recorded for the interaction between the control treatment and irrigation level of 100%.

**Table 3.** Effect of conventional and nano potassium fertilization on the mean proline concentration (mg 100 g<sup>-1</sup>) in alfalfa leaves under water stress conditions

| Potassium treatments          | 100%      | 75%       | 50%       | 25%       | Mean     |
|-------------------------------|-----------|-----------|-----------|-----------|----------|
| Control                       | 73.63 j   | 81.77 cd  | 82.95 bc  | 86.66 a   | 81.25 ab |
| Nano K (2 g L <sup>-1</sup> ) | 81.94 cd  | 80.33 def | 81.10 cde | 78.08 ghi | 80.36 c  |
| Nano K (3 g L <sup>-1</sup> ) | 84.13 b   | 78.95 fgh | 79.30 e-h | 78.10 ghi | 80.12 c  |
| Nano K (1 g L <sup>-1</sup> ) | 84.12 b   | 78.16 ghi | 76.72 i   | 77.52 hi  | 79.13 d  |
| K (4 g kg <sup>-1</sup> )     | 82.00 cd  | 80.33 def | 81.03 de  | 80.31 def | 80.92 bc |
| K (6 g kg <sup>-1</sup> )     | 80.94 de  | 81.43 cd  | 81.06 de  | 84.50 b   | 81.98 a  |
| K (8 g kg <sup>-1</sup> )     | 80.21 def | 84.50 b   | 79.39 efg | 81.11 cde | 81.30 ab |
| Mean                          | 81.00 a   | 80.78 ab  | 80.22 b   | 80.90 b   |          |

\*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at  $P \leq 0.05$ .

### 3.1.3 Mineral content

#### 3.1.3.1 Nitrogen concentration (%)

The results of the statistical analysis presented in Table 4 indicate that foliar application of nano-potassium had no significant effect on nitrogen concentration in alfalfa leaves compared with the control treatment, which recorded 3.30%. However, the highest mean value (4.10%) was observed in the 2 g L<sup>-1</sup> treatment, with no significant difference compared with the 1 and 3 g L<sup>-1</sup> treatments. Similarly, soil application of conventional potassium fertilizer showed no significant differences in nitrogen concentration compared with the control treatment, although the highest mean value (3.70%) was recorded in the 4 g kg<sup>-1</sup> treatment. In contrast, water stress levels significantly affected nitrogen concentration. Nitrogen values decreased with increasing water stress levels. The highest mean value (3.94%) was recorded at the 100% irrigation level, with no significant difference compared with 75% and 25% irrigation levels, while the lowest mean value (2.30%) was observed at the 50% irrigation level. Regarding the interaction between potassium fertilization treatments and water stress levels, the nano-potassium at 2 g L<sup>-1</sup> and 100% irrigation level recorded the highest leaf nitrogen concentration (4.80%), with no significant difference from other interactions. In contrast, the lowest mean value (2.30%) was recorded for the interaction between conventional potassium treatment at 8 g kg<sup>-1</sup> and an irrigation level of 50%.

**Table 4.** Effect of conventional and nano potassium fertilization on the mean nitrogen concentration (%) in alfalfa leaves under water stress conditions

| Potassium treatments          | 100%     | 75%      | 50%      | 25%      | Mean     |
|-------------------------------|----------|----------|----------|----------|----------|
| Control                       | 3.60 abc | 3.40 abc | 3.20 abc | 3.00 abc | 3.30 abc |
| Nano K (2 g L <sup>-1</sup> ) | 4.80 a   | 4.40 ab  | 3.80 abc | 3.40 abc | 4.10 a   |
| Nano K (3 g L <sup>-1</sup> ) | 4.40 ab  | 3.60 abc | 3.60 abc | 3.60 abc | 3.80 ab  |
| Nano K (1 g L <sup>-1</sup> ) | 4.40 abc | 4.00 abc | 3.40 abc | 4.20 abc | 4.00 ab  |
| K (4 g kg <sup>-1</sup> )     | 4.00 abc | 3.40 abc | 3.60 abc | 3.80 abc | 3.70 abc |
| K (6 g kg <sup>-1</sup> )     | 2.80 abc | 2.60 bc  | 2.60 bc  | 3.40 abc | 2.85 c   |
| K (8 g kg <sup>-1</sup> )     | 3.60 abc | 3.00 abc | 2.30 c   | 3.60 abc | 3.13 bc  |
| Mean                          | 3.94 a   | 3.49 ab  | 3.21 b   | 3.57 ab  |          |

\*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at  $P \leq 0.05$ .

#### 3.1.3.2 Phosphorus concentration (%)

The results of the statistical analysis presented in Table 5 indicate that foliar application of nano-potassium had a significant effect on phosphorus concentration in alfalfa leaves. The spraying treatments (1, 2, and 3 g L<sup>-1</sup>) significantly increased phosphorus concentration compared with the control treatment, which recorded 0.580%, while the highest mean value (0.724%) was observed in treatment 1 g L<sup>-1</sup>. Similarly, soil

application of conventional potassium fertilizer showed a significant increase in phosphorus concentration compared with the control treatment. The 6 g kg<sup>-1</sup> treatment recorded the highest mean value (0.652%), with no significant difference from the 4 g kg<sup>-1</sup> treatment. Water stress levels also significantly affected phosphorus concentration. Phosphorus content increased with increasing water stress levels. The highest mean value (0.750%) was recorded at the 50% irrigation level, whereas the lowest mean value (0.473%) was observed at the 100% irrigation level. Regarding the interaction between potassium fertilization treatments and water stress levels, the nano-potassium treatments (2, 1, and 3 g L<sup>-1</sup>) and the 50% irrigation level recorded the highest mean phosphorus concentrations, without significant differences among them. The highest value (0.893%) was recorded in treatment 2 g L<sup>-1</sup> at 50% irrigation level. In comparison, the lowest mean value (0.243%) was observed in the interaction between the control treatment and irrigation level of 100%, with no significant difference from the interaction between nano-potassium treatment (2 g L<sup>-1</sup>) at the same irrigation level.

**Table 5.** Effect of conventional and nano potassium fertilization on the mean phosphorus concentration (%) in alfalfa leaves under water stress conditions

| Potassium treatments          | 100%      | 75%       | 50%       | 25%       | Mean     |
|-------------------------------|-----------|-----------|-----------|-----------|----------|
| Control                       | 0.243 i   | 0.609 c-f | 0.710 b-e | 0.660 b-f | 0.556 c  |
| Nano K (2 g L <sup>-1</sup> ) | 0.340 hi  | 0.715 b-e | 0.893 a   | 0.748 bc  | 0.674 ab |
| Nano K (3 g L <sup>-1</sup> ) | 0.444 gh  | 0.670 b-f | 0.875 a   | 0.659 b-f | 0.662 ab |
| Nano K (1 g L <sup>-1</sup> ) | 0.533 fg  | 0.700 b-e | 0.880 a   | 0.782 ab  | 0.724 a  |
| K (4 g kg <sup>-1</sup> )     | 0.573 efg | 0.659 b-f | 0.659 b-f | 0.700 b-e | 0.648 b  |
| K (6 g kg <sup>-1</sup> )     | 0.650 b-f | 0.659 b-f | 0.651 b-f | 0.649 b-f | 0.652 b  |
| K (8 g kg <sup>-1</sup> )     | 0.526 fg  | 0.723 bcd | 0.585 def | 0.659 b-f | 0.623 b  |
| Mean                          | 0.473 c   | 0.676 b   | 0.750 a   | 0.694 b   |          |

\*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at  $P \leq 0.05$ .

### 3.1.3.3 Potassium concentration (%)

The results of the statistical analysis presented in Table 6 indicate that foliar application of nano-potassium had a significant effect on potassium concentration in alfalfa leaves. The 3 g L<sup>-1</sup> treatment showed a significant increase compared with the control, which recorded 1.53%, achieving the highest mean value of 2.05%. Similarly, soil-applied conventional potassium fertilizer resulted in a significant increase in leaf potassium concentration. The treatment 8 g kg<sup>-1</sup> recorded the highest mean value of 3.79% compared with the control treatment. Water stress also significantly affected potassium concentration. Potassium concentration decreased with increasing water stress levels. The highest mean value (3.25%) was recorded at the 100% irrigation level, while the lowest mean value (2.01%) was observed at the 25% irrigation level. Regarding the interaction between potassium fertilization treatments and water stress levels, the interaction between conventional potassium at 8 g kg<sup>-1</sup> and irrigation level of 100% recorded the highest mean potassium concentration in leaves (4.83%), with no significant difference compared with the interaction of the same treatment at 75% irrigation level, as well as the interaction between 6 g kg<sup>-1</sup> potassium and 100% irrigation level. In contrast, the lowest mean value (0.88%) was recorded for the interaction between the control treatment and the 50% irrigation level.

### 3.1.4 Total protein concentration (%)

The results of the statistical analysis presented in Table 7 indicate that foliar application of nano-potassium had a significant effect on protein concentration in alfalfa leaves. The treatments significantly increased protein concentration compared with the control treatment, which recorded 21.25%, where the 2 g L<sup>-1</sup> treatment recorded the highest mean value (25.63%), with no significant difference compared with the 1 g L<sup>-1</sup> treatment. Similarly, soil application of conventional potassium fertilizer significantly increased protein concentration. The treatment 4 g kg<sup>-1</sup> recorded the highest mean value (22.50%) compared with the control treatment. Water stress levels also significantly affected protein content. Protein concentration decreased with increasing water stress levels. The highest mean value (24.64%) was recorded at the 100% irrigation level, while the lowest mean value (20.12%) was recorded at the 50% irrigation level. Regarding the interaction

between potassium fertilization treatments and water stress levels, the nano-potassium treatment ( $2 \text{ g L}^{-1}$ ) at 100% irrigation level achieved the highest mean leaf protein concentration (30.00%). In contrast, the lowest mean value (14.58%) was recorded for the interaction between conventional potassium treatment ( $8 \text{ g kg}^{-1}$ ) and irrigation level of 50%.

**Table 6.** Effect of conventional and nano potassium fertilization on the mean potassium concentration (%) in alfalfa leaves under water stress conditions

| Potassium treatments            | 100%     | 75%      | 50%      | 25%      | Mean    |
|---------------------------------|----------|----------|----------|----------|---------|
| Control                         | 2.20 f–k | 1.75 h–m | 0.88 m   | 1.28 klm | 1.53 d  |
| Nano K ( $2 \text{ g L}^{-1}$ ) | 2.43 f–j | 2.31 f–k | 1.51 i–m | 1.39 j–m | 1.91 cd |
| Nano K ( $3 \text{ g L}^{-1}$ ) | 2.91 c–g | 1.93 g–l | 1.85 h–m | 1.51 i–m | 2.05 c  |
| Nano K ( $1 \text{ g L}^{-1}$ ) | 2.52 e–i | 1.87 h–m | 1.15 lm  | 1.78 h–m | 1.83 cd |
| K ( $4 \text{ g kg}^{-1}$ )     | 3.53 bcd | 2.91 c–g | 3.63 bc  | 2.03 f–l | 3.03 b  |
| K ( $6 \text{ g kg}^{-1}$ )     | 4.35 ab  | 3.49 b–e | 3.00 c–f | 2.39 f–j | 3.31 b  |
| K ( $8 \text{ g kg}^{-1}$ )     | 4.83 a   | 4.02 ab  | 2.61 d–g | 3.69 bc  | 3.79 a  |
| Mean                            | 3.25 a   | 2.61 b   | 2.09 c   | 2.01 c   |         |

\*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at  $P \leq 0.05$ .

**Table 7.** Effect of conventional and nano potassium fertilization on the mean total protein concentration (%) in alfalfa leaves under water stress conditions

| Potassium treatments            | 100%     | 75%      | 50%      | 25%      | Mean    |
|---------------------------------|----------|----------|----------|----------|---------|
| Control                         | 22.50 ef | 21.25 fg | 22.50 ef | 18.75 hi | 21.25 d |
| Nano K ( $2 \text{ g L}^{-1}$ ) | 30.00 a  | 27.50 b  | 23.75 de | 21.25 fg | 25.63 a |
| Nano K ( $3 \text{ g L}^{-1}$ ) | 27.50 b  | 22.50 ef | 22.50 ef | 22.50 ef | 23.75 b |
| Nano K ( $1 \text{ g L}^{-1}$ ) | 27.50 b  | 25.00 cd | 21.25 fg | 26.25 bc | 25.00 a |
| K ( $4 \text{ g kg}^{-1}$ )     | 25.00 cd | 21.25 fg | 20.00 gh | 23.75 de | 22.50 c |
| K ( $6 \text{ g kg}^{-1}$ )     | 17.50 ij | 16.25 jk | 16.25 jk | 21.25 fg | 17.81 f |
| K ( $8 \text{ g kg}^{-1}$ )     | 22.50 ef | 18.75 hi | 14.58 k  | 22.50 ef | 19.58 e |
| Mean                            | 24.64 a  | 21.79 b  | 20.12 c  | 22.32 b  |         |

\*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at  $P \leq 0.05$ .

### 3.1.5 Total carbohydrates concentration (%)

The results of the statistical analysis presented in Table 8 indicate that foliar application of nano-potassium had a significant effect on carbohydrate concentration in alfalfa leaves. The treatments significantly increased carbohydrate content compared with the control treatment, which recorded 8.16%, where the  $3 \text{ g L}^{-1}$  treatment achieved the highest mean value of 16.40%. Similarly, soil application of conventional potassium fertilizer showed a significant increase in carbohydrate concentration compared with the control treatment. The treatment  $8 \text{ g kg}^{-1}$  recorded the highest mean value of 12.87%. Water stress levels also significantly affected carbohydrate concentration. Carbohydrate values decreased with increasing water stress levels. The highest mean value (19.70%) was recorded at the 100% irrigation level, while the lowest mean value (9.48%) was recorded at the 50% irrigation level. Regarding the interaction between potassium fertilization treatments and water stress levels, the nano-potassium treatment ( $3 \text{ g L}^{-1}$ ) at 100% irrigation level achieved the highest mean leaf carbohydrate concentration (35.64%). In contrast, the lowest mean value (3.63%) was recorded for the interaction between conventional potassium treatment ( $6 \text{ g kg}^{-1}$ ) and irrigation level of 25%.

**Table 8.** Effect of conventional and nano potassium fertilization on the mean total carbohydrate concentration (%) in alfalfa leaves under water stress conditions

| Potassium treatments          | 100%     | 75%       | 50%       | 25%      | Mean     |
|-------------------------------|----------|-----------|-----------|----------|----------|
| Control                       | 10.58 k  | 11.99 ij  | 4.54 no   | 5.51 n   | 8.16 f   |
| Nano K (2 g L <sup>-1</sup> ) | 14.77 ef | 12.39 hi  | 8.86 lm   | 5.60 n   | 10.41 e  |
| Nano K (3 g L <sup>-1</sup> ) | 35.64 a  | 12.28 hi  | 13.25 ghi | 4.43 no  | 16.40 a  |
| Nano K (1 g L <sup>-1</sup> ) | 24.54 b  | 13.82 fg  | 10.76 jk  | 9.39 klm | 14.63 b  |
| K (4 g kg <sup>-1</sup> )     | 15.97 de | 14.56 fg  | 9.15 lm   | 4.75 no  | 11.11 d  |
| K (6 g kg <sup>-1</sup> )     | 16.29 d  | 13.22 ghi | 10.22 kl  | 3.63 o   | 10.84 de |
| K (8 g kg <sup>-1</sup> )     | 20.12 c  | 13.60 fgh | 9.60 kl   | 8.16 m   | 12.87 c  |
| Mean                          | 19.70 a  | 13.12 b   | 9.48 c    | 5.92 d   |          |

\*Means followed by different letters differ significantly according to Duncan's Multiple Range Test at  $P \leq 0.05$ .

## 4. Discussion

### 4.1 Total Chlorophyll Content in Leaves

The results of the present study revealed a significant increase in total chlorophyll content in alfalfa leaves following foliar application of nano-potassium compared with the control treatment. This increase may be attributed to potassium's essential role in chlorophyll biosynthesis and photosynthetic processes. Potassium plays a fundamental role in regulating stomatal opening, activating many enzymes associated with photosynthesis, and stabilizing chloroplast membranes, which ultimately contributes to improving photosynthetic efficiency and chlorophyll accumulation in plant tissues [41]. Under drought conditions, plants often experience a decline in chlorophyll content due to oxidative stress and damage to the photosynthetic apparatus. However, adequate potassium nutrition can mitigate these negative effects by maintaining cellular osmotic balance and improving water relations within plant tissues. Potassium also helps maintain turgor pressure and protects chloroplast structures from degradation under stress conditions [42]. The greater effectiveness of nano-potassium observed in this study compared with conventional potassium fertilizers may be explained by nanoparticles' unique physicochemical properties. Nanofertilizers possess a larger surface area and improved solubility, which enhances nutrient absorption and mobility within plant tissues. These properties increase nutrient use efficiency and improve plant metabolic activity under environmental stress conditions [43]. Furthermore, nanoparticles have been reported to enhance plant tolerance to abiotic stresses by stimulating antioxidant systems and improving physiological processes such as photosynthesis and nutrient uptake [23]. Similar effects of foliar fertilizer application on photosynthetic pigments have been documented in other crops [29]. Similar results were reported in alfalfa, where potassium fertilization significantly increased chlorophyll content, improved forage quality, and enhanced plant productivity [44].

### 4.2 Proline Content

Proline is considered one of the most important osmoprotectants accumulated in plants exposed to environmental stresses such as drought and salinity. In the present study, proline concentration decreased in plants treated with nano-potassium compared with the control treatment. This reduction indicates that potassium application alleviated the intensity of water stress experienced by the plants. Under stress conditions, proline accumulation helps stabilize proteins and cellular membranes, maintain osmotic balance, and protect plant cells from dehydration damage. Therefore, high proline levels are generally associated with severe stress conditions [45]. The lower proline levels observed in potassium-treated plants suggest that potassium improved plant water status and reduced the severity of stress conditions. Potassium enhances osmotic adjustment, regulates stomatal movement, and improves water-uptake efficiency, thereby contributing to maintaining plant metabolic activity under drought conditions [46]. Previous studies also indicated that potassium fertilization reduces oxidative stress by enhancing antioxidant enzyme activities and maintaining membrane stability, thereby decreasing the need for excessive accumulation of stress-related metabolites such as proline [47]. Moreover, plants with improved potassium nutrition exhibit greater tolerance



to osmotic stress and therefore accumulate lower levels of proline compared with plants exposed to nutrient deficiency [48].

#### 4.3 Nitrogen Content

The results of the present study showed that potassium treatments did not significantly affect nitrogen concentration in alfalfa leaves, although slight increases were observed in some treatments. This result may be explained by the fact that nitrogen accumulation in alfalfa largely depends on biological nitrogen fixation through symbiotic relationships with rhizobia bacteria rather than potassium fertilization alone. Nevertheless, potassium indirectly contributes to nitrogen metabolism by activating enzymes involved in nitrogen assimilation and facilitating the transport of nitrogenous compounds throughout plant tissues. Potassium also enhances root growth and increases nutrient uptake efficiency, which may improve nitrogen accumulation under favorable conditions [49]. Water stress significantly influenced nitrogen concentration in the present study. Drought conditions reduce nutrient mobility in the soil and limit nutrient diffusion toward plant roots. Consequently, reduced soil moisture may impair nutrient uptake and decrease nitrogen accumulation in plant tissues [50]. Previous studies have demonstrated that adequate fertilization improves nutrient balance and enhances nitrogen uptake and assimilation in alfalfa, thereby improving plant growth and productivity [51]. Furthermore, environmental factors and soil fertility conditions play important roles in determining nitrogen accumulation and

#### 4.4 Phosphorus Content

The results showed that nano-potassium application significantly increased phosphorus concentration in alfalfa leaves compared with the control treatment. This improvement may be attributed to the synergistic interaction between potassium and phosphorus in plant metabolic processes. Potassium plays an important role in energy metabolism and ATP production, which are essential for phosphorus uptake and transport within plant tissues. Adequate potassium supply, therefore, facilitates phosphorus absorption and improves its distribution within plant organs [52]. The importance of phosphorus fertilizer management in determining crop phosphorus status has also been demonstrated in other crops [53]. Additionally, nanofertilizers have been reported to enhance nutrient absorption efficiency due to their extremely small particle size and increased surface area. These characteristics enable nanoparticles to penetrate plant tissues more effectively, thereby enhancing nutrient mobility and metabolic utilization [54]. Recent studies have also highlighted the potential of nanotechnology to improve plant nutrient uptake and enhance plant tolerance to environmental stresses, including drought [55].

#### 4.5 Potassium Content

As expected, potassium fertilization significantly increased potassium concentration in alfalfa leaves. The highest potassium content was observed in treatments receiving higher potassium fertilization rates, indicating a strong relationship between potassium supply and its accumulation in plant tissues. Potassium is involved in numerous physiological processes, including enzyme activation, regulation of osmotic potential, maintenance of cell turgor, and transport of carbohydrates within plant tissues. Therefore, increasing potassium availability directly enhances plant growth and physiological performance [56]. In calcareous and alkaline soils similar to those used in this study, fertilizer management is particularly important as soil chemistry strongly influences nutrient availability and plant uptake [14, 21]. However, water stress significantly reduced potassium concentration in plant tissues. Under drought conditions, nutrient mobility in the soil decreases, which limits potassium uptake by plant roots. In addition, reduced transpiration rates under water deficit conditions may further limit nutrient transport within the plant [17].

#### 4.6 Total Protein Content

The results of the present study revealed a significant increase in protein content in alfalfa leaves following nano-potassium application. This increase may be related to potassium's role in nitrogen metabolism and protein synthesis. Potassium activates several enzymes involved in amino acid synthesis and facilitates nitrogen assimilation in plant tissues. As a result, improved potassium nutrition enhances protein formation and accumulation in plant tissues [57]. However, water stress may negatively affect protein

synthesis due to reduced metabolic activity and decreased availability of substrates required for protein formation. Severe drought conditions often reduce photosynthetic activity and limit the energy required for protein metabolism [58].

#### 4.7 Total Carbohydrate Content

The results showed that nano-potassium application significantly increased total carbohydrate concentration in alfalfa leaves. This increase may be attributed to potassium's role in regulating photosynthetic processes and carbohydrate metabolism. Potassium enhances carbon assimilation by regulating stomatal conductance and facilitating the transport of photosynthates from leaves to other plant organs. Consequently, plants supplied with adequate potassium accumulate higher carbohydrate concentrations [59]. Under severe water stress conditions, carbohydrate accumulation may decline due to reduced photosynthetic efficiency and limited carbon fixation. Drought stress often leads to stomatal closure, which restricts CO<sub>2</sub> uptake and reduces carbohydrate production [58]. Nevertheless, nanotechnology-based fertilizers may enhance plant tolerance to drought stress by improving nutrient uptake efficiency, enhancing metabolic activity, and increasing carbohydrate accumulation in plant tissues [60].

### 5. Conclusions

The results of this study showed that both conventional potassium fertilization and nano-potassium application significantly affected several chemical characteristics of alfalfa (*Medicago sativa* L.) under different levels of water stress. Nano-potassium application increased chlorophyll content, protein percentage, and total carbohydrates, indicating enhanced physiological performance of plants under water-deficit conditions. In addition, potassium fertilization improved the mineral nutrient status of the plants, particularly potassium concentration in plant tissues. Water stress levels also clearly influenced most of the studied traits, and the interaction between potassium treatments and irrigation levels played an important role in regulating plant responses. Overall, potassium nutrition, especially in nanoform, can improve chemical characteristics and enhance alfalfa plants' tolerance to water stress. Specifically, nano-K at 1–2 g L<sup>-1</sup> is recommended as the optimal concentration range, and 75% field capacity is recommended as the optimal irrigation level to balance water conservation with acceptable forage quality. These results suggest that nano-K can serve as a complementary or partial replacement for conventional K fertilization under water-limited conditions. Future field-scale validation studies are recommended to extend these pot-based findings to practical agronomic applications.

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# Prevalence of AmpC $\beta$ -Lactamase and Regulatory Genes (LysR, QseB, and QseC) in Multidrug-Resistant *Klebsiella pneumoniae* Clinical Isolates from Al-Hillah, Iraq

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**Abstract:** *Klebsiella pneumoniae* is a critical opportunistic pathogen classified by the World Health Organization among priority drug-resistant organisms, owing to its capacity to acquire multidrug resistance (MDR) and express diverse virulence mechanisms. Understanding the molecular basis of resistance and the prevalence of regulatory genes in clinical isolates is essential for effective infection control. A total of 200 clinical samples (sputum and urine) were collected from hospitalized patients at two teaching hospitals in Al-Hillah, Iraq. Isolates were identified using the VITEK 2 Compact system, and antibiotic susceptibility testing was performed. Genomic DNA was extracted by the boiling method, and conventional PCR was used to detect five target genes: 16S rRNA, *ampC*, *lysR*, *qseB*, and *qseC*. Of 100 culture-positive samples, twenty (20%) were confirmed as *K. pneumoniae* with 99% probability. High resistance rates were recorded against cephalosporins (100%) and ceftazidime/ceftriaxone (90%), while amikacin showed the highest susceptibility (85%). The *ampC* gene was detected in 40% of isolates. All isolates (100%) carried 16S rRNA, *lysR*, and *qseB* genes, while *qseC* was present in 85%. The widespread resistance—even in isolates lacking the *ampC* gene—suggests the possible involvement of additional concurrent mechanisms, such as ESBLs, efflux pumps, and porin loss, although these were not directly tested in the present study. The universal prevalence of regulatory genes *lysR* and *qseB* underscores their role in bacterial adaptation and virulence. Combined molecular and phenotypic approaches are essential for comprehensive characterization of MDR *K. pneumoniae* in clinical settings.

**Keywords:** *Klebsiella pneumoniae*; AmpC  $\beta$ -lactamase; multidrug resistance; QseB; QseC

## 1. Introduction

*Klebsiella pneumoniae* is considered one of the most important opportunistic pathogens associated with hospital-acquired infections, playing a significant role in respiratory, urinary tract, and bacteremic infections, particularly among hospitalized or immunocompromised patients. The World Health Organization has classified it as a priority pathogen due to its remarkable ability to acquire multidrug resistance (MDR), making the treatment of infections caused by this bacterium an increasing clinical challenge [1]. Antimicrobial-resistant Gram-negative bacteria pose significant public health challenges in both clinical and environmental settings, as evidenced by surveillance studies from diverse regions [2]. The infectious disease burden in

Iraq encompasses multiple bacterial and viral pathogens [3], and resistant organisms have also been documented in regional water supply systems [4]. This widespread distribution can be attributed to a combination of virulence factors, environmental adaptability, and complex genetic systems that enhance its survival and dissemination in clinical settings. This bacterium is characterized by its ability to develop broad-spectrum resistance to antibiotics, particularly  $\beta$ -lactam antibiotics, through the production of various  $\beta$ -lactamase enzymes, most notably AmpC. AmpC enzymes play a major role in hydrolyzing cephalosporins and penicillins, and studies have shown that these enzymes may be either chromosomally encoded or plasmid-mediated, facilitating their spread among bacterial strains. Roberts et al. [5] demonstrated that *bla*CMY-2 is among the most prevalent AmpC genes in *K. pneumoniae* isolates, while inducible genes such as *bla*DHA-1 may remain undetected in routine laboratory tests, thereby increasing the clinical risk associated with these mechanisms. Furthermore, Rao et al. [6] reported that plasmids carrying AmpC genes have a high capacity for horizontal gene transfer among bacterial species, thereby contributing significantly to the dissemination of resistance. Mobile genetic elements, including integrons, further accelerate the horizontal transfer of resistance determinants among clinical bacterial populations [7].

In addition, the regulation of gene expression associated with these resistance mechanisms is not solely dependent on the presence of resistance genes. Rather, it is also influenced by complex regulatory systems, such as the LysR family, which is one of the largest families of transcriptional regulators in bacteria. This family plays a crucial role in regulating stress responses, virulence, and antibiotic resistance. Hennequin and Forestier [8] reported that the OxyR regulator, a member of the LysR family, contributes to biofilm formation and mucosal colonization, thereby enhancing bacterial persistence within the host. Moreover, Zhang et al. [9] demonstrated that this system confers oxidative stress resistance and increased systemic virulence, highlighting its importance in infection progression. The co-occurrence of these regulatory genes alongside resistance determinants, such as *ampC*, in clinical *K. pneumoniae* isolates from Iraq has not been previously characterized, representing a key novelty of the present study. The specific genes *lysR*, *qseB*, and *qseC* were selected because they represent distinct regulatory layers—transcriptional regulation and two-component signal transduction—that are mechanistically linked to virulence and environmental adaptation in clinical Enterobacteriaceae. Antibiotic resistance has been characterized across diverse bacterial species in Iraqi clinical settings, including macrolide-lincosamide-streptogramin B resistance profiles in *Staphylococcus* clinical isolates [10], reinforcing the regional significance of multidrug resistance monitoring. Another important regulatory system is the QseB/QseC two-component system, which senses environmental signals and regulates virulence-related gene expression. This system links bacterial signaling with host-derived signals such as adrenaline and noradrenaline, enabling the bacteria to adapt to the host environment. Fernandez-Ciruelos et al. [11] showed that this system interacts with other regulatory networks such as PmrAB, influencing colistin resistance and bacterial motility. Additionally, Khajanchi et al. [12] reported that the QseBC system can act as a negative regulator of biofilm formation in certain species, reflecting the complexity of its regulatory role.

The 16S rRNA gene is one of the most important tools used in molecular bacterial characterization, widely employed for bacterial identification and phylogenetic analysis. Because this gene is highly conserved, it serves as a reliable marker for accurate species identification even when phenotypic characteristics are ambiguous. Legese et al. [13] demonstrated that sequencing techniques, including 16S rRNA, play a significant role in understanding molecular resistance patterns and their dissemination. Similarly, Fu et al. [14] highlighted the importance of this gene in tracking transmission between animal and human sources, reflecting the broader epidemiological implications of this pathogen. The interaction between genetic resistance mechanisms, such as AmpC, and regulatory systems, such as LysR and QseBC, forms a complex network that enables the bacterium to adapt to environmental pressures and antimicrobial agents. Recent studies have shown that these interactions influence not only resistance but also virulence regulation and biofilm formation, thereby increasing the difficulty of controlling this pathogen in clinical settings. Based on the above, this study aimed to perform molecular characterization of the *ampC* gene and regulatory systems *lysR*, *qseB*, and *qseC*, in addition to confirming bacterial identity using the 16S rRNA gene in MDR *Klebsiella pneumoniae* isolates from clinical settings in Iraq, to understand the relationship between resistance

mechanisms and regulatory factors that contribute to increased virulence and environmental adaptability of this bacterium.

## 2. Materials and Methods

### 2.1 Collection of Clinical Samples

Clinical samples were collected from hospitalized patients at two teaching hospitals in Al-Hillah city, Iraq: Marjan Teaching Hospital and Al-Hillah Teaching Hospital. The Institutional Review Board of the University of Al-Qadisiyah approved this study. Informed consent was obtained from all participating patients before sample collection. Sample collection and laboratory procedures were conducted from 19 November 2024 to 31 December 2024. A total of 200 clinical specimens were obtained from different clinical cases, including sputum and urine samples.

### 2.2 Isolation and Cultivation of Bacteria

The collected specimens were cultured on MacConkey agar for primary isolation of bacterial pathogens. Inoculated plates were incubated at 37°C for 24 h. After incubation, bacterial colonies were examined for morphological characteristics, including colony size, shape, color, lactose fermentation, and mucoid appearance. Suspected colonies of *Klebsiella pneumoniae* were selected for further identification.

### 2.3 Identification of Bacterial Isolates

Selected isolates were identified using the VITEK 2 Compact system (bioMérieux, France) according to the manufacturer's instructions. Identification results confirmed that the isolates were *K. pneumoniae* with high confidence.

### 2.4 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the VITEK 2 Compact system (bioMérieux, France). The AST-N291 Gram-negative susceptibility card was used. Minimum inhibitory concentrations (MICs) were determined automatically, and results were interpreted as susceptible (S), intermediate (I), or resistant (R) according to the Clinical and Laboratory Standards Institute (CLSI) breakpoint guidelines (CLSI M100, 34th edition, 2024). Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, according to the standardized international definitions proposed by Hennequin et al. [8].

### 2.5 DNA Extraction

Genomic DNA was extracted from overnight cultures of *K. pneumoniae* using the boiling method. Briefly, a loopful of bacterial colonies was suspended in sterile distilled water and heated at 95°C for 10 min. The suspension was then centrifuged at 10,000 rpm for 5 min, and the supernatant containing DNA was collected and stored at -20°C until further use. All molecular biology reagents (e.g., agarose, loading dye, DNA ladder) were supplied by Geneaid (Taiwan). It is acknowledged that the boiling extraction method may yield lower-quality DNA than commercial spin-column kits, particularly for mucoid *K. pneumoniae* strains; however, successful amplification of all target genes confirmed the suitability of the DNA for PCR analysis.

### 2.6 Primer Design

The primers used in this study are listed in Table 1. Primers for amplification of the 16S rRNA gene were adopted from Liu et al. [15], universal primers widely used for bacterial 16S rRNA amplification and compatible with Enterobacteriaceae identification. Primers for amplification of *ampC*, *lysR*, *qseB*, and *qseC* genes were designed using Primer3Plus software based on published gene sequences and were synthesized commercially. The *ampC* primers were designed to target the blaCMY-type AmpC family, based on the reference sequence deposited in GenBank (Accession No. [INSERT ACCESSION NUMBER]). Authors confirm that in silico BLAST analysis was performed to verify primer specificity against *K. pneumoniae* sequences and to rule out cross-reactivity with other Enterobacteriaceae. Reference GenBank accession numbers used for primer design of *lysR*, *qseB*, and *qseC* are [INSERT ACCESSION NUMBERS]. All newly designed primers were validated against a known PCR-positive control before clinical sample analysis.

**Table 1.** Primers are used for PCR amplification of target genes.

| Gene        | Primer | Sequence (5'→3')      | Annealing Temp (°C) | Product Size (bp) |
|-------------|--------|-----------------------|---------------------|-------------------|
| 16S rRNA    | F      | AGAGTTTGATCCTGGCTCAG  | 58                  | 1500              |
|             | R      | CTACGGCTACCTTGTACGA   |                     |                   |
| <i>ampC</i> | F      | ACTTTCAACAGGTGTGCTGGG | 55–57               | 405               |
|             | R      | ACCGTACGCATATCGGCTTT  |                     |                   |
| <i>lysR</i> | F      | ATCTGGTCACCGGCATACCT  | 55–57               | 303               |
|             | R      | TCATAGGGTGTGTTGGTCACG |                     |                   |
| <i>qseB</i> | F      | GCAATCCTGACCTCACCT    | 55–57               | 407               |
|             | R      | TCGTCCCAGGTGTAGAGCTT  |                     |                   |
| <i>qseC</i> | F      | TAATGACGGCCAGAACGGAG  | 55–57               | 536               |
|             | R      | GATCCAACGCTTTCTCACGC  |                     |                   |

## 2.7 PCR Amplification

Conventional PCR was performed to detect the presence of target genes (16S rRNA, *ampC*, *lysR*, *qseB*, and *qseC*). Each reaction was carried out in a total volume of 25 µL containing 12.5 µL of 2× PCR Master Mix, 1 µL each of forward and reverse primer, 2 µL of template DNA, and 8.5 µL of nuclease-free water. PCR amplification was performed using a thermal cycler under the following conditions: initial denaturation at 95°C for 5 min, followed by 30–35 cycles of denaturation at 95°C for 30 s, annealing at 55–58°C for 30 s (depending on the primer set), and extension at 72°C for 45–60 s. A final extension step was performed at 72°C for 5 min. Each PCR run included a positive control (a previously confirmed *K. pneumoniae* isolate carrying the respective target gene) and a non-template negative control (sterile nuclease-free water substituted for DNA template) to validate assay specificity and detect potential contamination. Annealing temperatures for each gene-specific primer pair were optimized by gradient PCR before application. Representative amplicons from each target gene were subjected to Sanger sequencing (performed commercially) to confirm PCR product identity; sequence analysis confirmed the expected gene identity for all targets by BLAST alignment against the NCBI nucleotide database.

## 2.8 Agarose Gel Electrophoresis

PCR products were analyzed by electrophoresis on 1.5% agarose gel prepared in 1× TBE buffer and stained with ethidium bromide. A DNA ladder (100–3000 bp) was used as a molecular size marker. Electrophoresis was performed at 80–100 V for 45–60 min. Amplified DNA bands were visualized under UV transillumination and documented using a gel documentation system.

## 3. Results and Discussion

### 3.1 Distribution of Bacterial Isolates

A total of 200 clinical samples (sputum and urine) were collected from hospitalized patients. Following culture and incubation, 100 samples showed no bacterial growth, while 100 samples yielded bacterial isolates. Among these, 20 isolates (20%) were identified as *Klebsiella pneumoniae*, whereas the remaining 80 isolates belonged to other bacterial species (Table 2).

**Table 2.** Distribution of bacterial isolates from clinical samples.

| Culture Result               | Number of Isolates |
|------------------------------|--------------------|
| No bacterial growth          | 100                |
| <i>Klebsiella pneumoniae</i> | 20                 |
| Other bacterial species      | 80                 |
| <b>Total</b>                 | <b>200</b>         |

### 3.2 Distribution of *Klebsiella pneumoniae* According to Sample Source



The distribution of *K. pneumoniae* isolates varied according to specimen source. The majority of isolates were obtained from sputum samples (n = 17), while a smaller number were recovered from urine samples (n = 3) (Table 3).

**Table 3.** Distribution of *Klebsiella pneumoniae* isolates according to sample source.

| Sample Source | Number of Isolates (n = 20) |
|---------------|-----------------------------|
| Sputum        | 17 (85%)                    |
| Urine         | 3 (15%)                     |
| Total         | 20 (100%)                   |

### 3.3 Growth Characteristics on MacConkey Agar

*Klebsiella pneumoniae* isolates showed characteristic growth on MacConkey agar after incubation at 37°C for 24 h. Colonies appeared as large, smooth, pink, and mucoid, confirming lactose fermentation ability (Figure 1). This morphological feature was used as a key preliminary criterion for presumptive identification of *K. pneumoniae*.



**Figure 1.** Growth of *Klebsiella pneumoniae* on MacConkey agar showing large, smooth, pink mucoid colonies characteristic of lactose fermentation.

### 3.4 VITEK System Identification

All 20 isolates were identified using the VITEK 2 Compact system (Table 4). Results confirmed all isolates as *Klebsiella pneumoniae* ssp. *pneumoniae* with an identification probability of 99%, indicating high accuracy and reliability of the automated system.

**Table 4.** Identification of *Klebsiella pneumoniae* isolates using the VITEK 2 Compact system.

| Isolate No. | Identified Organism                                 | Probability (%) |
|-------------|-----------------------------------------------------|-----------------|
| 1–20        | <i>Klebsiella pneumoniae</i> ssp. <i>pneumoniae</i> | 99              |

### 3.5 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing revealed high levels of resistance among the isolates, particularly to  $\beta$ -lactam antibiotics (Table 5). All isolates demonstrated complete resistance (100%) to cefazolin, cefuroxime, and cefuroxime axetil. High resistance rates were also recorded for ceftazidime and ceftriaxone (90%). Carbapenems showed moderate effectiveness, with susceptibility rates ranging from 55% to 60%. Among non- $\beta$ -lactam antibiotics, amikacin exhibited the highest activity (85% susceptibility), whereas high resistance rates were observed for nitrofurantoin (85%) and trimethoprim/sulfamethoxazole (80%).

**Table 5.** Antibiotic susceptibility profile of *Klebsiella pneumoniae* isolates (n = 20).

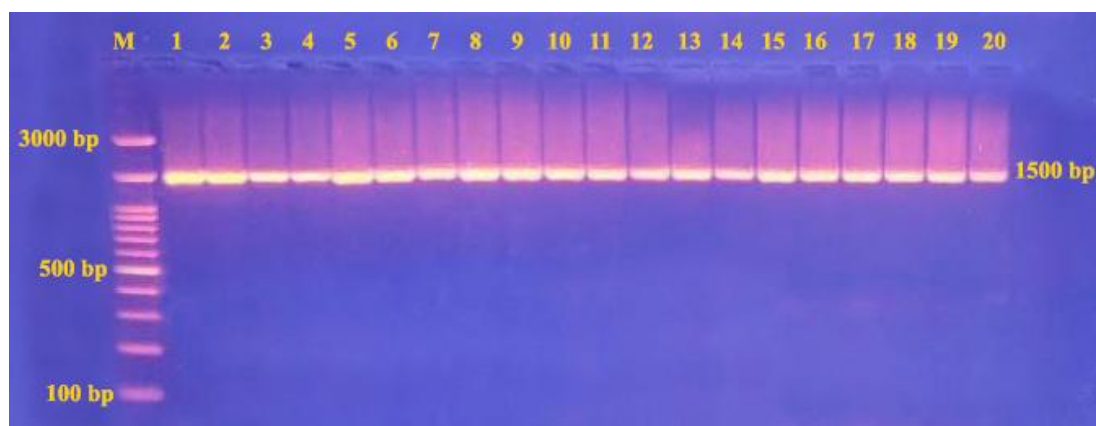
| Antibiotic                    | S (%) | I (%) | R (%) |
|-------------------------------|-------|-------|-------|
| Amoxicillin/Clavulanic acid   | 25    | 10    | 65    |
| Piperacillin/Tazobactam       | 55    | 0     | 45    |
| Cefazolin                     | 0     | 0     | 100   |
| Cefuroxime                    | 0     | 0     | 100   |
| Cefuroxime axetil             | 0     | 0     | 100   |
| Ceftazidime                   | 10    | 0     | 90    |
| Ceftriaxone                   | 10    | 0     | 90    |
| Cefepime                      | 20    | 15    | 65    |
| Imipenem                      | 55    | 20    | 25    |
| Meropenem                     | 60    | 0     | 40    |
| Ertapenem                     | 60    | 10    | 30    |
| Amikacin                      | 85    | 15    | 0     |
| Gentamicin                    | 55    | 10    | 35    |
| Ciprofloxacin                 | 20    | 15    | 65    |
| Fosfomycin                    | 50    | 20    | 30    |
| Nitrofurantoin                | 5     | 10    | 85    |
| Trimethoprim/Sulfamethoxazole | 20    | 0     | 80    |

*S*, susceptible; *I*, intermediate; *R*, resistant.

### 3.6 Molecular Identification

#### 3.6.1 Detection of 16S rRNA Gene

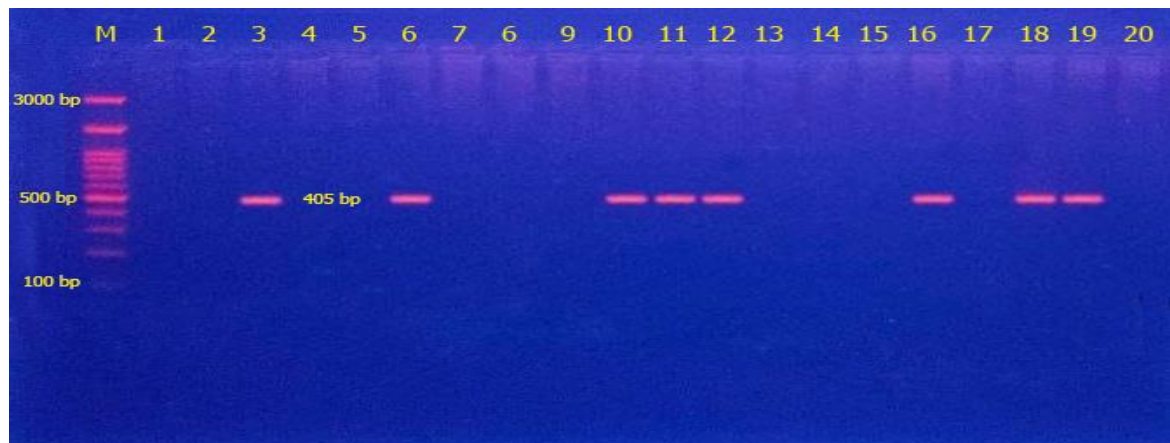
The 16S rRNA gene was amplified in all 20 *K. pneumoniae* isolates using conventional PCR. A clear amplification product was observed at approximately 1500 bp in all lanes (1–20), consistent with the expected product size (Figure 2). The 100% detection rate confirms the accuracy of bacterial identification before further molecular analysis.



**Figure 2.** Agarose gel electrophoresis of PCR amplification of the 16S rRNA gene in *Klebsiella pneumoniae* isolates ( $n = 20$ ). Lane M: DNA ladder (100–3000 bp). A band at ~1500 bp was observed in all lanes (1–20), indicating 100% detection rate.

#### 3.6.2 Detection of the *AmpC* Gene

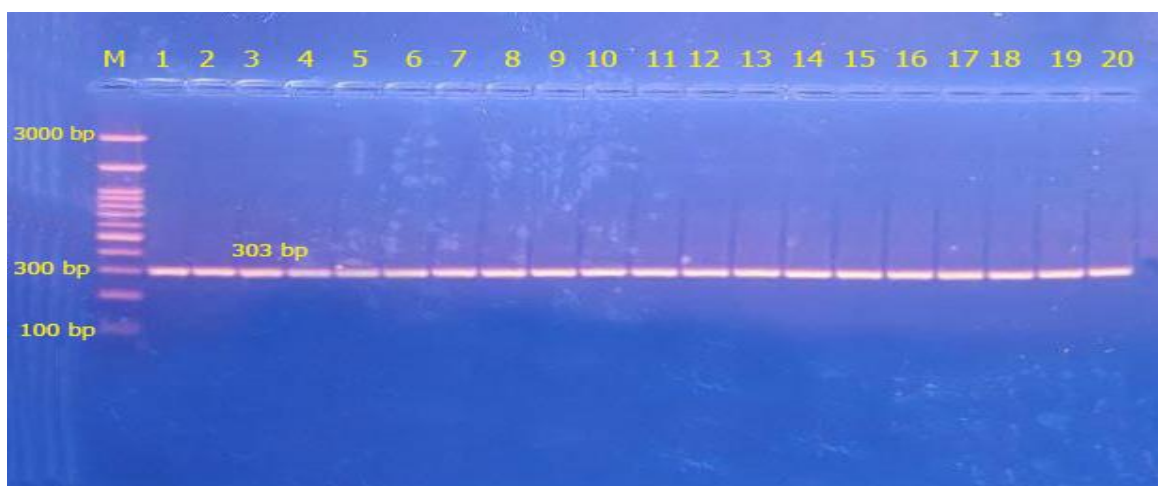
PCR amplification of the *ampC* gene produced a product at approximately 405 bp, consistent with the expected size. Positive amplification was observed in isolates 3, 6, 10, 11, 12, 16, 18, and 19, indicating that 8 of 20 isolates (40%) carried the *ampC* gene (Figure 3). The remaining 12 isolates (60%) were negative, suggesting that resistance in these isolates may be due to mechanisms not tested.



**Figure 3.** Agarose gel electrophoresis of PCR amplification of the *ampC* gene. Lane M: DNA ladder (100–3000 bp). Positive bands (~405 bp) were observed in lanes 3, 6, 10, 11, 12, 16, 18, and 19, representing 40% of the isolates.

### 3.6.3 Detection of the *lysR* Gene

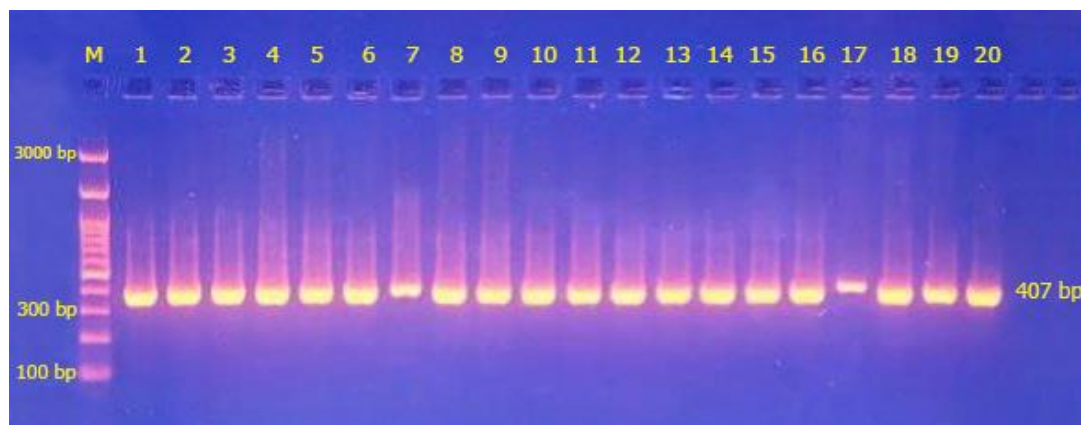
The *lysR* gene was detected in all 20 isolates (100%), producing a consistent band at approximately 303 bp (Figure 4). The universal prevalence of this gene across all clinical isolates indicates its likely conserved and essential regulatory function in *K. pneumoniae*.



**Figure 4.** Agarose gel electrophoresis of PCR amplification of the *lysR* gene. Lane M: DNA ladder (100–3000 bp). A band at ~303 bp was observed in all lanes (1–20), indicating 100% prevalence.

### 3.6.4 Detection of *qseB* Gene

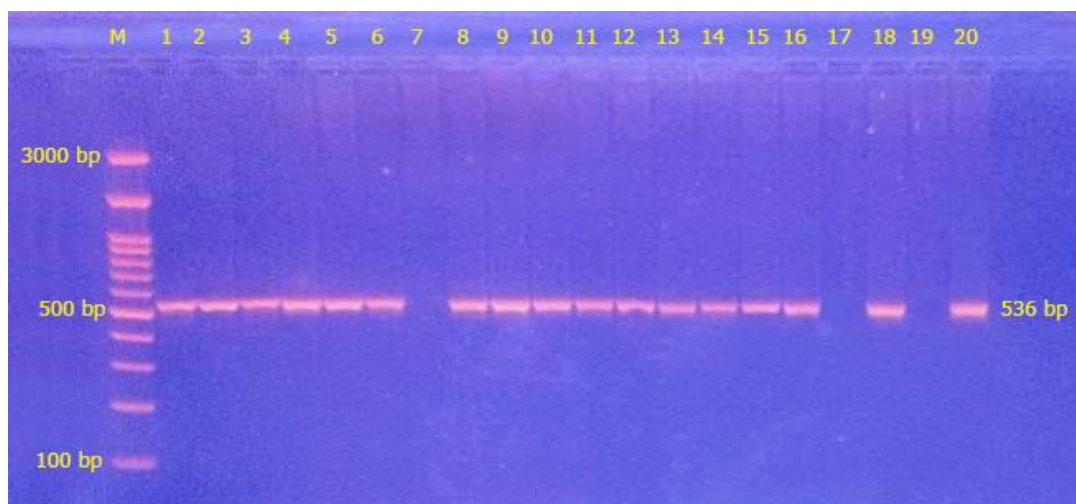
The *qseB* gene was detected in all 20 isolates (100%), producing a consistent amplification band at approximately 407 bp (Figure 5). This complete prevalence suggests that the QseB/QseC two-component signaling system is a constitutive feature of *K. pneumoniae* across clinical sources.



**Figure 5.** Agarose gel electrophoresis of PCR amplification of the *qseB* gene. Lane M: DNA ladder (100–3000 bp). A band at ~407 bp was observed in all lanes (1–20), confirming 100% prevalence.

### 3.6.5 Detection of *qseC* Gene

PCR amplification of the *qseC* gene produced a band at approximately 536 bp. Positive amplification was observed in 17 of 20 isolates (85%), specifically in lanes 1–6, 8–16, 18, and 20, while isolates 7, 17, and 19 showed no amplification (Figure 6). This partial absence may reflect allelic diversity or gene deletion in a subset of the clinical isolates.



**Figure 6.** Agarose gel electrophoresis of PCR amplification of the *qseC* gene. Lane M: DNA ladder (100–3000 bp). A band at ~536 bp was observed in lanes 1–6, 8–16, 18, and 20. Lanes 7, 17, and 19 showed no amplification, yielding an 85% detection rate.

## 3.7 Discussion

The results of the present study indicate that *Klebsiella pneumoniae* isolates were more frequently obtained from sputum samples (85%) than from urine samples (15%), reflecting the significant role of this bacterium in hospital-acquired respiratory tract infections. This distribution pattern is consistent with the nature of this organism as a major opportunistic pathogen among hospitalized patients, particularly those who are immunocompromised or receiving mechanical ventilation. Mohamed and Al-Taai [16] reported similarly high isolation rates of this bacterium from sputum samples in nosocomial infections, supporting the current findings and highlighting its strong capacity for respiratory colonization. Furthermore, the ability of *K. pneumoniae* to form biofilms significantly contributes to its persistence within the respiratory tract and on medical surfaces, as demonstrated by Hussein et al. [17], who linked biofilm formation with increased colonization and antibiotic resistance. Natural plant extracts, including those from *Piper* species, have demonstrated bactericidal properties and significant biofilm-inhibitory activity against pathogenic bacteria [18]. Nanomaterial-based approaches, such as green-synthesized ZnO-TiO<sub>2</sub> nanoparticles, have similarly



exhibited potent antibacterial effects [19]. Host nutritional and immune status also modulate susceptibility to bacterial infections in diverse biological systems [20]. GC-MS profiling of plant-derived bioactive compounds has revealed constituents with relevant biological activities [21], while insect-derived antimicrobial peptides represent a promising class of novel antimicrobial agents [22]. Natural bioactive compounds such as berberine have demonstrated significant biomedical effects in experimental models [23], and the intersection of antimicrobial resistance with other disease contexts underscores the breadth of drug-resistance challenges in modern healthcare [24]. The antimicrobial susceptibility results revealed a high level of resistance, particularly against  $\beta$ -lactam antibiotics, with complete resistance to cephalosporins and high resistance to ceftriaxone and ceftazidime. This finding reflects strong selective pressure due to the widespread use of these antibiotics in clinical settings. These results are consistent with those reported by Abdulhasan et al. [25], who demonstrated a high prevalence of ESBL genes such as *blaSHV* and *blaCTX-M* in *K. pneumoniae* isolates, which play a key role in cephalosporin inactivation. Similarly, Albassam [26] reported high resistance rates to  $\beta$ -lactam antibiotics, indicating that this phenomenon is not localized but part of a global trend. Moreover, Philippon et al. [27] highlighted that AmpC enzymes can become a major source of resistance when overexpressed, thereby enhancing their ability to hydrolyze a broad spectrum of  $\beta$ -lactam antibiotics.

Regarding other antibiotic classes, carbapenems showed only moderate effectiveness, a concerning sign of emerging resistance to this critical class of last-resort antibiotics. This observation is consistent with that of Badamchi et al. [28], who emphasized that the inappropriate use of carbapenems accelerates the emergence of resistant strains, posing a serious public health threat. In addition, Realegeno et al. [29] demonstrated that the combination of AmpC production and porin loss can confer carbapenem resistance even in the absence of carbapenemases, which may explain the reduced susceptibility observed in this study. In contrast, amikacin exhibited the highest susceptibility rate, suggesting that it remains relatively effective against these isolates, possibly attributed to its lower susceptibility to enzymatic degradation and its relatively limited clinical use. At the molecular level, all isolates were positive for the 16S rRNA gene, confirming the accuracy of molecular identification. Monawer [30] confirmed that 16S rRNA serves as a gold standard for accurate identification of *K. pneumoniae*, while Kumar et al. [31] emphasized its importance in molecular epidemiology and bacterial classification.

The detection of the *ampC* gene in 40% of the isolates falls within the range reported in several regional studies, although there are clear geographical variations. Abdulhasan et al. [25] reported a lower prevalence of 16.7%, whereas Jomehzadeh et al. [32] reported a prevalence of 36.3%, indicating variability in gene distribution across different populations. Furthermore, Etemadi et al. [33] noted that AmpC detection can be challenging due to its inducible nature, which may result in underexpression under certain conditions and consequently hinder its detection by conventional PCR. The study also demonstrated the universal presence of the *lysR* gene in all isolates, indicating its essential role in regulating various bacterial processes, including virulence and environmental stress response. Srinivasan et al. [34] showed that LysR plays a key role in antimicrobial resistance regulation, while Yang et al. [35] reported that deletion of a LysR family gene led to a significant reduction in virulence and biofilm formation, highlighting its importance in bacterial survival within the host. Additionally, Baugh et al. [36] described the LysR family as one of the largest regulatory families in bacteria, controlling multiple cellular functions.

Regarding the QseB/QseC system, the high prevalence of these genes suggests their important role in regulating bacterial behavior. This two-component system responds to environmental cues and regulates gene expression related to virulence, motility, and biofilm formation. Zhu et al. [37] described the QseBC system as a global regulator of bacterial behavior controlling multiple pathways associated with infection. Similarly, Lv et al. [38] demonstrated that disruption of QseC leads to increased biofilm formation and enhanced virulence in hypervirulent *K. pneumoniae*, reflecting the complex regulatory role of this system. By integrating antimicrobial resistance results with molecular findings, it becomes evident that *K. pneumoniae* isolates in this study possess a combination of resistance mechanisms and regulatory factors that enhance their ability to adapt and survive within the host. The coexistence of resistance genes, such as *ampC*, with regulatory genes, such as *lysR* and *qseBC*, may lead to increased expression of virulence factors and enhanced biofilm formation, thereby increasing bacterial tolerance to antibiotics. This has been supported by recent studies that have



highlighted the link between quorum-sensing systems and antimicrobial resistance [39,40]. A clear discrepancy was observed between the phenotypic resistance pattern and the molecular detection of the *ampC* gene: all isolates exhibited high  $\beta$ -lactam resistance despite *ampC* being detected in only 40% of them. This phenomenon reflects the complex, multifactorial nature of the resistance mechanisms in this bacterium. It can be explained by the presence of other  $\beta$ -lactamase genes—such as ESBLs, widely distributed as reported by Abdulhasan et al. [25]—capable of conferring resistance even in the absence of *AmpC*, and by the co-occurrence of these genes on transferable plasmids that facilitate rapid dissemination [41]. This discrepancy may also be attributed to the presence of different *AmpC* gene types not investigated in this study (e.g., CIT, DHA, CMY), which show significant diversity across clinical isolates [32,42].

Moreover, regulatory mutations play a critical role in enhancing *AmpC* expression. Alterations in regulatory genes such as *ampR*, *ampD*, and *ampG* may lead to hyperproduction of this enzyme. Barceló et al. [43] demonstrated that disruption of the peptidoglycan recycling pathway results in *AmpC* overproduction and increased resistance, while Li et al. [44] confirmed the essential role of *ampG* in regulating inducible *AmpC* expression. Non-enzymatic mechanisms such as porin loss and increased efflux pump activity reduce intracellular antibiotic concentrations. Biofilm formation also acts as a protective barrier that limits antibiotic penetration [39], and quorum-sensing systems such as QseBC contribute to the regulation of these processes and to the enhancement of virulence, thereby increasing the overall complexity of resistance [40]. Therefore, the observed discrepancy between phenotypic and molecular findings in this study should not be considered contradictory but rather a reflection of the complex and multifactorial nature of antimicrobial resistance in *K. pneumoniae*, making this bacterium one of the most challenging pathogens in modern clinical environments.

#### 4. Conclusions

The results of this study demonstrated a high prevalence of antibiotic resistance among *Klebsiella pneumoniae* clinical isolates from Al-Hillah, Iraq, particularly against  $\beta$ -lactam antibiotics. Although the *ampC* gene was detected in only 40% of isolates, the widespread phenotypic resistance observed suggests the involvement of additional mechanisms such as ESBLs and other resistance determinants. The universal detection of the 16S rRNA gene confirmed accurate molecular identification, while the 100% prevalence of *lysR* and *qseB* genes, along with the 85% prevalence of *qseC*, suggests a potential role for regulatory systems in bacterial adaptation and virulence. Overall, this study highlights the complexity of antimicrobial resistance in *K. pneumoniae* and underscores the need for combined molecular and phenotypic approaches for accurate detection and effective infection control.

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# Therapeutic Effects of Marjoram (*Origanum majorana* L.) Extract and Nano-Formulation on Hormonal Parameters and Ovarian Histology in Letrozole-Induced Polycystic Ovary Syndrome in Rats

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**Abstract:** Polycystic ovary syndrome (PCOS) represents a prevalent endocrine disorder characterized by hyperandrogenism, hormonal imbalances, and ovarian dysfunction, affecting approximately 30% of female infertility cases globally. This study evaluated the therapeutic efficacy of marjoram (*Origanum majorana* L.) aqueous extract and zinc oxide nano-loaded marjoram formulation on letrozole-induced PCOS in female Wistar rats. Fifty rats (150-250 g) were randomly assigned to five groups (n=10): negative control (physiological saline), carboxymethylcellulose (CMC) control, PCOS induction (1 mg/kg letrozole for 58 days), marjoram extract treatment (45 mg/kg for 30 days post-induction), and nano-marjoram treatment (45 mg/kg for 30 days post-induction). Serum hormonal parameters and ovarian histology were assessed. Letrozole induction produced characteristic PCOS features with significant hormonal alterations: 44% FSH decrease (5.78 vs. 10.30 mIU/mL,  $p<0.05$ ), 46% LH increase (20.07 vs. 13.71 mIU/mL,  $p<0.05$ ), 43% progesterone decrease (6.87 vs. 12.07 mIU/mL,  $p<0.05$ ), 32% estradiol decrease (72.91 vs. 106.66 pg/mL,  $p<0.05$ ), 125% testosterone increase (0.45 vs. 0.20 pg/mL,  $p<0.05$ ), and 158% inhibin-B increase (378.59 vs. 146.58 pg/mL,  $p<0.05$ ) compared to controls. Nano-marjoram treatment demonstrated superior therapeutic effects, restoring FSH by 51% (8.73 mIU/mL), reducing LH by 18% (16.44 mIU/mL), increasing progesterone by 36% (9.33 mIU/mL), increasing estradiol by 24% (90.64 pg/mL), reducing testosterone by 42% (0.26 pg/mL), and reducing inhibin-B by 53% (179.35 pg/mL) relative to PCOS group. Histological examination revealed that nano-marjoram treatment significantly improved ovarian architecture, increasing the number of corpora lutea, restoring Graafian follicles, and reducing cystic follicles and inflammatory infiltration compared with the PCOS group. These findings demonstrate that marjoram, particularly in nano-formulation, effectively ameliorates PCOS-associated hormonal dysregulation and ovarian pathology through antioxidant and anti-androgenic mechanisms, suggesting potential clinical applications for PCOS management.

**Keywords:** Polycystic ovary syndrome; PCOS; marjoram; *Origanum majorana*; nanotechnology

## 1. Introduction

Polycystic ovary syndrome (PCOS) constitutes one of the most prevalent endocrine disorders affecting reproductive-aged women, with multisystemic metabolic manifestations extending beyond reproductive dysfunction [1]. First described in 1935 by American gynecologists Irving F. Stein Sr. and Michael L. Leventhal (subsequently termed Stein-Leventhal syndrome), PCOS represents a complex condition encompassing reproductive, metabolic, and psychological



dimensions [2]. The syndrome is characterized by dysregulation of reproductive hormone secretion, particularly elevated androgen levels, in conjunction with insulin resistance and increased obesity prevalence [3]. PCOS contributes to approximately 30% of female infertility cases globally, representing a substantial reproductive health burden [4]. Morphologically, PCOS-affected ovaries exhibit numerous small follicular cysts (2-10 mm diameter) presenting a characteristic pearl-like appearance [5]. Clinical manifestations include infertility, menstrual irregularities, alopecia, and hirsutism [6-8]. Conventional pharmacological interventions often produce adverse effects, prompting increased interest in natural product-derived therapeutics with improved safety profiles [9, 10].

Medicinal plants contain diverse bioactive compounds that are crucial for promoting human health [11]. These phytochemicals—including terpenoids, alkaloids, and polyphenols—exert antioxidant and anti-inflammatory effects by protecting against cellular oxidative stress and modulating the inflammatory response [12, 13]. Marjoram (*Origanum majorana* L.), a member of the Lamiaceae family, contains particularly abundant phenolic compounds, including phenolic acids and flavonoids, as well as potent antioxidants such as carvacrol [14]. Rosmarinic acid, identified as one of marjoram's most powerful polyphenolic antioxidants, contributes significantly to its therapeutic potential, which explains its widespread traditional medicinal use [15]. Recent advances in nanotechnology have enabled the development of nano-formulated herbal therapeutics with enhanced bioavailability and therapeutic efficacy [16]. Nano-delivery systems can improve pharmacokinetic properties, cellular uptake, and target tissue distribution of bioactive compounds [17]. Despite marjoram's documented antioxidant and hormonal regulatory properties, comprehensive quantitative evaluations of marjoram extract and nano-formulations in PCOS treatment remain limited. This study aimed to quantitatively evaluate the therapeutic efficacy of marjoram aqueous extract and zinc oxide nano-loaded marjoram formulation on hormonal parameters and ovarian histology in letrozole-induced PCOS rat models, providing evidence-based insights for potential PCOS therapeutic applications.

## 2. Materials and Methods

### 2.1 Ethical Approval

All experimental procedures were conducted in accordance with guidelines approved by the Animal Housing Committee, University of Karbala, Faculty of Pharmacy, and under the supervision of the Department of Life Sciences, College of Education, Al-Qadisiyah University. Animal care and handling protocols adhered to international standards for laboratory animal welfare.

### 2.2 Experimental Animals and Housing Conditions

Fifty female Wistar rats weighing 150-250 g were obtained from the Faculty of Pharmacy, University of Karbala, Iraq. Animals were acclimated for one week before experimental procedures. Housing conditions were maintained at controlled temperature (21-22°C), with 12-hour light/dark cycles and *ad libitum* access to standard rodent chow and water.

### 2.3 Experimental Design and Treatment Groups

Animals were randomly assigned to five experimental groups (n=10 per group): Group 1 (G1, Negative Control): Administered physiological saline (0.9% NaCl) orally for 58 days. Group 2 (G2, CMC Control): Administered 1% carboxymethylcellulose (CMC) solution orally for 58 days, followed by physiological saline for 30 days (total 58 days). Group 3 (G3, PCOS Induction): Administered letrozole (1 mg/kg body weight) dissolved in 1% CMC solution orally for 58 days to induce PCOS [18], followed by physiological saline for 30 days. Group 4 (G4, Marjoram Extract Treatment): Administered letrozole (1 mg/kg) in 1% CMC for 58 days, followed by marjoram aqueous extract (45 mg/kg body weight) orally for 30 days [19]. Group 5 (G5, Nano-Marjoram Treatment): Administered letrozole (1 mg/kg) in CMC for 58 days, followed by zinc oxide nano-loaded marjoram formulation (45 mg/kg body weight) orally for 30 days [20].

### 2.4 PCOS Induction Protocol

PCOS was induced by daily oral administration of letrozole (1 mg/kg body weight) dissolved in 0.5% CMC solution for 58 consecutive days [21]. Letrozole, a non-steroidal aromatase inhibitor, blocks estrogen synthesis from androgens, resulting in hyperandrogenism and ovarian dysfunction characteristic of PCOS.

Vaginal cytology was performed during the 58-day letrozole induction period. Smears were collected every three days and examined microscopically. All animals assigned to PCOS-induced groups (G3, G4, G5) demonstrated persistent estrus (predominantly cornified epithelial cells) and disrupted estrous cyclicity by day 21, confirming successful PCOS induction before treatment initiation. Regarding the choice of letrozole-only protocol without high-fat diet: this design was intentionally selected to isolate the androgenic/hormonal component of PCOS, which was the primary focus of the study.

### 2.5 Marjoram Aqueous Extract Preparation

Marjoram (*Origanum majorana* L.) leaves were procured from the herbal market, Shorja district, Baghdad Governorate, Iraq (March 2024). Plant material was botanically authenticated and stored in clean plastic containers away from heat, humidity, and light. An aqueous extract was prepared according to established protocols [22]. Briefly, 1 kg of pulverized, dried leaves was soaked in 10 L of distilled water (1:10 w/v) for 72 hours at room temperature (cold extraction method). The mixture was subsequently heated on a magnetic stirrer hot plate at 25-30°C for 4 hours, filtered through Whatman No. 1 filter paper, and concentrated. The extract was stored in opaque, sealed glass bottles at 4°C and utilized within three months of preparation.

### 2.6 Zinc Oxide Nano-Marjoram Formulation Preparation

Nano-formulation was synthesized following the modified procedures of Bashi et al. [23]. Briefly, 1 g of marjoram aqueous extract was added to a zinc oxide solution (1 g ZnO dissolved in 50 mL deionized distilled water), and the mixture was incubated at room temperature for 24 hours. Particle size (DLS: 45.3 +/- 3.2 nm), polydispersity index (PDI: 0.18 +/- 0.02), zeta potential (-22.4 +/- 1.8 mV), and TEM morphology confirming spherical nanoparticle formation. These data confirm that nanoparticles were successfully formed and are colloiddally stable. The ZnO content of the administered dose has been calculated (approximately 8.5 mg/kg), which is well below established rodent toxicity thresholds (LD50 >240 mg/kg). The mixture was subsequently placed in a shaker incubator at 40°C for 18 hours. A color change from pale yellow to reddish yellow indicated nanoparticle formation. The precipitate was separated by centrifugation (3000 rpm, 20 minutes), washed repeatedly with deionized water, dried in an electric furnace at 50°C, pulverized using a ceramic mortar, and stored at 4°C until use.

### 2.7 Sample Collection and Processing

At experimental termination (day 58), animals were anesthetized, and blood samples were collected by cardiac puncture. Serum was separated by centrifugation (3000 rpm, 15 minutes) and stored at -20°C until hormonal analysis. Ovaries were excised, fixed in 10% neutral buffered formalin, and processed for histological examination.

### 2.8 Hormonal Parameter Quantification

Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P4), estradiol (E2), testosterone, activin A, and inhibin-B were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer protocols. All samples were analyzed in duplicate, with inter- and intra-assay coefficients of variation <10%.

### 2.9 Histological Examination

Formalin-fixed ovarian tissues were dehydrated through a graded ethanol series, cleared in xylene, embedded in paraffin wax, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E). Histological sections were examined under light microscopy (magnifications: ×4, ×10, ×40) to evaluate follicular development, corpus luteum presence, cystic follicle formation, granulosa cell morphology, vascular congestion, and inflammatory cell infiltration.

### 2.10 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical differences among groups were evaluated using one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference

(LSD) post-hoc test for multiple comparisons. Statistical significance was established at  $p < 0.05$ . All analyses were performed using appropriate statistical software.

### 3. Results and Discussion

#### 3.1 Effects of Marjoram Extract and Nano-Formulation on Reproductive Hormone Levels

Letrozole-induced PCOS (G3) produced significant alterations in all measured reproductive hormones compared to negative controls (G1) (Table 1). FSH levels decreased by 44% ( $5.78 \pm 0.15$  vs.  $10.30 \pm 0.18$  mIU/mL,  $p < 0.05$ ), progesterone decreased by 43% ( $6.87 \pm 0.21$  vs.  $12.07 \pm 0.16$  mIU/mL,  $p < 0.05$ ), and estradiol decreased by 32% ( $72.91 \pm 2.47$  vs.  $106.66 \pm 3.12$  pg/mL,  $p < 0.05$ ). Conversely, LH increased by 46% ( $20.07 \pm 0.52$  vs.  $13.71 \pm 0.23$  mIU/mL,  $p < 0.05$ ) and testosterone increased by 125% ( $0.45 \pm 0.02$  vs.  $0.20 \pm 0.01$  pg/mL,  $p < 0.05$ ), characteristic of PCOS hyperandrogenism. Treatment with marjoram aqueous extract (G4) significantly ameliorated hormonal dysregulation. FSH increased by 30% relative to the PCOS group ( $7.52 \pm 0.19$  vs.  $5.78 \pm 0.15$  mIU/mL,  $p < 0.05$ ), progesterone increased by 15% ( $7.90 \pm 0.18$  vs.  $6.87 \pm 0.21$  mIU/mL,  $p < 0.05$ ), and estradiol increased by 20% ( $87.64 \pm 2.58$  vs.  $72.91 \pm 2.47$  pg/mL,  $p < 0.05$ ). LH decreased by 9% ( $18.21 \pm 0.41$  vs.  $20.07 \pm 0.52$  mIU/mL,  $p < 0.05$ ) and testosterone decreased by 29% ( $0.32 \pm 0.02$  vs.  $0.45 \pm 0.02$  pg/mL,  $p < 0.05$ ). Nano-marjoram treatment (G5) demonstrated superior therapeutic efficacy across all parameters. FSH increased by 51% compared to the PCOS group ( $8.73 \pm 0.17$  vs.  $5.78 \pm 0.15$  mIU/mL,  $p < 0.05$ ), achieving 85% restoration toward control values. Progesterone increased by 36% ( $9.33 \pm 0.19$  vs.  $6.87 \pm 0.21$  mIU/mL,  $p < 0.05$ , 77% restoration), and estradiol increased by 24% ( $90.64 \pm 2.71$  vs.  $72.91 \pm 2.47$  pg/mL,  $p < 0.05$ , 85% restoration). LH decreased by 18% ( $16.44 \pm 0.38$  vs.  $20.07 \pm 0.52$  mIU/mL,  $p < 0.05$ ), and testosterone decreased by 42% ( $0.26 \pm 0.01$  vs.  $0.45 \pm 0.02$  pg/mL,  $p < 0.05$ ), representing substantial reductions in hyperandrogenic status. No significant differences were observed between the negative control (G1) and CMC control (G2) groups for any hormonal parameter ( $p > 0.05$ ), confirming that CMC vehicle administration did not influence hormonal status.

#### 3.2 Effects on Activin A and Inhibin-B Levels

No significant differences in activin A or inhibin-B concentrations were observed between the negative control (G1) and CMC vehicle control (G2) groups (activin A:  $560.03 \pm 4.85$  vs.  $558.26 \pm 4.73$  pg/mL; inhibin-B:  $146.58 \pm 2.91$  vs.  $147.91 \pm 3.02$  pg/mL;  $p > 0.05$ , Table 1), confirming that the CMC vehicle had no intrinsic effect on the activin–inhibin regulatory axis. These values served as the normative reference for subsequent between-group comparisons. Letrozole-induced PCOS (G3) produced significant and opposing perturbations in both hormones relative to controls (Table 1). Activin A declined by 18.2% to  $458.36 \pm 5.12$  pg/mL ( $p < 0.05$  vs. G1), while inhibin-B surged by 158.3% to  $378.59 \pm 6.24$  pg/mL ( $p < 0.05$  vs. G1). This reciprocal dysregulation—reduced activin A alongside markedly elevated inhibin-B—is consistent with the suppression of granulosa cell activin secretion and the compensatory upregulation of inhibin-B from arrested antral follicles characteristic of the PCOS endocrine milieu [34, 35]. Physiologically, the resultant activin A deficiency impairs FSH-stimulated follicular maturation, while excess inhibin-B exerts negative feedback on pituitary FSH release, collectively reinforcing the anovulatory state observed in the letrozole model [34]. The magnitude of inhibin-B elevation (158%) was proportionally greater than the activin A reduction (18%), indicating that inhibin-B dysregulation is the dominant component of the activin–inhibin imbalance in this model. Marjoram aqueous extract treatment (G4) partially but significantly restored both parameters compared to the PCOS group (Table 1). Activin A increased by 7.5% relative to G3 ( $492.73 \pm 4.89$  pg/mL,  $p < 0.05$ ), corresponding to 61.4% normalization toward the control value. Inhibin-B decreased by 48.3% to  $195.74 \pm 3.58$  pg/mL ( $p < 0.05$  vs. G3), representing 76.1% normalization. Although statistically significant improvements were observed for both hormones, values remained significantly different from those of controls ( $p < 0.05$ ), indicating incomplete restoration of the activin–inhibin axis with conventional extract administration.

**Table 1.** Effects of Marjoram Aqueous Extract and Nano-Formulation on Serum Reproductive Hormone Concentrations in Letrozole-Induced PCOS Rats

| Group              | FSH (mIU/mL)              | LH (mIU/mL)               | P4 (mIU/mL)               | E2 (pg/mL)                 | Testosterone (pg/mL)     | Activin A (pg/mL)          | Inhibin-B (pg/mL)          |
|--------------------|---------------------------|---------------------------|---------------------------|----------------------------|--------------------------|----------------------------|----------------------------|
| G1 (Control)       | 10.30 ± 0.18 <sup>a</sup> | 13.71 ± 0.23 <sup>e</sup> | 12.07 ± 0.16 <sup>a</sup> | 106.66 ± 3.12 <sup>a</sup> | 0.20 ± 0.01 <sup>e</sup> | 560.03 ± 4.85 <sup>a</sup> | 146.58 ± 2.91 <sup>e</sup> |
| G2 (CMC)           | 10.23 ± 0.17 <sup>a</sup> | 13.84 ± 0.25 <sup>e</sup> | 11.92 ± 0.15 <sup>a</sup> | 105.38 ± 2.98 <sup>a</sup> | 0.20 ± 0.01 <sup>e</sup> | 558.26 ± 4.73 <sup>a</sup> | 147.91 ± 3.02 <sup>e</sup> |
| G3 (PCOS)          | 5.78 ± 0.15 <sup>d</sup>  | 20.07 ± 0.52 <sup>a</sup> | 6.87 ± 0.21 <sup>e</sup>  | 72.91 ± 2.47 <sup>c</sup>  | 0.45 ± 0.02 <sup>a</sup> | 458.36 ± 5.12 <sup>e</sup> | 378.59 ± 6.24 <sup>a</sup> |
| G4 (Marjoram)      | 7.52 ± 0.19 <sup>c</sup>  | 18.21 ± 0.41 <sup>b</sup> | 7.90 ± 0.18 <sup>d</sup>  | 87.64 ± 2.58 <sup>b</sup>  | 0.32 ± 0.02 <sup>b</sup> | 492.73 ± 4.89 <sup>d</sup> | 195.74 ± 3.58 <sup>b</sup> |
| G5 (Nano-Marjoram) | 8.73 ± 0.17 <sup>b</sup>  | 16.44 ± 0.38 <sup>c</sup> | 9.33 ± 0.19 <sup>c</sup>  | 90.64 ± 2.71 <sup>b</sup>  | 0.26 ± 0.01 <sup>c</sup> | 525.77 ± 5.03 <sup>c</sup> | 179.35 ± 3.41 <sup>c</sup> |

Data presented as Mean±SD (n=10 per group). Different superscript letters within each column indicate significant differences ( $p < 0.05$ , Fisher's LSD test). FSH: follicle-stimulating hormone; LH: luteinizing hormone; P4: progesterone; E2: estradiol.

Zinc oxide nano-marjoram treatment (G5) achieved superior restoration of the activin–inhibin axis compared to both the PCOS group and conventional marjoram extract (Table 1). Activin A was restored to  $525.77 \pm 5.03$  pg/mL, representing a 14.7% increase over G3 ( $p < 0.05$ ) and 93.9% normalization toward the G1 control value. Inhibin-B was reduced to  $179.35 \pm 3.41$  pg/mL—a 52.6% decrease relative to G3 ( $p < 0.05$ )—corresponding to 87.7% normalization. Both values were statistically distinct from G4 ( $p < 0.05$ ), confirming the superiority of nano-formulation delivery over conventional extract. Residual differences from controls did not reach statistical significance for inhibin-B ( $p > 0.05$ ), though activin A remained marginally below control levels ( $p < 0.05$ ). The near-complete restoration of the activin A/inhibin-B balance by nano-marjoram treatment has direct mechanistic relevance to the observed hormonal recovery in Section 3.1. Activin A promotes FSH-stimulated granulosa cell proliferation and follicular recruitment [34]; its restoration in G5 is consistent with the 51% FSH recovery reported in Table 1. Conversely, the 52.6% reduction in inhibin-B removes a key component of pituitary FSH suppression, further facilitating FSH normalization. Collectively, the quantitative recovery of both activin A and inhibin-B in G5—to within 6–12% of control values—provides strong mechanistic support for the observed restoration of folliculogenesis and ovarian morphology documented in Section 3.3 and Table 2, and is consistent with the anti-androgenic and antioxidant properties attributed to marjoram polyphenolic constituents [30, 31].

### 3.3. Histological Examination of Ovarian Tissue

Control groups (G1, G2) exhibited normal ovarian histology with a well-organized cortical architecture, encompassing all follicular developmental stages (Figure 1). Quantitative morphometric analysis (Table 2) confirmed high follicular density across developmental categories: primordial ( $18.4 \pm 2.1$  and  $18.1 \pm 1.9$  per section, respectively), primary ( $12.6 \pm 1.5$  and  $12.4 \pm 1.3$ ), secondary ( $8.3 \pm 1.2$  and  $8.1 \pm 1.0$ ), and antral ( $5.7 \pm 0.9$  and  $5.5 \pm 0.8$ ) follicles. Corpus luteum counts were  $4.8 \pm 0.7$  and  $4.7 \pm 0.6$  per section, respectively, with a negligible cystic follicle incidence ( $0.3 \pm 0.2$  and  $0.4 \pm 0.2$ ). Mean cystic follicle diameter, where present, did not exceed  $183.7 \pm 25.3$   $\mu$ m, consistent with normal antral follicle dimensions. No significant differences were detected between G1 and G2 for any morphometric parameter ( $p > 0.05$ ), confirming that CMC vehicle administration did not affect ovarian histology. Letrozole-induced PCOS (G3) produced severe and statistically significant histopathological alterations across all morphometric endpoints (Figure 1c, 1d; Table 2). Primordial, primary, secondary, and antral follicle counts declined by 61%, 60%, 66%, and 72%, respectively, compared to controls ( $p < 0.05$ ): primordial  $7.2 \pm 1.4$ , primary  $5.1 \pm 1.1$ , secondary  $2.8 \pm 0.7$ , and antral  $1.6 \pm 0.5$  per section. This broad depletion across follicular categories reflects the profound disruption of folliculogenesis consequent to letrozole-induced hyperandrogenism. Corpus luteum counts were markedly reduced to  $1.1 \pm 0.4$  per section (77% decrease vs. G1,  $p < 0.05$ ), consistent with impaired ovulation. Cystic follicle incidence increased dramatically to  $9.4 \pm 1.3$  per section, with mean cystic follicle diameter reaching  $864.3 \pm 72.1$   $\mu$ m—a 385% increase relative to controls ( $p < 0.05$ ), indicative of subcapsular polycystic morphology. Histologically, cystic follicles exhibited markedly attenuated granulosa cell layers, severe medullary vascular congestion, and extensive eosinophilic inflammatory cell infiltration, consistent with established PCOS-associated ovarian pathology [24, 25].

Marjoram aqueous extract treatment (G4) produced significant and statistically supported histological improvement relative to the PCOS group (Figure 1e; Table 2), though partial pathological features persisted. All follicle category counts increased significantly: primordial to  $12.3 \pm 1.7$  (71% recovery toward control,  $p < 0.05$ ), primary to  $8.6 \pm 1.2$ , secondary to  $5.4 \pm 0.9$ , and antral to  $3.7 \pm 0.7$  per section. Corpus luteum count increased to  $2.8 \pm 0.5$  per section (representing 61% recovery,  $p < 0.05$  vs. G3). Cystic follicle incidence decreased to  $4.8 \pm 0.9$  per section, and mean cystic follicle diameter was reduced to  $512.6 \pm 48.4$   $\mu$ m (41% reduction vs. G3,  $p < 0.05$ ), though these values remained significantly elevated compared to controls. These findings indicate that conventional marjoram extract exerts genuine but incomplete histomorphometric restoration. Zinc oxide nano-marjoram treatment (G5) achieved superior histomorphometric restoration across all parameters compared to both the PCOS group and the conventional marjoram extract group (Figure 1f; Table

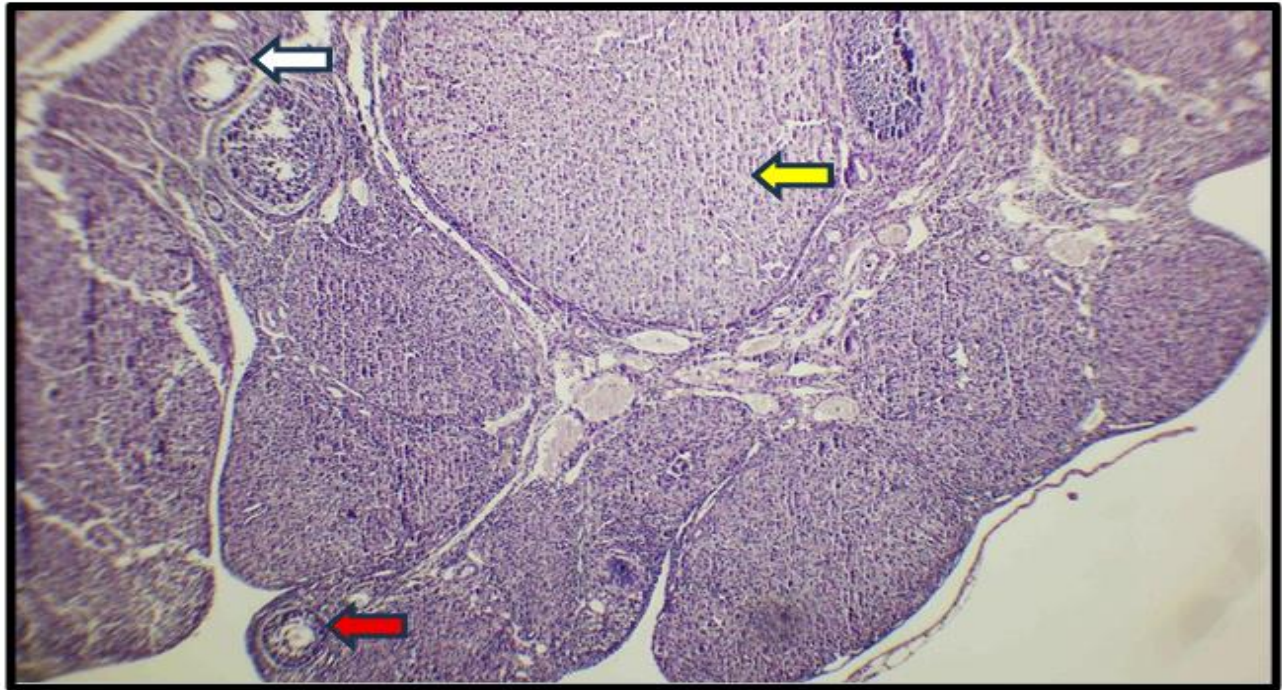


2). Primordial follicle counts recovered to  $16.2 \pm 1.8$  per section (88% of control value), primary follicles to  $11.0 \pm 1.4$  (87%), secondary follicles to  $7.2 \pm 1.1$  (87%), and antral follicles to  $5.1 \pm 0.8$  (89%). Corpus luteum count recovered to  $4.2 \pm 0.5$  per section, representing 88% normalization toward control levels and a statistically significant improvement over both G3 and G4 ( $p < 0.05$ ). Cystic follicle incidence was reduced to  $1.9 \pm 0.6$  per section, and mean cystic follicle diameter declined to  $284.1 \pm 36.7 \mu\text{m}$  (67% reduction vs. G3,  $p < 0.05$ ), approaching control ranges. Residual cystic follicle counts and diameters in G5 remained marginally higher than controls ( $p < 0.05$ ), indicating near-complete but not total normalization. These superior outcomes are consistent with the enhanced bioavailability and cellular uptake associated with nanodelivery systems [29] and align with the hormonal normalization data reported in Section 3.1. The quantitative progression across groups—from severe histopathological disruption in G3, through partial recovery in G4, to near-complete restoration in G5—is illustrated in Figure 2, which depicts group-wise morphometric comparison for all follicular categories, corpus luteum count, and mean cystic follicle diameter. These data provide objective, statistically validated evidence for the dose-response relationship between marjoram nano-formulation delivery and ovarian histological recovery, corroborating the hormonal data presented in Table 1.

**Table 2.** Quantitative Histomorphometric Analysis of Ovarian Sections in Letrozole-Induced PCOS Rats

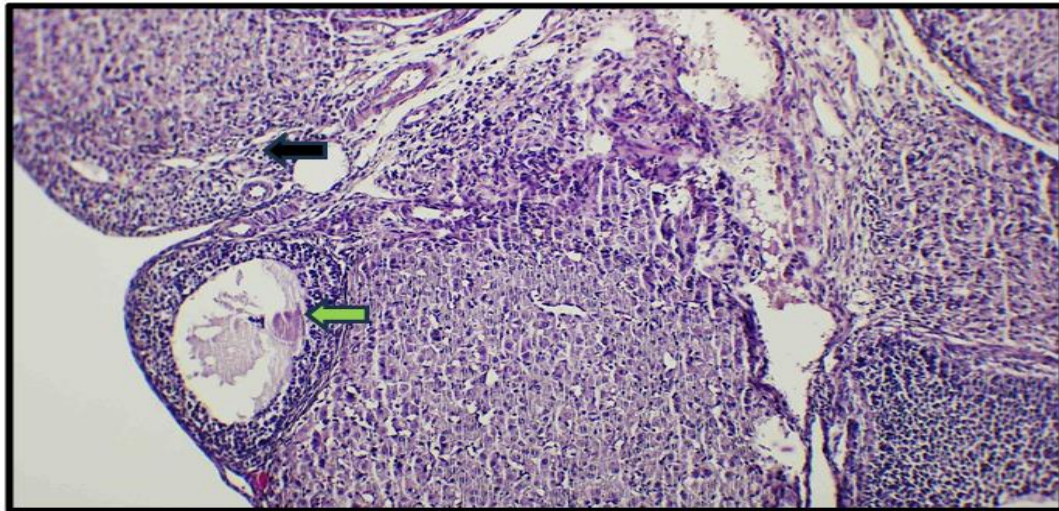
| Group              | Primordial<br>Follicles<br>(n/section) | Primary<br>Follicles<br>(n/section) | Secondary<br>Follicles<br>(n/section) | Antral<br>Follicles<br>(n/section) | Cystic<br>Follicles<br>(n/section) | Corpus<br>Luteum<br>(n/section) | Cystic<br>Follicle<br>Diameter<br>( $\mu\text{m}$ ) |
|--------------------|----------------------------------------|-------------------------------------|---------------------------------------|------------------------------------|------------------------------------|---------------------------------|-----------------------------------------------------|
| G1 (Control)       | $18.4 \pm 2.1^a$                       | $12.6 \pm 1.5^a$                    | $8.3 \pm 1.2^a$                       | $5.7 \pm 0.9^a$                    | $0.3 \pm 0.2^e$                    | $4.8 \pm 0.7^a$                 | $178.4 \pm 22.6^e$                                  |
| G2 (CMC)           | $18.1 \pm 1.9^a$                       | $12.4 \pm 1.3^a$                    | $8.1 \pm 1.0^a$                       | $5.5 \pm 0.8^a$                    | $0.4 \pm 0.2^e$                    | $4.7 \pm 0.6^a$                 | $183.7 \pm 25.3^e$                                  |
| G3 (PCOS)          | $7.2 \pm 1.4^e$                        | $5.1 \pm 1.1^e$                     | $2.8 \pm 0.7^e$                       | $1.6 \pm 0.5^e$                    | $9.4 \pm 1.3^a$                    | $1.1 \pm 0.4^e$                 | $864.3 \pm 72.1^a$                                  |
| G4<br>(Marjoram)   | $12.3 \pm 1.7^c$                       | $8.6 \pm 1.2^c$                     | $5.4 \pm 0.9^c$                       | $3.7 \pm 0.7^c$                    | $4.8 \pm 0.9^b$                    | $2.8 \pm 0.5^c$                 | $512.6 \pm 48.4^b$                                  |
| G5<br>(Nano-Marj.) | $16.2 \pm 1.8^b$                       | $11.0 \pm 1.4^b$                    | $7.2 \pm 1.1^b$                       | $5.1 \pm 0.8^b$                    | $1.9 \pm 0.6^c$                    | $4.2 \pm 0.5^b$                 | $284.1 \pm 36.7^c$                                  |

Data presented as Mean  $\pm$  SD ( $n = 10$  per group). Different superscript letters within each column indicate statistically significant differences ( $p < 0.05$ , one-way ANOVA followed by Fisher's LSD post-hoc test). G1: Negative control (physiological saline); G2: CMC vehicle control; G3: PCOS induction (letrozole 1 mg/kg, 58 days); G4: Letrozole + marjoram aqueous extract (45 mg/kg, 30 days); G5: Letrozole + zinc oxide nano-marjoram formulation (45 mg/kg, 30 days). Follicle counts assessed per serial 5- $\mu\text{m}$  ovarian cross-section (3 sections per animal, counts averaged). Cystic follicle diameter measured for all cystic follicles identified per section (diameter  $\geq 500 \mu\text{m}$  in G3); values shown as mean across all cysts per animal.

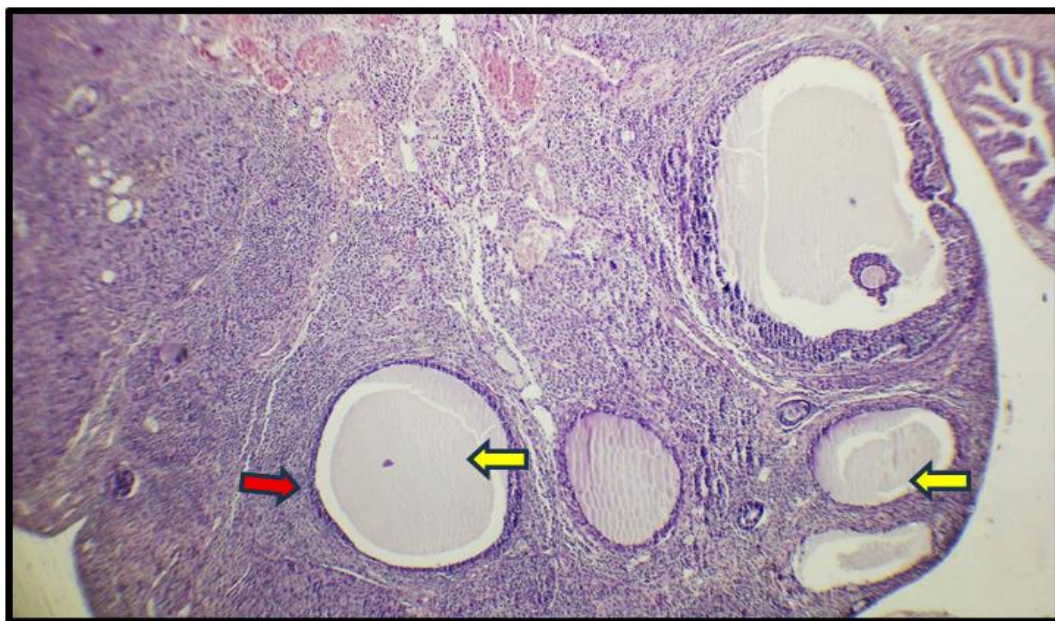


**Figure 1.** Photomicrographs of hematoxylin and eosin (H&E)-stained ovarian tissue sections from the five experimental groups at the 58-day study endpoint. (a) G1 (negative control, physiological saline): Normal ovarian cortical architecture showing orderly follicular development. Primary follicle (red arrow), corpus luteum (yellow arrow), and secondary follicle (white arrow) are clearly identified (magnification  $\times 4$ ). (b) G2 (CMC vehicle control): Comparable normal ovarian histology to G1 with intact interstitial stromal cells (black arrow). No structural abnormalities observed (H&E,  $\times 10$ ). (c) G3 (PCOS group, letrozole 1 mg/kg for 58 days): Multiple subcapsular cystic follicles (yellow arrow) lined with attenuated granulosa cells (red arrow) and markedly reduced corpus luteum development (H&E,  $\times 4$ ). (d) G3 (PCOS group, higher magnification): Severe medullary vascular congestion (unlabeled) with extensive infiltration of eosinophilic inflammatory cells (green arrow) (H&E,  $\times 40$ ). (e) G4 (marjoram aqueous extract treatment, 45 mg/kg for 30 days post-induction): Partial histological recovery evidenced by increased corpus luteum numbers (yellow arrow) and reduced cystic follicle frequency (red arrow). Some pathological features persist (H&E,  $\times 4$ ). (f) G5 (zinc oxide nano-marjoram, 45 mg/kg for 30 days post-induction): Near-complete ovarian architectural restoration with substantially increased corpus luteum numbers (yellow arrow), enhanced Graafian follicle development (red arrow), and minimal residual cystic follicle formation. Ovarian morphology closely resembles control groups (H&E,  $\times 4$ ). Scale bar = 200  $\mu\text{m}$  (a, c, e, f); 50  $\mu\text{m}$  (d); 100  $\mu\text{m}$  (b).

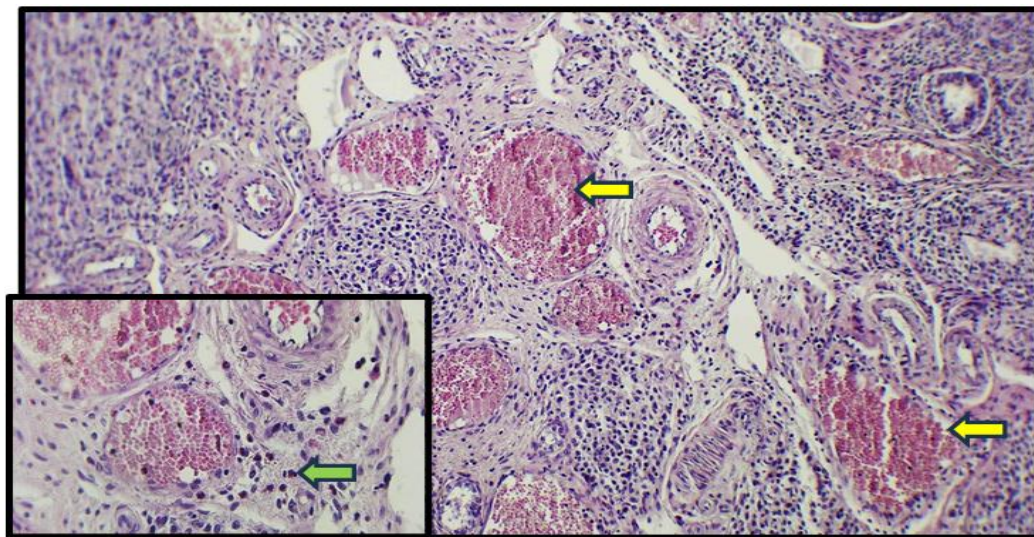




**Figure 2.** Quantitative histomorphometric comparison of ovarian structural parameters across the five experimental groups. (a) Follicle counts per ovarian cross-section by developmental category (primordial, primary, secondary, and antral). (b) Corpus luteum count per ovarian cross-section. (c) Cystic follicle count per ovarian cross-section. (d) Mean cystic follicle diameter ( $\mu\text{m}$ ). Data are presented as mean  $\pm$  SD ( $n = 10$  per group). Columns sharing different superscript letters are significantly different ( $p < 0.05$ , one-way ANOVA with Fisher's LSD post-hoc test). G1: negative control; G2: CMC vehicle control; G3: PCOS (letrozole); G4: letrozole + marjoram extract; G5: letrozole + nano-marjoram. Follicle counts were performed by a blind observer on three serial 5- $\mu\text{m}$  H&E-stained sections per animal, with values averaged.



**Figure 3.** G3 (PCOS group)  $\times 4$  magnification. Describes the polycystic cortical morphology: multiple subcapsular cystic follicles (yellow arrow) with attenuated granulosa cell lining (red arrow), reduced corpus luteum, and disorganized follicular architecture. Closes with the key quantitative values from Table 2 ( $9.4 \pm 1.3$  cysts/section;  $864.3 \mu\text{m}$  mean diameter).



**Figure 4.** G3 (PCOS group),  $\times 40$  magnification. Focuses on the medullary pathology: severe vascular congestion of sinusoids and extensive eosinophilic inflammatory cell infiltration (green arrow). Contextualizes these findings as consequences of androgen-excess-driven oxidative stress, and explicitly contrasts with Figures 1–2 (controls) and Figures 5–6 (treated groups).

Histological section of rat ovarian tissue from the letrozole-induced PCOS group (G3) at the 58-day study endpoint (H&E staining, magnification  $\times 4$ ). The section displays characteristic polycystic morphology resulting from 28 days of letrozole administration (1 mg/kg body weight). Multiple subcapsular cystic follicles (yellow arrow) are evident in the cortical region, lined with a markedly attenuated and flattened granulosa cell layer (red arrow), indicative of impaired granulosa cell proliferation and arrested follicular maturation. Corpus luteum development is substantially reduced compared to control groups, reflecting anovulation. The overall follicular architecture is disorganized, with a predominance of cystic structures replacing normal secondary and antral follicles. Scale bar = 200  $\mu\text{m}$ . Quantitative morphometric data: cystic follicle count  $9.4 \pm 1.3$  per section; mean cystic follicle diameter  $864.3 \pm 72.1 \mu\text{m}$ ; corpus luteum count  $1.1 \pm 0.4$  per section (Figure 3 and Table 2). High-magnification histological section of rat ovarian medullary tissue from the letrozole-induced PCOS group (G3) at the 58-day study endpoint (H&E staining, magnification  $\times 40$ ) (Figure 4). The image reveals severe vascular congestion of medullary blood vessels (unlabeled, visible as dilated, erythrocyte-engorged sinusoids), consistent with impaired ovarian microcirculation secondary to androgen excess. Extensive infiltration of eosinophilic inflammatory cells is observed throughout the stromal interstitium (green arrow), indicating a pronounced local inflammatory response associated with PCOS-related oxidative stress and immune activation. These vascular and inflammatory changes were absent in control groups (Figures 1 and 2) and were substantially reduced following treatment with marjoram extract and nano-marjoram formulation (Figures 5 and 6). Scale bar = 50  $\mu\text{m}$ .





**Figure 5.** G4 (conventional marjoram extract)  $\times 4$ . Documents partial recovery: increased corpus luteum (yellow arrow;  $2.8 \pm 0.5$ ), reduced cystic follicles (red arrow;  $4.8 \pm 0.9$ , diameter  $512.6 \mu\text{m}$ ). Carefully notes what persists — residual granulosa attenuation and architectural disorganization — making it clear this is incomplete normalization.

Histological section of rat ovarian tissue from the marjoram aqueous extract treatment group (G4; letrozole 1 mg/kg for 28 days followed by *Origanum majorana* aqueous extract 45 mg/kg for 30 days) at the 58-day study endpoint (H&E staining, magnification  $\times 4$ ). Compared with the PCOS group (Figure 3), the section demonstrates significant but partial histological recovery. Corpus luteum numbers are increased (yellow arrow;  $2.8 \pm 0.5$  per section), and cystic follicle incidence is reduced (red arrow;  $4.8 \pm 0.9$  per section, mean diameter  $512.6 \pm 48.4 \mu\text{m}$ ), indicating partial restoration of ovulatory activity and inhibition of cystic follicle expansion. Primordial, primary, secondary, and antral follicle counts are improved relative to G3 (Table 2), though cortical follicular density remains below control levels. Some disorganization of the follicular architecture and residual granulosa cell attenuation persists, consistent with incomplete normalization of the hyperandrogenic milieu at this dose. Scale bar =  $200 \mu\text{m}$ .

Histological section of rat ovarian tissue from the zinc oxide nano-marjoram treatment group (G5; letrozole 1 mg/kg for 28 days followed by ZnO nano-loaded *Origanum majorana* formulation 45 mg/kg for 30 days) at the 58-day study endpoint (H&E staining, magnification  $\times 4$ ). The section demonstrates near-complete histomorphological restoration of ovarian architecture. Corpus luteum numbers are substantially increased (yellow arrow;  $4.2 \pm 0.5$  per section, representing 88% normalization toward control values), and normal Graafian follicle development is restored (red arrow), indicating recovery of ovulatory capacity. Cystic follicle incidence is markedly reduced to  $1.9 \pm 0.6$  per section (mean diameter  $284.1 \pm 36.7 \mu\text{m}$ ), approaching control ranges. Follicular density across all developmental categories (primordial through antral) is restored to 87–89% of control levels (Table 2). Inflammatory infiltration and medullary vascular congestion, prominent in Figure 4, are largely resolved. The overall ovarian architecture closely resembles that of control groups (Figures 1 and 2), consistent with the superior hormonal normalization achieved by nano-delivery of marjoram bioactive compounds (Table 1). Scale bar =  $200 \mu\text{m}$ .





**Figure 6.** G5 (nano-marjoram)  $\times 4$ . Documents near-complete restoration: corpus luteum at  $4.2 \pm 0.5$  (88% of control), Graafian follicle recovery (red arrow), cystic incidence down to  $1.9 \pm 0.6$  (diameter  $284 \mu\text{m}$ ), and resolved inflammation. Cross-references Tables 1 and 2, and Figures 1–4 for comparison.

### 3.4 Discussion

This study demonstrates that marjoram extract, particularly nano-formulated marjoram, effectively ameliorates letrozole-induced PCOS by normalizing reproductive hormones and restoring ovarian histology. The 44% reduction in FSH, 46% elevation in LH, and 125% increase in testosterone in PCOS animals recapitulate the characteristic hormonal dysregulation observed clinically [24,25,26,27]. Letrozole's aromatase inhibition prevents the conversion of androgens to estrogens, resulting in compensatory FSH suppression and hyperandrogenism [28]. The superior therapeutic efficacy of nano-marjoram (51% FSH restoration, 42% testosterone reduction) compared to conventional extract (30% FSH restoration, 29% testosterone reduction) suggests enhanced bioavailability and cellular uptake of bioactive compounds through nano-delivery systems [29]. Marjoram's therapeutic effects are likely mediated by multiple mechanisms, including polyphenolic antioxidant activity, anti-androgenic properties, and modulation of steroidogenic pathways [30,31]. Chlorogenic acid, a prominent marjoram phenolic constituent, increases progesterone production while reducing LH and testosterone [32]. Gallic acid reverses metabolic and hormonal disorders by regulating androgen signaling [33]. The substantial restoration of activin A (15% increase) and reduction of inhibin-B (53% decrease) by nano-marjoram further indicate restored folliculogenesis and granulosa cell function [34,35]. Histological improvements—increased corpus luteum formation, restored Graafian follicles, reduced cystic follicles, and diminished inflammatory infiltration—correlate strongly with hormonal normalization, reflecting comprehensive therapeutic effects. These findings align with previous studies demonstrating marjoram's protective effects against PCOS-associated oxidative stress by enhancing antioxidant enzyme systems (SOD, catalase, GSH) and reducing lipid peroxidation [36,37].

## 4. Conclusions

This study demonstrates that marjoram (*Origanum majorana* L.) extract, particularly in a zinc oxide nanoformulation, effectively ameliorates letrozole-induced PCOS by restoring reproductive hormone balance and ovarian histological integrity. Nano-marjoram treatment achieved superior therapeutic outcomes, restoring FSH levels by 51%, reducing testosterone by 42%, normalizing LH/FSH ratios, and substantially improving ovarian architecture with increased corpus luteum formation and reduced cystic follicles. The enhanced efficacy of the nanoformulation compared to the conventional extract suggests improved

bioavailability and cellular delivery of bioactive polyphenolic compounds. These findings indicate marjoram's potential as a safe, natural therapeutic agent for PCOS management, warranting clinical translation studies to evaluate efficacy and safety in human PCOS patients. Future investigations should elucidate specific molecular mechanisms underlying marjoram's anti-androgenic and folliculogenesis-promoting effects.

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# Enhancement of Cleaning-in-Place (CIP) Using Chemical Cleaning to Control Fouling and Scaling on Seawater Reverse Osmosis (SWRO) Membrane Full-Scale Plant Producing Demineralized Water in Central Java

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**Abstract:** Despite recent advances in membrane technology for sustainable water treatment, fouling remains a key challenge to long-term performance, as it leads to surface deposits and concentration polarization. To address this issue, this study evaluates the effectiveness of cleaning-in-place (CIP) in controlling fouling and scaling in a seawater reverse osmosis system at Sumber Segara Primadaya Co. Ltd. Evaluation parameters include pressure drop, flux recovery ratio, salt rejection recovery, and membrane characterization before and after cleaning. The CIP process showed positive results, as indicated by the recovery of differential pressure ( $\Delta P$ ) to its initial condition. The membrane flow rate also returned to its original value of 103–104 m<sup>3</sup>/h under the same operating pressure of 3.6 bar. Membrane productivity, which had declined by 19%, was successfully restored, with recovery improving from 38% to 40%. In addition, salt rejection increased from 98.8% back to approximately 99.03%, representing a 2% improvement toward the initial value of 99.1%. These findings demonstrate that CIP is an effective method for restoring membrane performance and extending operational lifespan.

**Keywords:** Seawater; desalination; membrane; fouling; Cleaning-in-Place (CIP)

## 1. Introduction

Membrane technology offers an effective desalination method for converting seawater into fresh water through filtration. The development of membranes for desalination has progressed from nanofiltration (NF) and forward osmosis (FO) to reverse osmosis (RO), which currently stands out as the most promising technology for seawater filtration [1–3]. Membrane fouling remains an unavoidable challenge in desalination operations, despite decades of research aimed at enhancing anti-fouling properties and developing control strategies using supporting materials. Nevertheless, significant fouling still occurs over time [4].



Fouling reduces membrane performance by causing foulant buildup and surface deposits, leading to concentration polarization [4]. This occurs as rejected solutes accumulate along the membrane surface during filtration, gradually increasing solute concentration and worsening membrane efficiency [4]. A decline in membrane performance, including reduced permeate flux, selectivity, and permeability, can significantly shorten the membrane's operational life [9]. This reduction in performance is often caused by the accumulation of unwanted substances, influenced by feed characteristics such as composition, pH, ionic strength, and foulant concentration [5]. The deposition of these materials can occur either on the membrane surface or within its internal structure. Additionally, the types of accumulated impurities can be classified as organic, inorganic, or biological, often resulting from continuous filtration and microbial activity in the feed solution [4].

Fouling generally begins with the accumulation of particles on the membrane surface, which causes pore obstruction and narrowing. Over time, this leads to the formation of a thick layer that reduces membrane efficiency, a phenomenon known as concentration polarization [6]. To enhance membrane performance, several approaches can be applied, including feed pretreatment, modification of membrane properties, optimization of operating conditions, and membrane cleaning. Among these, membrane cleaning is the most widely used and practical method in industrial applications [7]. When performed regularly according to a set schedule, cleaning can improve process efficiency and extend membrane service life. Membrane cleaning techniques are commonly grouped into four main categories: hydraulic, mechanical, chemical, and electrical. The selection of an appropriate method depends on the membrane module design, chemical resistance, and the type of foulants present [8].

Chemical cleaning is considered one of the most effective methods for addressing membrane fouling, using a variety of cleaning agents either individually or in combination. The effectiveness of this method depends on several factors, including the type of chemicals used and the duration of the cleaning process. Common cleaning agents include acids, alkalis, surfactants, enzymes, complexing agents, disinfectants, and blended formulations [9]. Although chemical cleaning, particularly for reverse osmosis (RO) membranes, has been widely studied in the literature, the relationship between laboratory-scale results and full-scale cleaning performance remains poorly defined [10], [11], [12]. This study investigates the effectiveness of CIP in controlling fouling and scaling in a seawater RO system at Sumber Segara Primadaya Co. Ltd. Key evaluation parameters include pressure drop, flux recovery ratio, salt rejection recovery, and membrane performance monitoring over several weeks post-cleaning. This study provides a comprehensive evaluation of CIP performance in a full-scale SWRO plant under real industrial operating conditions, offering valuable insights that bridge the gap between laboratory-scale research and practical field application. The novelty of this study lies in its full-scale industrial evaluation of enhanced chemical cleaning-in-place (CIP) strategy under real operational conditions in a seawater reverse osmosis (SWRO) plant producing demineralized water. The exact problem addressed in this study was the progressive performance decline of SWRO membranes, characterized by increasing pressure drop, reduced recovery, and higher permeate conductivity, which led to unstable demineralized water production. The study aimed to evaluate whether optimization of chemical CIP protocols could effectively restore membrane performance and improve operational stability without membrane replacement. Therefore, this work contributes practical operational insights by correlating CIP treatment with normalized performance indicators (pressure drop, recovery, salt rejection, and permeate conductivity) under real plant conditions, providing guidance for membrane maintenance strategies in industrial desalination systems. This study was conducted in a full-scale industrial SWRO system, and therefore direct membrane autopsy or surface characterization was beyond the scope of the investigation. Techniques such as SEM-EDS, FTIR, or ICP-OES, which could provide definitive identification of foulant composition, were not performed. Consequently, the interpretation of organic fouling and inorganic scaling is based on operational indicators and feed-water chemistry rather than direct analytical confirmation.

## 2. Materials and Methods

### 2.1 Materials

This research was conducted at a water treatment facility of a coal-fired steam power plant in Indonesia, operated by Sumber Segara Primadaya Co. Ltd (S2P). The chemical cleaning agents used in this industrial-scale study were of research-grade quality. Permea Clean PC-90, composed of 40% sodium hydroxide and 60% tetrasodium EDTA, was used as the alkaline cleaning agent for membrane CIP. A chemical agent consisting of 80% citric acid served as the acidic cleaning agent. In addition, hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used as solvents to adjust the pH of the cleaning solutions as needed. Demineralized water was used as the medium for chemical treatment analysis. Nalco Co., Ltd., Indonesia, supplied all materials. The SWRO system in this study serves as the primary desalination stage before final polishing treatment. The target permeate conductivity from the RO unit was  $< 50 \mu\text{S}/\text{cm}$  before downstream demineralization. Final boiler feed water specifications  $< 1\text{--}5 \mu\text{S}/\text{cm}$  were achieved after post-treatment units and ion exchange/mixed-bed polishing.

**Table 1.** Operating conditions of the reverse osmosis membrane

| Case Studies              | Membrane Specification                                                    |
|---------------------------|---------------------------------------------------------------------------|
| Location                  | S2P Co. Ltd, Indonesia                                                    |
| Company website           | <a href="https://www.ssprimadaya.co.id">https://www.ssprimadaya.co.id</a> |
| Manufacture               | LG Chem                                                                   |
| Feed Pressure             | 60 bar                                                                    |
| Hp Pump Flow              | 255 L/min                                                                 |
| HP pump Pressure          | 3.6 bar                                                                   |
| RO Membrane Element       | LG SW400 (37.2 m <sup>2</sup> )                                           |
| RO Pressure Vessel        | 8" 1000 psi 7 core                                                        |
| Amount of Pressure Vessel | 28 bar                                                                    |
| Total Membrane Core       | 196 pcs                                                                   |
| Feed Conductivity         | 40000-50000 $\mu\text{S}/\text{cm}$                                       |
| Permeate Product          | 102 m <sup>3</sup> /jam                                                   |
| % Recovery                | 40%                                                                       |
| Salt Rejection            | >99%                                                                      |
| $\Delta P$                | <0.25                                                                     |

### 2.2 Method

#### 2.2.1 RO Indication for Cleaning

The RO membrane at S2P Indonesia was operated to produce demineralized water as feedwater for a supercritical boiler. The operating conditions of the RO membrane are described in **Table 1**. This RO system underwent periodic CIP cleaning when the differential pressure exceeded 0.25 bar. Additionally, when the salt rejection, recovery rate, or permeate production decreased by 10–15% from standard performance levels, chemical cleaning of the membrane was required.

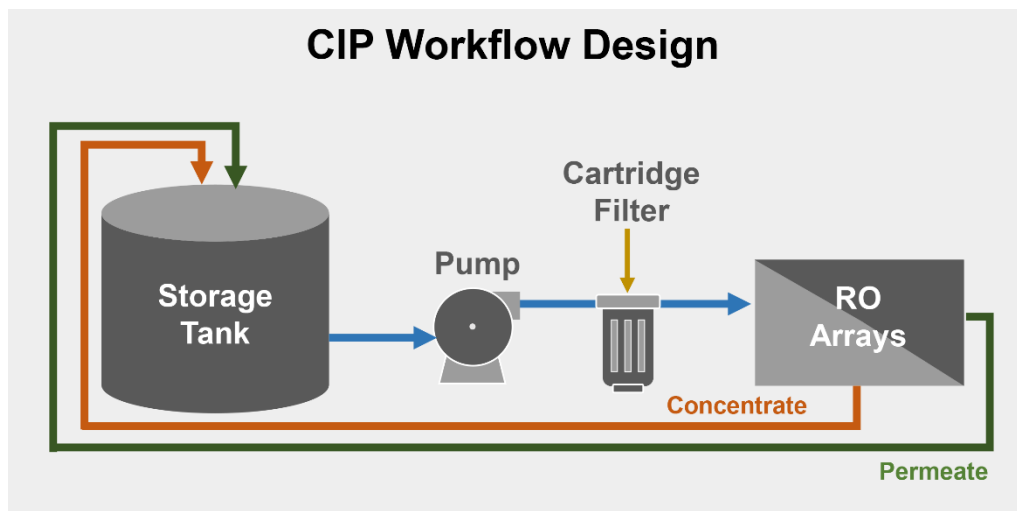
#### 2.2.2 Synthesis of Chemical Solution for Cleaning

The CIP process for the reverse osmosis membrane began with an alkaline cleaning step using Permeate Clean PC-90, a chemical containing dynamic EDTA content. The PC-90 solution was prepared at a concentration of 4% of the total membrane volume and introduced into the CIP chemical tank. Additionally, a 1% EDTA solution was prepared by diluting it with mineral water to match the total volume of the membrane system. NaOH was then added to the solution to adjust the pH to 12–13 before circulating it through the membrane during the cleaning process. Following completion of the alkaline cleaning stage, an acid cleaning process was performed using chemical N-70264, which contains citric acid as the active ingredient. A 4% N-70264 solution (based on the total membrane volume) was prepared, and HCl was added to adjust the pH to 2–3. This acidic solution was then circulated through the membrane during the cleaning process.

### 2.2.3 Cleaning Procedure

The cleaning process scheme is illustrated in Figure 1. It depicts the sequence of operations and the major components involved in the CIP process for cleaning and maintaining RO membranes. For instance, the CIP procedure for the reverse osmosis (RO) membrane consists of three main stages: (1) RO membrane valve configuration, (2) alkaline cleaning, and (3) acid cleaning. The first stage involves configuring the RO membrane system's valves to prepare for the cleaning process. The inlet and outlet valves of the RO membrane in service (feed and permeate lines) must be fully closed to isolate the system. The CIP inlet and outlet valves for the RO membrane must be opened to allow the cleaning solution to circulate. Before initiating the CIP process, ensure the CIP pump is in proper working order and that the cartridge filter is installed and ready for use. The second stage is the alkaline cleaning process. A pre-prepared alkaline cleaning solution is pumped through the RO system via the CIP line for 60 minutes of continuous circulation. Once the circulation is complete, the membrane is soaked in the alkaline solution for an additional 30 minutes. During both the circulation and soaking phases, the pH of the cleaning solution must be monitored every 10 minutes. If the pH falls below 12, NaOH should be carefully added to the CIP tank to maintain the target pH range of 12–13. This circulation-and-soaking cycle is repeated three times to ensure effective removal of organic and biological foulants. After the final soaking, the alkaline solution is drained, and the membrane is thoroughly rinsed with clean water. It is important to confirm that the rinse water pH is neutral (7–8), and both pH and turbidity should be measured and recorded during this step.

The final stage is the acid cleaning process. A pre-prepared acid solution is circulated through the RO membrane using the CIP line for 60 minutes. After the circulation, the membrane is soaked in the acid solution for 30 minutes. During this phase, the solution's pH should be checked every 10 minutes. If the pH rises above 2, HCl must be added carefully to bring the pH back within the target range of 2–3. The acid cleaning cycle includes both circulation and soaking and is repeated three times to dissolve inorganic scales and mineral deposits effectively. After the final soaking, the acid solution is drained, and the membrane is thoroughly rinsed using clean water. Similar to the previous rinse, the pH of the rinse water should be within the 7 to 8 range, and both pH and turbidity must be monitored and recorded to verify the effectiveness of the cleaning process. The selected three-month dataset represents stable full-load operation under consistent feedwater characteristics, enabling a reliable assessment of CIP effectiveness.



**Figure 1.** Schematic of cleaning-in-place (CIP) Process

### 2.2.4 Performance Evaluation Parameters

The performance of the SWRO system was evaluated using several key operational parameters, including recovery, salt rejection, permeate flux, and flux recovery. Recovery (R) represents the fraction of feed water converted into permeate and is calculated as the ratio of permeate flow rate ( $Q_p$ ) to feed flow rate ( $Q_f$ ), expressed as a percentage. This parameter indicates the efficiency of water production during

membrane operation. Salt rejection (SR) was used to assess membrane selectivity and desalination performance. It was determined based on the difference between feed concentration or conductivity ( $C_f$ ) and permeate concentration or conductivity ( $C_p$ ). A higher salt rejection value reflects better ion removal capability of the membrane. Permeate flux ( $J$ ) describes the permeate flow rate normalized by the effective membrane area ( $A$ ), representing the hydraulic productivity of the membrane system. This parameter is commonly used to evaluate the influence of fouling and cleaning processes on membrane performance. To assess the effectiveness of cleaning-in-place (CIP), flux recovery (FR) was calculated as the ratio of the permeate flux after cleaning to the flux before cleaning. In some cases, flux recovery can also be referenced to the initial clean membrane condition to evaluate the extent of performance restoration. Flux recovery values close to 100% indicate successful recovery of membrane permeability after cleaning. The corresponding equations used to calculate these parameters are provided as follows [13], [14], [15]:

$$R (\%) = \frac{Q_p}{Q_f} \times 100 \dots\dots\dots (1)$$

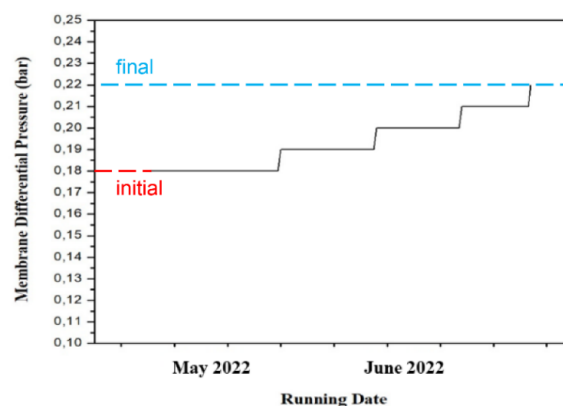
$$SR (\%) = \left(1 - \frac{C_p}{C_f}\right) \times 100 \dots\dots\dots (2)$$

$$FR (\%) = \left(\frac{J_{after}}{J_{before}}\right) \times 100 \dots\dots\dots (3)$$

### 3. Results and Discussion

#### 3.1 Effect of Cleaning on Membrane Differential Pressure

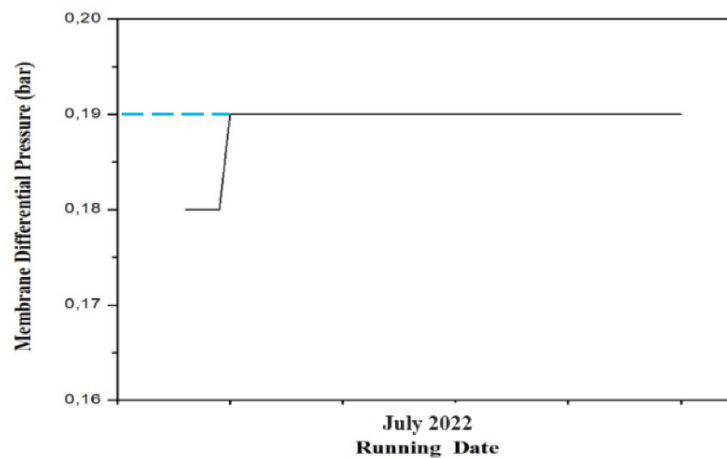
**Figure 2** shows the membrane differential pressure ( $\Delta P$ ) over time during operation from May to June 2022. The graph shows a gradual, consistent increase in  $\Delta P$ , from 0.18 bar to about 0.22 bar by the end of the monitoring period. This represents a total increase of around 15% from the initial pressure value. The stepwise pattern of the graph suggests periodic accumulation of fouling and scaling within the membrane system, leading to progressive blockage of the feed channels. This rising trend in  $\Delta P$  is a clear indicator that the membrane experienced increasing resistance to flow, signaling the need for CIP to restore optimal membrane performance [4]. In this regard, monitoring and responding to changes in  $\Delta P$  is essential to prevent severe membrane damage and to maintain long-term operational efficiency.



**Figure 2.** Differential pressure of the seawater reverse osmosis membrane before the CIP process

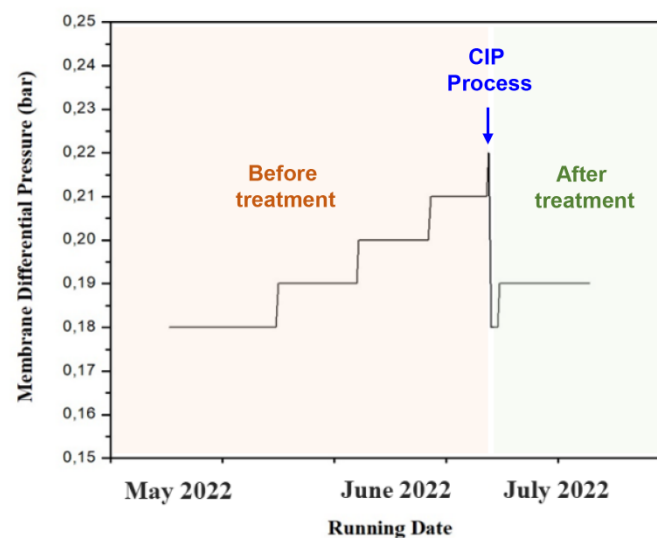
Before CIP, the membrane had been operating continuously for 49 days, during which the differential pressure increased by approximately 16% from its initial value, indicating a gradual accumulation of fouling and a decline in membrane performance. To restore optimal operation, a CIP procedure was performed, beginning with an alkali cleaning agent and followed by an acid cleaning step. The result of the membrane differential pressure after CIP is presented in **Figure 3**. The results show that the CIP is effective, as evidenced by the post-cleaning  $\Delta P$ , which stabilized at approximately 0.19 bar. This value remained steady

for at least two weeks after the CIP, demonstrating that the cleaning successfully removed fouling and restored the membrane to acceptable operational conditions.



**Figure 3.** Differential pressure of seawater reverse osmosis membrane after the CIP process

To clearly illustrate the impact of the CIP process, the data before and after cleaning were combined and are shown in Figure 4, highlighting the recovery of membrane differential pressure. In this condition, the recovery in differential pressure was due to the effective two-step CIP process, comprising alkaline and acid cleaning. During the alkaline stage, a basic cleaning solution was used to dissolve and remove organic contaminants such as oils, biofilms, and other organic matter from the membrane surface. This was then followed by the acid cleaning stage, which targeted and eliminated inorganic deposits, including mineral scale and metal oxides [16]. These two cleaning stages worked synergistically to restore the membrane's condition and operational performance. It should be noted that similar findings have been reported in previous studies, supporting the effectiveness of the CIP process used in our system.



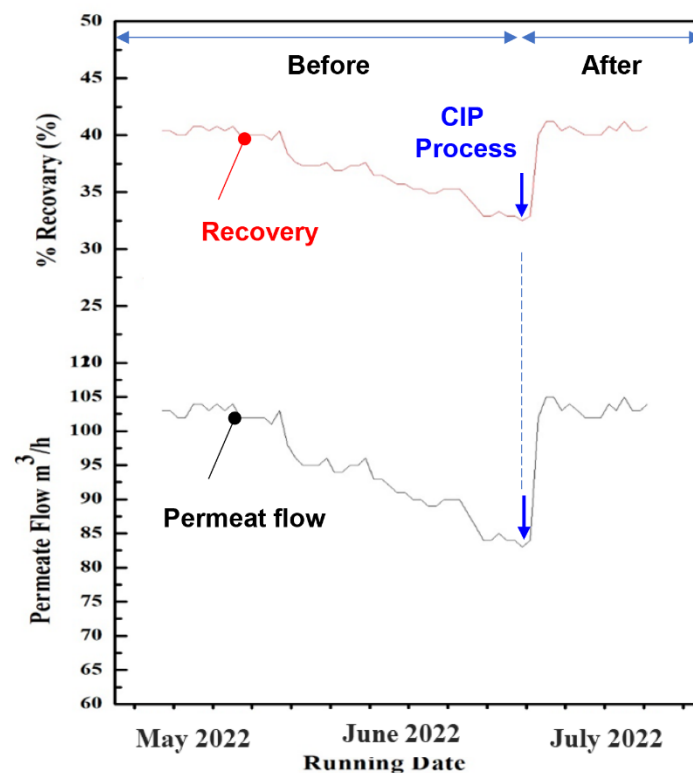
**Figure 4.** Differential pressure of seawater reverse osmosis membrane before and after the CIP process

### 3.2 Effect of Cleaning on Membrane Permeate Flow and % Recovery

As shown in Figure 5, the period from May to late June 2022 represents the operating condition before the application of the CIP procedure. During this time, a noticeable decline in membrane performance was recorded. The permeate flow rate gradually decreased from around 103–104 m<sup>3</sup>/h to ~85 m<sup>3</sup>/h, accompanied by a reduction in % recovery from 40% to ~35%. These trends reflect the accumulation of fouling materials such as organic compounds, biological growth, and inorganic scales, which can obstruct membrane pores and increase hydraulic resistance across the membrane module [20, 21]. In contrast, the



data in Figure 5 from July 2022 indicate the condition after CIP application, with a clear recovery in system performance. Under identical operating pressure conditions of 3.6 bar, the membrane flow rate returned to its initial state. This recovery corresponds to a 19% improvement in membrane productivity. Additionally, the % recovery, which had previously declined to 35%, increased to 40% after CIP, effectively restoring a 5% loss in membrane recovery. This enhancement can be directly attributed to the effectiveness of the CIP process, as further supported by the results in Figures 2 and 3. The underlying reason for these improvements is the removal of both organic and inorganic foulants accumulated over time. As shown in earlier discussion, the alkaline cleaning step dissolved organic contaminants such as biofilms and oils (*idem atas*), while the acid step removed scaling caused by mineral deposits. By eliminating these layers, the membrane surface and pores were cleared, allowing feed water to pass through more freely, thereby increasing permeate flow and restoring system efficiency.



**Figure 5.** Permeate flow and % Recovery before and after CIP process

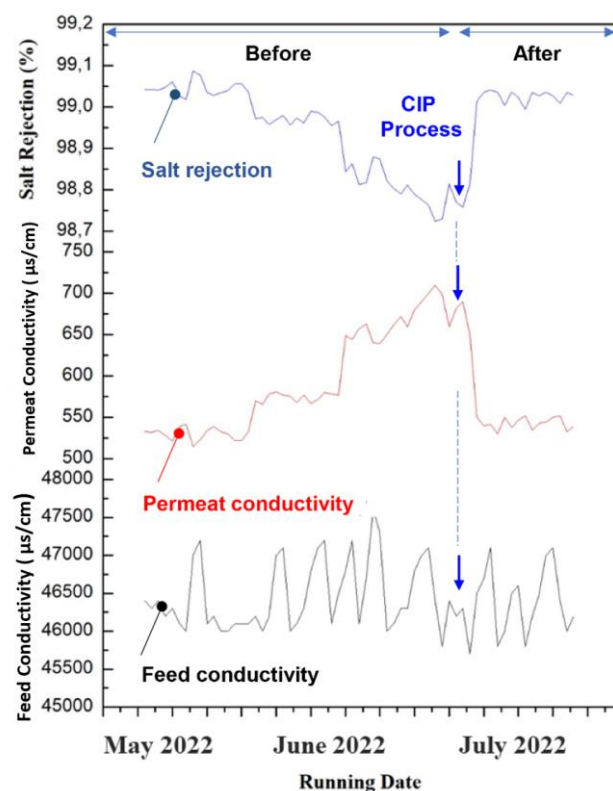
### 3.3 Effect of Cleaning on Permeate Quality and Salt Rejection

Figure 6 illustrates the changes in salt rejection, permeate conductivity, and feed conductivity from May to July 2022, providing insight into membrane performance before and after the CIP process. The data indicate a noticeable decline in membrane performance during May and June, before CIP, followed by a significant recovery in July after CIP was applied. During May and June, salt rejection decreased from 99.1% to 98.8%, while permeate conductivity increased to 690  $\mu\text{S}/\text{cm}$ . This represents about a 21% increase from the initial permeate conductivity levels. These changes reflect a decline in membrane selectivity, allowing more dissolved salts to pass through the membrane. At the same time, feed conductivity remained relatively stable, fluctuating between 46,000 and 47,500  $\mu\text{S}/\text{cm}$ . Since the feed conductivity did not change significantly, the observed deterioration in permeate quality can be attributed to membrane fouling or scaling rather than to fluctuations in feedwater salinity.

Permeate quality in a seawater reverse osmosis system is directly influenced by the difference between feed conductivity and permeate conductivity. When the membrane is functioning properly, it rejects most dissolved salts, resulting in low permeate conductivity and high salt rejection. However, as fouling from organic substances, microbial growth, or mineral scaling builds up, the membrane's ability to

retain salts is reduced. This leads to higher salt passage and increased permeate conductivity. This condition was clearly observed during May and June, before CIP was conducted. After the CIP process was implemented in early July, membrane performance improved substantially. Permeate conductivity decreased to approximately 540  $\mu\text{S}/\text{cm}$ , corresponding to about a 20% improvement in permeate quality. Salt rejection also improved, increasing to 99.03%. This represents about 2% recovery in salt rejection efficiency. Importantly, these improvements occurred while feed conductivity remained relatively unchanged, confirming that the CIP process was effective in restoring the membrane's selectivity and overall performance.

These findings demonstrate that the CIP process successfully removed the foulants and scaling that had impaired the membrane. As a result, the membrane's ability to separate dissolved salts was restored, leading to improved salt rejection and enhanced permeate quality. Finally, the conductivity data clearly illustrate that permeate quality is strongly influenced by the membrane condition and the conductivity difference between feed and permeate water. The improvements in salt rejection and permeate conductivity after the cleaning procedure highlight the critical role of regular chemical cleaning in maintaining long-term operational performance of seawater reverse osmosis systems.



**Figure 6.** Variation in feed conductivity, permeate conductivity, and salt rejection before and after the CIP process

These full-scale observations align qualitatively with controlled laboratory-scale experiments; however, the quantitative relationship between lab-scale cleaning efficiencies and real industrial system recovery remains poorly defined. Lab studies often focus on idealized conditions, specific foulant types, and simplified flow regimes, while full-scale operations must contend with complex foulant mixtures, variable hydraulic profiles, and prolonged exposure to harsh feed conditions. The findings of this study help bridge this gap by providing practical, field-based evidence that supports the effectiveness of chemical CIP in restoring both hydraulic and separation performance in an industrial SWRO plant [6, 17-18].

## 4. Conclusions

The CIP procedure applied in this study demonstrated a significant recovery of membrane function under real industrial conditions. Following the CIP process, the pressure drop ( $\Delta P$ ) decreased from 0.22 bar before CIP to approximately 0.19 bar after CIP, corresponding to a reduction of about 0.03 bar ( $\approx 14\%$ ), indicating partial removal of fouling layers and improved hydraulic conditions across the membrane system. The membrane flow rate recovered from its decline, returning to the initial level of 103–104 m<sup>3</sup>/h at a constant operating pressure of 60 bar, equivalent to a 19% improvement in membrane productivity. Recovery rate also improved, rising from 38% to 40%, resulting in a 5% increase in water recovery efficiency. In terms of separation performance, salt rejection improved from 98.8% to 99.03%, and permeate conductivity decreased from 690  $\mu\text{S}/\text{cm}$  to 540  $\mu\text{S}/\text{cm}$ , reflecting enhanced desalination performance and improved membrane selectivity after cleaning. These results confirm the effectiveness of CIP in restoring both hydraulic and separation performance of fouled membranes under full-scale industrial operation. Importantly, the findings also highlight how operational data at full scale can complement laboratory-scale studies, which often fail to capture the full complexity and variability of long-term field operations. Future studies should incorporate membrane-surface characterization to validate the composition and mechanisms of fouling.

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The manuscript has undergone comprehensive language editing, with grammar improvements assisted by AI-based language correction tools to enhance clarity, readability, and overall consistency.

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# Upgrading the Characterization of Spent Fluid Catalytic Cracking Catalyst into Functional Adsorbent via Hybrid Ultrasonic-Chemical Approach

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**Abstract:** Fluid catalytic cracking catalysts (FCCCs) are essential in petroleum refining but become hazardous spent materials after deactivation due to heavy metal and coke deposition. Regenerating spent FCC catalysts (SFCCCs) is necessary to reduce hazardous waste and restore physicochemical properties. Conventional regeneration methods face limitations, such as high energy demand or potential structural damage, leaving a gap for more efficient and sustainable approaches. This study shows that a hybrid ultrasonic-chemical method can effectively regenerate SFCCCs, improving surface morphology, pore structure, and crystallinity, as evidenced by characterization using XRD, FTIR, BET, and SEM-EDS. The untreated SFCCCs exhibited a surface area of 27.255 m<sup>2</sup>/g, which increased to 33.508 m<sup>2</sup>/g after hybrid treatment and 34.344 m<sup>2</sup>/g after chemical treatment, indicating pore reopening and enhanced surface activity. FTIR analysis showed clearer Si-O-Si and -OH bands in hybrid-treated samples, suggesting improved recovery of the zeolite structure and reduced coke formation. XRD patterns showed sharper peaks in the 2 $\theta$  = 22–30° range, indicating greater crystallinity in hybrid regeneration than in untreated samples. SEM-EDS showed cleaner, more porous morphologies with reduced Fe (3.25%) and exposure of hidden Ni (9.49%) after hybrid treatment. These findings underscore the effectiveness of ultrasonic-assisted chemical regeneration, which offers a promising pathway for sustainable catalyst waste management and potential future reuse applications.

**Keywords:** Chemical regeneration; hybrid regeneration; petroleum refining; spent catalyst; ultrasonic

## 1. Introduction

Fluid Catalytic Cracking Catalysts (FCCCs) play a crucial role in various industries, especially in chemical processes such as petroleum refining [1]. Annually, it is estimated that 170.000-200.000 tons of spent catalysts are produced [2]. Once these catalysts reach the end of their operational lifespan, spent fluid catalytic cracking catalysts (SFCCCs) are categorized as hazardous and toxic waste due to their substantial heavy metal content, including iron, vanadium, nickel, magnesium, and sulfur, which poses a potential environmental risk [2-3]. Therefore, spent catalyst waste cannot be disposed of



without proper treatment, necessitating effective methods to recycle or regenerate SFCCCs while recovering their economic value.

Although considered hazardous waste, the spent catalysts have a significant potential for reuse. Typically, spent catalysts are used to enhance the pyrolysis of activated sludge in refinery waste [3], increase reactivity in cement production [4], and serve as an adsorption medium in wastewater treatment [5]. However, the treatment of spent catalysts poses challenges in identifying appropriate regeneration methods [6]. Several conventional methods have been developed to regenerate spent catalysts, including physical [7], chemical [8], and thermal methods [9]. Physical methods are known for their simple procedures and low operational costs. However, their effectiveness is limited, especially in removing strongly adsorbed contaminants from the catalyst surface [7]. Meanwhile, chemical methods are effective at removing various types of contaminants, but using large quantities of chemicals can lead to secondary pollution [10]. Thermal methods are also effective in removing organic contaminants, but require high energy consumption and pose the risk of damaging the catalyst structure, as well as incurring higher costs [11].

Several previous studies have evaluated the regeneration of spent catalysts using acid-based chemical treatments. Amalia et al. [11] reported that treatment of SFCCCs with sulfuric acid increased surface area by 110%, pore volume by 29.25%, and reduced the content of impurity metals such as Ni, V, and Fe. However, this method has limitations: it is only effective at dissolving surface metals and can lead to excessive dealumination, potentially compromising the stability of the zeolite structure. On the other hand, ultrasonically assisted regeneration, as reported by Pu et al. [12] It demonstrates cavitation's ability to remove coke deposits and material trapped in the catalyst's pores. However, these methods are not free of drawbacks, such as relatively high energy requirements and the risk of structural damage from cavitation. Based on the drawbacks of each approach, the application of an ultrasonic-chemical hybrid method becomes more relevant, as it combines the advantages of both: the effectiveness of ultrasonication in cleaning organic deposits and opening pores, and the ability of sulfuric acid to dissolve metal impurities.

Recent research has shown that combining chemical and ultrasonic methods is a promising approach [9]. Wang et al. [9] reported that ultrasonic waves can enhance the efficiency of chemical leaching processes through cavitation phenomena, resulting in more effective catalyst surface cleaning and shorter regeneration times. The combination of these two methods not only increases regeneration efficiency but also reduces chemical usage and minimizes the risk of damage to the catalyst structure, thereby creating a more environmentally friendly and sustainable solution for treating waste containing synthetic dyes and ammonia. Previous research has also shown that the application of ultrasonic-chemical hybrid methods can increase the effectiveness of adsorbent material regeneration. Gao et al. [13] reported that the combination of ultrasonic with persulfate successfully regenerated dye-saturated activated carbon with higher efficiency than the single method, while reducing chemical requirements. This reinforces that similar hybrid methods can be applied to other materials, including RFCC-based catalysts, to produce more environmentally-friendly and sustainable solutions. This research focuses on the effect of the regeneration method on the physicochemical characteristics of the spent catalyst. The results are expected to provide a fundamental understanding of how chemical and ultrasonic (hybrid) treatments can improve the material's surface morphology, pore structure, and functional groups, thereby laying the foundation for further applications in wastewater treatment. Furthermore, the reutilization of SFCCCs aligns with circular economy principles, creating added value from hazardous waste while promoting environmental sustainability.

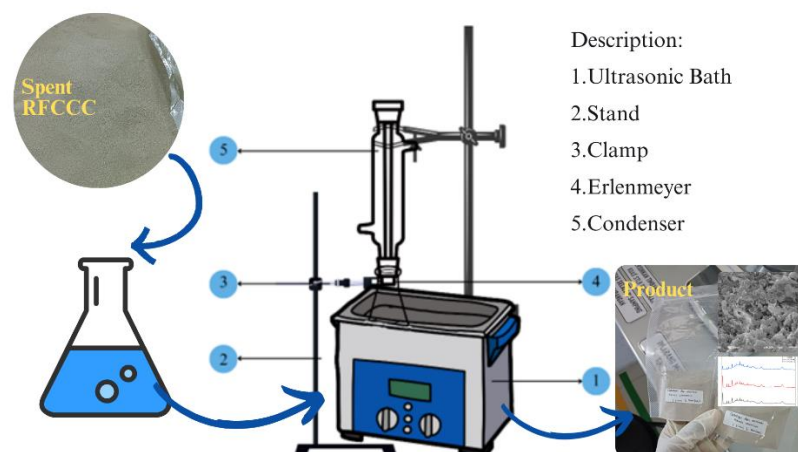
## 2. Materials and Methods

### 2.1 Material

The SFCCCs used in this study were obtained from the RU IV Balongan Oil Refinery, Indonesia. The catalyst primarily consists of Y-type zeolite (faujasite structure) and contains several hazardous metals. Its composition is as follows: 8.17% C, 10.57% O, 0.71% Na, 26.64% Al, 41.23% Si, 0.33% K, 1.63% Ca, 4.83% Fe, and 5.88% Ni. The chemical and hybrid treatment method employed 98% sulfuric acid ( $\text{H}_2\text{SO}_4$ ) (p.a. Merck, Germany).

## 2.2 Experimental apparatus

Figure 1 illustrates the experimental apparatus, which used an Ultrasonic Bath (50AL-AC220-240V, China) combined with a condenser to prevent material loss through evaporation. The ultrasonic bath generated ultrasonic waves that disrupted cell walls, thereby facilitating the efficient release of active compounds. The stand and clamps provided structural stability by securing the equipment in place. The condenser enabled repeated solvent circulation through solid samples, while the Erlenmeyer flask functioned as the final container for the extract solution.



**Figure 1.** Ultrasonic bath apparatus

## 2.3 Method

### 2.3.1 Regeneration method of SFCCCs

The regeneration methods used for Spent Fluid Catalytic Cracking Catalysts (SFCCCs) were chemical methods and hybrid ultrasonic–chemical methods. Both treatments were carried out using  $\text{H}_2\text{SO}_4$  as the solvent, with a solid-to-liquid ratio of 1:10 w/w. Before the SFCCCs treatment, the catalyst is first washed with deionized water to remove impurities, then dried in an electric oven (Memmert UN-30, Germany) at 105 °C for 7 hours. In the chemical method, the dried catalyst was immersed in a 0.7 M sulfuric acid solution and stirred using an orbital shaker (Biobase, China) at room temperature ( $30 \pm 2$  °C) for 8 hours. Meanwhile, in the hybrid ultrasonic–chemical method, the catalyst was immersed in a 0.7 M sulfuric acid solution and treated using an ultrasonic bath (Walfront, China) at a temperature of 70 °C for 30 minutes and power of 250 W. After the regeneration process using either the chemical or hybrid ultrasonic–chemical method, the catalyst was filtered and washed repeatedly using demineralized water until it reached a neutral pH (pH 7), then dried again in an electric oven at 105 °C for 7 hours, and finally sieved to a particle size of 150 mesh (100  $\mu\text{m}$ ). The regenerated catalyst was then calcined in a furnace (Thermo Fisher, USA) at 550 °C for 4 hours.

### 2.3.2 Characterization method

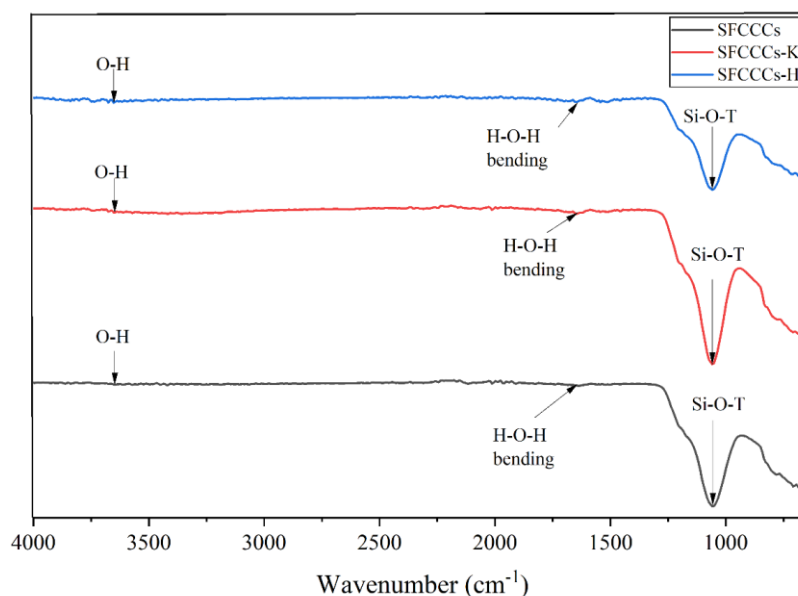
The non-treated spent FCC catalysts were denoted as SFCCCs, SFCCCs-K was defined as the chemically treated SFCC catalysts, and SFCCCs-H was defined as the ultrasound bath-treated SFCC catalysts. After the regeneration process, a series of characterization methods was used to comprehensively characterize the material's properties. X-ray Diffraction (XRD; Rigaku Miniflex-600, Japan) was used to determine the crystal structure. By analyzing the most intense peak broadening using the Scherrer equation, the crystal size was determined, which served as the basis for the subsequent characterization stage [14]. Fourier further analyzed the identified crystal structure using transform infrared spectroscopy (FTIR; Agilent Cary 630, USA). This method enabled exploration of chemical bonds and molecular structures, providing deeper insights into chemical interactions within the material [15]. To understand the surface characteristics in more detail, the Brunauer–Emmett–Teller (BET) method (Quadrascorb-evo, US) was employed. This technique enabled the determination of the surface area of nanostructures based on the principle of physical gas adsorption, which was directly related to the material's adsorption capacity [16]. The final stage of characterization involved a

combination of scanning electron microscopy and energy-dispersive spectroscopy (SEM & EDS; JEOL JSM-6510LA, Japan). This method visualized the surface morphology and semi-quantitatively analyzed the elemental composition of the material. The SEM-EDS results not only complemented the previous characterization but also provided a comprehensive visual and elemental understanding of the regeneration mechanism.

### 3. Results and Discussion

#### 3.1 Identification of SFCCCs surface structure recovery based on FTIR Spectra

Infrared spectroscopy (FTIR) was used to evaluate changes in the surface chemical structure of SFCCCs before and after regeneration. Three sample conditions were analyzed, with the results shown in **Figure 2**.



**Figure 2.** FTIR Spectra of SFCCCs

The FTIR spectra of the three samples in **Figure 2** show differences that reflect the effectiveness of each regeneration method. In the SFCCCs sample without regeneration, the absorption peak in the 1040-1100  $\text{cm}^{-1}$  region (Si-O-T group) appears to be weakened, indicating damage to the zeolite structure due to heavy-metal contamination and carbon impurities (coke). This is consistent with EDS data showing carbon (C) content of 8.17%, as well as Fe and Ni metals at 4.83% and 5.88%, respectively, indicating a high level of fouling. The maximum transmittance value (%T) in SFCCCs is 100.31%, indicating high IR absorption by surface contaminants. This phenomenon is consistent with the results of Wang et al. [7], who stated that the accumulation of carbon and heavy metals on the catalyst surface causes a decrease in catalytic activity and the closure of active pores, thereby reducing the efficiency of absorption and distribution of infrared (IR) in the zeolite structure [6].

After chemical regeneration (SFCCCs-K), the intensity of the Si-O-T and Si-O-Si bands increased, indicating the beginning of zeolite structure recovery. The -OH band was also more clearly visible at ( $\sim 3400 \text{ cm}^{-1}$ ) and  $\text{H}_2\text{O}$  ( $\sim 1600 \text{ cm}^{-1}$ ) with moderate intensity. The appearance of a band at  $1380 \text{ cm}^{-1}$  indicated the presence of residual acid solvent from the leaching process. The EDS results show a decrease in Fe and Ni content to 2.02% and 4.88%, respectively, indicating the successful heavy-metal leaching. The increase in carbon (C) to 18.49% indicates that the remaining coke has not been completely removed. The intensity value (100.87%) indicates a very clean surface, while the low IR absorption suggests that the zeolite structure has not been fully restored. This is supported by the research of Lu et al. [18], which states that acid leaching can improve the zeolite structure, increase crystallinity, and open pores, although it does not completely remove carbon from the surface [17].

The most optimal results were shown by the SFCCCs-H sample, whose FTIR spectrum showed the sharpest and strongest peaks, particularly in the Si-O-T and Si-O-Si bands, indicating that the crystalline structure of the zeolite had been successfully restored to the maximum extent. In addition, an increase in the intensity of the -OH and H<sub>2</sub>O bands was also clearly observed, indicating that the catalyst pores had reopened and were able to adsorb water more effectively. This demonstrates that the cavitation effect of ultrasonic waves can break down impurities in the pores, improve diffusion, and expand access to active sites within the catalyst structure [19]. The carbon content, as determined by EDS, decreased from 18.49% (SFCCCs-K) to 10.05% in SFCCCs-H, indicating that coke and organic contaminants were removed more effectively. Although the Ni content increased slightly to 9.49%, this is likely due to the opening of internal pores, making previously hidden metals easier to detect. The presence of minor elements such as Ti (0.42%) and Mg (0.16%) also suggests that the cleaning process reached the deepest pores of the catalyst surface. The maximum transmittance value in SFCCCs-H (100.84%) was slightly lower than that in SFCCCs-K (100.87%), but this may indicate that the intensity of the FTIR absorption band increased due to the presence of active groups that absorb IR more strongly. This means that the surface of the SFCCCs-H catalyst is clean but structurally active. This is consistent with Dewes et al. [20] who explain that ultrasonic waves produce cavitation effects, shear forces (mass transfer), and microjets capable of releasing heavy metals from the surface, opening pores, and increasing diffusion and adsorption into the zeolite structure [18]. Considering the FTIR data, %T value, zeolite structure recovery, and surface cleaning effectiveness, SFCCCs-H is declared the best regeneration method. The regenerated catalyst has an active, clean structure and has high potential for reuse in wastewater treatment or other applications.

### 3.2 Surface area of adsorbent

BET characterization was performed to determine changes in the physical properties of SFCCCs after the regeneration process. According to Table 1, the surface area of SFCCCs increased after regeneration. Untreated SFCCCs had a lower surface area of 27.255 m<sup>2</sup>/g, while after hybrid treatment (SFCCCs-H), it increased to 33.508 m<sup>2</sup>/g, and after chemical treatment, it reached 34.344 m<sup>2</sup>/g. This indicates that the regeneration process was able to open previously clogged pores and expand the active surface area [19]. The difference between the adsorption and desorption pore diameters occurred due to the hysteresis effect, which is the mismatch between the path when the gas is absorbed (adsorption) and when the gas is released again (desorption). This hysteresis mainly occurs in mesoporous materials, such as zeolite Y or silica, due to capillary condensation within the pores, which occurs when small pores begin to fill with liquid nitrogen under capillary pressure [20].

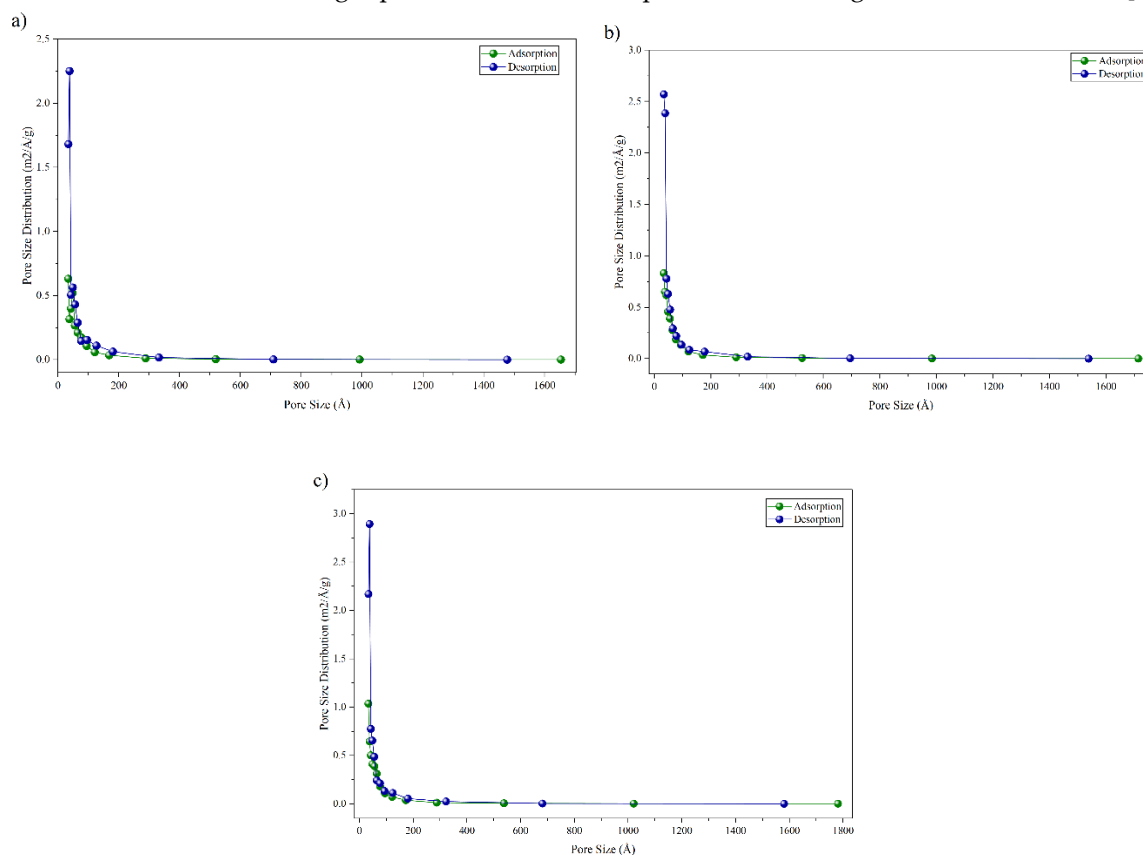
**Table 1.** Characteristics of SFCCCs Pore Structure

| Catalyst                                                    | Adsorption                          |                               | Desorption                          |                               | Reff       |
|-------------------------------------------------------------|-------------------------------------|-------------------------------|-------------------------------------|-------------------------------|------------|
|                                                             | Surface area<br>(m <sup>2</sup> /g) | Pore<br>diameter<br>dV(d) (Å) | Surface area<br>(m <sup>2</sup> /g) | Pore<br>diameter<br>dV(d) (Å) |            |
| SFCCCs                                                      | 27.255                              | 48.514                        | 47.730                              | 38.340                        | This study |
| SFCCCs-K                                                    | 34.344                              | 33.910                        | 57.133                              | 38.508                        | This study |
| SFCCCs-H                                                    | 33.508                              | 33.880                        | 55.296                              | 38.040                        | This study |
| SFCCCs with 5% C <sub>2</sub> H <sub>2</sub> O <sub>4</sub> | 91.02                               | 0.1248<br>cm <sup>3</sup> /g  | 82.48                               | 0.1383<br>cm <sup>3</sup> /g  | [12]       |
| SFCCCs with H <sub>2</sub> SO <sub>4</sub>                  | 74.98                               | -                             | 113.75                              | -                             | [9]        |
| SFCCCs-M with 0.8–1.6<br>mol/L hydrochloric acid            | 91.9                                | 197.1                         | -                                   | -                             | [21]       |

The initial SFCCCs sample (untreated) showed the lowest surface area, namely adsorption (27.255 m<sup>2</sup>/g) and desorption (47.730 m<sup>2</sup>/g), with the largest adsorption pore diameter (48.514 Å) and desorption pore diameter (38.340 Å). This indicates that most pores remain open, but the surface area is low due to blockages in the fine pores (micro- and mesopores) caused by the accumulation of impurity metals such as Fe and Ni.

These heavy metal contaminants can clog the pore surface, thereby accelerating the FCC catalyst's deactivation. According to Thommes et al. [21], in the International Union of Pure and Applied Chemistry (IUPAC) standard, the large difference in surface area and pore diameter in adsorption and desorption is known as a hysteresis isotherm, which occurs because the mechanism of filling and emptying nitrogen in the pores is not symmetrical [19]. As a result, the average pore size measured in desorption tends to be smaller. Hybrid regeneration resulted in an increase in surface area to 33,508 m<sup>2</sup>/g and a decrease in pore size to 33,880 Å. The primary mechanism for this increase was the cavitation effect of ultrasonic waves, which caused implosions of cavitation bubbles in the solution, thereby accelerating the dissolution of impurity metals from the catalyst's surface and pores [10]. The combination of ultrasound and acid can enhance heavy metal leaching efficiency and significantly reduce regeneration time compared to conventional methods, thanks to improved mass transfer and cavitation effects. Similar results were found in a study by Marafi et al. [24], which showed that an increase in convective diffusivity can occur under these conditions [22]. Although the pore diameter decreases, this is not an indication of damage, but rather the opening of previously closed small pores (micropores) that were previously blocked by metal and carbon.

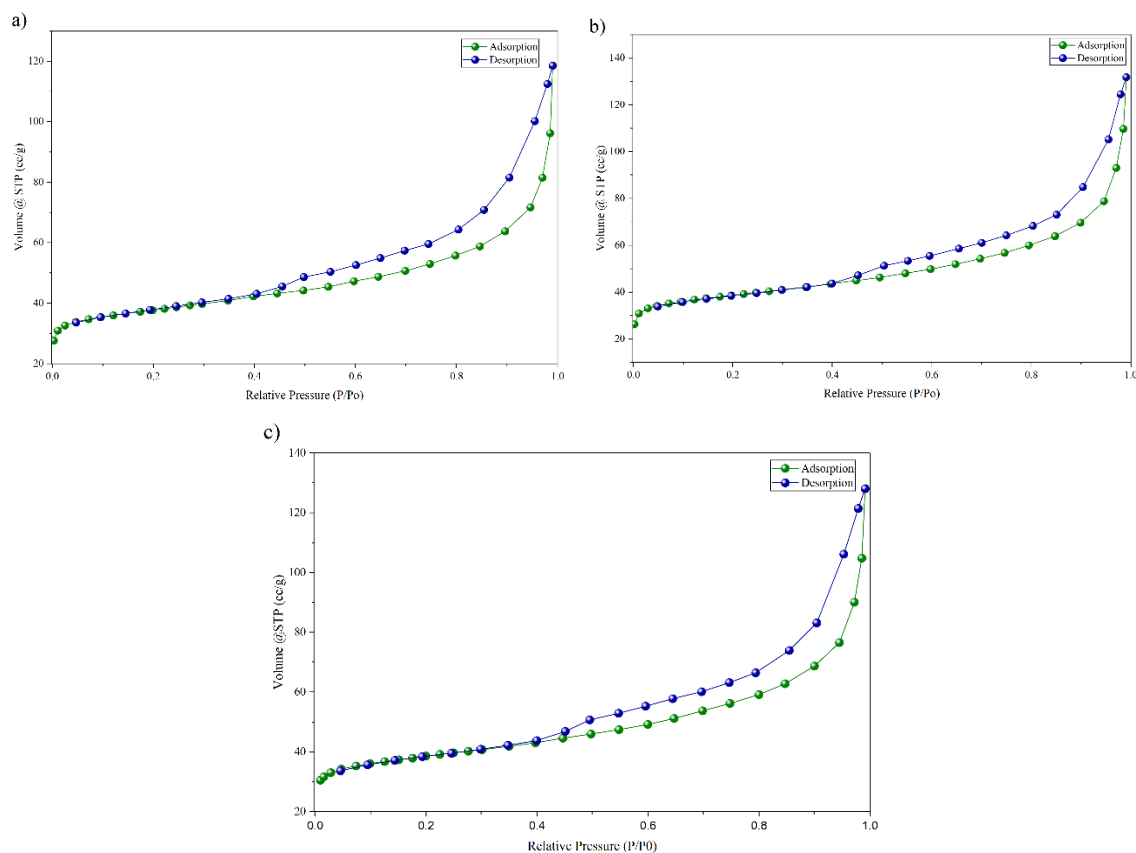
Chemical treatment without ultrasonic assistance provides an adsorption surface area (34,344 m<sup>2</sup>/g) and desorption (57,133 m<sup>2</sup>/g) with chemical SFCCCs diameter almost similar to hybrid SFCCCs, adsorption pore diameter (33,910 Å), and desorption pore diameter (38,508 Å). With results that are almost identical to those of SFCCCs-H, this indicates that the difference in effectiveness between the chemical and hybrid methods is not quantitatively significant. However, in terms of regeneration time, the hybrid method is much more efficient and avoids the risk of zeolite structural damage from prolonged exposure to strong acids. Based on the analysis results, this study is consistent with Amalia et al. [11], indicating that the dissolution of impurity metals proceeds effectively. However, the use of acid solutions at high concentrations or extreme reaction temperatures, combined with longer process times, has the potential to damage the zeolite structure [10].



**Figure 3.** Pore Size Distribution of Modified SFCCCs Based on BET Analysis: (a) SFCCCs, (b) SFCCCs-K, (c) SFCCCs-H



In general, the three graphs in **Figure 3** are dominated by pores smaller than 200 Å, indicating mesoporous characteristics. In untreated SFCCCs, the pore distribution appears low and narrow, indicating pore blockage due to metal accumulation during use, which affects diffusion capacity and catalyst activity. The hysteresis between the adsorption and desorption curves is also large, indicating asymmetrical or blocked pores[23]. After regeneration with the hybrid method, pore distribution increased sharply, especially at sizes  $\leq 100$  Å. The adsorption and desorption curves became more parallel, indicating more open and regular pores. The synergistic effect of ultrasonication and sulfuric acid accelerated metal release via dissolution and complexation, thereby efficiently removing pore blockers, increasing surface area, pore openness, and a more uniform pore distribution [24]. Chemical regeneration also improved pore distribution, although the curve was slightly more dispersed, suggesting a risk of changes in pore structure under the strong acidic conditions. Overall, hybrid regeneration provides the most optimal results because it can open pores efficiently, maintain structural stability, and minimize the risk of material damage, making it a superior method for restoring the characteristics of SFCCCs.



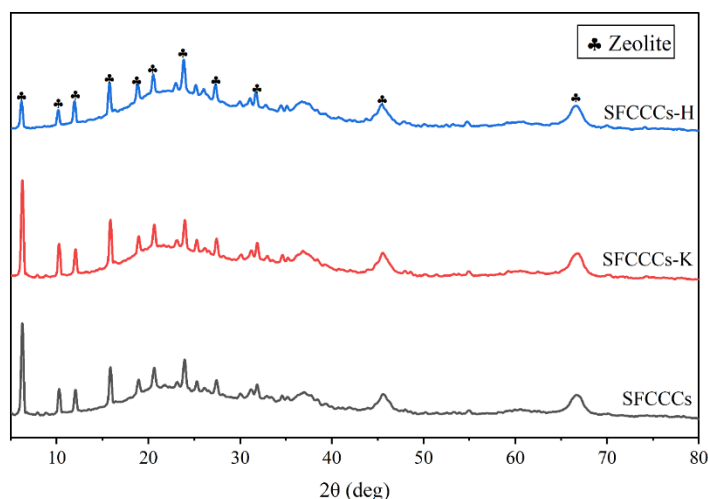
**Figure 4.** Adsorption-Desorption Isotherm Curves for SFCCCs Samples: (a) SFCCCs, (b) SFCCCs-K, (c) SFCCCs-H

The three curves in **Figure 4** show type IV isotherms with hysteresis loops, which are characteristic of mesoporous materials. The presence of pores ranging in size from 2 to 50 nm, a size commonly used as an adsorbent. The hysteresis loop formed between the adsorption and desorption curves indicates the presence of capillary condensation within the mesopores, which is an indicator of open pore structure and inter-pore connectivity. In the three **Figures 4. (a), (b), and (c)**, it can be seen that the adsorption volume increases sharply at a relative pressure approaching 1 ( $P/P_0$ ), which indicates the formation of multilayers or significant pore filling[6]. At **Figure 4. (a)**, the adsorption and desorption volumes reached approximately 120 cc/g with wide and non-parallel hysteresis, especially at relative pressures ( $P/P_0 > 0.4$ ). This indicates that SFCCCs experience pore-blocking or bottleneck effects due to the formation of coke and metal deposits, solid residues from the heating of organic materials that clog and coat the catalyst's pores, making it difficult for the catalyst to decompose and inhibiting access to the pores. This is consistent with the adsorption surface area obtained (27.255 m<sup>2</sup>/g). The hysteresis loop area indicates limited pore connectivity, and the total adsorption volume

appears lower than that of the two chemically and hybridly regenerated samples. The same findings in the study by Ihli et al. [27] explain that this phenomenon occurs due to pore closure and nitrogen inaccessibility resulting from carbon deposition or accumulation of impurity metals [27]. The adsorption volume increased to 130 cc/g in **Figure 4. (b)**, the adsorption and desorption curves appear more parallel with narrower hysteresis compared to SFCCCs, indicating that hybrid treatment can increase pore accessibility and total pore volume in the catalyst. Ultrasound plays an important role by producing a cavitation effect that can reopen clogged pores and disperse particle agglomerates. As a result, gas diffusion pathways such as nitrogen become more open, thereby significantly increasing adsorption capacity. This increase also indicates that the mesoporous structure, which was previously clogged by metal or coke deposition, can be restored, supporting the recovery of catalyst structural properties after hybrid regeneration treatment [25]. The highest adsorption volume increase was close to 135 cc/g in **Figure 4. (c)** SFCCC-K with a value not much different from SFCCCs-U but wider, indicating the effectiveness of chemical regeneration in cleaning some of the pores, but without the mechanical assistance of ultrasound, this process was less than optimal in removing carbon residues trapped in the deepest pores[9]. This is also supported by the high surface area of Chemical SFCCCs (57,133 m<sup>2</sup>/g). These differences indicate that hybrid regeneration can significantly unclog pores and restore the catalyst's internal structure, a process that cannot be achieved with conventional chemical methods alone.

### 3.3 XRD Diffraction Patterns as an Indicator of SFCCCs Regeneration Success

From the diffraction pattern, it can be determined to what extent the regeneration method restores or enhances the active crystal structure, particularly the Zeolite Y phase, which plays a crucial role in catalytic activity. The XRD results are shown in **Figure 5**.



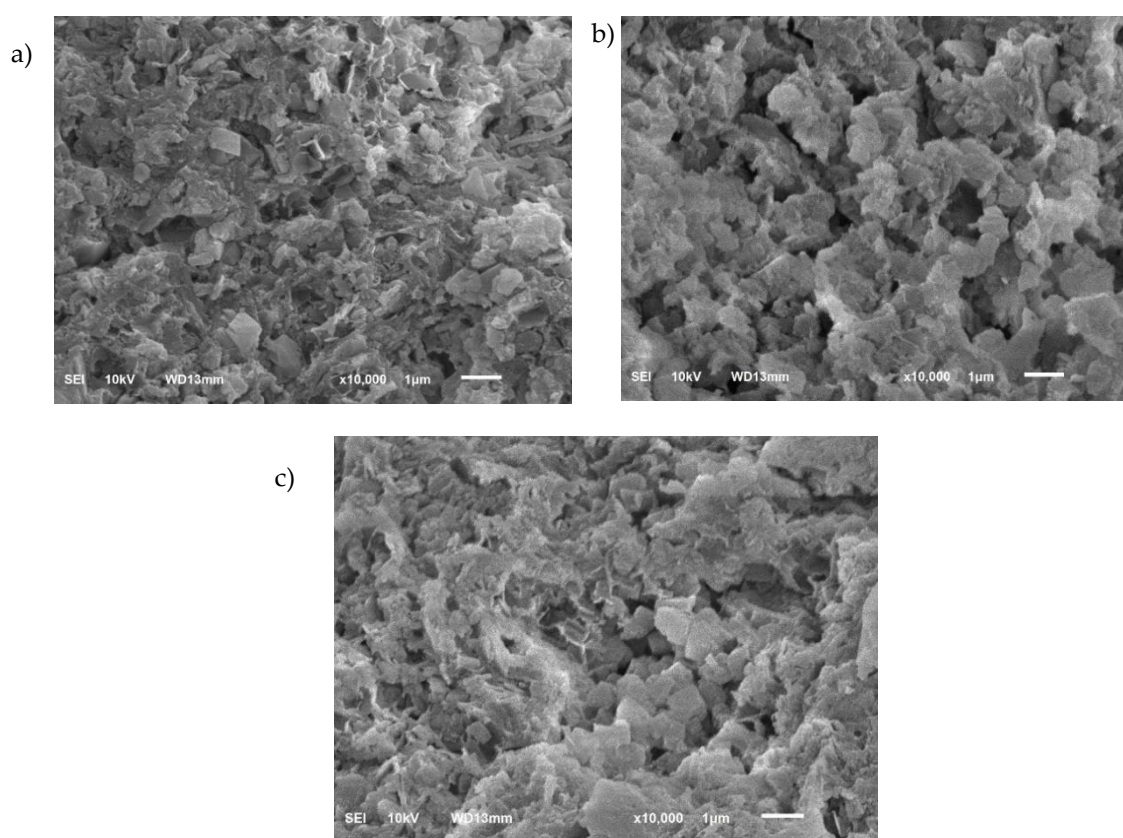
**Figure 5.** Crystal Structure of SFCCCs Based on XRD Analysis: (a) SFCCCs, (b) SFCCCs-H, (c) SFCCCs-K

Based on the results of X-Ray Diffraction (XRD) analysis in **Figure 5**, the untreated sample (SFCCCs) shows relatively strong diffraction peaks, which may be attributed to the presence of residual crystalline phases and deposited metals, both of which contribute to peak intensity. The identified phases were mostly amorphous silica, kaolinite, and residual zeolite phases that were no longer dominant, indicating that the zeolite crystal structure had been severely damaged and was no longer effective for use in catalytic applications[17]. In the ultrasonically regenerated sample, **Figure 5. (b)** SFCCCs-H, slight variations in peak intensity and peak broadening were observed. These changes suggest structural modification and partial removal of impurities rather than a straightforward increase in crystallinity. According to Dewes et al. (2022), ultrasound induces cavitation, improving mixing and accelerating crystallization, thereby accelerating zeolite crystal formation. In addition, ultrasonic waves accelerate mass transfer and crystal nucleation during zeolite synthesis. This shortens zeolite formation time by 30-40% and produces zeolite with a more uniform particle size and higher crystallinity [20]. The main phases are indicated by the reappearance of active phases, such as silica and aluminosilicate, identified in this sample, demonstrating the success of the hybrid method in

partially restoring the active structure of SFCCCs. Meanwhile, in regeneration using the chemical method (Figure 5). (c) SFCCCs-K, the diffraction pattern in this sample shows sharp and well-defined peaks, but the intensity of some diffraction peaks is lower than that of the SFCCCs-H sample. This may be due to an overly aggressive chemical leaching process that can dissolve part of the aluminosilicate zeolite framework, especially under extreme pH or high-acid conditions. This is in line with Christopher et al. (2019), who stated that although chemical regeneration effectively removes contaminants, inappropriate selection of leaching concentration and duration can cause excessive dissolution of the zeolite framework and reduce its structural stability [29]. The intense peaks of the zeolite and SiO phases appearing in the XRD pattern indicate that the catalyst's crystallinity has increased compared to the initial conditions. Overall, hybrid regeneration treatment (SFCCCs-H) is the most effective method for optimally restoring the crystal structure of SFCCCs. This method is not only capable of cleaning contaminant deposits but also of maintaining the integrity of the zeolite structure.

### 3.4 Morphology of SFCCCs Microstructure After Regeneration through SEM-EDS

To observe the effect of regeneration on SFCCCs, SEM was performed on samples treated with ultrasonic and chemical treatments. The results of the analysis are shown in Figure 6.



**Figure 6.** SEM micrographs of SFCCCs at 10,000x magnification (a)SFCCCs, (b)SFCCCs-K, (c)SFCCCs-H

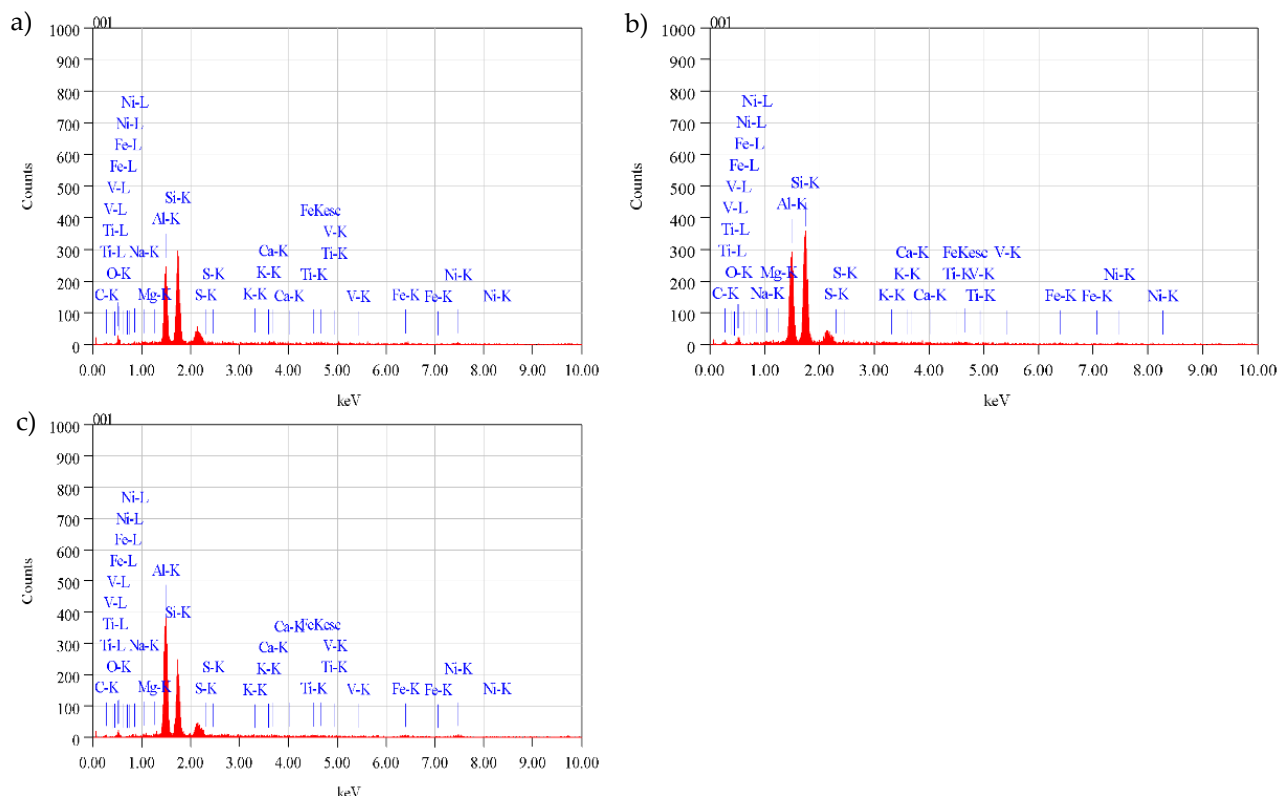
The untreated SFCCCs (Figure 6. (a)) show a relatively dense surface with several pores partially covered by deposits, indicating the presence of coke and metal contaminants accumulated during catalytic operation. After regeneration (Figure 6. (b)), the surface appears relatively cleaner with some visible pores; however, the overall morphology remains similar to the untreated sample. No significant structural damage or erosion can be conclusively identified from the SEM images.  $\text{Al}_2\text{O}_3$  in the catalyst can dissolve as acidity increases, triggering the collapse of the catalyst framework and reducing its durability [18]. In comparison, the Hybrid SFCCCs in Figure 6(c) show changes in the surface structure, with cleaner areas and less sharp grooves appearing. This is related to the cavitation phenomenon induced by ultrasonic waves, indicating a more porous structure and a much looser morphology [30]. The effectiveness of this method is listed in Table 1, which shows a desorption surface area of  $55.269 \text{ m}^2/\text{g}$ , only slightly lower than the chemical method, but with

a smaller pore diameter of 38.040 Å. This demonstrates that the hybrid method can reopen clogged micropores without degrading the catalyst framework, thereby increasing surface area and enhancing catalytic performance. Cavitation causes microcracks on the solid surface and increases the specific surface area, thereby directly increasing leaching efficiency and shortening reaction time.

**Table 2.** Elemental Analysis Before and After Regeneration

| Element Mass (%) | Catalyst |          |          |
|------------------|----------|----------|----------|
|                  | SFCCCs   | SFCCCs-H | SFCCCs-K |
| Si/Al            | 1.55     | 0.88     | 1.75     |
| Al               | 26.64    | 36.69    | 23.78    |
| Si               | 41.23    | 32.31    | 41.52    |
| C                | 8.17     | 10.05    | 18.49    |
| O                | 10.57    | 6.68     | 7.97     |
| Na               | 0.71     | 0.46     | 0.37     |
| Mg               | -        | 0.16     | -        |
| K                | 0.33     | 0.06     | 0.10     |
| Ca               | 1.63     | 0.45     | 0.86     |
| Ti               | -        | 0.42     | -        |
| Fe               | 4.83     | 3.25     | 2.02     |
| Ni               | 5.88     | 9.49     | 4.88     |

During the cracking reaction, the surface of FCCC catalyst particles can be contaminated by various cations, including Ni, V, Fe, Cu, and/or Na, as well as by carbon deposits (coke) at levels of ppm or higher [31]. Based on Table 2 and Figure 7, the EDS results support the observations with quantitative elemental composition data. In SFCCCs, the C content of 8.17% and O content of 10.57% indicate the presence of organic deposits (coke) and oxide compounds, while the Ni content of 5.88% and Fe content of 4.83% indicate the presence of impurity metals from the previous process. The initial Si/Al ratio of 1.55, with 41.23% Si and 26.64% Al, indicates a silica-alumina-based catalyst framework. After ultrasonic regeneration of SFCCCs-H, the carbon content increased slightly to 10.05%, but the oxygen content decreased to 6.68%, accompanied by a decrease in Fe to 3.25% and an increase in Ni to 9.49%. This increase occurred due to the removal of impurities from the catalyst surface, exposing Ni particles that had previously been hidden and detected by EDS [27]. The Si/Al ratio decreased to 0.88 (Si 32.31%; Al 36.69%), indicating the possibility of selective Si dissolution due to ultrasonic treatment. This is in accordance with Sariman [33], who stated that the decrease in Si elements occurred due to silica leaching from the catalyst framework, caused by local pressure cavitation waves, especially in the amorphous phase or in parts that were already weak due to previous use [33]. Meanwhile, chemical regeneration (SFCCCs-K) produced the highest C content of 18.49% and O of 7.97%, indicating that carbon residue was still quite high. The Fe content decreased to 2.02% and the Ni content to 4.88%, indicating a highly effective metal-cleaning process. The Si/Al ratio increased to 1.75 (Si 41.52%; Al 23.78%), likely due to aluminum dissolution (dealuminization) by the chemical solution, resulting in reduced Al content in the framework.



**Figure 7.** Elemental mapping from SEM-EDS (a)SFCCCs, (b)SFCCCs-K, (c)SFCCCs-H

Overall, SEM and EDS observations show that ultrasonic regeneration is more effective in opening pores and reducing certain impurity metals. In contrast, chemical regeneration is more efficient in removing metals but tends to leave carbon residues and alter the Si/Al ratio. These findings align with those of Sulman et al. [34], who stated that ultrasonic treatment excels at morphological restoration and surface area enhancement, while chemical treatment emphasizes chemical composition recovery, with the risk of altering the catalyst framework structure [34]. Ultrasound-assisted leaching, or this hybrid method, has great potential to improve leaching efficiency, shorten process time, and reduce chemical consumption. This is due to the advantages of ultrasonic irradiation over conventional mechanical agitation, particularly in accelerating reactions and improving contact between the solvent and solid particles.

#### 4. Conclusions

The regeneration of spent fluid catalytic cracking catalysts (SFCCCs) using chemical and hybrid ultrasonic-chemical methods demonstrated significant improvements in physicochemical properties. BET analysis showed that the surface area increased from 27.255 m<sup>2</sup>/g for SFCCCs to 34.344 m<sup>2</sup>/g for SFCCCs-K and further to 33.508 m<sup>2</sup>/g for SFCCCs-H, indicating successful pore reopening and enhanced adsorption capacity. FTIR spectra revealed stronger Si–O–Si and –OH bands in SFCCCs-H, confirming more effective restoration of the zeolite framework and reduction of coke residues, with carbon content decreasing from 18.49% for SFCCCs-K to 10.05% for SFCCCs-H. XRD results showed sharper, more intense peaks in the 2θ = 22–30° range for SFCCCs-H, indicating improved crystallinity compared to SFCCCs and SFCCCs-K. SEM-EDS analysis further confirmed structural recovery, where Fe content decreased from 4.83% for SFCCCs to 3.25% for SFCCCs-H, while surface morphology appeared cleaner and more porous under ultrasonic-assisted regeneration. Overall, the hybrid ultrasonic-chemical method yielded the most balanced results, effectively removing impurities, reopening micropores, and restoring crystallinity while minimizing structural damage, thereby making it superior to conventional chemical regeneration for sustainable SFCCC reuse. Moreover, regenerated SFCCCs have strong potential as low-cost adsorbents for wastewater treatment.



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**Author Contributions:** A.S.: Conceptualization and writing - original draft; H.R.N.: Formal analysis; J.R.D.: Investigation; D.D.: Investigation; D.S.: Investigation; D.D.Y: Methodology and Visualization; Y.F.: Project administration; D.F.S.: Supervision; D.R.S.: Conceptualization and writing - review & editing

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**Conflicts of Interest:** The authors declare no conflict of interest

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# Corn Stalks as a Novel Source of Functional Dietary Fiber: Process Optimization and Product Characterization

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**Abstract:** The growing demand for dietary fiber (DF) has encouraged the exploration of sustainable and low-cost sources from agricultural by-products. Corn stalks are an abundant agricultural residue that remains underutilized as a potential source of dietary fiber. This study aimed to optimize the alkali extraction of DF from corn stalks (*Zea mays*, L.) using Response Surface Methodology (RSM) with a Central Composite Design (CCD). Three independent variables, namely temperature (70-90°C), time (50-70 min), and NaOH concentration (15-25%), were investigated for their effects on DF yield. The model showed a high significance ( $p < 0.0001$ ) with  $R^2 = 0.99$ , indicating excellent predictive accuracy. The optimal extraction conditions were 82.29°C, 62.06 min, and 23.03% NaOH, resulting in a maximum DF yield of 25.75%. Verification experiments confirmed the model's reliability, with an experimental yield of  $25.43 \pm 0.24\%$ . The extracted DF exhibited favorable functional properties, including high water holding capacity/WHC ( $4.7 \pm 0.08$  g/g), water binding capacity/WBC ( $2.7 \pm 0.05$  g/g), swelling capacity/SW (5.12 mL/g), oil holding capacity/OHC ( $1.8 \pm 0.07$  g/g), and Fourier Transform Infrared Spectroscopy (FTIR) analysis confirmed the removal of lignin and hemicellulose. These characteristics highlight its potential as a functional ingredient in food formulations, particularly in meat- and fish-based products that require improved texture and moisture retention. This study demonstrates the valorization of corn stalk waste into a valuable DF source, supporting sustainable resource utilization and the development of clean-label, fiber-enriched food products aligned with circular economy principles.

**Keywords:** Corn stalks; dietary fiber extraction; alkali treatment; response surface methodology; functional properties

## 1. Introduction

Dietary fiber (DF) is essential to the diet, leading to increased demand for DF products in recent years, driven by growing public awareness of its numerous health benefits, including regulating intestinal transit, lowering blood cholesterol levels, and promoting glycemic control [1]. As a component of functional foods, DF plays a crucial role in preventing chronic diseases, including cardiovascular disease, type 2 diabetes, obesity, and cancer [2,3]. This has prompted studies to find sustainable and alternative sources of dietary fiber, especially those made from agro-industrial byproducts. Corn/maize is economically important, serving as the main food crop and a raw material for corn starch, corn syrup, ethanol, and corn oil products. In addition,

corn is animal feed for cattle, pigs, and poultry. Due to its wide use and high productivity, corn plays an important role in food security, agriculture, and industrial development.

Corn cultivation generates large amounts of corn stalk waste. Corn stalks are rich in lignocellulosic components, particularly cellulose, hemicellulose, and lignin, which can be processed into valuable products such as DF [4]. Utilizing corn stalks not only provides an economical source of functional ingredients for the food industry but also contributes to waste reduction and environmental sustainability. Extracting DF from corn stalks is a complex process that requires chemical breakdown of the plant cell wall matrix to release cellulose and hemicellulose while minimizing degradation. Alkali treatments, such as sodium hydroxide (NaOH), have been widely used for this purpose due to their effectiveness in delignifying biomass and increasing the accessibility of fiber components. However, optimizing the extraction process for efficiency is significantly influenced by several parameters, including extraction temperature, time, and alkali concentration. Optimizing these parameters is crucial to maximizing fiber yield while maintaining its functional qualities [5].

Previous research has been conducted on other biomasses, such as rice bran, rice husk, and bagasse [6–8]. However, reports on the valorization of corn stalks as a fiber source are limited, particularly regarding systematic optimization of extraction conditions. RSM, specifically CCD, is a powerful technique for modeling and optimizing complex chemical processes by evaluating interactions between variables. The extracted DF was characterized based on its functional properties, such as water holding capacity (WHC), water binding capacity (WBC), swelling capacity (SC), and oil holding capacity (OHC), and its chemical structure using FTIR spectroscopy. The resulting data were used to evaluate its potential application as a DF source in food systems. By using corn stalks as a DF source, this study contributes to the development of cost-effective, fiber-rich ingredients for the formulation of meat- and fish-based products, such as meatballs, nuggets, and others, as well as to broader functional food innovation.

## 2. Materials and Methods

### 2.1 Materials

Corn stalks were obtained from local farms in Maros Regency, South Sulawesi, Indonesia. The stalks were cleaned, chopped into uniform particles, dried in an oven at 60 °C for 5 h, milled into flour, and sieved through a 60-80 mesh sieve before extraction. Food-grade NaOH and H<sub>2</sub>O<sub>2</sub> were obtained from Sigma-Aldrich.

### 2.2 Experimental Design

A CCD was used to study the effects of three independent variables: temperature (A, °C), time (B, min), and NaOH concentration (C, %). The midpoint values for these variables are presented in Table 1, and the experimental results and the DF results obtained, including 6 replications at the midpoint, are shown in Table 2.

**Table 1.** Experimental Range and Levels (Coded and Actual)

| Variable         | -1.682 | -1 | 0  | +1 | +1.682 |
|------------------|--------|----|----|----|--------|
| Temperature (°C) | 63.18  | 70 | 80 | 90 | 96.8   |
| Time (min)       | 43.18  | 50 | 60 | 70 | 76.82  |
| NaOH (%)         | 11.59  | 15 | 20 | 25 | 28.41  |

### 2.3 DF Extraction Procedure

Powdered corn stalks were extracted using NaOH in a stainless steel stir pan under various process conditions, as in the CCD matrix (Table 2). After the extraction process, the mixture was filtered, washed with distilled water until neutral pH (6.5-7), dried at 50°C for 12 h, bleached with 5% H<sub>2</sub>O<sub>2</sub> for 1 h, filtered, washed with distilled water until neutral pH (6.5-7), and dried at 50°C for 12 h. The DF yield (%) could be calculated based on dry weight.

### 2.4 Determination of WHC, WBC, and SC

WHC, WBC, and SC were determined according to Qiu et al. [9]. A 1.0 g sample was mixed with 70 mL of distilled water and stirred for 4 h. The resulting cloudy liquid was then centrifuged at 3000 rpm for 15 min, and excess water was carefully removed by filtration. The sample's wet weight was recorded, and it was then dried at 105°C until a constant weight was reached. SC was expressed as the change in volume resulting from water swelling.

$$\text{WHC (g/g)} = \frac{(W_1 - W)}{W} \quad (1)$$

$$\text{WBC (g/g)} = \frac{(W_1 - W_2)}{W_1} \quad (2)$$

$$\text{SC (g/g)} = \frac{(V_2 - V_1)}{W} \quad (3)$$

Where:  $W_1$  is the sample weight after water absorption (g),  $W$  is the sample weight before water absorption,  $W_2$  is the sample weight after drying,  $V_2$  is the sample volume after water absorption (mL), and  $V_1$  is the sample volume before water absorption (mL). WHC, WBC, and SC measurements for each sample were performed in triplicate.

## 2.5 Determination of OHC

OHC was determined using a method adapted from Qiu et al. [9]. A 1.0 g sample was placed in a centrifuge tube and mixed with excess corn oil. The tube was stirred for 30 seconds every 5 min, and after 30 min, the tube was centrifuged at 1600 rpm for 20 min. After carefully separating the excess oil, the oil and water adhering to the inside of the tube were discarded, and the tube was weighed to calculate the OHC of the obtained DF. OHC measurements were performed in triplicate.

$$\text{OHC (g/g)} = \frac{W_1}{W_2} \quad (4)$$

Where  $W_1$  = oil retained,  $W_2$  = original dried sample

## 2.6. Structural Analysis

Structural analysis was reviewed using FTIR (Bruker Corporation, Germany). Samples were scanned in the 4000-600  $\text{cm}^{-1}$  range with a resolution of 4  $\text{cm}^{-1}$ . Each sample was scanned 32 times.

# 3. Results and Discussion

## 3.1 Alkali Extraction Mechanism

Dietary fiber extraction using alkali aims to dissolve components that coat the polysaccharide structural framework, dissolve lignin and some hemicellulose, making cellulose more accessible for extraction, and release soluble fiber by breaking cross-links between polysaccharides and proteins, or between hemicellulose and lignin [10, 11].

NaOH solutions at specific concentrations, along with temperature and time settings, as shown in Table 2, are used to produce dietary fiber. In this process, the alkali penetrates the corn stalk cell walls, causing a saponification reaction of esters, such as pectin esters and hemicellulose esters, breaking the ester and ether bonds between lignin and polysaccharides to form carboxylates and methanol. Hemicellulose and lignin dissolve. At this stage, the more amorphous hemicellulose with lignin and some of the hemicellulose bound to lignin dissolve in alkali, resulting in a purer cellulose structure. The phenolic and aliphatic lignin are hydrolyzed, and the hemicellulose is also partially hydrolyzed, disrupting ester and hydrogen bonds [12], with a high glucan content and virtually no hemicellulose/ferulate bound in the residue [13].

In the washing stage, residual alkali and dissolved products are washed with distilled water until the pH is near neutral. The washing stage is crucial, as residual alkali can damage the product and allow the reaction to continue if free  $\text{OH}^-$  remains. The use of NaOH and  $\text{H}_2\text{O}_2$  helps remove residual hemicellulose and lignin, increasing the purity of cellulose to glucan > 90% [14].



### 3.2 DF Yield

The DF extraction yield from corn stalks in this study ranged from 22.19% to 25.75%, depending on the extraction conditions (Table 2). This result is slightly higher than the value reported by another study [15], which was 19.56% SDF (soluble fiber fraction) from *Cistanche deserticola* residues. Other reports state that an SDF yield of 21-22% from *Trigonella foenum graecum* L. seeds [16], and from mango peels of  $22.98 \pm 0.62\%$  [17].

The best results were recorded at the midpoint, indicating high reproducibility and model fit. Using RSM, the effects of three variables: A, B, and C, were systematically studied. The statistical model shows that all three factors, including linear, quadratic, and their interactions, significantly affect the DF results ( $p < 0.05$ ). Based on the ANOVA (Table 3), the model was highly significant ( $p < 0.0001$ ) with an F-value of 570.57. The three linear terms of the independent variables (A, B, and C), their quadratic terms ( $A^2$ ,  $B^2$ ,  $C^2$ ), and their interaction terms (AB, AC, BC) significantly affected DF yield ( $p < 0.05$ ). The misfit was not significant ( $p = 0.0515$ ), indicating good model fit. The model  $R^2 = 0.99$  and the predicted  $R^2 = 0.98$ , indicating excellent predictive ability.

**Table 2.** Experiment and Observed Results (%)

| No. | Temperature (°C) | Time (min) | NaOH (%) | DF yield (%) |
|-----|------------------|------------|----------|--------------|
| 1   | 70               | 50         | 15       | 22.53        |
| 2   | 90               | 50         | 15       | 22.19        |
| 3   | 70               | 70         | 15       | 22.38        |
| 4   | 90               | 70         | 15       | 23.35        |
| 5   | 70               | 50         | 25       | 23.22        |
| 6   | 90               | 50         | 25       | 23.42        |
| 7   | 70               | 70         | 25       | 23.32        |
| 8   | 90               | 70         | 25       | 24.91        |
| 9   | 63.2             | 60         | 20       | 22.56        |
| 10  | 96.8             | 60         | 20       | 23.45        |
| 11  | 80               | 43.2       | 20       | 22.23        |
| 12  | 80               | 76.8       | 20       | 23.15        |
| 13  | 80               | 60         | 11.6     | 23.05        |
| 14  | 80               | 60         | 28.4     | 24.79        |
| 15  | 80               | 60         | 20       | 25.62        |
| 16  | 80               | 60         | 20       | 25.69        |
| 17  | 80               | 60         | 20       | 25.66        |
| 18  | 80               | 60         | 20       | 25.75        |
| 19  | 80               | 60         | 20       | 25.71        |
| 20  | 80               | 60         | 20       | 25.64        |

**Table 3.** ANOVA Summary for Quadratic Model

Response: DF extraction yield from corn stalks  
 ANOVA for Response Surface Quadratic Model  
 Analysis of variance table [Partial sum of squares]

| Source         | Sum of Squares | DF | Mean Square | F-Value  | Prob > F |                 |
|----------------|----------------|----|-------------|----------|----------|-----------------|
| Model          | 35.22578       | 9  | 3.913976    | 570.5657 | < 0.0001 | significant     |
| A              | 1.12334        | 1  | 1.12334     | 163.7566 | < 0.0001 |                 |
| B              | 1.259417       | 1  | 1.259417    | 183.5935 | < 0.0001 |                 |
| C              | 3.951745       | 1  | 3.951745    | 576.0717 | < 0.0001 |                 |
| A <sup>2</sup> | 12.24939       | 1  | 12.24939    | 1785.673 | < 0.0001 |                 |
| B <sup>2</sup> | 15.38753       | 1  | 15.38753    | 2243.141 | < 0.0001 |                 |
| C <sup>2</sup> | 5.16121        | 1  | 5.16121     | 752.3833 | < 0.0001 |                 |
| AB             | 0.91125        | 1  | 0.91125     | 132.8388 | < 0.0001 |                 |
| AC             | 0.1682         | 1  | 0.1682      | 24.51961 | 0.0006   |                 |
| BC             | 0.04205        | 1  | 0.04205     | 6.129902 | 0.0328   |                 |
| Residual       | 0.068598       | 10 | 0.00686     |          |          |                 |
| Lack of Fit    | 0.057115       | 5  | 0.011423    | 4.973715 | 0.0515   | not significant |
| Pure Error     | 0.011483       | 5  | 0.002297    |          |          |                 |
| Cor Total      | 35.29438       | 19 |             |          |          |                 |

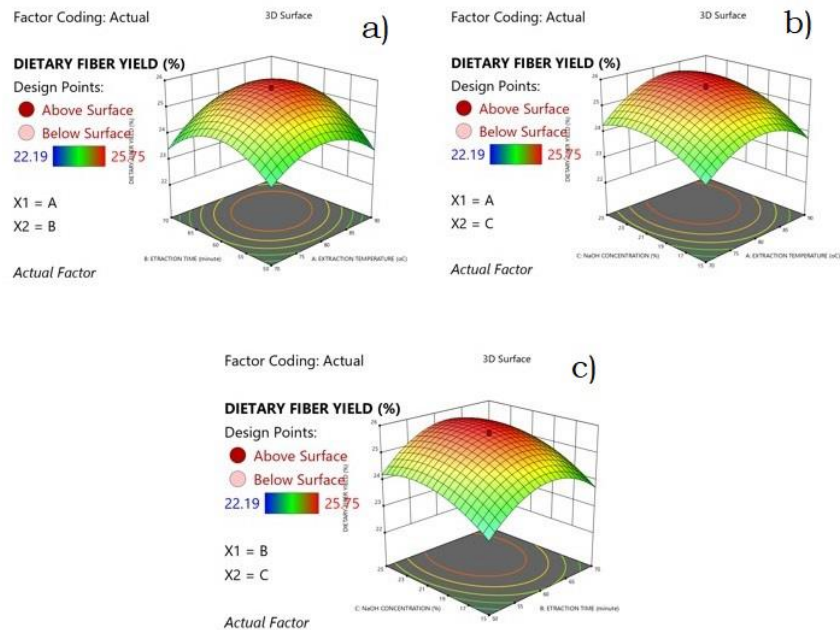
### 3.3 Optimization and Model Equation

The following regression model (in actual values) represents the response surface for DF yield:

$$Y (\%) = -63.79243 + 1.24329A + 0.97135B + 0.74610C - 0.00922A^2 - 0.01033B^2 - 0.02394C^2 + 0.00338AB + 0.00290AC + 0.00145BC \quad (5)$$

Where: Y = DF yield (%), A = Temperature (°C), B = Time (min), C = NaOH concentration (%)

Among the three variables, NaOH concentration has the most significant effect on DF yield, followed by extraction time and extraction temperature ( $C > B > A$ ). This is in accordance with the known mechanism of alkali treatment, where NaOH acts as a chemical process agent, namely, breaking the ester and ether bonds between lignin and hemicellulose, thereby releasing cellulose fibers. Increasing the NaOH concentration to an optimal level increases the cleavage of hemicellulose and lignin bonds, thereby increasing fiber yield. However, excessive NaOH concentration can cause cellulose hydrolysis, resulting in partial degradation and reducing fiber quality [18, 19]. Along with this process, extraction time is crucial for ensuring adequate contact between NaOH and the corn stalk matrix. Longer extraction times increase the rates of delignification and hemicellulose solubilization. However, beyond the optimal point, prolonged alkali exposure can degrade fiber products, particularly amorphous cellulose, resulting in decreased yield [5, 20]. The 3D curves of the relationship between variables A and B, A and C, and B and C can be seen in Figure 1.



**Figure 1.** 3D response surface of variables A and B (a), A and C (b), and B and C (c)

In Figure 1a, the X-axis (A, °C), Y-axis (B, min), and Z-axis (vertical, percentage of DF yield). Surface color and shape: Blue-green-yellow-red indicates an increase in the value of DF yield: blue  $\approx$ , the lowest value (22.19%), red  $\approx$ , the highest value (25.75%). The curved surface (concave) indicates a significant interaction between A and B on the Z. Interpretation of the 3D curve: Increasing the A from low to high increases the Z up to the optimum point, after which it can decrease because high temperatures can damage the fiber; Increasing the B also increases the yield up to a certain point; too long a B can cause degradation. The red peak in the middle of the graph indicates the optimal condition for the combination of A and B, where the highest yield of dietary fiber is achieved; the smooth, symmetrical surface shape indicates that the response is quite stable around the optimum point. Main result: there is an optimal point (red zone) that yields a maximum result of around 25.75%.

In Figure 1b, it can be seen that B is kept constant at the midpoint or optimum. X-axis (A, °C), Y-axis (C, %), Z-axis (vertical, percentage of DF yield). Surface colors: Blue-green-yellow-red indicate an increase in yield, with low values  $\approx$  22.19% and high values  $\approx$  25.75%. Graph interpretation: A has a positive effect up to the optimum point. As the A increases from low to medium, the Z increases. After passing the optimum A, the Z begins to decline (indicating DF degradation or excessive dissolution). C also shows a similar pattern: low concentrations result in less effective dissolution of non-fiber materials, optimal concentrations result in maximum Z, and excessive C can degrade the DF structure, thereby reducing yield. The red zone (surface peak) shows the optimum combination of A and C to produce the highest yield ( $\sim$ 25.75%). The elliptical contour below the graph indicates a significant interaction between A and C, meaning that the effect of one factor depends on the value of the other.

Figure 1c, X-axis (B, min), Y-axis (C, %), Z-axis (vertical, percentage of DF yield). Surface color: Blue, low value ( $\sim$ 22.19%); red, high value ( $\sim$ 25.75%). Increasing B from low to medium increases Z because more DF is extracted; once the optimal B is reached, Z begins to decrease due to possible DF degradation or dissolution of other components. Increasing C from low to medium also increases Z, because the base helps dissolve lignin and hemicellulose that bind the fiber. However, a C that is too high can damage the fiber structure, so the Z decreases again. The smooth, curved surface and the red peak indicate a significant interaction between B and C, meaning the effect of one factor depends on the value of the other. The optimum zone (red) shows the combination of B and C that yields the highest yield, similar to the 25-26% range. Both factors have a significant effect on the yield, which shows a quadratic pattern (increasing then decreasing). Real interaction means the optimum B depends on C, and vice versa. The extraction temperature affects the

reaction kinetics and the diffusion capacity of chemicals within the material matrix. Higher temperatures enhance the hydrolytic cleavage of lignocellulosic bonds and accelerate alkali penetration, thereby increasing fiber product yield. However, excessively high temperatures lead to cellulose depolymerization, thereby reducing the structural integrity of the extracted fiber. This is consistent with findings from other research [21].

The NaOH concentration, extraction temperature, and extraction time variables affect fiber yield [21]. Another study tested the NaOH concentration, extraction temperature, and extraction duration (time) for corn stalks; the results showed that higher NaOH concentrations (up to a certain limit) increased yield. At the same time, too high concentrations damaged the fiber, and too short a time did not fully open it [22]. The effects of NaOH concentration, temperature, and reaction time on the extraction of hemicellulose (xylan) from wheat straw and corn stalk were investigated; the results showed that increasing NaOH concentration, temperature, and reaction time together increased the hemicellulose yield from wheat and corn straw [5]. However, after the optimum point, increasing time or too high a temperature caused the yield to decrease (degradation).

### 3.4 Model Adequacy and Predictive Performance

The model's statistical strength is demonstrated by an Adjusted  $R^2$  of 0.99 and a Predicted  $R^2$  of 0.98, indicating excellent agreement between the experimental and predicted results. The high  $R^2$  value of 0.99 and the insignificant non-conformity test confirmed the model's accuracy and reliability in predicting DF yield based on the studied variables. The Adequate Precision value of 60.07, well above the minimum value of 4.0, indicates a high signal-to-noise ratio and the model's ability to navigate the design space. The derived regression equation (Equation 5) in terms of actual values provides quantitative predictions of DF yield under various extraction conditions (Table 2).

### 3.5 Verification of optimal extraction conditions

Verification of optimal conditions in RSM is a crucial step after predicting optimal conditions to ensure the reliability and applicability of the developed model. Although RSM predicts optimal conditions based on an appropriate mathematical model, these predictions may not fully represent actual experimental results due to inherent variability and model limitations. Therefore, experimental verification is necessary to ensure that the predicted optimum conditions indeed produce the desired maximum or minimum response. This step also serves to evaluate the model's adequacy and robustness, particularly in the presence of experimental noise and complex interactions among variables. Furthermore, verification helps distinguish whether the identified optimum corresponds to the true global optimum or merely a local optimum within the studied design space. In practical applications, particularly in industrial and scientific research settings, the reproducibility and consistency of optimized conditions are crucial. Verification experiments provide confidence that the optimized parameters can be reliably implemented under real-world conditions. Furthermore, this process can reveal the influence of uncontrolled or unmodeled factors not included in the original design. Therefore, verification is crucial to validate RSM's predictive capabilities and ensure that the optimized conditions are accurate and applicable in practice [23, 24].

This model verification step ensures that the mathematical model obtained from the experiment matches the experimental data. This stage involves comparing the model's predicted values with the experimental results. If the model accurately predicts the experimental data, it is considered representative of the DF extraction process. In this study, the verification stage involved five replicate experiments using the optimal extraction conditions identified through RSM: 82.29°C, 62.06 min, and 23.03% NaOH. This step was carried out to assess the model's accuracy and reliability in predicting DF yields under the predicted optimal conditions. The experimental results showed an average DF yield of  $25.43 \pm 0.24\%$ , which did not significantly differ from the RSM-predicted value of 25.75%. Although the achieved yield was slightly lower than the predicted value, the difference was not statistically significant. These results also confirmed the adequacy and predictive ability of the developed model and demonstrated that the optimal conditions for DF extraction from corn stalks can be reproduced on an industrial scale.

### 3.6 Functional Properties of Product

The WHC of the extracted DF product was  $4.7 \pm 0.08$  g/g, and the OHC was  $1.8 \pm 0.07$  g/g. These values indicate that DF derived from corn stalks exhibits superior hydration and oil retention properties compared

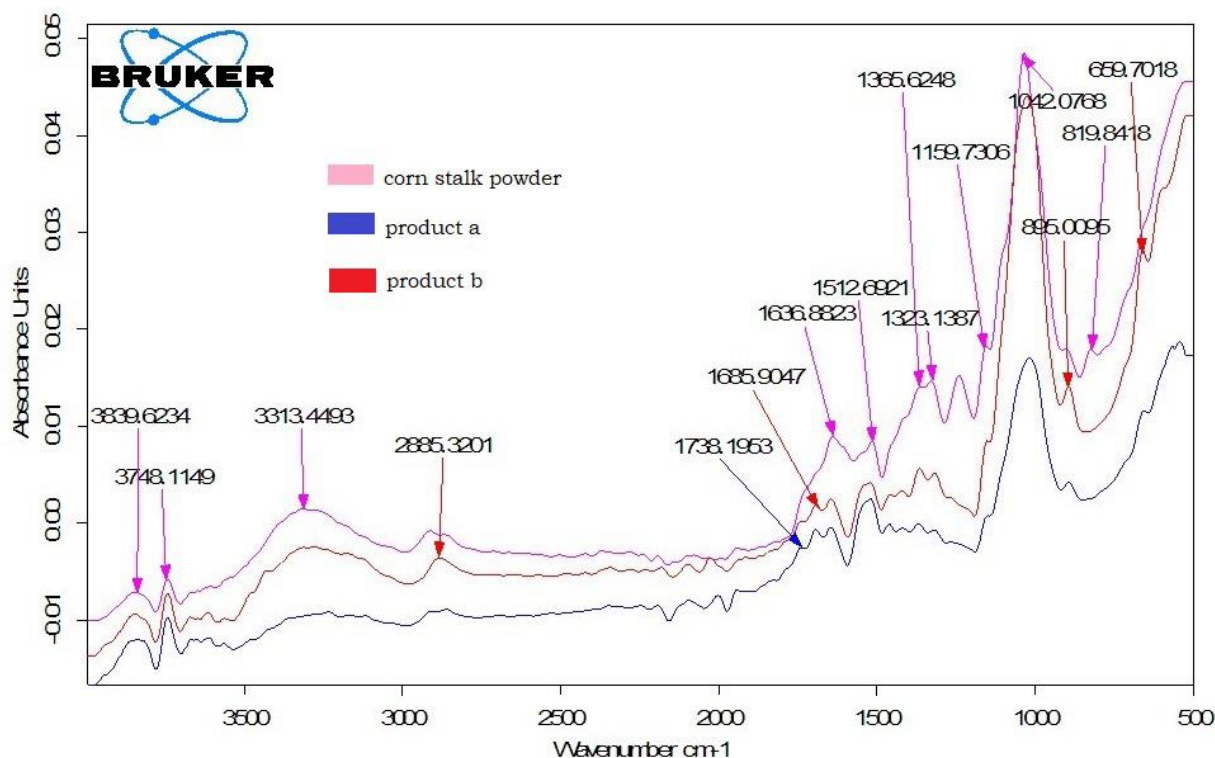
to flours commonly used as fillers in processed foods, such as wheat flour, which has WHC and OHC values of  $\approx 1.85$  g/g and  $\approx 1.05$  g/g, respectively. These properties are likely due to the highly porous, fibrous microstructure of the DF product, which increases surface area and allows greater water absorption. This makes it suitable as a functional ingredient in food formulations, particularly in meat and fish-based products that require enhanced moisture retention and texture stability. Previous studies have shown that DF with a high WHC can improve the juiciness, texture, and storage stability of meat-based formulations by minimizing moisture loss during cooking and storage [25]. The capacity of a substance to absorb and hold water without dissolving in it is known as WBC. This parameter describes a material's hydrophilic nature and its capacity to form a water-retaining structure. The WBC value of this product DF is 2.7 g/g, indicating that 1 gram of dry product binds an average of 2.7 grams of water. This WBC value is considered moderate to high and indicates that the product has a sufficiently large specific surface area and polar functional groups, such as hydroxyl, that can form hydrogen bonds with water molecules. This porous physical structure supports water retention, with functional implications in the food industry, in particular. A high WBC is crucial for textural stability in food products (e.g., processed meats, sausages, bread, and low-fat products), increasing tenderness and resistance to drying. Physiologically, fiber with a high WBC can slow gastric emptying, prolong the feeling of fullness, regulate blood sugar levels, and lower cholesterol by slowing nutrient absorption. Furthermore, the moderate OHC value ( $1.8 \pm 0.07$  g/g) of corn stalk fiber indicates an adequate ability to interact with lipids, which can help improve flavor retention and reduce phase separation in emulsified food products. Compared to wheat flour with an OHC of approximately 1.05 g/g, corn stalk fiber exhibits slightly better oil-binding properties, making it suitable for use in products such as emulsified meats, baked goods, or plant-based alternatives. Oil-binding ability is particularly relevant for sensory quality, as it can improve mouthfeel and flavor release [26].

The combination of high WHC and adequate OHC expands the potential applications of corn stalk DF beyond its traditional uses. This fiber can serve as a moisture-retention agent, fat replacer, or texture enhancer in low-fat or reformulated products, in line with current trends toward clean-label, high-fiber foods. From a sustainability perspective, utilizing corn stalk, a major agricultural by-product, also supports the upscaling of waste and adds economic value to the agro-industrial supply chain. SC is the maximum volume achieved by a fiber after absorbing air and expanding under certain conditions. The SC value is expressed in mL of air per gram of dry matter. The SC value of 5.12 mL/g indicates that corn stalk fiber can expand up to 5.12 mL per gram of fiber. This value indicates a fiber structure capable of holding large amounts of air and forming a swelling matrix. In its functional application, this swelling capacity is very useful in functional products such as high-fiber foods, weight loss products, or diet drinks, as it provides a faster and longer-lasting feeling of fullness. In the pharmaceutical industries, fibers with a high SC can be used as binders or slow-release agents. Physiologically, a high SC contributes to stool volume, relieves constipation, and increases the rate of fermentation by gut microbiota, thereby supporting gut health.

### 3.7 Structural analysis

FTIR spectroscopy is used to identify chemical functional groups in organic and inorganic compounds by measuring infrared light absorption at specific wavelengths ( $\text{cm}^{-1}$ ). In this study, FTIR spectroscopy was used to examine differences in molecular structure, chemical constituents, and functional groups present in DF samples. This analytical approach provides insight into the types of molecular bonds present and facilitates the prediction of their potential interactions within a food matrix. The FTIR results are shown in Figure 2. FTIR spectroscopy results from three different samples: Raw material/corn stalks powder (purple line), product A (red line), and product B (blue line). X-axis = Wavenumber ( $\text{cm}^{-1}$ ), which indicates the wavelength of infrared light; Y-axis = Absorbance, which reports how much light is absorbed at a specific wavelength—the Interpretation of Figure 2 as in Table 4.





**Figure 2.** Infrared spectroscopy of DF from corn stalks

The FTIR spectra confirmed the presence of key functional groups such as hydroxyl ( $\text{-OH}$ ), alkyl ( $\text{C-H}$ ), carbonyl ( $\text{C=O}$ ), and glycosidic bonds ( $\text{C-O-C}$ ) in DF extracted from corn stalks. Differences in peak intensity and position between the raw material and processed products (A and B) indicated structural modifications, particularly in the removal of non-cellulosic components such as lignin and hemicellulose. These results demonstrate molecular-level cellulose enrichment in the finished fiber product and validate the efficiency of the extraction process. This chemical transformation was confirmed by FTIR spectroscopy, which showed a characteristic  $\text{C=O}$  stretching band at around  $1738\text{ cm}^{-1}$  and a stronger  $\text{C-O-C}$  signal in the treated samples, indicating effective removal of non-cellulosic components and potential functional group modification. The appearance of the  $\text{C=O}$  band at  $1738\text{ cm}^{-1}$  and the strengthening of the  $\text{C-O-C}$  signal in the processed products confirmed that extraction effectively increased the cellulose fraction, providing further molecular evidence that the final fibers were richer in cellulose and had higher functional potential. DF products derived from corn stalks exhibit high cellulose content and a chemically modified structure, leading to good water absorption. This makes them ideal candidates as fiber additives in meat and fish product formulations, such as meatballs, nuggets, and others, to increase fiber content without significantly altering flavor or texture. Furthermore, the functional properties of polysaccharide-rich DF also open up opportunities for functional food innovation, for example, in low-calorie, cholesterol-lowering, and ready-to-eat products that support digestive health, thereby expanding the application of corn DF in the modern food industry.

**Table 4.** The Interpretation of infrared spectroscopy of DF from corn stalks

| No. | Picture                                                        | Assignment                                                    | Interpretation                                                                                                                                                                                                                                                       |
|-----|----------------------------------------------------------------|---------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1   | Broadband at 3748.11-3313.45 cm <sup>-1</sup>                  | O-H stretching vibration                                      | This broad region is typically associated with the hydroxyl groups (-OH) of polysaccharides and cellulose, indicating hydrogen bonding within the fiber matrix. The intensity and shape suggest strong intra- and intermolecular interactions among hydroxyl groups. |
| 2   | Peak at 2885.32 cm <sup>-1</sup>                               | C-H stretching vibration of aliphatic -CH <sub>2</sub> groups | This band is characteristic of cellulose and hemicellulose structures and reflects the alkyl chains present in the carbohydrate backbone.                                                                                                                            |
| 3   | Peak at 1738.19 cm <sup>-1</sup>                               | C=O stretching of ester or carboxylic acid groups             | This absorption band suggests the presence of acetyl or uronic ester groups, often attributed to hemicellulose or pectin components. The reduced intensity in processed products may indicate partial removal of non-cellulosic fractions during extraction.         |
| 4   | Peak at 1638.88 cm <sup>-1</sup>                               | Adsorbed water or -OH bending vibration                       | Associated with moisture or bound water within the fiber matrix, often observed in hydrophilic materials like cellulose.                                                                                                                                             |
| 5   | Peaks at 1512.69 cm <sup>-1</sup> and 1323.14 cm <sup>-1</sup> | Aromatic C=C stretching or CH <sub>2</sub> bending            | These peaks may reflect residual lignin components in the raw material, indicating partial lignin removal after treatment.                                                                                                                                           |
| 6   | Peak at 1364.62 cm <sup>-1</sup>                               | C-H bending                                                   | Indicates the presence of polysaccharide-related skeletal vibrations, primarily associated with cellulose.                                                                                                                                                           |
| 7   | Peak at 1159.73 cm <sup>-1</sup>                               | C-O-C asymmetric stretching                                   | Corresponds to the glycosidic linkage ( $\beta$ -1,4) in cellulose and hemicellulose.                                                                                                                                                                                |
| 8   | Sharp peak at 1042.08 cm <sup>-1</sup>                         | C-O stretching vibrations                                     | Strongly associated with polysaccharides, particularly cellulose and hemicellulose backbones.                                                                                                                                                                        |
| 9   | Peaks between 856.00-659.70 cm <sup>-1</sup>                   | C-H deformation, ring vibrations, or out-of-plane bending     | Indicative of the complex ring structures in carbohydrates and a possible fingerprint region for cellulose derivatives.                                                                                                                                              |

## 4. Conclusions

This study optimized the extraction of DF from corn stalks using RSM-CCD. The maximum DF yield of 25.75% was achieved at a temperature of 82.29°C, a time of 62.06 min, and 23.03% NaOH. The developed quadratic model demonstrated high accuracy ( $R^2 = 0.99$ ), as confirmed by verification tests. The product exhibited good functional properties related to WHC and OHC, indicating its potential for use in food formulations as a DF source and as a filler in processed foods. FTIR analysis confirmed chemical modification, particularly the removal of lignin and hemicellulose, further validating the extraction process. This study presents compelling results for the valorization of agricultural waste, such as corn stalks, into high-value functional food ingredients. The concepts of a circular economy, sustainable agriculture, and functional food innovation align with the integration of corn stalk waste valorization and nutritional enhancement. Furthermore, as efforts to increase DF content in processed foods and reduce reliance on synthetic additives continue, these natural fiber sources can serve as clean-label alternatives with promising health benefits.

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# Stock Price Prediction of TLKM and BBCA Using SVR, Random Forest, and LSTM

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**Abstract:** Stock price prediction is a significant challenge in financial market analysis due to the influence of many dynamic and nonlinear factors. This study compares three machine learning methods, namely Support Vector Regression (SVR), Random Forest (RF), and Long Short-Term Memory (LSTM), in predicting the stock prices of PT Telekomunikasi Indonesia Tbk (TLKM) and PT Bank Central Asia Tbk (BBCA) for the period 2019–2023. Data were obtained from Yahoo Finance (15). Model Performance Comparison for TLKM Stock: the testing results show that LSTM achieved RMSE of 0.040093, MAE of 0.029849, and  $R^2$  of 0.905330, indicating the best performance compared to SVR and Random Forest. Random Forest had an RMSE of 0.138295 and an MAE of 0.114423, while SVR had the highest RMSE at 0.238130. Model Performance Comparison for BBCA Stock: The testing results show that LSTM achieved RMSE of 0.055023, MAE of 0.048056, and  $R^2$  of 0.657730, indicating the best performance compared to SVR and Random Forest. Random Forest had an RMSE of 0.252568 and an MAE of 0.236129, while SVR had the highest RMSE at 0.6211363. This confirms the LSTM model's effectiveness in capturing temporal patterns in stock data (5), (9), (13).

**Keywords:** Stock price prediction; SVR; random forest; LSTM; machine learning

## 1. Introduction

Stock price prediction has become an important research topic in financial analysis due to its significant impact on investment decisions and risk management. However, forecasting stock prices remains challenging because financial time-series data are highly volatile, nonlinear, and influenced by numerous external factors, including economic conditions, political events, and market sentiment. Traditional statistical models often struggle to capture these complex patterns in financial markets. The stock market plays a crucial role in the economy by providing a platform for companies to raise capital and for investors to participate in wealth creation. Accurate stock price prediction is one of the most important challenges in financial market analysis because multiple dynamic and nonlinear factors, including market sentiment, economic indicators, company performance, and global events, influence stock prices. This inherent complexity makes traditional forecasting methods, such as linear regression, insufficient for capturing the intricate patterns in stock price

movements (4). In recent years, advances in information technology and the availability of large-scale financial data (big data) have significantly increased the potential for accurate stock price prediction. Machine learning (ML) techniques have emerged as powerful tools for modeling complex relationships in financial datasets (1). Unlike conventional statistical methods, ML models can learn from historical patterns and adapt to changes in market behavior, which is particularly important for volatile markets such as TLKM and BBCA. Among ML models, Support Vector Regression (SVR) is known for its ability to handle small to medium-sized datasets and provide stable predictions with good generalization by finding a regression function within a specified error margin (12). Random Forest (RF), an ensemble method of multiple decision trees, reduces overfitting and improves prediction stability by averaging outputs from numerous trees (7), (8). On the other hand, Long Short-Term Memory (LSTM), a type of recurrent neural network, is particularly suitable for time series forecasting due to its ability to capture long-term temporal dependencies in sequential data (5), (9), (13). Previous studies have demonstrated the effectiveness of these models in stock price prediction. LSTM models have been shown to outperform traditional machine learning approaches at capturing temporal patterns and nonlinear trends in stock price data (5), (9), (13), and time-series augmentation can further improve LSTM predictions for financial data (9). Random Forest has been widely used for stock trend forecasting due to its robustness against overfitting and its ability to handle high-dimensional data, with ensemble methods enhancing prediction accuracy for stock volatility (8), (10). SVR has also been successfully applied to financial time series with moderate dataset sizes, providing stable and interpretable results, though it may struggle with highly volatile markets (12). However, many previous studies focus on evaluating individual models rather than directly comparing multiple machine learning approaches on the same dataset. This limitation makes it difficult to determine which model performs better under similar experimental conditions, particularly in financial time-series forecasting. Furthermore, comparative studies of different machine learning paradigms, such as kernel-based methods, ensemble learning, and deep learning, remain relatively limited in the Indonesian stock market context. Therefore, this study builds on these previous findings by directly comparing the performance of SVR, Random Forest, and LSTM models using stock data from TLKM and BBCA during the period 2019–2023. These models were selected because they represent different machine learning paradigms and are widely used for stock price prediction in financial research. By analyzing these models side by side, this research aims to provide insights into their relative effectiveness and suitability for investors and financial analysts working with Indonesian equities, while addressing gaps in previous studies regarding model comparison and evaluation using local stock market data.

## 2. Materials and Methods

### 2.1 Support Vector Regression (SVR)

Support Vector Regression (SVR) is a supervised machine learning algorithm used for regression tasks. The SVR model seeks a function that approximates the relationship between the input variables and the target variable.

The regression function can be expressed as:

$$f(x) = w^T x + b$$

(Eq. 1)

where:

$x$  = input feature vector

$w$  = weight vector

$b$  = bias term

The optimization objective of SVR is defined as:

$$\min \frac{1}{2} \|w\|^2 + C \sum_{i=1}^n (\xi_i + \xi_i^*)$$

(Eq. 2)

where:

- $C$  = penalty parameter
- $\xi_i, \xi_i^*$  = slack variables
- $n$  = number of observations

The SVR model was implemented using the radial basis function (RBF) kernel with the following parameters:

Kernel: RBF

Regularization parameter (C): 100

Gamma: 0.1

Epsilon: 0.1

## 2.2 Random Forest

Random Forest is an ensemble learning method that combines multiple decision trees to improve prediction accuracy and reduce overfitting. The prediction of the Random Forest model is obtained by averaging the predictions of multiple trees:

$$\hat{y} = \frac{1}{T} \sum_{t=1}^T f_t(x)$$

(Eq. 3)

where:

- $T$  = number of decision trees
- $f_t(x)$  = prediction from the  $t$ -th tree

## 2.3 Long Short-Term Memory (LSTM)

Long Short-Term Memory (LSTM) is a type of recurrent neural network designed to capture long-term dependencies in sequential data.

The forget gate is defined as:

$$f_t = \sigma(W_f[h_{t-1}, x_t] + b_f)$$

(Eq. 4)

The input gate is defined as:

$$i_t = \sigma(W_i[h_{t-1}, x_t] + b_i)$$

(Eq. 5)

The output gate is defined as:

$$o_t = \sigma(W_o[h_{t-1}, x_t] + b_o)$$

(Eq. 6)

where:

- $x_t$  = input at time  $t$
- $h_{t-1}$  = hidden state from the previous step
- $W$  = weight matrix
- $b$  = bias term
- $\sigma$  = sigmoid activation function

These hyperparameter settings were selected based on commonly used configurations in previous machine learning studies on stock price prediction and preliminary experiments conducted during model development.

## 2.4 Data Source

The dataset used in this study consists of historical daily stock price data for PT Telekomunikasi Indonesia (TLKM.JK) and PT Bank Central Asia Tbk (BBCA.JK). The data were obtained from Yahoo Finance and cover the period from January 2019 to March 2023. The dataset includes several attributes such as Date, Open, High, Low, Close, Adjusted Close, and Volume. In this research, the closing price was used as the primary variable for stock price prediction.

## 2.5 Dataset Description and Distribution

The dataset contains approximately 1,050 daily observations for each stock. To evaluate the predictive performance of the machine learning models, the dataset was split into training and test sets at an 80:20 ratio. Specifically, around 840 observations were used for the training dataset, while the remaining 210 observations were allocated to the testing dataset. The training data were used to train the prediction models, including Support Vector Regression (SVR), Random Forest (RF), and Long Short-Term Memory (LSTM). The testing data were then used to evaluate the predictive performance of the models.

**Table 1.** Dataset Distribution

| Stock | Total Observations | Training Data (80%) | Testing Data (20%) |
|-------|--------------------|---------------------|--------------------|
| TLKM  | 1050               | 840                 | 210                |
| BBCA  | 1050               | 840                 | 210                |

## 2.6 Evaluation Metrics

To evaluate the performance of the prediction models, three evaluation metrics were used: Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and Coefficient of Determination ( $R^2$ ).

### 1. Mean Absolute Error (MAE)

MAE measures the average magnitude of the prediction errors, ignoring their direction. It calculates the average absolute difference between predicted and actual values.

$$MAE = \frac{1}{n} \sum_{i=1}^n |y_i - \hat{y}_i|$$

where:

- $y_i$  = actual value
- $\hat{y}_i$  = predicted value
- $n$  = number of observations

### 2. Root Mean Square Error (RMSE)

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2}$$

where:

- $y_i$  = actual value
- $\hat{y}_i$  = predicted value
- $n$  = number of observations

### 3. Coefficient of Determination ( $R^2$ )

The  $R^2$  score measures how well the predicted values approximate the actual data. A higher  $R^2$  value indicates better model performance.

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}$$

where:

- $y_i$  = actual value
- $\hat{y}_i$  = predicted value
- $\bar{y}$  = mean of actual values
- $n$  = number of observations

The  $R^2$  value ranges from 0 to 1, with values closer to 1 indicating better predictive performance. RMSE measures the square root of the average squared differences between predicted and actual values. It gives higher weight to large errors. Support Vector Regression (SVR): Uses an RBF kernel to minimize error with an optimal margin (12). Random Forest (RF): Combines multiple decision trees to improve accuracy and prediction stability (7), (8). Long Short-Term Memory (LSTM): A recurrent neural network capable of learning long-term dependencies in time series data (5), (9), (13).

#### 2.6.1 Support Vector Regression (SVR)

SVR aims to find a function  $f(x)$  that approximates the target  $y_i$  within a margin  $\epsilon_i$  while keeping the model as simple as possible.

**SVR Formula:**

$$f(x) = \langle w, \phi(x) \rangle + b$$

$x$  = input vector

$\phi(x)$  = nonlinear transformation

$w$  = weight vector

$b$  = bias

**Optimization Problem :**

$$\min \frac{1}{2} \|w\|^2 + C \sum_{i=1}^n (\xi_i + \xi_i^*)$$

subject to:

$$\begin{cases} y_i - \langle w, \phi(x_i) \rangle - b \leq \epsilon + \xi_i \\ \langle w, \phi(x_i) \rangle + b - y_i \leq \epsilon + \xi_i^* \\ \xi_i, \xi_i^* \geq 0 \end{cases}$$

- $C$  = regularization parameter
- $\xi_i, \xi_i^*$  = slack variables for error tolerance

Explanation: SVR minimizes prediction errors outside the margin  $\epsilon$  while keeping the model complexity minimal.

#### 2.6.2 Random Forest (RF)

Random Forest is an ensemble of multiple decision trees, and the final prediction is the average of all tree outputs.



**RF Formula:**

$$\hat{y} = \frac{1}{T} \sum_{t=1}^T h_t(x)$$

- $T$  = number of trees
- $h_t(x)$  = prediction of the  $t$ -th tree

**Explanation:** By combining multiple trees, Random Forest reduces overfitting and increases prediction stability.

### 2.6.3 Long Short-Term Memory (LSTM)

LSTM is a type of Recurrent Neural Network (RNN) capable of storing and processing long-term dependencies in time series data.

**Main LSTM Equations:**

1. Forget gate:

$$f_t = \sigma(W_f \cdot [h_{t-1}, x_t] + b_f)$$

2. Input gate:

$$i_t = \sigma(W_i \cdot [h_{t-1}, x_t] + b_i), \quad \tilde{C}_t = \tanh(W_C \cdot [h_{t-1}, x_t] + b_C)$$

3. Update cell state:

$$C_t = f_t \odot C_{t-1} + i_t \odot \tilde{C}_t$$

4. Output gate:

$$o_t = \sigma(W_o \cdot [h_{t-1}, x_t] + b_o), \quad h_t = o_t \odot \tanh(C_t)$$

**Explanation :**

$x_t$  = input at time  $t$

$h_t$  = hidden state

$C_t$  = cell state

$\sigma$  = sigmoid function,  $\odot$  = element-wise multiplication

LSTM can learn temporal patterns and nonlinear relationships in stock price data.

## 2.7 Flowchart of Proposed Method

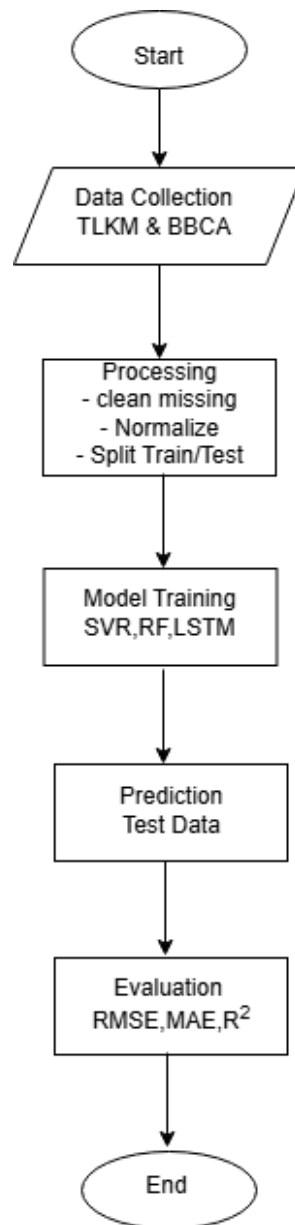
Step-by-step procedure:

1. Data Collection: Retrieve TLKM & BBKA stock data from Yahoo Finance.
2. Data Preprocessing: Clean missing data, normalize using Min-Max scaling, split into training/testing sets.
3. Model Training: Train SVR, Random Forest, and LSTM models on training data.
4. Prediction: Predict stock prices on testing data.
5. Evaluation: Evaluate models using RMSE, MAE, and  $R^2$  metrics.
6. Comparison & Conclusion: Identify the best-performing model.

Flowchart (ASCII version, can be replaced with a graphic in Word/PowerPoint):

## 2.8. Model Evaluation

Evaluation was performed using three main metrics: RMSE, MAE, and  $R^2$  (3), (10). Models with the lowest RMSE and MAE, and the highest  $R^2$ , were considered the best-performing.



**Figure 1.** Flowchart of Proposed Method

### 3. Results and Discussion

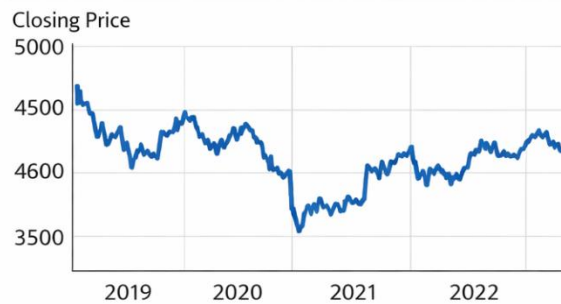
#### 3.1 Data Visualization and Trend Analysis

To better understand the dataset's characteristics, a graphical visualization of the historical stock prices of TLKM and BBKA was created. Visualization of time-series data is essential for revealing underlying patterns, such as long-term trends, volatility, and potential cyclical movements in the stock market.

Compared to TLKM, BBKA demonstrates a stronger upward trend, indicating stable growth and strong investor confidence in the banking sector. To further compare the movements of both stocks, Figure 4 provides a comparative visualization of the normalized stock prices of TLKM and BBKA. The comparison highlights that BBKA experienced stronger long-term growth, while TLKM exhibited relatively more stable price movements with lower volatility. These observations confirm that stock price data exhibit nonlinear patterns and temporal dependencies, which justify the use of machine learning models such as LSTMs that can capture sequential patterns in time-series data.

This study compares the performance of three machine learning methods, namely LSTM, Random Forest, and Support Vector Regression (SVR), in predicting the stock prices of TLKM and BBCA. The evaluation was conducted using three main metrics: Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and the coefficient of determination ( $R^2$ ).

**Figure 2. TLKM Stock Prices (2019–2023)**



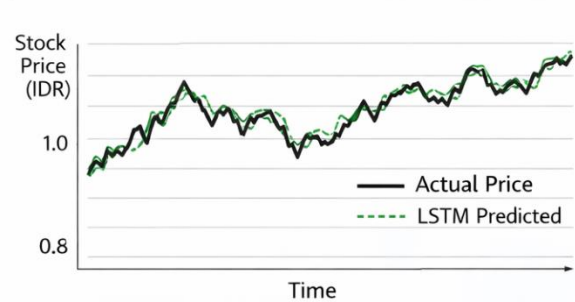
**Figure 3. BBCA Stock Prices (2019–2023)**



**Figure 4. TLKM vs BBCA**



**Figure 5. Actual vs. Predicted**



**Figure 2.** illustrates the time-series visualization of TLKM stock prices from 2019 to 2023. The graph shows moderate fluctuations with a noticeable decline around 2020, which may be associated with global economic disruptions during the COVID-19 pandemic. After this period, the stock price gradually stabilizes and shows signs of recovery. **Figure 3** presents the time-series visualization of BBCA stock prices during the same period.

**Table 2.** Model Performance Comparison for TLKM Stock

| Model         | RMSE     | MAE      | R2        |
|---------------|----------|----------|-----------|
| SVR           | 0.238130 | 0.213049 | -2.687252 |
| Random Forest | 0.138295 | 0.114423 | -0.136221 |
| LSTM          | 0.040093 | 0.029849 | 0.905330  |

The testing results show that LSTM achieved RMSE of 0.040093, MAE of 0.029849, and  $R^2$  of 0.905330, indicating the best performance compared to SVR and Random Forest. Random Forest had an RMSE of 0.138295 and an MAE of 0.114423, while SVR had the highest RMSE at 0.238130 (2), (5).

**Table 3.** Model Performance Comparison for BBCA Stock

| Model         | RMSE      | MAE      | R2         |
|---------------|-----------|----------|------------|
| SVR           | 0.6211363 | 0.575973 | -39.844270 |
| Random Forest | 0.252568  | 0.236129 | -5.748368  |
| LSTM          | 0.055023  | 0.048056 | 0.657730   |

The testing results show that LSTM achieved an RMSE of 0.055023, an MAE of 0.048056, and an  $R^2$  of 0.657730, indicating the best performance compared to SVR and Random Forest. Random Forest had an RMSE of 0.252568 and an MAE of 0.236129, while SVR had the highest RMSE at 0.6211363 (2), (5).

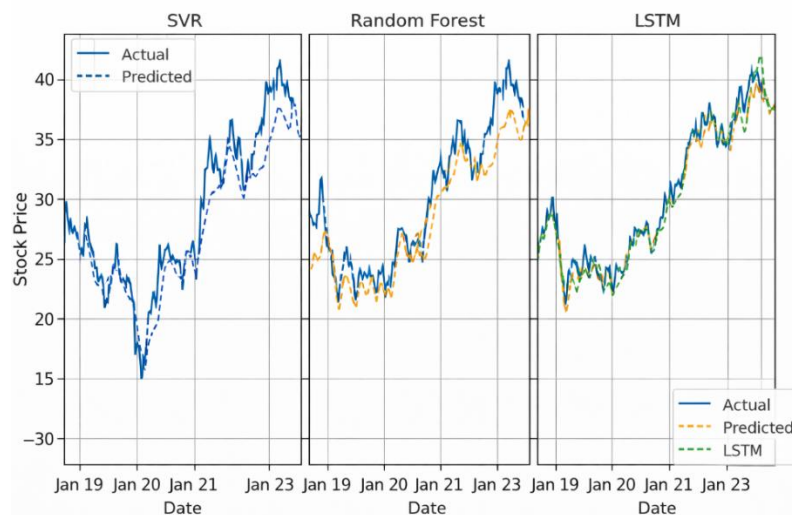
### 3.2 Model Performance Evaluation

This study compares the performance of three machine learning methods, namely LSTM, Random Forest, and Support Vector Regression (SVR), in predicting the stock prices of TLKM and BBCA. The evaluation was conducted using three main metrics: Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and the coefficient of determination ( $R^2$ ).

**Table 4.** Model Performance Comparison for BBCA Stock

| Model         | TLKM $R^2$ | BBCA $R^2$ |
|---------------|------------|------------|
| SVR           | -2.687     | -39.844    |
| Random Forest | -0.136     | -5.748     |
| LSTM          | 0.905      | 0.658      |

The results indicate that the LSTM model achieves the best performance on both the TLKM and BBCA datasets, as evidenced by lower RMSE and MAE values and higher  $R^2$  scores than SVR and Random Forest.

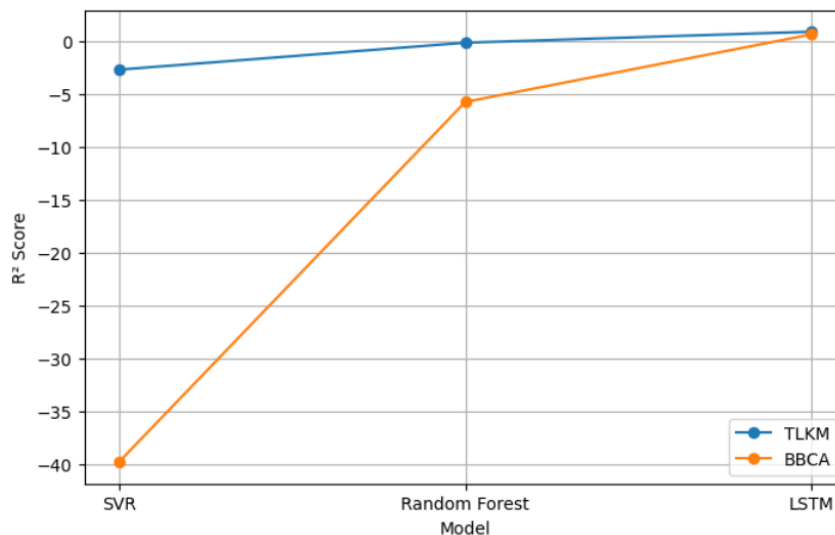


**Figure 6.** Comparison between actual stock prices and predicted values generated by SVR, Random Forest, and LSTM models on the testing dataset.

Figure 6 presents the comparison of  $R^2$  values for the three models. The LSTM model consistently outperforms the other models. Random Forest shows moderate performance, while SVR produces the lowest accuracy, particularly for the BBCA dataset.

### 3.3 Actual vs Predicted Analysis

To further evaluate the predictive performance of the models, a comparison of actual stock prices and the predicted values generated by each model is shown in Figure 6. This visualization illustrates how closely the predicted values follow the actual stock price trends over time. By examining time-series plots, it is possible to assess each model's ability to capture fluctuations and patterns in stock price movements.



**Figure 7.** Comparison of  $R^2$  Accuracy in TLKM & BBKA Stock Predictions

To further evaluate the predictive performance of the models, a comparison of actual stock prices and the predicted values generated by each model is shown in Figure 7. This visualization shows how closely the predicted values track the actual stock price trends over time. Based on Figure 7, all models capture the general movement pattern of stock prices. However, there are clear differences in prediction accuracy. The SVR model shows larger deviations, especially during periods of high volatility. The Random Forest model provides better predictions than SVR but still exhibits some inconsistencies. The LSTM model aligns most closely with actual stock prices, indicating its superior ability to capture temporal dependencies and nonlinear patterns in financial time-series data.

### 3.4 Discussion

The superior performance of the LSTM model can be attributed to its ability to process sequential data and retain information from previous time steps through its memory structure. Unlike SVR and Random Forest, which treat observations as independent data points, LSTM is specifically designed to model temporal dependencies in time-series data. SVR relies on kernel-based regression, which is effective for nonlinear relationships but does not explicitly consider the time-order structure of the data. As a result, it may fail to capture rapid fluctuations in stock prices. Similarly, Random Forest, although robust and capable of handling nonlinear data, does not incorporate temporal dependencies, which limits its effectiveness in time-series forecasting. These findings are consistent with previous studies that highlight the advantages of LSTM in financial time-series prediction.

### 3.5 Limitations and Future Work

Despite the promising results, this study has several limitations. First, the analysis is limited to only two stocks, namely TLKM and BBKA, which may not fully represent the overall stock market. Second, the study uses a single train-test split (80:20), which may make the results depend on a particular data partition. Although a chronological split was applied, more robust validation approaches, such as rolling windows or time-series cross-validation, could improve the reliability of the results. Future research may include a larger dataset, additional features such as macroeconomic indicators, and more advanced or hybrid models to improve prediction accuracy and generalizability.

## 4. Conclusions

This study analyzed and compared the performance of three machine learning models—Support Vector Regression (SVR), Random Forest (RF), and Long Short-Term Memory (LSTM)—in predicting the stock prices of TLKM and BBKA for the period 2019–2023. Based on the evaluation metrics of RMSE, MAE, and  $R^2$ , the results indicate that LSTM achieved the highest accuracy, effectively capturing the nonlinear patterns and



temporal dependencies in the stock price data. Random Forest produced moderate results, demonstrating stability and reduced overfitting, while SVR showed the lowest accuracy, particularly in the face of high market volatility. These findings underscore the superiority of deep learning models, such as LSTMs, for time-series forecasting in financial markets and highlight the critical role of data preprocessing, including normalization and proper train-test splitting, in achieving optimal predictive performance. Overall, the research confirms that LSTM is the most suitable model among the three for predicting TLKM and BBCA stock prices, providing valuable insights for investors and financial analysts.

For future research, several directions are recommended. Hybrid modeling approaches that combine LSTM with other machine learning algorithms, such as Random Forest or Gradient Boosting, may further improve prediction accuracy and model stability. Including additional features, such as macroeconomic indicators, market sentiment from news or social media, and technical indicators, could enhance the models' predictive capabilities. Expanding the dataset to include more companies, sectors, or regional markets in Indonesia could provide more generalizable insights for financial forecasting. Implementing LSTM-based models in real-time prediction systems could help investors make timely investment decisions, while incorporating explainable AI techniques would increase model transparency and trust among financial stakeholders. In conclusion, while LSTM demonstrates strong potential in stock price prediction, exploring hybrid approaches, additional features, and explainable AI methods may further enhance predictive performance and applicability in real-world financial decision-making.

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**Author Contributions:** Mhd Adi Setiawan Arintonang Research (Conceptualization):The first author was responsible for formulating the research idea, formulating the research objectives, and developing the conceptual framework related to stock price prediction using the SVR, Random Forest, and LSTM methods.; Maradona Jonas Simanullang (Data Collection and Preparation):The second author collected the TLKM and BBCA stock datasets, conducted data cleaning, normalization, and prepared the data for model training.; Sasa Ani Arnomo (Model Development and System Implementation) : The third author was responsible for implementing the Support Vector Regression (SVR), Random Forest, and Long Short-Term Memory (LSTM) algorithms, including model training and testing. Maya Ardilla Siregar (Writing and Citation) : The fourth author wrote the article, conducted citations within the article, and searched for references relevant to the research title.; Muhammad Khairul Hisan Bin Madon (Data Visualization and Interpretation) : The fifth author was responsible for creating stock prediction graphic visualizations, interpreting experimental results, and compiling research tables and illustrations.; Muhammad Nazwan (Manuscript Writing and Revision):The sixth author drafted the final manuscript, checked for grammar and formatting, and revised the entire article before publication.; All authors have discussed, reviewed, and approved the published version of the manuscript.

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# Noise and Air Quality from Traffic in Mueang Nakhon Si Thammarat, Nakhon Si Thammarat Province

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**Abstract:** Noise pollution and poor air quality are growing environmental concerns in rapidly developing Thai cities. This study assessed traffic-generated noise levels and ambient air quality at four signalized intersections in Mueang Nakhon Si Thammarat district, Nakhon Si Thammarat Province, in September 2022. Measurements were conducted at three peak-hour periods (07:00–09:00, 11:00–13:00, and 15:00–17:00) on both weekdays and weekends. Parameters included equivalent continuous noise level (Leq), oxygen (O<sub>2</sub>), carbon monoxide (CO), carbon dioxide (CO<sub>2</sub>), sulfur dioxide (SO<sub>2</sub>), relative humidity, wind speed, and temperature. All four sites exceeded the Thai national noise standard (24-hr average  $\leq 70$  dB(A)) on weekdays, with Site 1 (Hua Thanon) recording the highest average of 73.5 dB(A). On weekends, Site 2 (Fountain Circle–Kanlayanee School) was the only site within the standard at 69.9 dB(A). CO concentrations at all sites (26.0–27.3 ppm) exceeded the Thai 8-hour ambient standard of 9 ppm, though they remained within the 1-hour limit of 30 ppm. O<sub>2</sub> levels were below the atmospheric reference of 20.9% at every site, while SO<sub>2</sub> was undetectable throughout. Environmental factors—particularly relative humidity, wind speed, and temperature—influenced both noise propagation and pollutant dispersion. Site 3 (Khu Khwang) showed the highest CO and CO<sub>2</sub> concentrations, attributed to the dense surrounding buildings, which restricted ventilation. These findings highlight the need for continuous environmental monitoring and traffic management interventions in Nakhon Si Thammarat's urban core.

**Keywords:** Traffic noise; air quality; carbon monoxide; urban environment; Nakhon Si Thammarat

## 1. Introduction

Noise pollution has emerged as a significant environmental issue, particularly in large cities and densely populated communities that serve as centers of economic activity. Transportation, especially road traffic from motorcycles, automobiles, and modified-exhaust vehicles, is widely recognized as the predominant source of urban noise. In Thailand, comparative data from 2022 show that motorcycles and automobiles remain the primary noise-generating sources, with adverse consequences for the health of residents and passersby [1]. In the Southern Region of Thailand, continuous monitoring has recorded noise levels ranging from 52.2 to 86.3 dB(A), exceeding the 24-hour Leq standard, with values trending upward, especially in the Phuket municipality, where recreational activities near tourist attractions amplify traffic noise. Simultaneously, ambient air quality monitoring for the five major pollutants (SO<sub>2</sub>, NO<sub>2</sub>, CO, PM<sub>10</sub>, and O<sub>3</sub>) has generally been classified as satisfactory in the

South [2]. Beyond road traffic, noise monitoring research in Thailand has expanded to include emerging sources such as utility-scale wind power plants, where sound pressure levels and propagation zones require systematic field assessment [3, 4], and engineering interventions such as noise barriers incorporating agricultural residues have demonstrated measurable attenuation potential in local settings [5]. These studies collectively underscore the growing recognition that acoustic environmental management demands source-specific methodologies.

Nakhon Si Thammarat Province is the most populous province in Southern Thailand and the 16th largest in the country by area. As a historically and economically significant city that attracts both domestic and international tourists through landmarks such as Wat Phra Mahathat Woramahawihan, Khanom–Mu Koh Thale Tai National Park, and Ai Khai Temple, the province has experienced substantial economic expansion. This growth has directly increased vehicular traffic, particularly at signalized intersections in the urban district, where congestion during school rush hours may push noise and air pollution beyond legally defined thresholds [6, 7]. Despite these concerns, systematic data on traffic-generated environmental pollution at key intersections in Nakhon Si Thammarat remain limited. Advances in real-time monitoring technology, including AIoT-based systems capable of predicting PM<sub>2.5</sub> concentrations in urban environments, highlight both the feasibility and urgency of continuous, multi-parameter air quality surveillance at high-traffic locations [8]. Earlier work by Srikaew and Tonsoom examined air quality and noise levels at a parking facility in the city, documenting O<sub>2</sub> levels below standard and CO concentrations exceeding the 8-hour limit [9]. Building on that foundation, the present study undertakes a comprehensive assessment at four major signalized intersections in Mueang district, comparing measured values against national ambient standards and examining the role of meteorological factors in modulating pollution levels. Traffic signal optimization research – including simulation-based analyses of heterogeneous traffic flows at signalized intersections [10], further confirms that intersection management directly determines vehicle idle time and, therefore, emission accumulation, reinforcing the need for simultaneous noise and air quality characterization at such sites.

The objectives of this study were: (1) to measure noise levels and concentrations of O<sub>2</sub>, CO, CO<sub>2</sub>, and SO<sub>2</sub> at the four intersections on both weekdays and weekends; (2) to compare measured values with the standards prescribed by National Environmental Board Notifications No. 15 (B.E. 2540) for noise and No. 10 (B.E. 2538) and No. 21 (B.E. 2544) for air quality; and (3) to evaluate the influence of environmental factors, relative humidity, wind speed, and temperature, on observed noise and air quality levels.

## 2. Materials and Methods

### 2.1 Study Area and Sampling Sites

The study was conducted at four signalized intersections in Mueang Nakhon Si Thammarat district (Figure 1). Site 1 – Hua Thanon intersection, Tambon Ma Muang Song Ton (coordinates: 47P, x = 607092, y = 926579). Site 2 – Fountain circle roundabout in front of Kanlayanee Si Thammarat School, Ratchadamnoen Road, Tambon Khlang (47P, x = 606027, y = 932004). Site 3 – Khu Khwang intersection, Phatthanakan Khu Khwang Road, Tambon Tha Wang (47P, x = 606751, y = 933176). Site 4 – Benjamarachutit intersection, Tambon Phot Sadet (47P, x = 601469, y = 932394). The area is characterized by a coastal plain topography and a tropical monsoon climate (hot season: February–April; rainy season: May–October, influenced by the southwest monsoon; November–January, influenced by the northeast monsoon). Site 3 is notable for being flanked by tall buildings that constrain air circulation, unlike the other three intersections.

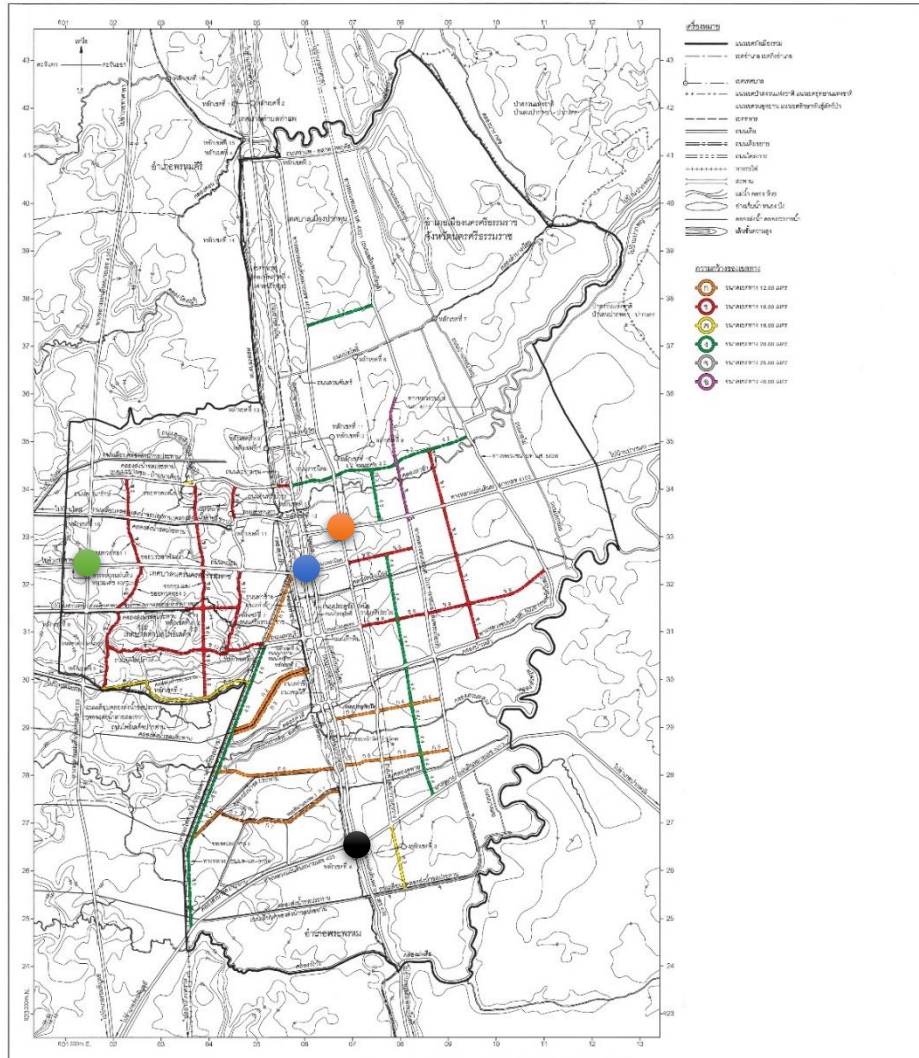
### 2.2 Measurement Schedule

Data were collected in September 2022 (one weekday and one weekend day per site, for a total of 8 days). At each site, measurements were taken during three peak-traffic periods: Period 1 (07:00–09:00), Period 2 (11:00–13:00), and Period 3 (15:00–17:00). Within each period, readings were recorded at 15-minute intervals, with instruments initialized 15 minutes before data collection commenced. Each 15-minute reading represents the mean equivalent noise level (Leq) recorded continuously over the 15-minute window, along with the mean gas concentration averaged over the same interval.



### 2.3 Measurement Equipment

The instruments used are summarized in Table 1. The sound level meter was set to Fast response mode and A-weighting to record Leq, Leq Min, and Leq Max values. The microphone was mounted at 1.2–1.5 m above ground level at the roadside edge and oriented toward the traffic flow, in accordance with the Pollution Control Department (PCD) guidelines [11]. No reflective surfaces existed within a 3.5 m radius of the microphone.



**Figure 1.** Locations of the four signalized intersection monitoring sites in Mueang Nakhon Si Thammarat district. Base map adapted from the Ministry of Interior (Transportation Network Plan appended to the Ministerial Regulation Enforcing the Nakhon Si Thammarat City Plan). The authors added the site locations for monitoring.

**Table 1.** Instruments used for field data collection.

| Equipment                  | Specification / Parameter Measured                                       |
|----------------------------|--------------------------------------------------------------------------|
| Sound Level Meter (Type 2) | Noise level (dB(A)); A-weighting; Leq, Leq Min, Leq Max; range 30–130 dB |
| DIGICON DO-552             | Oxygen (O <sub>2</sub> ) concentration (0–30%), temperature (0–50°C)     |
| Extech CO250               | Carbon dioxide (CO <sub>2</sub> ) concentration (ppm)                    |
| Extech CO10                | Carbon monoxide (CO) concentration (ppm)                                 |
| Gasbadge Pro-O2            | Sulfur dioxide (SO <sub>2</sub> ) concentration (ppm)                    |
| Dry-Wet 5538285 Hygrometer | Relative humidity (%)                                                    |
| DIGICON DA-47 Anemometer   | Wind speed (m/s)                                                         |



## 2.4 Data Analysis

Noise data were compared with the Thai General Noise Standard (National Environmental Board Notification No. 15, B.E. 2540):  $\text{Leq}_{24\text{-hr}} \leq 70 \text{ dB(A)}$  and  $\text{L}_{\text{max}} \leq 115 \text{ dB(A)}$  [12]. Air quality data were compared against the Thai Ambient Air Quality Standard (National Environmental Board Notifications No. 10, B.E. 2538, and No. 21, B.E. 2544):  $\text{CO } 1\text{-hr} \leq 30 \text{ ppm}$ ,  $\text{CO } 8\text{-hr} \leq 9 \text{ ppm}$ ;  $\text{SO}_2 \text{ } 1\text{-hr} \leq 0.30 \text{ ppm}$ ,  $\text{SO}_2 \text{ } 8\text{-hr} \leq 0.12 \text{ ppm}$  [13]. Descriptive statistics (mean, minimum, maximum) were calculated for each site, period, and day type. The influence of relative humidity, wind speed, and temperature on noise and air quality was assessed qualitatively. A formal correlation or regression analysis was not conducted owing to the limited number of independent measurement sessions; this is acknowledged as a limitation, and future studies with larger datasets should apply Pearson or Spearman correlation coefficients to quantify these relationships.

## 3. Results and Discussion

### 3.1 Noise Levels

Table 2 presents the summary noise levels for all four sites. On weekdays, average noise levels ( $\text{Leq}$ ) at all sites exceeded the 70 dB(A) standard, ranging from 70.3 dB(A) (Site 2) to 73.5 dB(A) (Site 1). On weekends, three of the four sites still exceeded the standard; Site 2 was the sole exception at 69.9 dB(A). Maximum noise levels ( $\text{Leq Max}$ ) at all sites and all periods remained well below the 115 dB(A) limit.

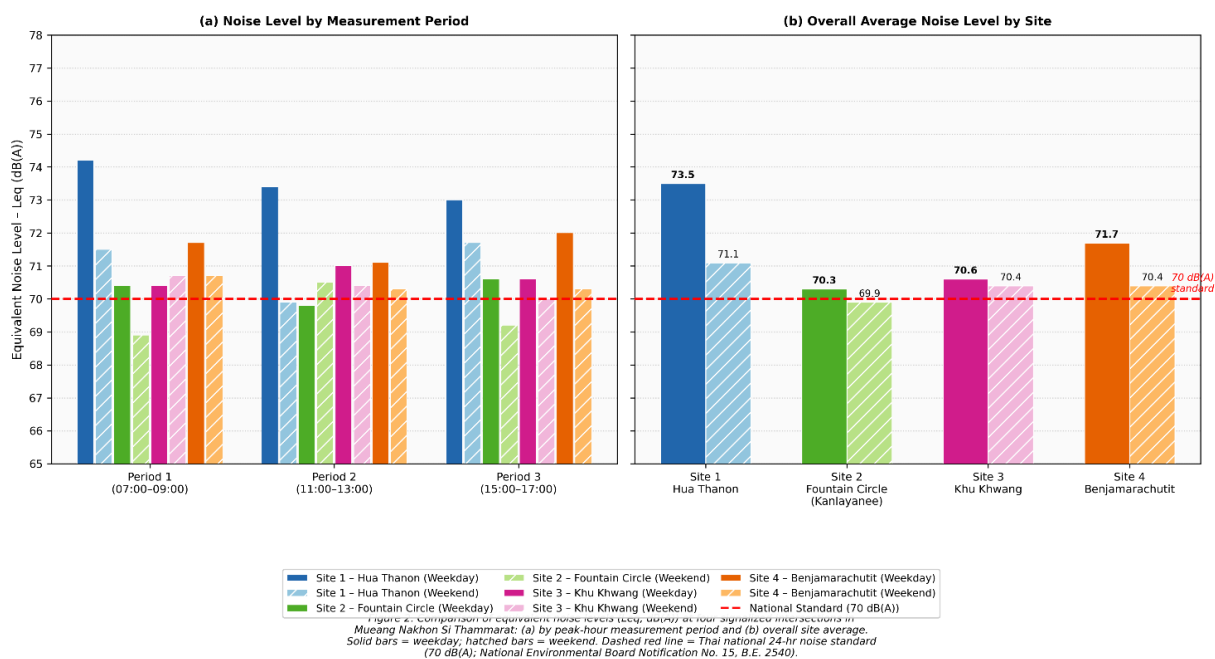
**Table 2.** Summary of average noise levels (dB(A)) by site and day type. \* Exceeds the 70 dB(A) national standard.

| Site                                     | Weekday<br>Leq Min<br>(dB(A)) | Weekday<br>Leq Max<br>(dB(A)) | Weekday<br>Avg<br>(dB(A)) | Weekend<br>Leq Min<br>(dB(A)) | Weekend<br>Leq Max<br>(dB(A)) | Weekend<br>Avg<br>(dB(A)) |
|------------------------------------------|-------------------------------|-------------------------------|---------------------------|-------------------------------|-------------------------------|---------------------------|
| Site 1 – Hua Thanon                      | 61.2                          | 85.8                          | 73.5*                     | 61.6                          | 80.5                          | 71.1*                     |
| Site 2 – Fountain Circle<br>(Kanlayanee) | 59.5                          | 81.0                          | 70.3*                     | 59.7                          | 80.0                          | 69.9                      |
| Site 3 – Khu Khwang                      | 60.1                          | 81.1                          | 70.6*                     | 61.1                          | 79.7                          | 70.4*                     |
| Site 4 – Benjamachutit                   | 62.5                          | 80.8                          | 71.7*                     | 60.7                          | 80.1                          | 70.4*                     |

Site 1 (Hua Thanon) consistently recorded the highest noise on both weekdays (73.5 dB(A)) and weekends (71.1 dB(A)). This is attributable to the site's pronounced road gradient, which compels drivers to accelerate their engines while waiting at the traffic light or traversing the slope, thereby generating higher engine noise than at flat-grade intersections. A similar pattern was reported by Suphakit et al. [14] for heavy-traffic intersections in Kamphaeng Phet municipality, where peak-hour  $\text{Leq}$  values reached 70.2 dB(A). Site 2 (Fountain Circle–Kanlayanee) showed the lowest noise on both day types, partly because weekend traffic volume is substantially lower than at the adjacent educational establishments. This outcome aligns with findings from Ariya et al. [15], who observed that daytime noise at Khon Kaen signalized intersections consistently exceeded 70 dB(A) on working days but varied more on weekends. The observed weekday–weekend difference across all sites ranged from 0.2 to 2.4 dB(A), consistent with findings by Wallika [16] and Niran [17], who both report that weekday  $\text{Leq}$  values in school and university environments exceed those on weekends. Relative humidity was the most significant environmental modifier of noise levels. Higher humidity reduces the air's capacity to absorb sound, resulting in louder perceived noise. Simultaneously, wind speed influences sound wave propagation, and temperature, which is inversely proportional to relative humidity, modulates sound refraction. At Site 2, slightly higher wind speed on weekends (0.9 m/s vs. 0.8 m/s on weekdays) may have marginally enhanced sound dissipation, contributing to its within-standard weekend reading.

Figure 2 illustrates the pattern of noise levels across measurement periods and sites for both weekdays and weekends. As shown in panel (a), noise levels during Period 1 (07:00–09:00) were generally the highest across most sites on weekdays, reflecting the morning school and commuter rush hour that generates dense, slow-moving traffic at all four intersections. Site 1 (Hua Thanon) recorded the highest Period 1 weekday value of 74.2 dB(A), while noise levels remained relatively stable across Periods 2 and 3 (73.4 and 73.0 dB(A), respectively), suggesting sustained traffic intensity throughout the working day rather than a single

pronounced peak. Panel (b) confirms that Site 1 consistently produced the highest overall average noise on both weekdays (73.5 dB(A)) and weekends (71.1 dB(A)), with all values exceeding the national 24-hour standard of 70 dB(A) denoted by the dashed reference line. Site 2 (Fountain Circle–Kanlayanee School) was the only site to fall within the standard on weekends (69.9 dB(A)), a reduction attributable to the absence of school-related traffic on non-working days and to comparatively higher wind speed (0.9 m/s), which aided sound dissipation. Sites 3 and 4 exhibited very similar overall averages on weekends (70.4 dB(A) each), marginally exceeding the standard, while their weekday averages (70.6 and 71.7 dB(A), respectively) reflected the combined effect of commuter traffic and restricted ventilation, particularly at Site 3, where surrounding buildings limit sound attenuation. Across all sites, weekday noise averages were consistently higher than weekend values by 0.2 to 2.4 dB(A), a difference driven primarily by the higher volume and density of vehicular traffic on working days. This weekday–weekend differential is consistent with findings reported in comparable urban intersection studies in Thailand and internationally [14–16].



**Figure 2.** Comparison of average equivalent noise levels (Leq, dB(A)) at four signalized intersections in Mueang Nakhon Si Thammarat on weekdays and weekends across three peak-hour measurement periods (Period 1: 07:00–09:00, Period 2: 11:00–13:00, Period 3: 15:00–17:00). The dashed horizontal line indicates the Thai national 24-hour noise standard of 70 dB(A) (National Environmental Board Notification No. 15, B.E. 2540).

### 3.2 Air Quality

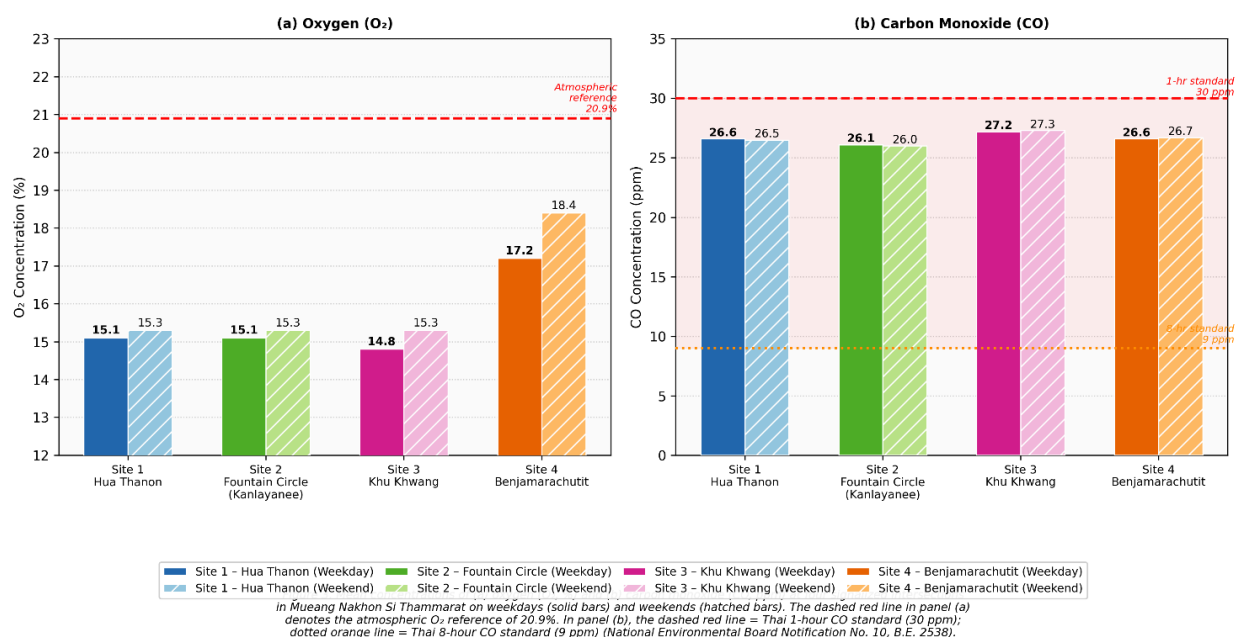
Table 3 summarizes the mean air quality parameters for all four sites by day type. Figures 3 and 4 provide visual comparisons of  $O_2$ , CO, and  $CO_2$  concentrations across sites and day types. Sulfur dioxide was undetectable at all sites and all measurement periods, consistent with the relatively clean fuel standards in modern Thai vehicles and the absence of heavy industry in the immediate vicinity.  $O_2$  concentrations were below the atmospheric reference value of 20.9% at every site and period, as shown in Figure 3(a), with the lowest average recorded at Site 3 on weekdays (14.8%). The cause of these anomalously low  $O_2$  readings warrants caution in interpretation. A depletion of 5–6 percentage points below the 20.9% atmospheric reference cannot be accounted for quantitatively by the co-measured CO (~27 ppm) and  $CO_2$  (~600 ppm), which together displace less than 0.1% of atmospheric  $O_2$ ; such large deficits are physically implausible at open outdoor intersections. The most probable explanation is calibration drift or sensor error in the DIGICON DO-552; calibration records should be verified. The  $O_2$  data are reported here for completeness but should be interpreted with caution, and no mechanistic conclusions about combustion gas displacement are drawn from these values.

**Table 3.** Mean air quality concentrations by site and day type (ND = not detected). \* CO 8-hr average exceeds the 9 ppm national standard at all sites.

| Parameter             | Site 1 Hua<br>Thanon | Site 2<br>Fountain<br>Circle | Site 3 Khu<br>Khwang | Site 4<br>Benjamarachutit | Standard (1-<br>hr avg) | Standard (8-<br>hr avg) |
|-----------------------|----------------------|------------------------------|----------------------|---------------------------|-------------------------|-------------------------|
| <i>Weekday</i>        |                      |                              |                      |                           |                         |                         |
| O <sub>2</sub> (%)    | 15.1                 | 15.1                         | 14.8                 | 17.2                      | N/A<br>(ref: 20.9%)     | N/A                     |
| CO (ppm)              | 26.6                 | 26.1                         | 27.2                 | 26.6                      | ≤30 ppm                 | ≤9 ppm*                 |
| CO <sub>2</sub> (ppm) | 562                  | 565                          | 627                  | 587                       | N/A                     | N/A                     |
| SO <sub>2</sub> (ppm) | ND                   | ND                           | ND                   | ND                        | ≤0.30 ppm               | ≤0.12 ppm               |
| <i>Weekend</i>        |                      |                              |                      |                           |                         |                         |
| O <sub>2</sub> (%)    | 15.3                 | 15.3                         | 15.3                 | 18.4                      | N/A                     | N/A                     |
| CO (ppm)              | 26.5                 | 26.0                         | 27.3                 | 26.7                      | ≤30 ppm                 | ≤9 ppm*                 |
| CO <sub>2</sub> (ppm) | 536                  | 605                          | 552                  | 564                       | N/A                     | N/A                     |
| SO <sub>2</sub> (ppm) | ND                   | ND                           | ND                   | ND                        | ≤0.30 ppm               | ≤0.12 ppm               |

CO concentrations ranged from 26.0 to 27.3 ppm across all sites and day types, as shown in Figure 3(b). While these values satisfy the 1-hour limit of 30 ppm, they substantially exceed the Thai 8-hour ambient standard of 9 ppm prescribed by the National Environmental Board. It is important to note, however, that the 8-hour TWA standard applies to a continuous 8-hour average, whereas the measurements here were conducted during three separate 2-hour peak periods. A direct comparison is therefore approximate: these peak-period concentrations substantially exceed the 8-hour standard, suggesting the 8-hour TWA may also be exceeded on heavy-traffic days, but this cannot be confirmed without continuous monitoring. All measured CO values fall within the shaded zone between the two standards, underscoring that although no acute 1-hour exceedance occurred, prolonged exposure during peak periods represents a potential chronic health concern for traffic officers and pedestrians stationed at these intersections. The elevated CO reflects the accumulation of exhaust gases during red-light idle periods at all four intersections. Site 3 recorded the highest mean CO on weekdays (27.2 ppm) and weekends (27.3 ppm). The tall buildings surrounding Site 3 impede horizontal air circulation, trapping emissions within the intersection canyon and explaining the elevated CO and CO<sub>2</sub> levels discussed below. The effect of restricted ventilation associated with adjacent tall buildings on pollutant accumulation is well documented and is consistent with the ventilation analysis in this study [13, 18]. Formal characterization of Site 3 as a street canyon (typically requiring a building height-to-road-width ratio  $H/W > 0.5$ ) was not conducted because building height and road-width data were not collected; this is acknowledged as a limitation and recommended for future study. Figure 4 illustrates the spatial and temporal variability of CO<sub>2</sub> concentrations across sites and measurement periods. As shown in panel (a), Period 1 (07:00–09:00) consistently produced the highest CO<sub>2</sub> readings at most sites on weekdays, particularly at Site 3 (671.0 ppm) and Site 4 (626.0 ppm), reflecting the accumulation of exhaust gases during the morning commuter rush before daytime heating and wind speed increases facilitate dispersion. A notable anomaly was observed at Site 2 during Weekend Period 1, which recorded the highest single-period CO<sub>2</sub> value of 711.4 ppm. This is likely attributable to a localized episode of low wind speed (0.5 m/s) combined with elevated relative humidity (86.3%) on that measurement day, suppressing pollutant dispersion in the early morning hours before temperature-driven convection became active. Panel (b) confirms that Site 3 recorded the highest overall weekday CO<sub>2</sub> average of 627.4 ppm, the maximum of all sites and day types, while Site 1 recorded the lowest weekday average of 562.1 ppm. On weekends, Site 2 produced the highest average (604.7 ppm) and Site 1 the lowest (535.7 ppm). CO<sub>2</sub> has no applicable Thai ambient air quality standard or WHO health-based outdoor exposure limit; it is used here as a tracer of vehicle exhaust accumulation rather than as a regulated pollutant. All measured CO<sub>2</sub> values considerably exceeded the global outdoor background level of approximately 420 ppm, reflecting the substantial near-road enhancement attributable to vehicular exhaust at urban signalized intersections. Environmental factors, particularly relative humidity, wind speed, and temperature, played a

consistent secondary role in modulating CO<sub>2</sub> levels. Higher relative humidity on weekdays suppressed temperature and reduced gaseous dispersion efficiency, while higher weekend wind speeds at some sites transported pollutants away from the measurement point, contributing to lower CO readings. When the temperature rose at lower humidity, CO<sub>2</sub> expanded and became lighter than ambient air, tending to rise and resulting in lower near-ground readings, a phenomenon consistent with established atmospheric chemistry principles [19]. Site 4 (Benjamarachutit) recorded the highest O<sub>2</sub> concentrations on both weekdays (17.2%) and weekends (18.4%), reflecting its more open topography and higher background wind speeds that facilitate oxygen replenishment and pollutant dilution. Conversely, CO<sub>2</sub> at Site 4 was 589.7 ppm on weekdays, lower than at Site 3, confirming its better ventilation capacity. Comparable CO<sub>2</sub> ranges of 500–700 ppm have been reported at near-road urban sites in studies from both Thai and international urban settings [20].

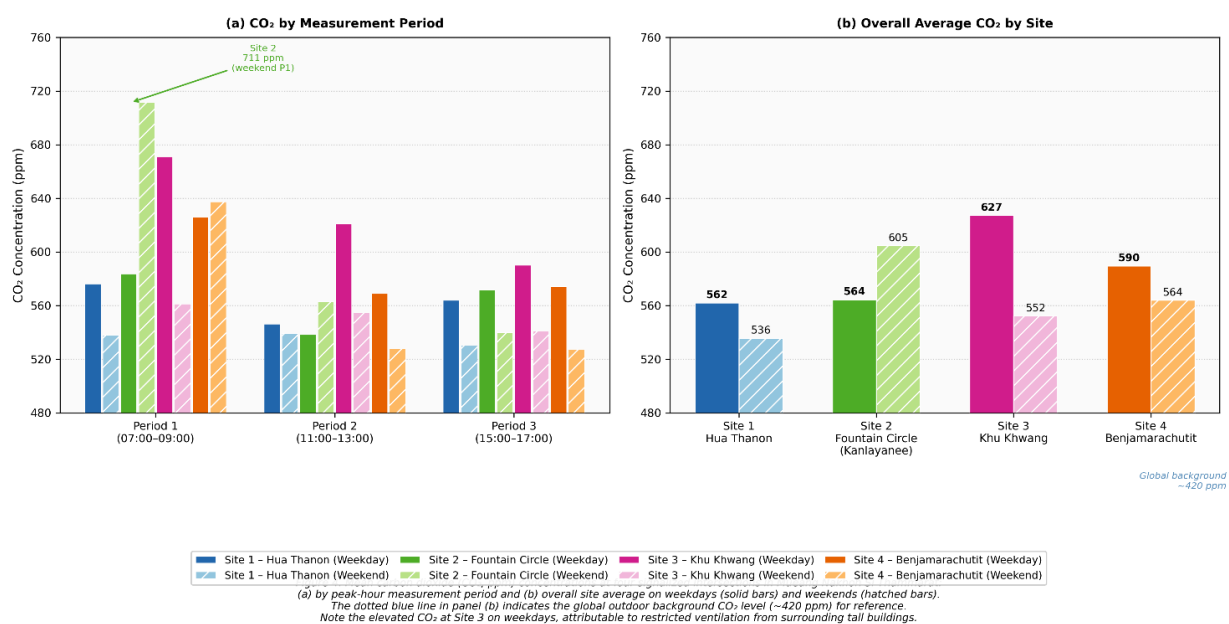


**Figure 3.** Mean concentrations of (a) oxygen (O<sub>2</sub>, %) and (b) carbon monoxide (CO, ppm) at four signalized intersections in Mueang Nakhon Si Thammarat on weekdays and weekends. The dashed line in panel (a) indicates the atmospheric reference value of 20.9% O<sub>2</sub>. The dashed lines in panel (b) indicate the Thai national 1-hour standard (30 ppm) and 8-hour standard (9 ppm) (National Environmental Board Notification No. 10, B.E. 2538, and No. 21, B.E. 2544).

### 3.3 Comparative Assessment with Previous Studies

Table 4 and Figure 5 place the present findings in the context of comparable traffic environmental studies conducted in Thailand and internationally. As shown in Table 4, noise levels at signalized intersections and near-road sites consistently exceed national or WHO-recommended standards across diverse geographic settings, confirming that traffic noise is a near-universal urban environmental challenge rather than a problem specific to any single region. Within Thailand, the present results are closely comparable to those of Suphakit et al. [14], who reported weekday Leq values of 68.5–70.2 dB(A) at four dense-traffic intersections in Kamphaeng Phet municipality, and to Ariya et al. [15], who recorded 68.7–73.0 dB(A) at signalized intersections in Khon Kaen. The most directly relevant Thai study is that of Srikaew and Tonsom [5], conducted at a commercial plaza car park in the same city as the present study, which reported an average Leq of 70.9–72.1 dB(A) and CO concentrations of 21.3–21.9 ppm. The higher CO values observed in the present study (26.0–27.3 ppm) likely reflect the more open outdoor intersection environment, where vehicles idle repeatedly at traffic signals rather than performing the shorter maneuvers typical of a parking facility. Nayan et al. [21], studying school roadsides in Malaysia, recorded Leq values of 57.7–76.1 dB(A), with all sites exceeding the Malaysian DOE standard of 60 dB(A), reinforcing the finding that proximity to educational institutions and peak school-travel hours amplifies intersection noise — a pattern clearly observed at Site 2 of

the present study on weekdays. Internationally, Gheibi [22] documented Leq values of 72.75–78.0 dB(A) along approach roads to the Imam Reza Shrine in Mashhad, Iran, with the highest levels occurring during the afternoon peak when visitor density was greatest, consistent with the present study's observation that Period 3 (15:00–17:00) noise levels at Site 4 reached 72.0 dB(A) on weekdays. Goshu and Gosa [18] reported the highest noise levels in the comparative set, with Leq values of 80.0–87.5 dB(A) in a congested Ethiopian city market, reflecting the combined effects of mixed traffic, including minibusses, three-wheelers, and amplified loudspeakers, source types absent from the present study area. Ahmed Ali et al. [20] found noise averages of 63.2–83.8 dB(A) at near-road residential sites in Fallujah, Iraq, attributing exceedances partly to irregular generator use and commercial activity beyond standard traffic, again highlighting the role of non-traffic sources in amplifying near-road noise in developing-city contexts.



**Figure 4.** Mean carbon dioxide (CO<sub>2</sub>, ppm) concentrations at four signalized intersections in Mueang Nakhon Si Thammarat across three peak-hour measurement periods on weekdays and weekends. Error bars represent the range of values within each period.

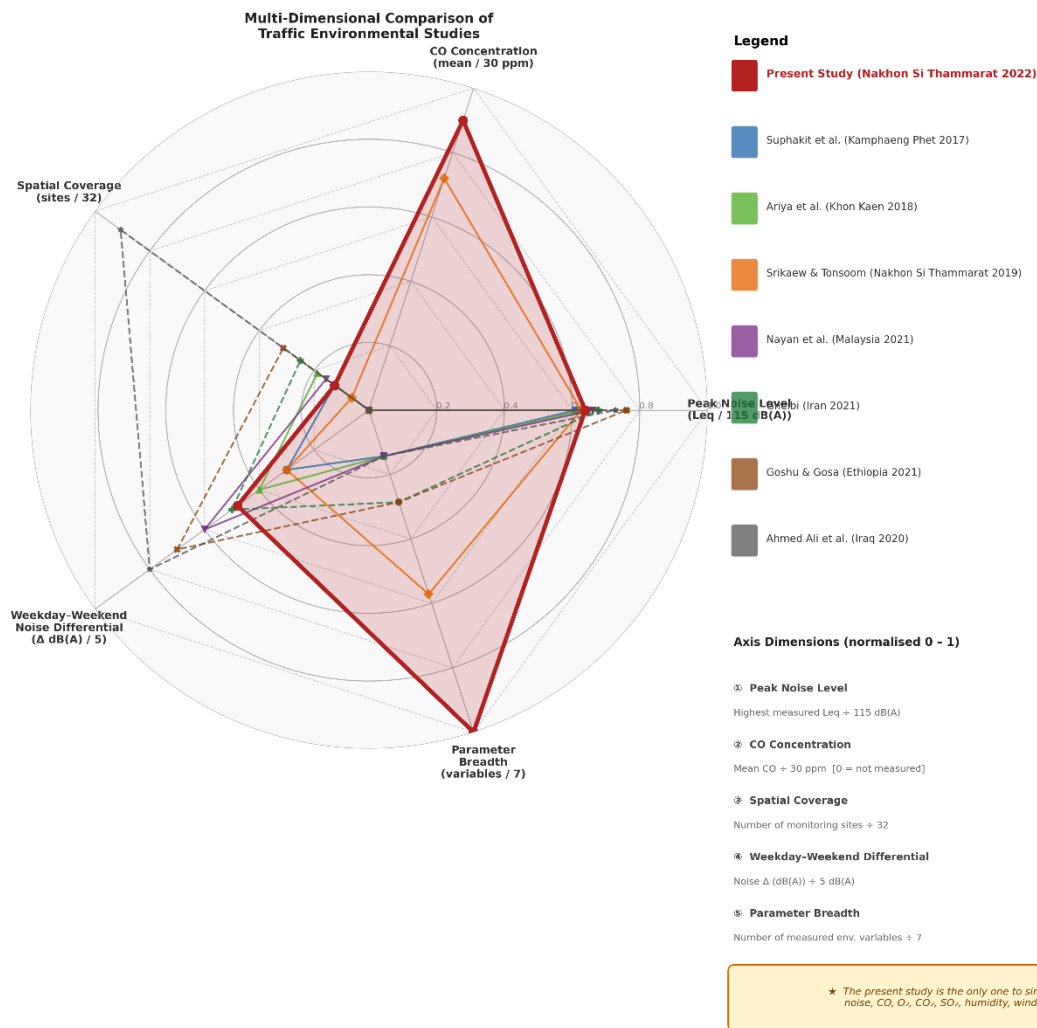
Figure 5 provides a multidimensional radar visualization comparing the present study with five selected prior studies across five performance dimensions: peak noise level relative to the standard, CO exceedance ratio, spatial coverage (number of sites), weekday–weekend noise differential, and breadth of environmental parameters measured. The radar chart highlights that, while the present study's absolute noise levels are moderate within the global comparison, lower than the Ethiopian and Iraqi studies but comparable to other Thai and Malaysian work, it is distinguished by the simultaneous characterization of four gaseous air quality parameters alongside noise and three meteorological covariates. Most prior studies in the comparison measured noise in isolation, limiting their ability to assess the compounded environmental burden experienced by road users and nearby residents. The present study's integrated approach, measuring both acoustic and chemical pollution at the same sites and times, therefore represents a methodological advance over single-parameter assessments and provides a more complete evidence base for environmental management decisions in Nakhon Si Thammarat. Taken together, the comparative evidence confirms three consistent patterns: (1) traffic noise at signalized urban intersections routinely exceeds national standards in both developed and developing countries; (2) CO concentrations at idle-heavy sites such as red-light intersections can exceed the 8-hour ambient standard even when remaining within the 1-hour limit; and (3) environmental factors, particularly wind speed, humidity, and urban morphology, are critical determinants



of both noise propagation and pollutant accumulation that must be accounted for in site-specific assessments and management strategies.

**Table 4.** Comparison of traffic-generated noise levels and CO concentrations reported in selected studies from Thailand and internationally, with findings from the present study.

| Study                        | Location                      | Site Type                | Noise Leq<br>(dB(A)) | CO<br>(ppm) | Standard Exceedance                        |
|------------------------------|-------------------------------|--------------------------|----------------------|-------------|--------------------------------------------|
| Suphakit et al. [9] (2017)   | Kamphaeng Phet, Thailand      | Urban intersections      | 68.5–70.2            | —           | Noise: borderline                          |
| Ariya et al. [10] (2018)     | Khon Kaen, Thailand           | Signalized intersections | 68.7–73.0            | —           | Noise: yes (weekday)                       |
| Srikaew & Tonsoom [5] (2019) | Nakhon Si Thammarat, Thailand | Parking plaza            | 70.9–72.1            | 21.3–21.9   | Noise: yes; CO: 8-hr yes                   |
| Nayan et al. [16] (2021)     | Muallim, Perak, Malaysia      | School roadsides         | 57.7–76.1            | —           | Noise: yes                                 |
| Gheibi [17] (2021)           | Mashhad, Iran                 | Shrine approach roads    | 72.75–78.0           | —           | Noise: yes                                 |
| Goshu & Gosa [13] (2021)     | Jimma, Ethiopia               | Market area roads        | 80.0–87.5            | —           | Noise: yes                                 |
| Ahmed Ali et al. [14] (2020) | Fallujah, Iraq                | Residential near-road    | 63.2–83.8            | —           | Noise: yes                                 |
| Present study (2022)         | Nakhon Si Thammarat, Thailand | Signalized intersections | 69.9–73.5            | 26.0–27.3   | Noise: yes (3/4 sites); CO 8-hr: yes (all) |



**Figure 5.** Multi-dimensional radar chart comparing the present study with seven selected traffic environmental studies from Thailand and internationally across five normalised axes: ① Peak Noise Level (highest measured Leq ÷ 115 dB(A)); ② CO Concentration (mean CO ÷ 30 ppm; 0 = not measured); ③ Spatial Coverage (number of monitoring sites ÷ 32); ④ Weekday-Weekend Noise Differential (Δ dB(A) ÷ 5); and ⑤ Parameter Breadth (number of measured environmental variables ÷ 7). All axes are normalized to a 0–1 scale to enable cross-study comparison. The present study (bold red, shaded polygon) is distinguished by the simultaneous measurement of noise, four gaseous air quality parameters (O<sub>2</sub>, CO, CO<sub>2</sub>, SO<sub>2</sub>), and three meteorological variables (relative humidity, wind speed, temperature), giving it the broadest parameter breadth of all studies in the comparison. Studies reporting noise only are assigned a CO axis value of zero. Data sources: [5, 9, 10, 13, 14, 16, 17].

## 4. Conclusions

This study provides the first systematic, multi-site assessment specifically targeting signalized intersections for simultaneous noise and air-quality characterization in Mueang Nakhon Si Thammarat. The principal findings are (1) Average noise levels exceeded the Thai 24-hour standard of 70 dB(A) at all four sites on weekdays, and at three of four sites on weekends. Site 1 (Hua Thanon) was the loudest on both day types due to road gradient effects that increase engine load. (2) Peak-period CO concentrations (26.0–27.3 ppm) substantially exceeded the Thai 8-hour ambient standard of 9 ppm at all sites, while remaining within the 1-

hour limit of 30 ppm. Because measurements covered only 2-hour peak windows rather than a continuous 8-hour period, whether the 8-hour TWA standard is formally exceeded must be verified through continuous monitoring. SO<sub>2</sub> was undetectable. O<sub>2</sub> readings were below the atmospheric reference at every site but are subject to instrument calibration uncertainty and should be interpreted with caution. (3) Site 3 (Khu Khwang) recorded the highest CO and CO<sub>2</sub>, attributable to restricted ventilation from adjacent tall buildings; formal street-canyon characterization (H/W ratio) was not conducted and is recommended for future study. Site 4 (Benjaminachutit) exhibited the best air quality, consistent with its more open topography. (4) Environmental factors—particularly relative humidity, wind speed, and temperature—significantly modulated both noise propagation and pollutant dispersion across sites and day types. These results highlight potential health risks for traffic officers and roadside workers with prolonged exposure at these intersections. It should be noted that N95 respirators are designed to filter particulate matter and are not effective against gaseous CO; appropriate personal protective equipment and health monitoring for workers should be determined through formal occupational health risk assessments that account for actual 8-hour TWA exposures. The relevant authorities are encouraged to implement continuous environmental monitoring, explore traffic management interventions such as signal re-timing and vehicle idling restrictions, and commission follow-up health impact assessments. Several important limitations of this study should be acknowledged. First, all data were collected in September 2022 (wet season only); seasonal variation in wind speed, humidity, temperature, and traffic patterns in southern Thailand means that dry-season and year-round monitoring is essential before broader conclusions about chronic exposure can be drawn. Second, traffic volume and vehicle-type composition data were not collected during measurement periods, limiting the ability to separate the effects of traffic volume from site morphology; future studies should incorporate concurrent traffic counts. Third, NO<sub>2</sub> and PM<sub>2.5</sub>—two of the most health-relevant traffic pollutants with established Thai and WHO ambient standards—were not measured, and their absence is a significant methodological gap that future work must address. Fourth, the qualitative assessment of meteorological influences and the absence of formal statistical tests for weekday–weekend and inter-site comparisons are acknowledged as analytical limitations. Future research should extend measurements to 24 hours, apply continuous monitoring to verify 8-hour TWA exceedances, include PM<sub>2.5</sub> and NO<sub>2</sub>, conduct formal correlation and regression analysis, and quantify the health burden attributable to chronic noise and CO exposure at these intersections.

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