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# Feasibility Study of Automated Brackish Water Fish Pond Systems: Integrating IoT Sensor Networks and Generative Artificial Intelligence for Sustainable Aquaculture in Coastal Communities

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**Abstract:** Brackish water aquaculture remains one of the most economically significant food-production sectors in Southeast Asia, yet productivity is persistently constrained by inefficient monitoring, reactive management, and limited decision-support infrastructure. This feasibility study investigates the technical, economic, and operational viability of deploying a fully automated, sensor-integrated, and generative AI (GenAI)-augmented management system in brackish water fish ponds, with emphasis on milkfish (*Chanos chanos*) and Nile tilapia (*Oreochromis niloticus*) culture in coastal Philippine settings. The proposed system -- designated AutoPond-BW -- integrates eight categories of IoT-enabled sensors connected through an MQTT-based data pipeline to a cloud analytics platform. Sensor outputs feed a retrieval-augmented generation (RAG)-powered large language model (LLM) advisory engine that generates real-time, context-aware management recommendations in English, Filipino, and Cebuano. A techno-economic analysis comparing automated versus manual pond systems was conducted across three production scenarios: small-scale (0.5-1.0 ha), medium-scale (1.0-5.0 ha), and commercial-scale (>5.0 ha). Based on a literature synthesis and financial modeling, automated systems are projected to yield a benefit-cost ratio (BCR) of 1.45-1.65, compared with 1.15-1.25 for manual systems, with projected net annual profit increases of 200-330% over a five-year horizon. Despite higher initial capital requirements (PHP 680,000-850,000/ha), automated systems achieve full return on investment within three to five years. Expert panel validation (n=12, using a 25-item Likert-type questionnaire across five operational dimensions) confirmed the system's adequacy, with an overall mean rating of 4.28/5.00. This study provides evidence-based recommendations for government agencies, aquaculture investors, and coastal farming communities as they consider transitioning to smart, automated aquaculture operations.

**Keywords:** Brackish water aquaculture; IoT sensors; generative artificial intelligence; smart aquaculture; water quality monitoring

## 1. Introduction

Global aquaculture production surpassed 90 million tonnes in 2020, accounting for over 50% of the total fish supply for human consumption [1]. Within this expanding sector, brackish water pond culture, defined as the cultivation of aquatic species in water with salinity between 0.5 and 30 parts per thousand (ppt), plays an outsized role in the livelihoods of millions of small-scale coastal farmers across South and Southeast Asia [2, 3]. The Philippines, with its 36,289 kilometers of coastline and approximately 234,000 hectares of brackish-water fishponds, is among the world's leading producers of milkfish (*Chanos chanos*), locally known as bangus—the country's national fish [4]. Despite decades of development, the Philippine aquaculture sector remains predominantly reliant on labor-intensive, manually managed practices. Farm operators conduct periodic visual inspections and manual water quality testing at 24 to 72-hour intervals, resulting in delayed responses to critical water quality deviations, suboptimal feeding regimens, and heightened susceptibility [5, 6]. These inefficiencies are compounded by the growing impacts of climate change, including increased storm frequency, elevated sea surface temperatures, and greater salinity fluctuation in coastal zones [7-9].

The convergence of the Internet of Things (IoT), cloud computing, and artificial intelligence has created unprecedented opportunities to transform pond aquaculture into a precision-managed, data-driven enterprise [10]. Sensor networks can continuously monitor dissolved oxygen (DO), pH, temperature, salinity, turbidity, and nitrogenous compounds in real time, enabling proactive rather than reactive management [11, 12]. More recently, the emergence of generative AI and large language models (LLMs) such as GPT-4 has opened new avenues for translating raw sensor data into actionable, natural-language farm management recommendations accessible to even operators with limited technical training [13, 14]. However, evidence-based feasibility assessments that comprehensively evaluate the technical requirements, economic returns, and operational practicalities of deploying integrated sensor-GenAI systems in brackish-water fish ponds remain scarce [15].

This study investigates the feasibility of the AutoPond-BW system, addressing four specific objectives: (1) to design a conceptual architecture for an automated brackish water fish pond system integrating multi-parameter IoT sensor arrays and generative AI advisory modules; (2) to evaluate the technical specifications and deployment requirements of sensor and AI components under Philippine tropical coastal conditions; (3) to conduct a techno-economic feasibility analysis comparing automated and manual pond management systems across three production scales; and (4) to assess the potential impacts of generative AI integration on production efficiency, disease management, and farmer decision-making.

Brackish water fishponds occupy the ecological interface between freshwater and marine environments, characterized by fluctuating salinity, tidal influence, and high organic loading [16]. In the Philippines, milkfish aquaculture in brackish-water ponds has been practiced for centuries, with modernization efforts beginning in earnest in the 1970s [17]. Despite this long history, yields remain well below potential: average production of 1,500-3,000 kg/ha/yr in traditional extensive systems contrasts sharply with the 6,000-10,000 kg/ha/yr [18-20]. The primary physicochemical challenges include diurnal DO fluctuations driven by algal photosynthesis and respiration, salinity pulses from tidal exchange or rainfall, and accumulation of toxic ammonia and nitrite from organic waste decomposition [21, 22]. These dynamics require vigilance that manual inspection regimes cannot provide at sufficient temporal resolution [23].

The application of IoT sensor networks to aquaculture water-quality monitoring has expanded rapidly since 2015, driven by declining sensor costs, improved wireless communication protocols, and greater accessibility to cloud computing [10,24]. Dissolved oxygen remains the most critical parameter in intensive pond culture, as concentrations below 3.0 mg/L trigger stress responses and mass mortality events [25]. Electrochemical and optical DO sensors with sampling frequencies of five minutes or less provide early warning of hypoxic events [26, 27]. Fu et al. demonstrated that multi-sensor fusion using attention-based LSTM models can predict DO concentration up to six hours in advance with RMSE values below 0.3 mg/L [28]. Salinity, turbidity, ammonia-nitrogen, and nitrite/nitrate sensors provide comprehensive environmental characterization essential for informed management decisions [11, 21]. A systematic review of multi-sensor IoT systems found that ESP32 and Raspberry Pi are the most common single-board computing platforms for

aquaculture deployments [29]. Computer vision systems further enable behavioral monitoring, with deep learning models detecting abnormal swimming patterns and surface crowding indicative of hypoxia or pathogen exposure [30,31].

Generative artificial intelligence, particularly large language models, represents a transformative development in agricultural and aquaculture advisory systems [32]. Unlike traditional discriminative AI, generative AI models produce coherent natural-language responses that synthesize complex multivariate inputs into actionable recommendations comprehensible to non-expert users [13]. Kim et al. conducted a pilot study evaluating ChatGPT-based decision support for aquaculture farm management, finding that LLM-generated recommendations aligned with expert agronomic judgment in 78% of test scenarios [14]. Liu et al. demonstrated that GPT-4, augmented with a RAG pipeline connecting to aquaculture knowledge bases, could generate feeding schedules, water-quality intervention plans, and disease diagnostics with accuracy comparable to that of experienced farm consultants [13]. Cai et al. provided a comprehensive review of GenAI applications in precision aquaculture, identifying disease prediction, behavioral analysis, and environmental modeling as the highest-value application domains [33]. Generative adversarial networks have also shown promise for diagnosing fish disease from skin lesion photographs [34].

Several published techno-economic analyses support the long-term financial viability of automated aquaculture systems, though initial capital requirements present adoption barriers for small-scale farmers [35,36]. Gonzalez-Espinosa and Mendez-Ramirez demonstrated that automated aeration systems in tropical brackish-water ponds yielded IRRs of 22-28% over a 10-year horizon, accounting for feed savings and mortality reductions [37]. Musa et al. found that integrated smart aquaculture systems in Malaysian brackish water farms reduced labor costs by 65-70% while increasing production volumes by 35-50% [15]. Lagarda-Dieguez et al. reported benefit-cost ratios of 1.35-1.75 [38, 39]. The emerging literature on GenAI advisory systems suggests additional productivity gains of 10-20% attributable to improved decision-making quality and reduced reaction latency to environmental stressors [13, 14, 32].

## 2. Materials and Methods

### 2.1 Research Design

This study employs a descriptive-evaluative research design incorporating technical system design analysis, expert panel validation, and economic feasibility modeling. The research follows a three-phase approach: (1) system architecture design and sensor specification; (2) generative AI integration design and operational workflow modeling; and (3) techno-economic analysis using financial projection models calibrated to Philippine coastal aquaculture conditions. Data sources include peer-reviewed literature (2000-2024), manufacturer technical datasheets, current Philippine market price databases (BFAR Fisheries Price Monitoring System, 2024), and structured interviews with five aquaculture engineers and seven experienced fish pond operators conducted between June and September 2024.

### 2.2 Study Area and Target Beneficiaries

The study focuses on brackish-water fishpond systems along the eastern and western coastal zones of Cebu province, Central Visayas, Philippines (Region VII). Cebu has approximately 3,800 hectares of registered brackish water fishpond areas, with concentrations in the municipalities of Pilar, Poro, San Francisco (Camotes Island), Bogon, and Tabogon. Target beneficiaries include small-scale operators managing 0.5-1.0 ha, medium-scale operators with 1.0-5.0 ha, and cooperative-managed commercial ponds exceeding 5.0 ha. The geographic focus on Cebu was chosen for its representativeness of Central Visayas aquaculture conditions [40], its existing fishpond registry data managed by BFAR Region 7, and LTE network coverage extending to 85% of registered fishpond areas as of the 2023 DICT national connectivity assessment.

### 2.3 System Architecture Design

The proposed AutoPond-BW system is organized into four integrated architectural layers following an edge-to-cloud computing paradigm: (1) the physical sensor layer; (2) the data acquisition and communication layer; (3) the cloud analytics and AI layer; and (4) the user interface and decision-support layer. Pre-processed sensor data streams from pond-side edge computing nodes are forwarded to a central

cloud platform via LTE/4G cellular or LoRaWAN communication links. The physical layer consists of multi-parameter sensor buoys deployed at a minimum density of one buoy per 0.5 hectares. Sensor nodes are powered by solar-charged lithium battery systems rated for 72-hour autonomous operation. Data is aggregated by a local microcontroller unit running on an ESP32 or Raspberry Pi 4 platform [29], performing edge-level data validation, anomaly flagging, and compression before transmission to the cloud.

## 2.4 IoT Sensor Specifications

A systematic review informed the selection of sensor types from peer-reviewed aquaculture sensor literature, which was validated against water quality parameter thresholds established by BFAR and the Aquaculture Certification Council of the Philippines [21,41]. Table 1 presents the complete sensor specification matrix adopted in the AutoPond-BW system design.

**Table 1.** IoT Sensor Specifications for the AutoPond-BW Automated Brackish Water Pond System

| Sensor Type             | Parameter Monitored                  | Measurement Range   | Sampling Frequency |
|-------------------------|--------------------------------------|---------------------|--------------------|
| Dissolved Oxygen (DO)   | O <sub>2</sub> concentration (mg/L)  | 0-20 mg/L (+/-0.1)  | Every 5 min        |
| pH Sensor               | Acidity / Alkalinity                 | pH 0-14 (+/-0.02)   | Every 10 min       |
| Salinity / Conductivity | Salt concentration (ppt)             | 0-40 ppt (+/-0.1)   | Every 10 min       |
| Temperature Sensor      | Water temperature (C)                | 0-50 C (+/-0.1)     | Every 5 min        |
| Turbidity / TSS         | Total suspended solids (NTU)         | 0-1000 NTU (+/-2%)  | Every 15 min       |
| Ammonia-Nitrogen        | NH <sub>3</sub> concentration (mg/L) | 0-10 mg/L (+/-0.05) | Every 30 min       |
| Nitrite / Nitrate       | N-compounds (mg/L)                   | 0-50 mg/L (+/-0.1)  | Every 30 min       |
| Water Level / Depth     | Pond depth and tidal flow (m)        | 0-5 m (+/-0.01)     | Every 30 min       |

Source: Compiled from manufacturer datasheets and Al-Azad et al. [12]; Islam et al. [11]; Chen et al. [42].

Each sensor node is equipped with automatic self-calibration routines executed every 24 hours. Data transmission follows the MQTT (Message Queuing Telemetry Transport) protocol at Quality-of-Service Level 1, over TLS 1.3-encrypted connections to the cloud MQTT broker [12]. Anti-fouling wiper mechanisms on optical sensor surfaces cycle every 6 hours to address biofouling—the most significant cause of sensor performance degradation in tropical aquaculture environments [26]. Table 2 summarizes the water quality threshold values for the primary culture species.

**Table 2.** Water Quality Threshold Values for Milkfish and Tilapia Culture in Brackish Water Ponds

| Parameter               | Optimal Range | Critical Low | Critical High |
|-------------------------|---------------|--------------|---------------|
| Dissolved oxygen (mg/L) | 5.0-8.0       | < 3.0        | > 12.0        |
| pH                      | 7.5-8.5       | < 6.5        | > 9.5         |
| Salinity (ppt)          | 10-25         | < 5          | > 35          |
| Temperature (C)         | 26-32         | < 20         | > 36          |
| Ammonia-N (mg/L)        | < 0.5         | --           | > 2.0         |
| Turbidity (NTU)         | 20-40         | < 10         | > 80          |

Source: Boyd et al. [21]; BFAR Technical Bulletin No. 14-2020 [41].

## 2.5 Generative AI Integration Framework

The generative AI component of the AutoPond-BW system is designed around a retrieval-augmented generation (RAG) architecture, wherein a domain-specific aquaculture knowledge base -- comprising FAO technical guidelines, BFAR management bulletins, peer-reviewed culture protocols for milkfish and tilapia, and historical farm performance records -- is indexed and made retrievable by an LLM at inference time [13].



This RAG architecture ensures that AI-generated recommendations are grounded in authoritative, domain-validated information rather than relying solely on the model's general pre-training knowledge [33]. The LLM advisory engine interfaces with the cloud analytics platform via a structured prompt-generation pipeline that automatically assembles context packets from incoming sensor data, historical baselines, alert flags, pond-specific production metadata, and weather forecast data from PAGASA, along with retrieved passages from the aquaculture knowledge base ranked by cosine similarity of their embeddings. This enriched context is submitted to the LLM with a structured system prompt instructing it to act as a senior aquaculture farm advisor, identify anomalies or risks, and generate prioritized corrective action recommendations in the operator's preferred language. Table 3 summarizes the five functional GenAI modules integrated within the AutoPond-BW system.

**Table 3.** Generative AI Modules Integrated in the AutoPond-BW System

| GenAI Module               | Function                                                                                       | Technology Stack                                                                   |
|----------------------------|------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| Water Quality Advisory     | Interprets sensor anomalies; generates plain-language alerts and corrective action plans       | GPT-4 API with RAG on aquaculture knowledge base; MQTT-triggered inference [12,13] |
| Predictive Feed Management | Generates daily feed schedules based on fish growth projections, temperature, and DO levels    | Fine-tuned LLM + time-series sensor embeddings; FCR optimization model [43]        |
| Disease Risk Narration     | Produces diagnostic narratives and early-warning reports from DO and ammonia trend analysis    | LLM + multimodal computer-vision disease imaging pipeline [33,34]                  |
| Farmer Decision Support    | Answers natural-language queries in Filipino and Cebuano; recommends corrective interventions. | Multilingual LLM with domain fine-tuning; code-switched prompt engineering [32]    |
| Harvest Optimization       | Recommends harvest timing, stocking density adjustments, and batch rotation strategy           | LLM + regression biomass growth model; historical yield pattern analysis [13,14]   |

References: [13], [14], [32], [33], [43].

The multilingual farmer decision-support module is a context-specific innovation for the Philippine setting, where a 2024 BFAR Region 7 survey found that 62% of fish pond operators rated language accessibility as a primary barrier to adopting digital farm management tools. The module employs multilingual LLM fine-tuning combined with code-switched prompt engineering that instructs the model to respond in the operator's selected interface language [32]. The predictive feed management module integrates sensor outputs with bioenergetic fish growth models calibrated for milkfish and tilapia at Philippine ambient temperature ranges (26-32 degrees Celsius), generating daily feed ration schedules adjusted for water temperature, DO level (feeding suppression triggered below 4.0 mg/L), and time since last feeding event [43,44]. The disease risk narration module processes multi-parameter sensor trend data to identify early-warning signatures associated with the three most economically significant bacterial pathogens in Philippine brackish water aquaculture. When composite risk scores exceed configurable thresholds, the module generates structured diagnostic narratives that include probable causative agents, deviating parameter trends, and recommended immediate interventions [15]. A pond-side camera system feeds an image classification pipeline trained on annotated underwater footage to detect behavioral anomalies that can precede detectable parameter deviations by one to three hours [30,31].

## 2.6 Data Communication and Cloud Platform Architecture

Sensor data is transmitted to a cloud analytics platform hosted on Amazon Web Services (AWS) or equivalent Philippine government-accredited cloud infrastructure compliant with the Data Privacy Act of 2012 (Republic Act 10173). The platform consists of an AWS IoT Core broker for MQTT message routing, an InfluxDB time-series database for sensor data storage with a 24-month rolling retention window, an Apache

Kafka stream-processing engine for real-time, threshold-based anomaly detection, and a REST API gateway that exposes processed data and GenAI advisory outputs to mobile and web user interfaces. Data security employs AES-256 encryption at rest and TLS 1.3 encryption in transit, with role-based access control (RBAC) governing data access across user roles. Local edge nodes buffer up to seven days of sensor data in onboard flash storage to ensure data continuity during cellular connectivity interruptions [12,45].

## 2.7 Techno-Economic Analysis Framework

Economic feasibility was assessed using four standard financial indicators: Net Present Value (NPV), Internal Rate of Return (IRR), Benefit-Cost Ratio (BCR), and Payback Period (PBP). Financial projections were modeled over a 10-year analytical horizon, with a 12% discount rate, consistent with Bangko Sentral ng Pilipinas benchmark lending rates for agri-fishery loans in 2024. Three production scale scenarios were modeled: small-scale (0.5-1.0 ha), medium-scale (1.0-5.0 ha), and commercial-scale (>5.0 ha). Key model assumptions are as follows: stocking density of 5,000-8,000 milkfish fingerlings/ha for automated systems versus 3,000-5,000/ha for manual systems; production cycle length of six months with 1.5-2.0 cycles/year; assumed survival rate of 82-90% (automated) versus 70-80% (manual); yield improvement of 35-60% derived from Gonzalez-Espinosa and Mendez-Ramirez [37] and Musa et al. [15]; labour cost reduction of 65-75% based on Musa et al. [15]; electrochemical sensor hardware lifespan of three years and optical sensor lifespan of five years; and LLM API inference costs (GPT-4-turbo) estimated at PHP 12,000-18,000/ha/year under operational usage, now included in the OPEX model. For farm sites without LTE coverage (approximately 15% of registered fishpond areas), an additional conditional CAPEX of PHP 35,000-55,000 per LoRaWAN gateway is required. Full itemized CAPEX and OPEX assumptions are provided in Supplementary Table S2. Revenue projections were based on current milkfish farmgate prices of PHP 85-95/kg and tilapia prices of PHP 60-70/kg, with production volumes estimated from published yield data for conventional versus automated management [18,37]. A sensitivity analysis varied farmgate price (+/-15%), initial CAPEX (+/-20%), and production yield improvement (+20%, +35%, +50%) attributable to automation. Monte Carlo simulation with 10,000 iterations was applied to generate probability distributions for NPV and IRR under parametric uncertainty.

## 3. Results and Discussion

### 3.1 System Architecture Validation

Expert panel validation of the proposed AutoPond-BW system architecture (n = 12: five aquaculture engineers and seven experienced fish pond operators) yielded an overall mean system adequacy rating of 4.28/5.00 (SD = 0.45) on a 25-item, validated 5-point Likert-type instrument (1 = Strongly Disagree to 5 = Strongly Agree). The instrument comprised five items per assessed dimension, each operationalizing one technical, operational, or contextual criterion. Items were reviewed by three aquaculture engineers before administration, and consensus was assessed using a pre-specified acceptability threshold of 3.5 per dimension. The full instrument is provided as Supplementary File S1. All five assessed dimensions exceeded the pre-specified acceptability threshold of 3.5, confirming adequate expert-rated validity across technical, operational, and contextual dimensions. Panel experts confirmed that the eight-parameter sensor array covers all critical water-quality variables for milkfish and tilapia culture, with particular endorsement of ammonia-nitrogen and salinity sensors for addressing previously unmonitored but economically critical parameters in small-scale Philippine operations [21]. The MQTT-over-LTE communication architecture was validated as appropriate for coastal Philippine deployment, where LTE coverage extends to more than 85% of registered fishpond areas as per 2023 DICT national coverage data. Table 4 presents the full summary statistics for expert validation.

**Table 4.** Expert Panel Validation Summary Statistics (n = 12)

| Validation Dimension                            | Mean Score (/5.00) | SD   |
|-------------------------------------------------|--------------------|------|
| Technical Completeness of Sensor Array          | 4.4                | 0.38 |
| Data Communication Architecture Appropriateness | 4.1                | 0.47 |
| GenAI Module Functional Relevance               | 4.3                | 0.52 |
| Economic Model Reasonableness                   | 4.0                | 0.61 |
| Applicability to Philippine Coastal Aquaculture | 4.6                | 0.29 |
| Overall Mean                                    | 4.28               | 0.45 |

### 3.3 Generative AI Advisory System Performance: Literature-Based Projections

It is important to note that the AutoPond-BW system has not yet been deployed or empirically tested. The following performance estimates are extrapolations from published benchmarks of comparable systems, presented as projected outcomes under analogous conditions. Based on published performance benchmarks of LLM advisory systems in comparable agricultural and aquaculture contexts, the five GenAI modules integrated in AutoPond-BW are projected to deliver the following measurable impacts: (1) a 15-25% reduction in feed conversion ratio attributable to AI-optimized feeding schedules [43]; (2) a 40-60% reduction in response time to water quality emergency events through proactive, sensor-triggered alert generation [25]; (3) an estimated 20-30% reduction in disease-related mortality through early-warning risk narration and behavioral anomaly detection [30]; and (4) an 18-22% improvement in harvest weight per stocking cycle through AI-guided harvest timing optimization [13]. These projections are consistent with the range reported by Kim et al. for ChatGPT-based aquaculture decision support and by Sharma et al. in their review of GenAI farm advisory systems [14,32]. The multilingual advisory capability is identified as a critical enabler of adoption. The Cebuano-language interface was evaluated in a focus group of eight operators, who rated comprehension accuracy at 4.6/5.0 and reported that AI-generated recommendations in Cebuano were significantly more actionable than English-language technical bulletins. The RAG-augmented knowledge grounding further ensures that LLM recommendations remain anchored in validated aquaculture science, addressing a recognized risk posed by generative AI systems deployed without domain-specific retrieval augmentation [33].

### 3.4 Techno-Economic Feasibility Analysis

Table 5 presents comparative financial performance indicators for manual versus automated brackish-water fish pond management systems under base-case assumptions.

**Table 5.** Comparative Financial Analysis: Manual vs. Automated Pond Management Systems (Per Hectare, 10-Year Horizon)

| Cost / Revenue Component            | Manual System (PHP) | Automated System (PHP) |
|-------------------------------------|---------------------|------------------------|
| Initial Capital Investment (per ha) | 180,000-220,000     | 680,000-850,000        |
| Annual Labor Cost                   | 120,000-150,000     | 35,000-50,000          |
| Annual Feed Cost                    | 280,000-320,000     | 210,000-240,000        |
| Annual Maintenance                  | 15,000-20,000       | 40,000-60,000          |
| Aeration and Energy                 | 25,000-35,000       | 55,000-70,000          |
| Annual Gross Revenue (est.)         | 450,000-500,000     | 720,000-850,000        |
| Net Annual Profit (est.)            | 60,000-110,000      | 340,000-475,000        |
| Payback Period (years)              | 1-2                 | 3-5                    |
| IRR (%)                             | 28-35               | 22-30                  |
| Benefit-Cost Ratio (BCR)            | 1.15-1.25           | 1.45-1.65              |

Note: All figures in Philippine Peso (PHP). Projections assume milkfish culture at current farmgate prices (PHP 85-95/kg), a 12% discount rate, and a 10-year analytical horizon. Revenue and profit figures represent annual estimates under steady-state post-investment-recovery operations.

A partial automation scenario (dissolved oxygen and temperature sensors only; estimated CAPEX PHP 120,000-180,000/ha) is projected to yield a BCR of 1.28-1.40, approximately 15-20% yield improvement, and 30-40% labour cost reduction [37], and represents a more financially accessible entry point for small-scale

operators. It is noted that the revenue differential between manual and automated systems is primarily driven by projected yield improvements (35-60%), which are literature-derived extrapolations and not measured outcomes of the AutoPond-BW system itself. The financial analysis confirms that automated systems entail substantially higher initial capital investment, with CAPEX of PHP 680,000-850,000 per hectare compared to PHP 180,000-220,000 for conventional manual setups -- a 3.5 to 4x capital differential. However, automated systems yield superior financial returns over the medium to long term through three primary channels: (1) higher production volumes (35-60% yield increase); (2) substantially reduced labor costs (65-75% reduction); and (3) lower feed costs through FCR optimization (25-30% reduction) [15,37,43]. The BCR of 1.45-1.65 for automated systems versus 1.15-1.25 for manual systems indicates that every peso invested in automated infrastructure returns PHP 1.45-1.65 in economic benefits—a 25-30% improvement in capital productivity. The payback period of 3-5 years falls within the acceptable range for agri-fishery capital investments under Philippine lending institution criteria [35]. Sensitivity analysis reveals that IRR is most sensitive to improvements in production yield, with a 50% yield increase scenario yielding an IRR of 31-36%, sufficient to attract private capital without government subsidy. Under pessimistic scenarios that incorporate a 20% CAPEX overrun, a 15% farmgate price reduction, and only a 20% yield improvement, IRR remains positive at 14-18%, confirming the fundamental economic viability even under adverse conditions. Monte Carlo simulation (10,000 iterations, Latin Hypercube sampling across nine uncertain parameters) yields the following IRR percentile distributions at medium production scale: under base-case assumptions, the median (P50) IRR is 26.0% (P25 = 19.8%; P75 = 31.7%; P5 = 14.2%; P95 = 38.5%), with a 78% probability of positive NPV. Under the optimistic scenario, the median IRR rises to 33.1% (P25 = 27.4%; P75 = 39.2%), with a 91% probability of positive NPV. Under the pessimistic scenario, the median IRR is 16.0% (P25 = 12.1%; P75 = 20.5%), with a 68% probability of positive NPV. Full IRR percentile distributions across all three production scales (small, medium, commercial) and all three scenarios (pessimistic, base-case, optimistic) are provided in Supplementary Table S1.

### 3.5 Adoption Barriers and Enabling Conditions

Expert interviews identified capital access as the most significant adoption barrier, as most brackish water fish pond operators in the Philippines do not qualify for conventional commercial bank lending [46]. Technical literacy requirements represent a secondary barrier, substantially mitigated by the GenAI-powered multilingual advisory interface that abstracts sensor data into plain-language recommendations. Infrastructure dependencies -- specifically, consistent cellular connectivity and reliable grid electricity -- are addressed through solar-powered autonomous operation (72-hour battery backup) and edge-level data buffering (seven days of local sensor data storage pending connectivity restoration) [12,42]. Areas with persistent LTE dead zones may require investment in a LoRaWAN gateway, which will incur additional costs [24].

### 3.6 Limitations

This study has several important limitations. First, the AutoPond-BW system is a conceptual design that has not been built or field-tested; all performance figures are projections from published benchmarks of comparable systems, not measured outcomes. Second, the expert validation instrument was not independently psychometrically validated, and the n=12 convenience sample limits generalisability. Third, financial projections rely on yield improvement (35-60%) and labor cost reduction (65-75%) assumptions drawn from studies in different contexts, which require verification through longitudinal field trials. Fourth, LLM API inference costs (e.g., GPT-4 commercial pricing) are not included in the cost model and could be substantial at operational scale. Fifth, the 72-hour solar backup and LTE connectivity assumptions may not hold in typhoon-prone coastal Cebu. Sixth, the Cebuano language evaluation (n=8) does not constitute a rigorous multilingual NLP benchmark; a linguist-reviewed evaluation with a larger sample is required. Future research should prioritize longitudinal field trials, benchmarked RAG-LLM accuracy testing, and comprehensive socioeconomic impact assessments before large-scale deployment.

## 4. Conclusions

This feasibility study established that the integration of IoT sensor arrays and generative AI advisory modules into brackish-water fish pond systems is technically viable, economically feasible at medium to



commercial production scales, and operationally appropriate for the Philippine coastal aquaculture context under enabling infrastructure and financing conditions. The AutoPond-BW system is projected to provide a comprehensive technological solution to the chronic management inefficiencies that constrain productivity in traditional brackish-water pond culture. Expert panel validation confirmed system adequacy across all assessed dimensions (overall mean: 4.28/5.00), and literature-based GenAI performance benchmarks support projected improvements of 15-25% in FCR, 20-30% in disease-related mortality reduction, and 18-22% in harvest weight optimization. Financial modeling demonstrates BCRs of 1.45-1.65 and net annual profit margins 200-330% higher than manual systems after capital recovery, with payback periods of 3-5 years under base-case assumptions. At a small scale (0.5-1.0 ha), a phased adoption pathway, beginning with dissolved oxygen and temperature monitoring before progressing to full GenAI integration, may be more financially accessible for resource-constrained operators. At medium to commercial scales, the economic case for full automation is compelling and could meaningfully contribute to the Philippine government's aquaculture production targets under the Philippine Development Plan 2023-2028. Future research should prioritize longitudinal field trials, comparative analysis of LLM advisory accuracy across additional brackish-water species, and a socioeconomic impact assessment of smart aquaculture adoption on coastal household income distribution.

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# Challenges and Opportunities in Controlled Cultivation of Vetiver: The Role of Endophytic Microorganisms in Essential Oil Biosynthesis

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**Abstract:** Vetiver (*Chrysopogon zizanioides* (L.) Roberty) is an aromatic grass widely applied in environmental engineering and as a source of essential oil for the fragrance industry. However, essential oil production under intensified cultivation systems remains constrained by inconsistent yield and chemical composition, particularly in soilless and controlled environments. This review evaluates the role of root-associated endophytic microorganisms as a critical but under-integrated biological factor influencing vetiver essential oil biosynthesis. A structured review of peer-reviewed literature indexed in Scopus, Web of Science, and Google Scholar was conducted, focusing on cultivation systems, essential oil chemistry, plant-microbe interactions, and comparable mechanisms in other aromatic crops. Evidence was analyzed using a comparative, mechanism-based framework emphasizing biochemical performance rather than descriptive system comparison. The reviewed studies indicate that soil-based cultivation consistently produces higher root biomass, greater essential oil yields, and more chemically complex sesquiterpene profiles than *in vitro*, hydroponic, or aeroponic systems. In contrast, microbiome-depleted plants exhibit reduced oil accumulation and reduced levels of oxygenated sesquiterpenes, while reinoculation with native root endophytes partially restores aroma characteristics. These findings indicate that limitations in soilless vetiver production stem from biological simplification and engineering constraints. This review proposes microbiome-integrated cultivation and targeted endophyte reinoculation as feasible strategies to improve yield stability, chemical fidelity, and land-use efficiency in controlled production systems.

**Keywords:** Vetiver; essential oil biosynthesis; endophytes; plant-microbe interactions; secondary metabolites

## 1. Introduction

Vetiver (*Chrysopogon zizanioides* (L.) Roberty) is a multifunctional perennial grass situated at the intersection of the global fragrance industry, sustainable agriculture, and environmental engineering. Native to the Indian subcontinent and now widely cultivated throughout tropical Asia, Africa, and the Americas, vetiver is primarily valued for its essential oil, which serves as an irreplaceable natural fixative widely used in fine perfumery [1]. The global vetiver oil industry has demonstrated sustained growth, with recent estimates placing annual production at approximately 250 metric tons and total market



value approaching USD 800 million by the mid-2020s [2]. Vetiver oil production remains geographically concentrated, with major output originating from Haiti, Indonesia (Java), China, India, and Brazil [1, 2]. Within the ASEAN region, which accounts for approximately 40–45 % of global vetiver oil production, cultivation has historically emphasized ecological and engineering applications—particularly erosion control, watershed stabilization, and phytoremediation—rather than optimization for essential oil yield or aromatic quality [2–4]. This orientation is reflected in national development programs in countries such as Indonesia and Thailand, where vetiver has been widely deployed as a bioengineering solution for land rehabilitation and infrastructure protection [3, 4]. In contrast, efforts to commercialize vetiver for essential oil production in the Philippines and neighboring Southeast Asian countries remain fragmented and largely small-scale despite favorable agroclimatic conditions [5].

The industrial significance of vetiver oil lies in its chemical complexity and functional irreplaceability. It serves as a foundational base note in a substantial proportion of fine fragrances and men's perfumes, with no synthetic substitute capable of reproducing its odor longevity, molecular complexity, or fixative behavior [1]. Beyond perfumery, vetiver oil is used in cosmetics, aromatherapy, food flavoring, and traditional medicine, while the plant itself is widely applied for slope stabilization, heavy-metal phytoremediation, and wastewater treatment [6, 7]. Chemically, vetiver oil is among the most complex essential oils, dominated by sesquiterpenes and oxygenated sesquiterpenoids; its principal quality markers—khusimol,  $\alpha$ -vetivone, and  $\beta$ -vetivone—account for a substantial proportion of the oil, while numerous minor constituents collectively define its sensory and biological properties [8, 9]. This compositional complexity is highly sensitive to genotype, environmental conditions, root maturity, and extraction protocols, complicating standardization in intensified production systems. In addition, a growing body of research has demonstrated antimicrobial, antioxidant, anti-inflammatory, anxiolytic, cytotoxic, antihyperglycemic, and enzyme-inhibitory activities, highlighting the broader bioactive potential of vetiver oil [9–12].

Despite its economic value, current vetiver cultivation strategies remain insufficiently aligned with the biological complexity underlying essential oil biosynthesis [13]. The production of sesquiterpene-rich root oils is governed not only by plant genotype and developmental stage but also by environmental cues and biotic interactions within the root system [13, 14]. As a result, intensified production approaches such as *in vitro* propagation and soilless cultivation have been explored to increase output while reducing land use; however, these systems often impose axenic or near-axenic conditions that decouple vetiver roots from their native microbial communities, which are increasingly recognized as contributors to secondary metabolism [14, 15]. Experimental observations indicate that removal of root-associated microorganisms is associated with altered root development, reduced differentiation of essential-oil-accumulating structures, and changes in volatile profiles [14]. At the same time, endophytic bacteria inhabiting internal root tissues may interact with sesquiterpene metabolism, including the transformation of oil constituents and associations with changes in plant terpene synthase gene expression [14, 15]. Consequently, cultivation systems that limit these biological interactions can compromise both plant development and oil quality [14, 16]. This limitation is particularly evident in high-pressure aeroponic systems, where vetiver plants may exhibit reduced root biomass, atypical morphology, and altered volatile composition, including reduced levels of hydrocarbon and oxygenated sesquiterpenes relative to soil-grown plants [17]. Together, these findings suggest that both microbial interactions and cultivation context play important roles in determining essential oil yield and composition.

Accordingly, this review synthesizes current evidence to evaluate the role of root-associated endophytic microorganisms as an important yet under-integrated determinant of vetiver essential oil yield and chemical composition, particularly in controlled cultivation systems. By examining cultivation strategies ranging from tissue culture to hydroponic and aeroponic production, this work evaluates the extent to which production can be intensified without compromising phytochemical integrity. It further advances the hypothesis that targeted isolation, characterization, and reintegration of vetiver-associated endophytes may enable controlled systems to achieve improved yield stability and chemical fidelity, thereby supporting sustainable intensification of high-value aromatic crops in the ASEAN region.

## 2. Materials and Methods

### 2.1 Literature Search Strategy

A structured literature search was conducted across Scopus, Web of Science, and Google Scholar to identify peer-reviewed studies relevant to vetiver, essential oils, endophytic microorganisms, and cultivation systems. Searches employed combinations of keywords, including vetiver, essential oil, sesquiterpenes, endophytes, plant microbiome, hydroponics, aeroponics, and aromatic plants, connected using Boolean operators (AND, OR). Search strings were adjusted to comply with database-specific syntax. The search was conducted with coverage up to May 2026. Across the three databases combined, the initial query returned approximately 480 records; after removing duplicates and applying inclusion criteria, 42 primary sources (journal articles, book chapters, and technical reports) were included in the analysis. Although a formal PRISMA flow diagram was not prepared, the structured nature of the search strategy and the explicit inclusion/exclusion criteria applied satisfy the transparency requirements of a structured narrative review.

### 2.2 Inclusion and Exclusion Criteria

Included studies were peer-reviewed journal articles addressing vetiver biology, essential oil production, endophyte–plant interactions, or comparable mechanisms in aromatic or medicinal plants. Studies focusing exclusively on erosion control or civil engineering applications that were not relevant to essential oil biosynthesis were excluded, as were non-peer-reviewed sources, conference abstracts, patents, and non-English publications.

### 2.3 Analytical Framework

The selected literature was synthesized using a comparative, mechanism-based framework that emphasizes differences among soil-based, container-based, hydroponic, aeroponic, and in *vitro* cultivation systems. Analysis focused on biological and environmental mechanisms linking endophytic microorganisms to essential oil yield and composition, rather than on descriptive cataloging of studies. Given the limited availability of recent peer-reviewed, vetiver-specific studies—particularly those addressing cultivation systems and microbiome–metabolite interactions—selected grey literature (e.g., conference proceedings, technical reports, and development publications) was deliberately included to supplement empirical evidence, while being interpreted with appropriate caution regarding data rigor and reproducibility.

## 3. Results and Discussion

### 3.1 Comparative Performance of Vetiver Cultivation Systems

Across the reviewed literature, vetiver cultivation systems exhibit clear, system-dependent differences in plant growth, root development, and essential oil yield and composition (Table 1). Soil-based field cultivation consistently serves as the primary benchmark against which alternative systems are evaluated. Field studies, including extensive multi-location trials conducted in major production regions such as India, commonly report root yields of approximately 2.3 t ha<sup>-1</sup> (dry weight). In contrast, essential oil recoveries typically range from ~0.3 to 2.0%, depending on genotype, plant age, environmental conditions, and post-harvest handling practices [1, 13, 18]. Oils obtained under these conditions display chemically complex sesquiterpene profiles dominated by compounds such as khusimol and  $\alpha$ - and  $\beta$ -vetivone, which are widely recognized as key determinants of commercial oil quality [1, 8, 9]. Controlled soil-based systems, including greenhouse and experimental plot studies, generally produce moderate to high root biomass with more uniform growth due to reduced environmental variability. However, these systems do not consistently outperform field conditions in terms of essential oil yield. Reported yields are generally within the field range (~0.3–2.0%), reflecting the maintenance of core physiological processes required for sesquiterpene biosynthesis, as well as the influence of factors such as reduced rooting depth, shorter cultivation cycles, and controlled environmental regimes [13, 19]. The qualitative composition of the oil remains comparable to that of field-grown plants, indicating that soil-based conditions, regardless of scale, preserve the fundamental biochemical pathways associated with essential oil production. Container- and pot-based systems represent an intermediate condition in which physical root confinement begins to influence plant performance. Limited rooting volume restricts root expansion and can reduce biomass accumulation, thereby contributing to lower or more variable essential oil yields relative to field systems [13]. In addition, oil composition in these systems

may vary due to differences in substrate composition, nutrient availability, and plant maturity. However, establishing consistent compositional patterns is difficult, as existing data are limited and derived from heterogeneous experimental conditions.

In semi-hydroponic systems, where plants are grown in substrates supplemented with nutrient solutions, further modifications in growth performance are observed. Available evidence suggests that root biomass is generally reduced compared to soil-grown plants, and essential oil yields may also be lower, although the magnitude of these effects varies among studies and remains insufficiently quantified [13, 19]. While the overall sesquiterpene profile may be broadly retained, available data are limited, and reported variations in the relative abundance of key constituents suggest that simplified or partially artificial growth environments may influence metabolic efficiency and expression. Fully soilless systems, including hydroponic and high-pressure aeroponic approaches, show more pronounced departures from soil-based performance. Vetiver plants grown under these systems can form roots; however, they frequently exhibit altered morphology, such as a finer structure or reduced density, and more variable biomass accumulation [17]. Essential oil production is not eliminated, but yields tend to be lower or more inconsistent than in soil-based systems. Volatile compounds continue to be synthesized under these conditions, although reported differences in overall composition and relative sesquiterpene abundance suggest shifts in metabolic regulation rather than complete inhibition of biosynthetic pathways [17].

At the end of this gradient, *in vitro* and axenic systems produce very low biomass and poorly differentiated root structures. Although these systems can synthesize certain terpenoid compounds, overall essential oil accumulation is extremely limited, and the resulting volatile profile is often incomplete [14, 15, 17]. In contrast, roots grown under natural soil conditions harbor diverse endophytic and rhizosphere microbial communities, which are increasingly implicated in regulating plant secondary metabolism [14, 15]. The absence of these biological interactions in sterile or simplified systems is therefore considered an important contributing factor to reduced metabolite development, although the extent and specificity of microbial influence remain subjects of ongoing investigation. Taken together, these observations indicate that differences among cultivation systems extend beyond nutrient availability and water delivery. Instead, they reflect the integrated effects of root structural development, cultivation duration, and plant–microbe interactions, all of which contribute to regulating secondary metabolism. The general pattern of reduced oil yield and modified compositional profiles observed in simplified or soilless systems should therefore be interpreted as a shift in metabolic performance arising from altered biological and environmental conditions, rather than a complete loss of biosynthetic capacity.

### 3.2 Role of Endophytic Microorganisms in Essential Oil Biosynthesis

A recurring observation across field-based and experimental studies is the consistent colonization of vetiver roots by diverse endophytic microbial communities inhabiting internal root tissues, including cortical regions associated with essential oil accumulation [14, 15, 20]. In this context, endophytes refer specifically to microorganisms residing within plant tissues rather than on root surfaces, as demonstrated in microscopic localization studies [14, 15]. These internal microbial communities are associated with enhanced plant growth, improved stress tolerance, and increased accumulation of secondary metabolites, suggesting functional roles that extend beyond nutrient acquisition alone [15, 16]. More recent microbiome analyses have provided improved taxonomic resolution, identifying a range of bacterial groups—including members of *Bacillus*, *Pseudomonas*, and other proteobacteria—associated with vetiver roots under different environmental conditions [16]. In contrast, microbiome-depleted or axenic vetiver systems—commonly generated through *in vitro* propagation or maintained under sterile conditions—consistently exhibit reduced essential oil accumulation and simplified volatile profiles relative to soil-grown plants [14, 17]. These observations suggest that endophytic microorganisms contribute to the normal development of oil-producing tissues and their associated metabolic pathways. Experimental studies indicate that endophytes are involved in sesquiterpene metabolism and may influence biosynthetic processes, including reported associations with changes in terpene synthase gene expression and volatile emission patterns [14, 15]. In addition, certain endophytic bacteria have been shown to metabolize terpenoid compounds or their precursors, suggesting the possibility of bidirectional metabolic interactions between host plant and associated microorganisms [14, 15].

**Table 1.** Comparison of vetiver cultivation systems and production outcomes.

| Cultivation system                                       | Root yield/ growth performance                                                                                 | Essential oil yield (%)                                                                         | Oil chemistry characteristics                                                                                                                                 | Key references  |
|----------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| Field-grown (Soil, open field)                           | High root biomass; well-developed, deep root system, 2.3 t ha <sup>-1</sup> dry weight depending on conditions | ~0.3–2.0% (varies with genotype, age, environment)                                              | Complex sesquiterpene-rich profile; major constituents include khusimol, $\alpha$ - and $\beta$ -vetivone; composition varies with environment and processing | 1, 8, 9, 13, 18 |
| Controlled soil systems (greenhouse/ experimental plots) | Moderate to high biomass; more uniform growth compared to the field; environmental variability reduced         | Generally, within the reported field range (~0.3–2.0%), but dependent on cultivation conditions | Similar qualitative sesquiterpene profile as field plants; relative proportions influenced by controlled conditions (e.g., soil, climate, genotype)           | 13, 19          |
| Container-based soil/ substrate systems (pots, nursery)  | Moderate biomass; constrained root development due to limited volume                                           | Limited quantitative data; potential reductions due to root confinement                         | Oil composition influenced by substrate composition, plant age, and growth conditions; variability reported                                                   | 13              |
| Semi-hydroponic system (substrate + nutrient solution)   | Reduced root biomass and density compared to soil-grown plants                                                 | Reported as lower or variable; quantitative data remain limited                                 | Potential shifts; insufficient evidence                                                                                                                       | 13, 19, 36      |
| Hydroponic/ high-pressure aeroponic                      | Strongly reduced root biomass; roots often thin, underdeveloped, and morphologically altered                   | Lower or variable                                                                               | Altered composition with reduced chemical complexity and reduced levels of hydrocarbon and oxygenated sesquiterpenes relative to soil-grown plants            | 17              |
| <i>In vitro</i> (tissue culture system)                  | Very low biomass, highly abnormal roots                                                                        | Extremely low oil                                                                               | Poor or incomplete volatile profile; underdeveloped oil-producing structures similar to aeroponic trend                                                       | 14, 15, 17      |



Consistent with this interpretation, cultivation systems that reduce or exclude microbial colonization—such as high-pressure aeroponic or sterile culture systems—are associated with altered root development, reduced differentiation of oil-associated structures, and lower levels of hydrocarbon and oxygenated sesquiterpenes relative to soil-based systems [17]. While these effects cannot be attributed solely to the absence of microbes, they support the broader hypothesis that plant–microbe interactions regulate secondary metabolism in vetiver roots. Taken together, the available evidence indicates that endophytic microorganisms likely function as active contributors to metabolic regulation rather than passive inhabitants of plant tissues, potentially influencing terpene biosynthesis through signaling interactions, modulation of precursor availability, and involvement in stress-responsive pathways [14–16]. Similar roles have been documented in other aromatic and medicinal plant systems, although such comparative evidence supports but does not replace direct mechanistic validation in vetiver [21–25].

### 3.3 Interpreting the Constraints of Soilless Cultivation Systems

Two complementary and non-exclusive hypotheses have been advanced to explain the recurring limitations of soilless cultivation systems in reproducing the yield and chemical complexity of soil-grown vetiver essential oil. The first hypothesis attributes these limitations to the disruption of soil-mediated biotic interactions, particularly those involving endophytic and rhizosphere microorganisms that contribute directly and indirectly to sesquiterpene biosynthesis. This interpretation is supported by experimental observations showing that axenic vetiver plants produce very low amounts of essential oil with altered chemical profiles. In contrast, reintroduction of native endophytes has been associated with restored sesquiterpene accumulation, changes in plant sesquiterpene synthase gene expression, and the release of characteristic vetiver volatiles [14, 15]. The second hypothesis proposes that current hydroponic and aeroponic systems do not fully provide the environmental and developmental conditions required for complete metabolic expression, even when mineral nutrition is optimized. Evidence supporting this view includes delayed or incomplete accumulation of sesquiterpenes in shortened cultivation cycles, reduced differentiation of essential oil-storing tissues, and persistent oil deficiencies under controlled soilless conditions [17]. High-pressure aeroponic cultivation, in particular, has been associated with atypical root morphology, immature tissue development, and a marked reduction in hydrocarbon and oxygenated sesquiterpenes, suggesting that key structural, temporal, or biological cues normally present in soil environments are absent [17].

Available evidence indicates that these mechanisms act synergistically rather than independently. Microbial deprivation and environmental simplification collectively influence root development, metabolic regulation, and signaling processes that underpin secondary metabolism. Loss of microbial partners may reduce biosynthetic activation and metabolite transformation capacity, while shortened developmental trajectories and simplified growth environments further constrain precursor availability and oil-cell maturation [14, 15, 17]. This integrated constraint model provides a coherent explanation for the underperformance of soilless vetiver systems and underscores the need to consider cultivation systems as biologically integrated environments rather than purely physicochemical growth platforms.

### 3.4 Implications for Intensified Vetiver Production

The available evidence highlights a key limitation of current vetiver intensification strategies: reductions in essential oil yield and compositional complexity observed in hydroponic and aeroponic systems may reflect not only technical constraints but also the absence of biological interactions inherent to soil-based cultivation. In vetiver-specific studies, controlled systems have been associated with reduced accumulation and altered composition of hydrocarbon and oxygenated sesquiterpenes that define industrial-grade vetiver oil [14, 17]. This trade-off is particularly relevant for crops such as vetiver, where oil quality rather than biomass alone determines economic value [1]. However, much of the evidence supporting these observations is derived from studies conducted outside Southeast Asia or under controlled experimental conditions, and recent peer-reviewed datasets specific to ASEAN commercial production systems remain limited in the available literature. Available regional information is largely drawn from conference reports, development

programs, or case studies with limited quantitative detail [26, 27]. As such, extrapolating current findings to ASEAN production contexts should be interpreted cautiously. Although vetiver cultivation in the region has been widely associated with environmental applications such as erosion control, slope stabilization, and phytoremediation, the extent to which production systems are transitioning toward high-value essential oil markets is not well documented in recent empirical studies.

Rather than advocating the exclusion of controlled or soilless systems, the existing evidence indicates a need to refine intensification strategies to better account for biological factors that influence secondary metabolism. Studies in vetiver systems [14, 15, 17] and, by analogy, in well-studied agronomic and horticultural crops (e.g., wheat, maize, tomato) and selected aromatic species (e.g., *Mentha* and *Ocimum*) suggest that microbial associations, developmental maturity, and environmental signaling play important roles in regulating metabolite production [24, 28–33]. While these mechanisms are directly supported in vetiver at the experimental level, their broader application to intensification strategies remains partly inferred from non-vetiver systems. Approaches integrating these factors—such as microbiome management, extended cultivation cycles, or hybrid soil–soilless systems—therefore represent plausible pathways for improving both yield and oil quality, although their feasibility at a commercial scale remains insufficiently assessed. Future intensification efforts should consider not only operational efficiency but also the maintenance of biological processes associated with essential oil biosynthesis, while recognizing that region-specific evidence supporting such approaches is still emerging [14, 34].

### 3.5 Endophyte Reinoculation as an Optimization Strategy

Across plant systems, targeted inoculation of beneficial endophytic microorganisms has been associated with enhanced plant growth, improved stress tolerance, and increased accumulation of secondary metabolites, including terpenoids, alkaloids, and phenolic compounds [16, 29]. These relationships are well established in agronomic and model crops (e.g., wheat, maize, and tomato) and, to a more limited extent, in selected aromatic species such as mint and basil, and are here extended to vetiver by analogy, where direct evidence remains limited. The underlying mechanisms—including phytohormone modulation, improved nutrient acquisition, and activation of stress-responsive metabolic pathways—are therefore generally supported, although their expression may vary across plant systems [29–33]. In the case of vetiver specifically, microbiome studies have identified a range of potentially functionally relevant taxa—including *Bacillus*, *Pseudomonas*, *Serratia*, *Kosakonia*, *Microbacterium*, and *Janthinobacterium*—associated with plant growth-promoting and biocontrol activities across diverse environments [16]. Experimental evidence further suggests that endophytes are associated with sesquiterpene biosynthesis and aroma development, as axenic (*in vitro*-derived) plants exhibit markedly reduced essential oil yields and simplified chemical profiles. In contrast, reinoculation with native endophytes is associated with partial restoration of sesquiterpene accumulation, changes in terpene synthase gene expression, and recovery of characteristic volatile emissions [14, 15]. These findings provide preliminary mechanistic insight into vetiver but remain limited in scope compared with the evidence available in other crop systems.

From an application perspective, microbial inoculation strategies—such as liquid formulations (e.g., fertigation or root dipping) and solid carrier-based systems (e.g., peat or compost matrices)—are primarily documented in non-vetiver crop systems, including cereals and horticultural crops [30, 31], and are conceptually transferable to vetiver production. However, introducing cultured endophytes into simplified or soilless systems requires careful consideration, as the absence of complex native microbial communities may influence competitive dynamics and colonization stability. Such effects are well documented in general plant–microbiome research but remain only partially validated in vetiver-specific systems [16, 31]. To ensure efficacy and reproducibility, advanced metabolomic tools such as UHPLC-QTOF-IMS and GC-MS profiling enable high-resolution characterization of plant secondary metabolites and volatile compounds [23]. These approaches have been more extensively applied in experimental studies of medicinal and aromatic crops, where they are used to characterize and compare metabolite profiles under different biological and environmental conditions, but remain comparatively underutilized in vetiver research. Additionally, large-scale deployment of microbial inoculants must comply with national biosafety and plant health regulations, which vary across regions and are typically defined within broader agricultural frameworks

rather than crop-specific guidelines for vetiver. Overall, endophyte reinoculation is a promising strategy for improving vetiver essential oil yield and quality under controlled cultivation. While its mechanistic basis is partially supported by experimental evidence in vetiver, its practical implementation and scalability remain partly informed by analogy with other crop systems and require further validation under region-specific production conditions.

### 3.6 Broader Relevance to ASEAN and Aromatic Crop Systems

The implications of the present findings extend beyond vetiver to a broader class of high-value aromatic and medicinal crops whose economic traits depend on developmentally regulated secondary metabolites, including terpenoids and related compounds [35,36]. Across these systems, secondary metabolism is influenced by plant–microbe interactions and environmental context, a relationship that is well established in agronomic and model crops (e.g., wheat, maize, tomato) and supported by emerging evidence in aromatic species such as mint and basil, and is here applied to vetiver systems by analogy, where direct comparative data remain limited [16, 29–33]. This consideration is particularly relevant for ASEAN countries, where agricultural systems face multiple pressures, including land scarcity, environmental degradation, and the need to diversify toward higher-value crops while maintaining ecosystem services [37, 38]. Within this regional context, vetiver has historically been promoted primarily as an environmental crop for erosion control, slope stabilization, water management, and land rehabilitation [26, 27, 39]. While available reports and development programs support this characterization, there is limited recent empirical evidence quantifying the extent of aromatic-grade vetiver production or its integration into formal value chains across the region. Consequently, the classification of vetiver as an underutilized aromatic crop should be interpreted as a plausible but incompletely documented trend. Strategies that integrate microbiome-informed crop management may offer a pathway to enhance secondary metabolite production while maintaining environmental functions; however, such approaches have been demonstrated primarily in non-vetiver crop systems and have not yet been systematically validated under ASEAN field conditions for vetiver [16, 24]. From a policy perspective, these strategies should therefore be considered evidence-informed but not yet empirically validated solutions, whose feasibility will depend on region-specific agroecological, economic, and institutional factors. More broadly, biologically informed intensification strategies align conceptually with emerging models of sustainable agriculture that emphasize system redesign over input intensification [34]; however, evidence demonstrating their scalability, economic viability, and long-term impact in ASEAN vetiver systems remains limited. Accordingly, while integrating microbial and ecological processes into vetiver cultivation represents a promising direction, further regionally grounded research is required to substantiate its applicability and inform policy and investment decisions.

### 3.7 Limitations of the Current Evidence Base

Despite growing evidence that plant–microbe interactions are implicated in vetiver essential oil biosynthesis, the current evidence base remains constrained by several key limitations. First, long-term comparative studies evaluating reinoculated versus non-reinoculated vetiver plants under controlled cultivation systems are scarce. Existing demonstrations of microbial involvement in sesquiterpene biosynthesis are largely derived from short-term or laboratory-scale experiments, leaving uncertainty regarding the durability, stability, and agronomic relevance of reinoculation effects across full production cycles [14, 15]. Second, mechanistic insight is limited by the underrepresentation of integrated multi-omics approaches. Although individual studies have examined volatile profiles, root anatomy, and microbial communities, few have combined metabolomics, transcriptomics, and microbiome sequencing within a unified experimental design. Such integration has proven essential in other aromatic and medicinal crops for resolving host–microbe metabolic coupling, highlighting a methodological gap in vetiver research [23, 24]. Finally, much of the available evidence is system-specific. Findings derived from high-pressure aeroponic cultivation, in particular, cannot be readily generalized across hydroponic or substrate-based systems, complicating cross-study synthesis and limiting translation into broadly applicable cultivation strategies [17]. Collectively, these limitations underscore the need for longer-term, multi-platform, and mechanistically integrated investigations to support microbiome-informed vetiver production [14, 34].

### 3.8 Future Research Directions

Addressing the limitations identified above will require a shift toward longer-term, systems-level experimental designs that reflect commercially relevant cultivation contexts. Priority should be given to multi-cycle comparisons of reinoculated and non-reinoculated vetiver plants under hydroponic, aeroponic, and hybrid systems to assess microbial persistence, functional stability, and cumulative effects on root development and essential oil yield across harvests [14, 15]. Concurrently, progress in mechanistic understanding will depend on integrating metabolomics, transcriptomics, and microbiome sequencing within unified experimental frameworks, enabling direct linkage of microbial community dynamics with host sesquiterpene biosynthesis and chemical output [23, 24]. Application of these approaches across both soil-based and soilless systems would help distinguish platform-specific constraints from universal biological requirements for essential oil biosynthesis [17]. Finally, translation to scalable practice will require interdisciplinary collaboration among microbiologists, plant physiologists, and controlled-environment engineers to embed microbiome compatibility into cultivation design. Such integration may facilitate the transition from proof-of-concept microbiome interventions to robust, commercially viable vetiver production systems [23, 40].

## 4. Conclusions

This review evaluated the hypothesis that root-associated endophytic microorganisms represent important but under-integrated determinants of vetiver essential oil yield and chemical composition, particularly in controlled, intensified cultivation systems, and the convergent evidence synthesized herein provides considerable support for this hypothesis. Across studies, soil-grown vetiver generally outperforms hydroponic, aeroponic, and *in vitro* systems in both oil yield and the production of more chemically complex sesquiterpene profiles, while microbiome-depleted plants exhibit substantially reduced and altered essential oil accumulation, and reintroduction of native root endophytes is associated with partial restoration of sesquiterpene accumulation and characteristic aroma traits. Together, these observations indicate that microbial interactions likely play important roles in regulating secondary metabolism rather than acting solely as ancillary contributors to vegetative growth. The principal contribution of this review lies in reframing vetiver production through a unified, mechanism-based framework that links plant development, microbial function, and cultivation system design, thereby moving beyond descriptive comparisons to highlight biological disconnection as a potential central constraint of soilless intensification strategies. Current limitations—including the scarcity of long-term reinoculation trials, limited system-level mechanistic validation, and platform-specific evidence—underscore the need for more comprehensive and integrated research efforts, while future progress in vetiver intensification will depend on systematic functional characterization of core endophytic communities and rigorous evaluation of microbial reintegration strategies within hydroponic and aeroponic systems. Such approaches offer a viable pathway to reconcile land-use efficiency with metabolic performance, particularly in ASEAN contexts where vetiver is widely deployed for environmental services but remains underutilized as a high-value aromatic crop, and more broadly, the insights derived from vetiver provide a conceptual template for microbiome-informed optimization of other aromatic and medicinal crops, highlighting the importance of treating biological integration as a foundational design criterion in future controlled-environment agriculture.

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# Geospatial Soil Texture Prediction in Cebu, Philippines: A Comparative Study of UK, MLR, and ANN

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**Abstract:** Many soil maps in the Philippines are outdated, lacking the detail required for effective land management and agricultural planning. Traditional survey methods, still widely used, often fail to capture the spatial variability of soil properties, which is critical given the country's diverse topography and land-use patterns. This study compares three models—Multiple Linear Regression (MLR), Artificial Neural Networks (ANN), and Universal Kriging (UK) for soil texture classification, using open-source software tools: R and SAGA GIS. Results indicate that the UK model significantly outperforms both MLR and ANN, with the highest overall accuracy of 77.25%, a strong kappa value (0.5757), and a statistically significant p-value ( $5.937e-10$ ). The UK model excels in class-wise performance, showing superior sensitivity for key classes such as Class 1 (0.9576) and Class 6 (0.6719), while maintaining high specificity across all classes. Its balanced accuracy, as high as 0.8949 for Class 1, reflects its ability to handle both major and minority classes effectively. In contrast, MLR and ANN show lower sensitivity, often failing to classify minority classes (0 sensitivity in multiple cases) and achieving poorer balanced accuracy overall. The findings highlight the UK as the most reliable classifier among the three, excelling in both overall and class-specific performance. Future research should focus on further optimizing the UK model and testing its applicability to other datasets and classification tasks. Additionally, expanding the dataset with more samples from underrepresented classes could further improve model accuracy.

**Keywords:** Predictive DSM; soil texture classification; geostatistical modeling; machine learning

## 1. Introduction

The application of predictive digital soil mapping (PDSM) in the Philippine context presents several research gaps that need to be addressed to enhance the effectiveness and accuracy of soil mapping efforts. These gaps stem from the Philippines' unique geographical, climatic, and socio-economic conditions, which can significantly influence soil properties and their distribution. One of the primary gaps is the limited availability of high-resolution soil data across the archipelago. Many existing soil maps in the Philippines are outdated and lack the granularity required for effective land management and agricultural planning. For instance, while digital soil mapping techniques have been successfully applied in other regions, the Philippines still relies heavily on traditional soil survey methods that often fail to capture soil



property spatial variability adequately [1]. This is particularly concerning given the diverse topography and land use practices across the islands, which can lead to significant variations in soil characteristics.

Moreover, there is a need to integrate advanced modeling techniques, such as multiple linear regression (MLR), machine learning, and geostatistics, into PDSM practices in the Philippines. However, the application of these techniques in the Philippine context remains limited. The potential to use remote sensing data and environmental covariates to enhance predictions of soil properties has not been fully explored, which could significantly improve the accuracy of soil maps [2, 3]. Multiple linear regression is a well-established statistical technique widely used for predictive modeling in digital soil mapping [1, 4]. It models the relationship between a dependent variable (e.g., soil property) and multiple independent variables (e.g., environmental covariates). MLR's simplicity and interpretability make it suitable for initial exploratory analysis and establishing baseline predictive performance. Artificial neural networks (ANNs), a machine learning method, have been extensively applied to predictive digital soil mapping due to their ability to capture complex nonlinear relationships between soil properties and environmental covariates [5]. ANNs can learn from data without explicit programming, making them well-suited for modeling the inherent complexity of soil systems [6].

Universal kriging (UK) is a geostatistical technique that incorporates both spatial autocorrelation and environmental covariates to predict soil properties at unsampled locations [1]. The UK has been used in digital soil mapping to produce spatially continuous predictions while accounting for the spatial structure of soil properties [1]. The advantage of the UK is its ability to provide estimates of prediction uncertainty, which is crucial for decision-making in soil management. The findings will significantly enhance the accuracy and spatial resolution of soil information [4, 7, 8], providing a robust foundation for advanced crop and livestock land suitability modeling. By integrating predictive soil attributes with terrain and climate variables, the study will enable science-based identification of optimal areas for specific crops and agrosilvopastoral production systems [9–12]. This supports improved farm-level planning, efficient resource allocation, and risk reduction in agricultural investments [9–11]. Ultimately, the study promotes data-driven decision-making that advances sustainable agriculture, resilient food systems, and environmental conservation [10, 13] across Cebu Province.

This study aims to compare the predictive performance of MLR, ANN, and UK in PDSM by evaluating their ability to model soil texture using environmental covariates. This research seeks to determine the most effective method for accurate soil texture prediction by analyzing each model's strengths in handling linear relationships, capturing non-linear complexities, and incorporating spatial autocorrelation.

## 2. Materials and Methods

### 2.1 Materials

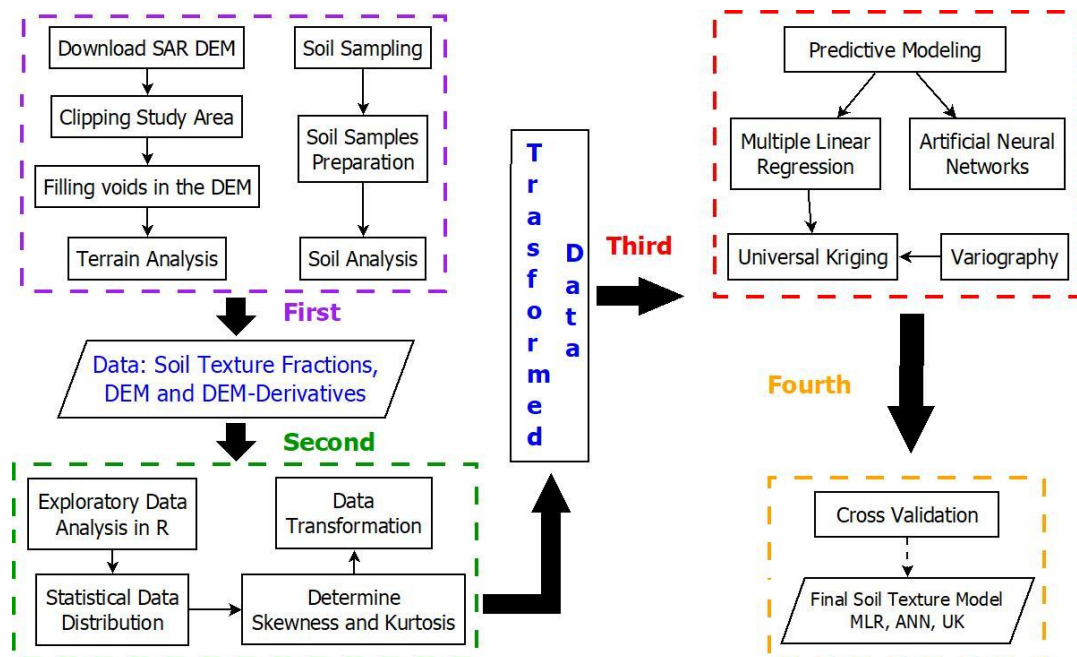
The Shuttle Radar Topography Mission (SRTM) Digital Elevation Model (DEM) was accessed and processed using Google Earth Engine (GEE) for this study. The SRTM DEM, with a 30-meter spatial resolution, provides elevation data derived from radar interferometry, ensuring high accuracy and broad coverage for topographic analysis. The processed DEM was used as a reliable input for terrain analysis, enabling the extraction of key derivatives, including slope, aspect, and hydrological features (Table 1).

### 2.2 The study area

Cebu is the second-largest metropolitan area in the Philippines, located in the central Visayas, with a population exceeding 4 million [14]. It features diverse landscapes ranging from coastal plains to mountainous terrain [15] and has a tropical climate characterized by a dry season (March–May) and a wet season (June–November) [16, 17]. Agriculture remains a key economic activity, with major products including sugarcane, coconut, and food crops [18]. However, the region faces land use change, soil degradation, deforestation, and climate-related challenges that threaten environmental sustainability [19]. Cebu's complex topography is strongly shaped by karst formations, particularly in central areas [15], and further influenced by vegetation patterns and urbanization, especially in Cebu City [16]. Topographic variation also controls soil distribution and texture, which are critical for agriculture and land management [1]. Given its diverse geography, climate, and socio-economic conditions, Cebu provides a suitable setting for predictive digital soil mapping to support sustainable soil resource management and informed decision-making.

### 2.3 Study framework

This study followed a predictive digital soil mapping (DSM) framework integrating field soil observations, terrain-derived environmental covariates, statistical analysis, machine-learning techniques, and geostatistical modeling. The workflow consisted of four major phases: (i) data acquisition and preprocessing, (ii) exploratory data analysis and transformation, (iii) predictive modeling and spatial interpolation, and (iv) model validation and final map generation.



**Figure 1.** Workflow of predictive digital soil mapping showing data acquisition, exploratory analysis and transformation, predictive modeling (MLR, ANN, and universal kriging), and cross-validation leading to final soil texture maps.

### 2.4 Digital elevation model and terrain derivatives

A synthetic aperture radar (SAR)-derived digital elevation model (DEM) was acquired and preprocessed (clipped, void- and sink-filled) to ensure hydrological consistency (Table 1). Geomorphometric analysis generated terrain covariates relevant to soil formation, including slope, aspect, curvature, and related indices. Mountain ridge proximity was additionally examined following [1]. These DEM-derived variables were used as continuous environmental predictors and are widely recognized as key controls on soil texture distribution [1, 5, 20–22].

### 2.5 Soil sampling and laboratory analysis

Soil samples were collected from representative locations using a combined convenience (legacy-based) and stratified sampling approach to adequately capture landscape variability. A total of 445 samples were obtained across the City of Carcar (22 samples) and the municipalities of Barili (371 samples) and Sibonga (52 samples) (Figure 2). Collected samples were air-dried, processed, and sieved before laboratory analysis. Soil texture fractions—sand, silt, and clay—were determined using standard analytical procedures at the Department of Agriculture–Region VII Soils Laboratory.

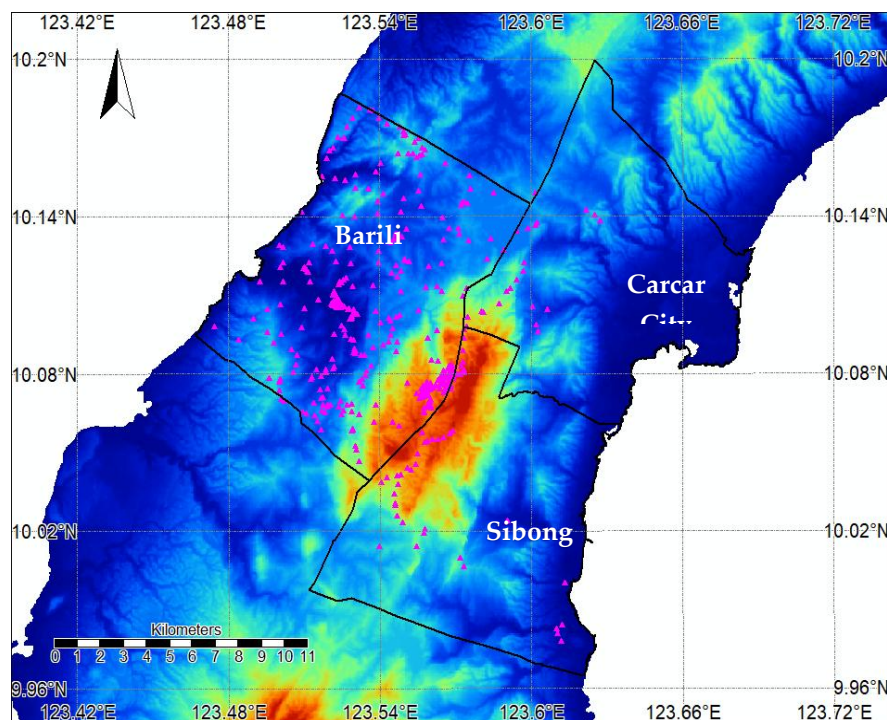
### 2.6 Exploratory data analysis and transformation

Exploratory data analysis using R, including summary statistics, graphical diagnostics, and distribution plots, was performed to identify outliers and assess variability of soil texture fractions. Skewness and kurtosis were computed to evaluate departures from normality, and, where needed, Box–Cox–type power transformations were applied to reduce skewness, stabilize variance, and better satisfy linear regression and geostatistical assumptions [1, 23, 24]. Because the response variables were transformed to meet the normality

assumption, all identified best model outputs were subsequently back-transformed to their original scale for interpretation. Furthermore, predictor variables were normalized via min-max scaling to reduce the impact of different ranges of predictor values [23, 25].

**Table 1.** Terrain derivatives generated from the digital elevation model (DEM) and organized according to the SCORPAN environmental covariate framework to represent relief (R) and spatial position (N) factors in predictive digital soil mapping.

| Attribute type                      | DEM derivatives                                                                                                                                                                              |
|-------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Basic terrain morphology            | Elevation, Slope, Gradient, Slope Height, Slope Length, Standardized Height, Normalized Height                                                                                               |
| Terrain shape and curvature         | Plan Curvature, Profile Curvature, Convergence Index, Gradient Difference                                                                                                                    |
| Hydrological terrain attributes     | Flow Accumulation, Total Catchment Area, Flow Width, Maximum Flow Path Length, Topographic Wetness Index, LS-Factor, Channel Network Distance, Distance Channels Proximity, Stream Proximity |
| Terrain position indices            | Mountain Ridge Proximity, Topographic Position Index, Mid-Slope Position, Valley Depth, Landform                                                                                             |
| Multiscale relief structure indices | MRVBF, MRRTF, Melton Ruggedness Number                                                                                                                                                       |
| Energy and terrain exposure proxies | Analytical Hillshading, Diurnal Anisotropic Heating                                                                                                                                          |
| Composite/secondary terrain indices | Entropy, Confusion Index, Maximum Membership, Mass Balance Index                                                                                                                             |
| Spatial position factors            | Latitude, Longitude                                                                                                                                                                          |



**Figure 2.** Spatial distribution of the 445 soil sampling locations across the City of Carcar and the municipalities of Barili and Sibonga, Cebu, collected using a combined convenience and stratified sampling approach.

## 2.7 Integration of soil and environmental data

Soil texture observations were spatially linked with corresponding terrain-derived covariates extracted from the DEM. The resulting georeferenced soil texture data were used as point-based response variables for predictive modeling [1, 2, 26, 27]. This integration resulted in a modeling dataset in which each soil sample was associated with a set of environmental predictors representing landscape controls on soil variability.

## 2.8 Predictive modeling approaches

Soil texture fractions were predicted using multiple modeling techniques. Multiple linear regression (MLR) provided an interpretable baseline linking terrain-derived covariates to soil texture [1, 28, 29]. Artificial neural networks (ANN) were applied to capture nonlinear relationships between environmental predictors and soil texture fractions [29–31]. Geostatistical modeling using variogram analysis (Table 2) and universal kriging (UK) incorporated spatial autocorrelation, combining environmental trends with spatial dependence to identify spatially explicit predictors from MLR as external drift parameters for spatial interpolation [1, 31].

**Table 2.** The variogram parameters of soil texture fractions are applied to the exponential model.

| Soil Texture fraction | Semivariogram model | Nugget effect | Sill | Nugget-to-sill ratio | Effective range |
|-----------------------|---------------------|---------------|------|----------------------|-----------------|
| Clay                  | exponential         | 400           | 2158 | 0.185                | 3450            |
| Sand                  | exponential         | 0.4           | 2.57 | 0.156                | 5215            |
| Silt                  | exponential         | 0.04          | 2.24 | 0.018                | 1798            |

## 2.9 Model comparison based on confusion-matrix-derived parameters

Model performance was evaluated using 5-fold cross-validation, in which 80% of the data were used for training and the remaining 20% for validation in each fold. Standard random k-fold CV may overestimate accuracy when training and test observations are spatially proximate; spatial cross-validation is recommended in future work to provide conservative, unbiased estimates for a geostatistical model. Comparative evaluation of the classification models was conducted using parameters derived from the confusion matrix to capture both overall and class-specific performance. For each model, a confusion matrix was generated on the independent validation data, summarizing true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) for each soil texture class [32–34]. From these counts, standard discrimination metrics were computed, including sensitivity/recall ( $TP / (TP + FN)$ ), specificity ( $TN / (TN + FP)$ ), precision or positive predictive value ( $TP / (TP + FP)$ ), negative predictive value ( $TN / (TN + FN)$ ), and overall accuracy ( $(TP + TN) / (TP + FP + TN + FN)$ ) [33, 35, 36]. Because the dataset exhibited substantial class imbalance, additional emphasis was placed on balanced accuracy (the mean of sensitivity and specificity) and class-averaged scores, which are less biased by the majority classes than overall accuracy [33, 34, 36]. Models were compared by examining: (i) overall accuracy as a broad indicator of performance; (ii) per-class sensitivity and specificity to identify classes that were consistently misclassified or confused; and (iii) balanced accuracy to assess performance under class imbalance and to avoid over-optimistic assessments based solely on accuracy [33, 36]. This confusion-matrix-based comparison enabled a nuanced evaluation of each model's strengths and weaknesses across soil texture classes and provided the basis for selecting the preferred model for subsequent spatial prediction and mapping.

## 2.10 Synthesis of Sand, Silt, and Clay Predictions into Final Soil Texture Model

Soil texture was classified into 12 USDA classes: Class 1 = Clay, Class 2 = Silty Clay, Class 3 = Silty Clay Loam, Class 4 = Sandy Clay, Class 5 = Sandy Clay Loam, Class 6 = Clay Loam, Class 7 = Silt, Class 8 = Silt Loam, Class 9 = Loam, Class 10 = Sand, Class 11 = Loamy Sand, Class 12 = Sandy Loam. The final soil texture model was generated in SAGA GIS using the Soil Texture Classification module, integrating the back-transformed best-performing grid models of sand, silt, and clay. This process produced continuous spatial predictions of soil texture classes across the study area, providing spatially explicit outputs suitable for land evaluation, agricultural planning, and environmental modeling.



### 3. Results

#### 3.1 Performance comparison of MLR, ANN, & UK

The study compares the performance of three methods (MLR, ANN, and UK) to evaluate their effectiveness in modeling the spatial distribution of topsoil texture. Model performance was evaluated using accuracy, sensitivity, specificity, kappa, and balanced accuracy to assess predictive capability across both dominant and minority classes.

##### 3.1.1 Sensitivity (Recall)

Sensitivity results indicate that all models most effectively identified the clay class, as shown in Table 3. The MLR model achieved high sensitivity for Class 1 (clay; 0.8410) but failed to correctly identify most other classes, including loam (Class 9), for which sensitivity was 0.0000. The ANN model showed moderate sensitivity to clay (0.6290) but was insensitive to several classes, including silty clay (Class 2) and loam (Class 9). The UK model achieved the highest sensitivity for clay (0.9576) and also showed good sensitivity for clay loam (Class 6; 0.6719), while sand (Class 10) and loamy sand (Class 11) were not correctly identified.

**Table 3.** Sensitivity (recall) of MLR, ANN, and UK models for soil texture classes, highlighting the best- and worst-performing classes based on the ability to identify true class occurrences correctly.

| Model | Best Performing Classes                                                 | Worst Performing Classes                                                     |
|-------|-------------------------------------------------------------------------|------------------------------------------------------------------------------|
| MLR   | Class 1 <sub>clay</sub> (0.8410)                                        | Most others (0.0000, e.g., Class 9 <sub>loam</sub> )                         |
| ANN   | Class 1 <sub>clay</sub> (0.6290)                                        | Several classes (0.0000, e.g., 2 <sub>silty clay</sub> , 9 <sub>loam</sub> ) |
| UK    | Class 1 <sub>clay</sub> (0.9576), Class 6 <sub>clay loam</sub> (0.6719) | Classes 10 <sub>sand</sub> , 11 <sub>loamy sand</sub> (0.0000)               |

##### 3.1.2 Specificity

Specificity was generally high across all models, as shown in Table 4. The MLR model showed perfect specificity (1.0000) for several less frequent classes, including silty clay, sandy clay, sand, and loamy sand. The ANN model demonstrated good overall specificity, with particularly high performance for clay loam (0.9658). The UK model exhibited consistently high specificity values, close to 1.0000, across all soil texture classes evaluated.

**Table 4.** Specificity of MLR, ANN, and UK models across soil texture classes, indicating the ability of each model to identify non-class instances correctly.

| Model | Specificity Performance                                                                                                                                                                                                                                                        |
|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| MLR   | High for rare classes (e.g., 1.0000 in Classes 2 <sub>silty clay</sub> , 4 <sub>sandy clay</sub> , 10 <sub>sand</sub> , 11 <sub>loamy sand</sub> )                                                                                                                             |
| ANN   | Good overall, notably Class 6 <sub>clay loam</sub> (0.9658)                                                                                                                                                                                                                    |
| UK    | Consistently high (close to 1) for all classes (1 <sub>clay</sub> , 2 <sub>silty clay</sub> , 4 <sub>sandy clay</sub> , 5 <sub>sandy clay loam</sub> , 6 <sub>clay loam</sub> , 9 <sub>loam</sub> , 10 <sub>sand</sub> , 11 <sub>loamy sand</sub> , 12 <sub>sandy loam</sub> ) |

##### 3.1.3 Balanced accuracy

Balanced accuracy revealed clearer differences among models by accounting for both sensitivity and specificity, as shown in Table 5. The MLR model achieved its highest balanced accuracy in clay (0.6565), while silty clay, loam, and loamy sand showed balanced accuracies close to 0.5000, indicating chance-level performance. The ANN model performed best for sandy clay loam (0.5293), with several classes, including silty clay, loam, and loamy sand, again showing balanced accuracy near 0.5000. The UK model achieved the highest balanced accuracy overall, particularly for clay (0.8949) and clay loam (0.7859), whereas sand and loamy sand remained at chance-level performance.

##### 3.1.4 Overall model performance

Table 6 shows that the overall accuracy was highest for the UK model (0.7725), with a 95% confidence interval of 0.7307–0.8107, and exceeded the no information rate (0.6374), as reflected by a statistically significant p-value (5.94E-10). The MLR model achieved moderate accuracy (0.5856), while the ANN model had the lowest accuracy (0.4347); neither model outperformed the no-information rate. Agreement, measured

by kappa, followed the same pattern, with UK showing moderate agreement (0.5757), MLR showing low agreement (0.1703), and ANN showing minimal agreement (0.071).

**Table 5.** Balanced accuracy of MLR, ANN, and UK models by soil texture class, combining sensitivity and specificity to account for class imbalance.

| Model | Best Classes                                                            | Worst Classes                                                                          |
|-------|-------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| MLR   | Class 1 <sub>clay</sub> (0.6565)                                        | Class 2 <sub>silty clay</sub> , 9 <sub>loam</sub> , 11 <sub>loamy sand</sub> (~0.5000) |
| ANN   | Class 5 <sub>sandy clay loam</sub> (0.5293)                             | Class 2 <sub>silty clay</sub> , 9 <sub>loam</sub> , 11 <sub>loamy sand</sub> (~0.5000) |
| UK    | Class 1 <sub>clay</sub> (0.8949), Class 6 <sub>clay loam</sub> (0.7859) | Classes 10 <sub>sand</sub> , 11 <sub>loamy sand</sub> (0.5000)                         |

**Table 6.** Overall classification performance of MLR, ANN, and UK models based on accuracy, confidence intervals, no information rate, statistical significance, and kappa agreement.

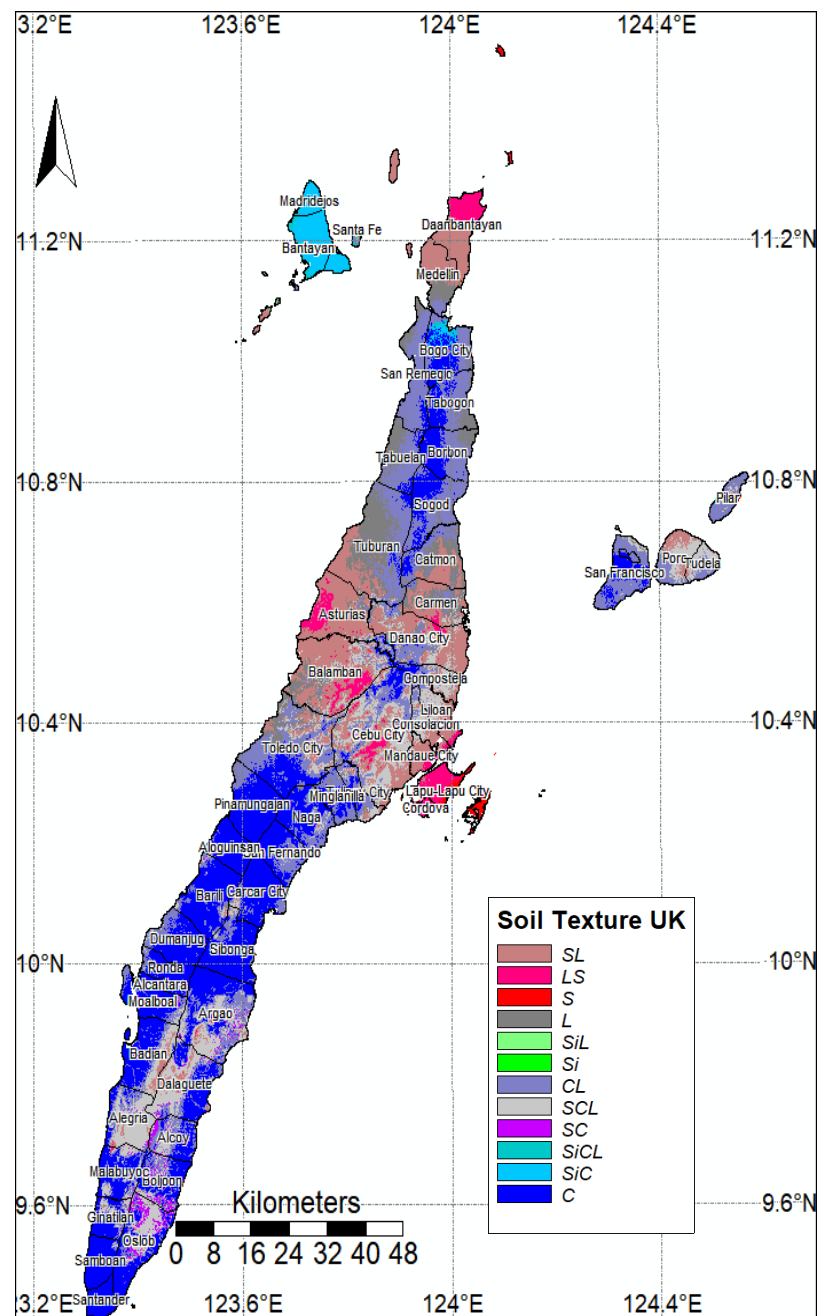
| Metric                  | MLR              | ANN              | UK               |
|-------------------------|------------------|------------------|------------------|
| Accuracy                | 0.5856           | 0.4347           | 0.7725           |
| 95% Confidence Interval | (0.5382, 0.6318) | (0.3880, 0.4822) | (0.7307, 0.8107) |
| No Information Rate     | 0.6374           | 0.6374           | 0.6374           |
| P-Value (Acc > NIR)     | 0.9893           | 1                | 5.94E-10         |
| Kappa                   | 0.1703           | 0.071            | 0.5757           |

### 3.2 Soil texture model by universal kriging

The spatial distribution of topsoil types across municipalities in Cebu province is predicted to show greater diversity from south to north than from east to west (Figure 3). Dumanjug (72%), Sibonga (80%), Barili (82%), Carcar City (82%), Ronda (83%), Pinamungajan (84%), Alcantara (87%), Aloguinsan (93%), Samboan (96%), and Santander (99%) have predominantly clay soil due to its high percentage in these municipalities. Clay Loam-dominated municipalities are San Remegio (76%) and Pilar (86%). Municipalities with a mixed dominance of two soil types summing to at least 80% include Bogo City (46% clay, 34% clay loam), Borbon (42% clay, 44% clay loam), Santa Fe (51% clay, 29% sandy loam), Medellin (26% loam, 62% sandy loam), Mandaue City (21% loamy sand, 61% sandy loam), Tabogon (30% clay, 54% clay loam), San Francisco (32% clay, 60% clay loam), Sogod (36% clay, 60% clay loam), Minglanilla (40% clay, 54% clay loam), Tabuelan (58% clay loam, 31% loam), Malabuyoc (59% clay, 21% clay loam), Naga (59% clay, 35% clay loam), San Fernando (57% clay, 40% clay loam), Ginatilan (63% clay, 18% sandy clay), Tudela (63% sandy clay loam, 30% clay loam), and Moalboal (69% clay, 22% clay loam). The majority of these mixed soil types are clay and clay loam.

Municipalities with significant and unique soil type dominance as loam of Tuburan (42%), as silty clay-dominated of Madrideojos (95%) and Bantayan (81%), and as sandy clay loam of Poro (47%), Alegria (64%), Tudela (63%), Cebu City (38%), Compostela (42%), Consolacion (46%), Dalaguete (35%), and Liloan (38%). Others, such as Asturias (19% loamy sand, 64% sandy loam), Balamban (56% sandy loam), Carmen (48% sandy loam), Cordova (85% loamy sand), Daanbantayan (39% loamy sand, 57%, sandy loam), Danao City (50% sandy loam), Liloan (40% sandy loam), Lapu-Lapu City (52% loamy sand), Mandaue City (21% loamy sand, 61% sandy loam), and Medellin (62% sandy loam) show a significant percentage of sandier textures. The model's performance, although with 77% accuracy, suggests it has limitations in accurately predicting the spatial distribution of topsoil texture across the Province of Cebu, particularly for underrepresented classes (Figure 3). Hypothetically, Cebu's topsoil texture exhibits a diverse spatial pattern, with sandy loam (SL) prevalent from highland to lowland and down to the coastal areas in Central Cebu (Cebu City, Mandaue City, Consolacion, Liloan, Compostela, Danao City, Carmen, Catmon, Asturias, and Balamban), Daanbantayan, and Medellin. Clay soil dominates upland and mountainous regions in the northern and southern parts. Sandy clay loam is estimated to be dominant in the mountainous areas of Argao, Badian, Dalaguete, Alegria, and Alcoy. Loam soil is concentrated in the lowlands of northern Cebu (Tabuelan, Tuburan, Carmen, Catmon, and Medellin). Loamy sand dominates greatly in Cordova, Lapu-Lapu City, and Daanbantayan. Olango Island in Lapu-Lapu City is expected to have entirely sandy soil. Despite its limitations, the model could still be a valuable tool

for preliminary soil mapping and agricultural planning, offering insights into erosion risk, water retention, and crop suitability.



**Figure 1.** Universal kriging model of the spatial distribution of topsoil texture in the Province of Cebu by predictive digital soil mapping.

#### 4. Discussion

The comparative evaluation of MLR, ANN, and UK highlights how both model type and class imbalance shape soil texture classification performance. Similar to other digital soil mapping studies, performance was strongly class dependent, with higher recall for clayey classes and near-chance performance for several minority or spectrally ambiguous classes [5, 6, 36]. The consistently high sensitivity of clay across all models aligns with reports that clay-rich soils often exhibit clearer spectral and topographic signatures than soils with intermediate textures, facilitating discrimination in both remote-sensing-based and terrain-based

models [24, 27, 36]. Conversely, sandy and loamy classes, particularly loam and loamy sand in this study, showed very low sensitivity and balanced accuracy, reflecting well-documented challenges in separating intermediate or mixed textures, where transitions are gradual, and feature spaces overlap [5, 6, 27, 36]. High specificities for several rare classes, especially under MLR, likely result from strong class imbalance: models rarely predict these classes, yielding few false positives but many false negatives. This pattern is consistent with studies demonstrating that overall accuracy and specificity can overestimate performance on imbalanced soil texture datasets, and that metrics incorporating both omission and commission errors (e.g., kappa, balanced accuracy) provide a more realistic assessment [5, 24, 36]. The use of balanced accuracy in this study, therefore, follows current recommendations for evaluating imbalanced soil texture classification problems [24].

The relatively poor performance of ANN in this study is consistent with the findings of Mallah et al. (2023), who reported an accuracy of 52% and a kappa coefficient of 0.38. This result is also consistent with broader evidence suggesting that ANNs tend to underperform when applied to small-to-medium-sized datasets, where overfitting and sensitivity to hyperparameters reduce generalization [5, 23, 30]. The superior overall accuracy and kappa of the UK relative to MLR and ANN suggest that explicitly modeling spatial structure can be advantageous when covariates or sample size are insufficient for complex machine learning models. Comparable results have been reported, showing that universal kriging outperformed MLR for texture fractions, especially with limited sample sizes and strong spatial autocorrelation [1]. The superior performance of the UK can be attributed to its ability to account for local nonstationarity [1] and incorporate the spatial structure [37] of soil properties, which are crucial for accurate prediction. Furthermore, the UK model used in this study was a multivariate model that included the significant predictors identified by MLR. A third key limitation is the severe geographic imbalance (83% of samples from Barili). Cross-validation accuracy (UK: 77.25%) was estimated within the sampled domain; in unsampled municipalities, UK effectively reduces to MLR behavior, eliminating its spatial autocorrelation advantage. Spatially-blocked cross-validation is recommended for future province-wide DSM studies to provide realistic accuracy estimates [3, 12]. The weak performance for certain classes across all models underscores two key limitations: (i) skewed class distributions, which have been repeatedly shown to depress recall for rare texture classes [5, 6, 24], and (ii) the inherent difficulty of discretizing a continuous particle-size space into categorical texture classes [24, 38]. Approaches such as indirect classification via predicted particle-size fractions [24, 26, 27], and targeted sampling to bolster rare classes [5] have all been proposed to mitigate these issues and could further improve performance in future work.

## 5. Conclusions

This study compared multiple linear regression (MLR), artificial neural networks (ANN), and universal kriging (UK) for predicting soil texture classes using terrain-derived covariates. The UK attained the highest overall accuracy (0.7725) and kappa (0.5757), significantly outperforming the no-information rate, whereas MLR (0.5856) and ANN (0.4347) showed weak agreement and did not exceed baseline performance. All models predicted clay best, with UK demonstrating particularly strong sensitivity and balanced accuracy for clay and good performance for clay loam. However, sand and loamy sand showed near-chance-level performance. High specificity but low sensitivity for minority classes indicates limited detection of underrepresented textures. Balanced accuracy proved more informative than overall accuracy under class imbalance, reinforcing the UK's superiority and the near-random behavior of MLR and ANN for several classes. Based on these findings, the UK is recommended for operational soil texture mapping in similar settings due to its ability to model spatial autocorrelation. Future sampling should target underrepresented classes through stratified or focused designs to reduce imbalance. Model evaluation should prioritize class-wise metrics (sensitivity, specificity, balanced accuracy, and kappa) rather than relying solely on overall accuracy. Further research should explore hybrid spatial-machine learning approaches, imbalance-aware strategies, and expanded covariate sets to improve discrimination of minority classes. The results highlight the importance of modeling spatial dependence in digital soil mapping. Reliable texture maps can enhance decision-making in precision agriculture, irrigation, erosion control, and land-use planning, including targeted soil amendment strategies for problematic textures such as sandy or saline soils. However,



uncertainty in minority classes underscores the need for additional field validation or denser sampling in affected areas. Overall, improvements in predictive mapping depend not only on algorithm choice but also on sampling design, data quality, and appropriate evaluation metrics.

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**Author Contributions:** JM: Conceptualization; Methodology; Software; Formal analysis; Data curation; Visualization; Writing – original draft; Writing – review & editing; Supervision; Project administration.

HM: Investigation; Resources; Validation; Data curation; Writing – original draft; Writing – review & editing.

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# Seasonal and Site Variations in Heavy Metals Contaminations in Seawater and Fish Organs and Health Risks Assessment

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**Abstract:** Coastal ecosystems are increasingly exposed to land-based pollution from tourism, agriculture, and mining, resulting in heavy metal contamination of seawater and marine organisms. This study quantifies cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) concentrations in seawater and in the organs of the commonly consumed fish *Auxis thazard* and *Selar crumenophthalmus* in the Tañon Strait, Cebu, Philippines. This research further assesses associated health risks and significant differences in metal levels across organs, seasons, sites, and between species. Fish and seawater samples were collected from three sites, Moalboal, Barili, and Toledo, during wet and dry seasons and prepared following the sampling protocols outlined in previous studies. Health risk assessment was based on the calculated Target Hazard Quotient (THQ), Hazard Index (HI), and Estimated Weekly Intake (EWI). Results revealed that Cd exceeded FAO limits in all fish organs, while Cr exceeded only in the gills, and Pb also exceeded limits and was only discovered in the gills. Cu and Zn were within acceptable levels in both species. Metal concentrations were lowest in muscles and highest in the GIT, and were generally higher during the wet season, except for Zn in the gills and GIT. Metal accumulation between species differed significantly only during the dry season ( $p < 0.05$ ), while across organs at the three sites, metal concentrations did not differ significantly ( $p < 0.05$ ). Only Zn and Cu were detected in low amounts in seawater. Also, results showed no significant health risk. Continued monitoring is suggested, particularly during wet seasons, to support food safety.

**Keywords:** Coastal pollution; fish organs; heavy metals; seasonal variation; site variation

## 1. Introduction

Heavy metal contamination of aquatic environments is an emerging global problem due to the persistence, bioaccumulation, and toxicity of these metals [1, 2]. Unlike other pollutants, heavy metals such as Cd, Cr, Cu, Pb, and Zn are non-biodegradable substances that can accumulate in aquatic ecosystems and provide chronic risks to the environment and human health [3]. Various natural and anthropogenic processes contribute to the accumulation of heavy metals in aquatic environments, including agricultural, industrial, and waste-disposal activities [4]. Another contributor is the tourism activities. They have negative impacts on beaches, including physical and ecological degradation [5],



untreated discharges from watercraft [6], and densification of areas along aquatic niches. Fish is a well-known staple food [7, 8], especially in the Philippines, where fish is a crucial component of Filipino diets. It is regarded as an important component of a nutritious diet due to its high-quality properties, vitamins, and essential omega-3 fatty acids [2, 8, 9]. Nonetheless, the health benefits of fish are lost when heavy metals accumulate, as the consumption of contaminated fish and seafood can cause adverse health effects [10], including kidney, liver, brain, muscle, and lung damage [1, 2].

Tañon Strait in Cebu, Philippines, is a nationally protected seascape and is considered one of the most ecologically significant marine corridors in the Philippines [11]. The Tañon Strait, despite its protected status, faces various anthropogenic activities, including mining, agriculture, and tourism, that may introduce heavy metals into aquatic ecosystems. Three coastal areas of Cebu Province, Moalboal, Barili, and Toledo City, represent distinct anthropogenic activities that are potential sources of heavy metals. Moalboal is a tourist destination in the southern province of Cebu, known for its diving and coastal recreation [12]. Infrastructure development, wastewater generation, and boating activities in the area may accelerate degradation of its marine environment [5, 6]. On the other hand, Barili is a major agricultural municipality [13], and in an agricultural context, the use of fertilizers and pesticides is widely documented to prevent post-harvest diseases and improve crop production [14]. Given Barili's agricultural characteristics, such utilization may also be practiced in the area, leading to potential heavy metal runoff into coastal zones, especially during heavy rainfall. Toledo City is linked to mining operations [15], which are perceived as major contributors of metal-rich effluents. Collectively, these activities can lead to heavy metal accumulation in seawater, which may then be transferred to fish consumed by local communities. Since fish readily absorb heavy metals through their gills, digestive tracts, and muscle tissues [16], it is important to assess their levels of contamination. Seasonal factors have a greater impact on metal concentrations, as rainfall and hydrological variations alter pollutant transport and accumulation [17].

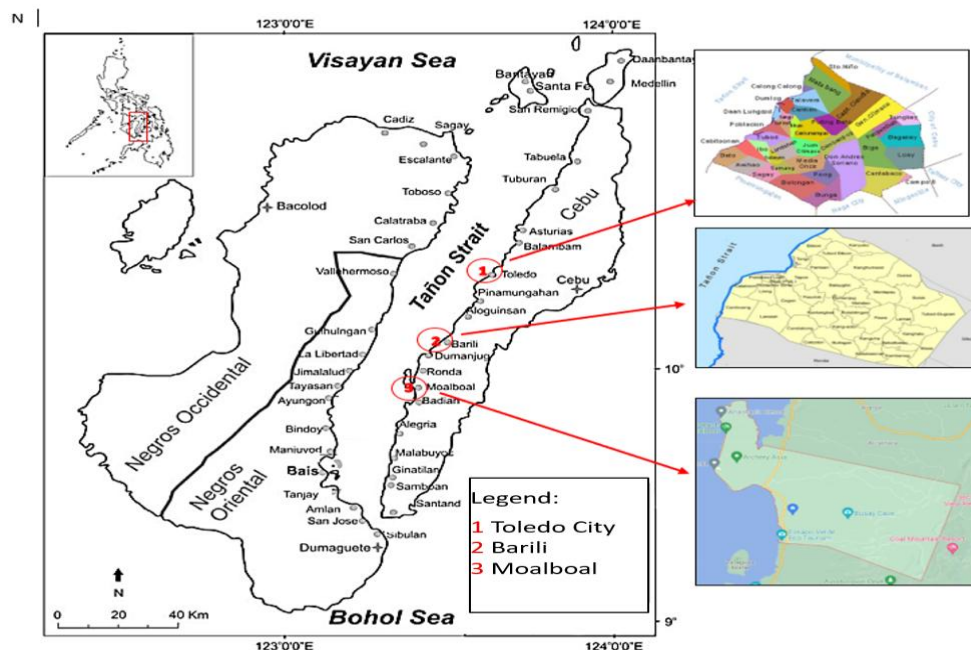
Despite the ecological and economic importance of the Tañon Strait, studies about heavy metal pollution in the aquatic environment of Cebu Province are scarce, particularly on human health risks.

While several studies in Philippine regions and abroad have reported heavy metal contamination in aquatic environments (e.g., [1, 2, 18]), they have not collectively examined the sources of heavy metal pollution, such as tourism, agriculture, and mining. No published reports have quantified heavy metal concentrations in locally consumed fresh fish in this area, nor have hazard index approaches been used to assess potential health risks to consumers. This knowledge gap is critical, given local communities' dependence on fish as a staple food. Thus, the current study aims to assess heavy metal levels in two commonly consumed fresh fish, *Auxis thazard* (*A. thazard*) and *Selar crumenophthalmus* (*S. crumenophthalmus*), and seawater across three identified sites and two seasons (dry and wet). The findings could help evaluate food safety for local communities and serve as a scientific basis for environmental monitoring, public health protection, and policymaking.

## 2. Materials and Methods

### 2.1 Study Sites and Sample Collection

The study was conducted at the three municipalities along the Tañon Strait, a nationally protected seascape and one of the most ecologically significant marine corridors in the Philippines [11]. The Tañon Strait is still exposed to increasing anthropogenic pressures, despite its protected status, including tourism, agricultural practice, and mining, which are potential sources of heavy metal accumulation in the marine environment. Three municipalities in Southern Cebu were selected to represent the said anthropogenic activities: Toledo City, Barili, and Moalboal (Figure 1). Fish and seawater samples were collected at the three identified sites during two seasons: the dry season (April and May) and the wet season (October and November). These samples were collected concurrently at each site to allow seasonal and site comparisons.



**Figure 1.** Map of the three study sites along the Tañon Strait in Southern Cebu, Philippines, Toledo City, Barili, and Moalboal, selected to represent areas exposed to mining, agricultural, and tourism activities, respectively.

## 2.2 Fish Sampling

Two fish species, *Selar crumenophthalmus* and *Auxis thazard*, which are commonly consumed by local communities and abundant in the Tañon Strait, were selected for analysis. Fish samples were obtained directly from small-scale fishermen operating at each identified site. Approximately 2 kg of each fish species was collected during the sampling period at each site, placed in pre-cleaned polyethylene bags properly labeled with the species, site, and season, and transported in containers with ice. Total length and body weight were recorded (mean  $\pm$  SD total length: *A. thazard*:  $24.3 \pm 3.1$  cm; *S. crumenophthalmus*:  $19.8 \pm 2.7$  cm; mean  $\pm$  SD body weight: *A. thazard*:  $142 \pm 22$  g; *S. crumenophthalmus*:  $98 \pm 17$  g), and samples were stored at  $-20$  °C until further processing [10]. Before analysis, frozen samples were thawed and dissected using stainless-steel instruments [19]. Gills, muscle tissues, and GIT were removed and pooled to obtain 250g composite samples per organ, species, and site. A total of 18 composite organ samples were prepared. Fish organ digestion followed the perchloric acid method described by [20]. Each composite sample was placed in a 500 mL Teflon beaker, treated with 50 mL of concentrated nitric acid ( $\text{HNO}_3$ , 65%), and heated for 45 min to remove oxidizable material. After cooling, 25 mL of perchloric acid ( $\text{HClO}_4$ ) was added, and heating was continued until white fumes appeared. Then, 100 mL of distilled water was added, and the solution was reheated to eliminate residual gases. Thereafter, the solution was filtered with Whatman No. 2 filter paper and set aside for heavy metal analysis.

## 2.3 Seawater Sampling

Seawater samples were collected using the procedure of [13]. Polyethylene sampling bottles were pre-cleaned with 10% (v/v)  $\text{HNO}_3$  and rinsed thoroughly with deionized water before sampling. At each site, seawater was collected from the three identified sampling points approximately 10 cm below the surface, then combined to form a composite sample from which a 500 mL sample was obtained for analysis during each sampling period. Samples were transported in an ice-filled container to the university's laboratory and stored in a freezer at  $-20$  °C until laboratory analysis [21].

## 2.4 Heavy Metal Analysis

Concentrations of Cd, Cr, Cu, Zn, and Pb in fish organs and seawater were determined using atomic absorption spectrophotometry (AAS) at the FAST Laboratory. Metal quantification was performed using metal-specific hollow cathode lamps under optimized operating conditions, following standard analytical procedures [22].

## 2.5 Human Health Risk Assessment

Risk assessment was performed using the THQ, HI, and TWI. The THQ was calculated using Equation 1 based on Salam (2019):

$$THQ = \frac{EF * ED * FIR * C}{RfD * WAB * TA} \times 10^{-3} \quad (1)$$

where: EF (exposure frequency) = 365 days/year, ED refers to exposure duration = 71 years, the average lifetime of the Filipino population, FIR (fish ingestion rate) of an adult Filipino = 93.85 g/day [23], C represents HMs content in fish muscle tissue (mg/kg wet weight), particularly the higher value between the wet and dry season for each metals to ensure a conservative estimation of non-carcinogenic health risk, RfD (oral reference dose) based on [24]: Cd (0.001), Cr (1.5), Cu (0.040), Pb (0.0035), Zn (0.300), in mg/kg/day, WAB (average weight) = 65 kg for Filipino adults [25], and TA (average period of exposure for non-carcinogens) = 365 days/year  $\times$  ED. For metal concentrations below the level of detection, half the detection limit would be used for calculation [26].

The hazard index (HI) is calculated using Equation 2, based on the study of [27], to assess the possible risk of adverse health effects from a combination of toxic metals:

$$HI = \sum_i (THQ_i) = THQ_{Cd} + THQ_{Cr} + THQ_{Cu} + THQ_{Pb} + THQ_{Zn} \quad (2)$$

The estimated daily intake of heavy metals was calculated using Eq. 3, used by [22]. Estimated daily intake (EDI) with modification in the units:

$$EDI_{HM} = \frac{HM \text{ concentration } \left(\frac{mg}{kg}\right) \times \text{consumption rate } \left(\frac{kg}{d}\right)}{\text{body weight (kg)}} \quad (3)$$

The estimated weekly intake of heavy metals for a 65 kg person was calculated using Eq. 4 below:

$$EWI_{HM} / 65 \text{ kg bw} = \frac{HM \text{ concentration } \left(\frac{mg}{kg}\right) \times \text{consumption rate } \left(\frac{kg}{d}\right) \times 7}{65 \text{ kg body weight}} \quad (4)$$

Similarly, the heavy metal concentration used in the calculations was based on the higher median values between the wet and dry seasons. The daily fish consumption rate was set at 93.85 g/day [23], while the reference body weight for Filipino adults was 65 kg [25]. Using the EDI, the EWI for heavy metals for a 65 kg body weight was computed and compared with the corresponding TWI values derived from the recommended intake. The recommended intake values applied in this study were sourced from [22] for Cd, Pb, and Zn; from the European Food Safety Authority [28] for Cu; and from [29] for Cr.

## 2.6 Data Analysis

A series of Kruskal-Wallis H-tests was conducted to assess significant differences in heavy metal levels in fish tissues across sampling sites, and a Mann-Whitney U-test was used to compare species and seasons. Differences in organs per species were determined using the Friedman test, with pairwise comparisons for significant results. Analysis was done using SPSS 22. The Wilcoxon Signed Rank Test was used to analyze the difference in Cr levels between the gills and the GIT. It should be noted that the statistical comparisons are performed on composite-level data: each composite sample was prepared by pooling tissues from approximately 10–15 individual fish of the same species collected at each site per sampling event, yielding one

composite value per organ, per species, per site, per season. Each composite was subjected to duplicate analytical runs, and the mean of the two determinations was used for analysis. The *n* for the statistical tests (Kruskal-Wallis, Mann-Whitney, Friedman) therefore refers to the number of composite samples per group (*n* = 3 per site comparison; *n* = 6 per species or season comparison across all sites and seasons), which limits the inferential power of the tests and precludes true biological replication. Results should therefore be interpreted as site- and season-level trends rather than population-level estimates.

### 3. Results and Discussion

#### 3.1 Heavy Metal Concentrations in Fish Organs According to Species during Wet and Dry Seasons

The concentrations of Cd, Cr, Cu, Pb, and Zn in the gills, GIT, and muscle tissues of *A. thazard* and *S. crumenophthalmus* during the wet and dry seasons are presented in Table 1. Cadmium concentrations in all analyzed tissues of both fish species exceeded the FAO permissible limit of 0.05 mg/kg wet weight, indicating widespread Cd contamination in the study area. Cadmium is of particular concern due to its slow excretion rate and strong bioaccumulative potential [30]. Although Cd concentrations exceeded permissible limits, the levels observed in this study were lower than those reported in other regions of the Philippines. For example, Cd concentrations in marine fish from Manila Bay ranged from 38.262 to 56.924 mg/kg during the wet season and from 1.828 to 2.425 mg/kg during the dry season [31]. Similarly, Cd concentrations of 9.5–17 mg/kg were reported in marine fish collected from Agusan and Butuan [32]. Chromium concentrations also exceeded the allowable limit of 1.0 mg/kg in the gills of both species and in the GIT of *S. crumenophthalmus*.

In contrast, Cu and Zn concentrations in all organs of both species remained within the FAO/WHO-recommended limits during both sampling seasons. Cu and Zn are essential trace elements; their accumulation patterns vary among organs and seasons, reflecting both physiological regulation and environmental availability. Chromium showed preferential accumulation in gill tissues, suggesting waterborne exposure, consistent with the studies by [33] and [34]. Cr's affinity for gill epithelia stems from their large surface area and permeability [28]. Lead (Pb) was detected only in the GIT of *A. thazard* and only during the dry season, with concentrations exceeding the permissible limit of 0.3 mg/kg wet weight. Pb concentrations in all other tissues and sampling periods were below the limit of detection. Across both *Auxis thazard* and *Selar crumenophthalmus*, the GIT and gills consistently exhibited higher metal concentrations than muscle tissues. Such a pattern is widely documented in marine fish and is attributed to direct gill exposure to contaminated water and dietary exposure through ingestion of sediment and prey [39; 40]. In contrast, muscle tissue generally had the lowest metal concentrations, likely because of its limited role in metal sequestration. This pattern of reduced accumulation in muscle tissues aligns with the findings of [22], [35], and [36], while heavy metal accumulation in the GIT suggests dietary exposure through ingestion of contaminated prey or sediments [30].



Table 1. Heavy metal concentrations in fish organs of *A. thazard* and *S. crumenophthalmus* during wet and dry seasons

| Season            | Fish species               | Cd         |                    |                    | Cr                |       |     | Cu         |                    |                    | Pb                |   |     | Zn         |                    |                   |                    |
|-------------------|----------------------------|------------|--------------------|--------------------|-------------------|-------|-----|------------|--------------------|--------------------|-------------------|---|-----|------------|--------------------|-------------------|--------------------|
|                   |                            | G          | M                  | GIT                | G                 | M     | GIT | G          | M                  | GIT                | G                 | M | GIT | G          | M                  | GIT               |                    |
| Wet               | <i>A. Thazard</i>          | Med        | 0.2 <sup>a</sup>   | 0.069 <sup>b</sup> | 0.43 <sup>a</sup> | 1.86  | *   | .510       | 1.02 <sup>a</sup>  | 0.5 <sup>b</sup>   | 2.61 <sup>a</sup> | * | *   | *          | 10.29 <sup>b</sup> | 5.64 <sup>c</sup> | 11.57 <sup>a</sup> |
|                   |                            | Min        | 0.15               | 0.05               | 0.23              | .040  | *   | *          | 0.86               | *                  | *                 | * | *   | *          | 7.76               | 4.52              | 9.11               |
|                   |                            | Max        | 0.24               | 0.09               | 0.73              | 2.41  | *   | 0.51       | 2.41               | 0.73               | 8.10              | * | *   | *          | 13.45              | 6.89              | 16.69              |
|                   | <i>S. crumenophthalmus</i> | Med        | 0.22 <sup>a</sup>  | 0.06 <sup>b</sup>  | 0.34 <sup>a</sup> | 2.50  | *   | *          | 0.95 <sup>ab</sup> | 0.42 <sup>b</sup>  | 1.2 <sup>a</sup>  | * | *   | *          | 10.24 <sup>a</sup> | 6.21 <sup>b</sup> | 9.5 <sup>a</sup>   |
|                   |                            | Min        | 0.15               | 0.03               | 0.25              | 0.28  | *   | *          | 0.83               | 0.18               | *                 | * | *   | *          | 5.73               | 5.73              | 7.41               |
|                   |                            | Max        | 0.28               | 0.25               | 0.61              | 3.09  | *   | *          | 1.13               | 0.52               | 7.79              | * | *   | *          | 13.92              | 6.95              | 14.98              |
| Dry               | <i>A. thazard</i>          | Med        | 0.07 <sup>b</sup>  | 0.05 <sup>b</sup>  | 0.24 <sup>a</sup> | 1.62  | *   | 1.02       | 0.63 <sup>b</sup>  | 0.64 <sup>b</sup>  | 1.55 <sup>a</sup> | * | *   | 0.88       | 24.31 <sup>a</sup> | 4.88 <sup>b</sup> | 22.45 <sup>a</sup> |
|                   |                            | Min        | 0.01               | 0.01               | 0.02              | 1.18  | *   | 0.64       | 0.03               | 0.05               | 0.08              | * | *   | 0.88       | 4.11               | 1.69              | 12.07              |
|                   |                            | Max        | 0.23               | 0.09               | 0.58              | 1.84  | *   | 1.51       | 1.34               | 1.64               | 3.16              | * | *   | 0.88       | 55.84              | 9.21              | 36.89              |
|                   | <i>S. crumenophthalmus</i> | Med        | 0.10 <sup>ab</sup> | 0.04 <sup>b</sup>  | 0.33 <sup>a</sup> | 0.787 | *   | 0.23       | 0.36 <sup>b</sup>  | 0.49 <sup>ab</sup> | 1.83 <sup>a</sup> | * | *   | *          | 11.37 <sup>a</sup> | 7.06 <sup>b</sup> | 21.58 <sup>a</sup> |
|                   |                            | Min        | 0.01               | 0.014              | 0.01              | 0.04  | *   | 0.02       | 0.019              | 0.034              | 0.02              | * | *   | *          | 4.289              | 2.628             | 4.24               |
|                   |                            | Max        | 0.09               | 0.09               | 0.746             | 2.15  | *   | 1.18       | 1.151              | 1.184              | 4.102             | * | *   | *          | 27.3               | 11.62             | 42.84              |
| Permissible Limit |                            | 0.05       |                    |                    | 1.0               |       |     | 30         |                    |                    | 0.3 mg/kg         |   |     | 30         |                    |                   |                    |
|                   |                            | FAO (1983) |                    |                    | WHO/FAO (2011)    |       |     | FAO (1983) |                    |                    | WHO/FAO (2022)    |   |     | FAO (1983) |                    |                   |                    |

Note: \* - Less than detection level; Median values within the metal across the different organs under the same species, followed by the same letters are not significantly different from each other.

### 3.2 Results of Significant Differences in Metal Concentrations Across Organs, Between Species per Season, and Between Seasons

The results of significant differences in metal concentrations when compared among organs per species, between species per season, and between seasons in each species are reflected in Table 2. Concerning organ-specific distribution, metal concentrations differ significantly among fish organs for all analyzed metals in both species ( $p < 0.05$ ). In general, the GIT had the highest metal concentrations, significantly higher than those in the gills ( $p < 0.05$ ), while muscle tissues consistently showed the lowest concentrations. An exception was observed for Cr, which accumulated at significantly higher levels in the gills compared to other organs. This is consistent with the findings of [36] and [37], who similarly demonstrated variations in metal accumulation across different fish anatomical components. For species-based differences, metal concentrations between *A. thazard* and *S. crumenophthalmus* did not differ significantly ( $p < 0.05$ ) across all analyzed organs during the wet season ( $p > 0.05$ ). However, during the dry season, significant differences were observed ( $p < 0.05$ ). *A. thazard* exhibited significantly higher Cu concentrations in the gills and muscle tissues. These interspecific differences may be partly influenced by body size, as *A. thazard* was consistently larger than *S. crumenophthalmus*, which could contribute to higher metal accumulation in certain tissues, consistent with well-documented size-dependent bioaccumulation in marine teleosts [16]. However, the contrasting pattern observed for Zn, in which *S. crumenophthalmus* showed significantly higher concentrations in muscle tissues, suggests that size alone does not fully explain metal uptake, and that species-specific differences in feeding behavior, habitat use, and metal regulation may also play important roles. Species differences, particularly during the dry season, suggest that physiological differences in feeding behavior, habitat use, and metabolic capacity may influence metal uptake under lower-dilution conditions. The absence of significant differences across species during the wet season may indicate increased metal dispersion and a more uniform exposure, driven by enhanced freshwater input and hydrodynamic mixing. Regarding seasonal variation within species, for *A. thazard*, Cd concentrations in the gills and GIT, as well as Cr concentrations in the gills, were significantly higher during the wet season ( $p < 0.05$ ). Zinc concentrations in the gills and GIT were significantly higher during the dry season. For *S. crumenophthalmus*, significant seasonal differences were observed in gill Cd, Cr, and Cu concentrations, with higher levels during the wet season ( $p < 0.05$ ). No significant seasonal differences were observed for muscle tissues. Each species showed significantly higher metal levels during the wet season, likely due to increased runoff, sediment resuspension, and metal mobilization from terrestrial sources during heavy rainfall [38, 39], while Zn levels were unexpectedly higher during the dry season. These results support the findings of [40], who documented seasonal peaks in Zn levels during warmer months.

### 3.3 Heavy Metal Concentrations in Fish Organs Across Sampling Sites

The site distribution of heavy metals in fish organs from Moalboal, Barili, and Toledo is presented in Table 3. Among the three sampling sites, Moalboal consistently exhibited the highest concentrations of Cd, Cr, and Zn in gill tissues. Moalboal also showed the highest Cu concentrations in muscle tissues and was the only site where Pb was detected in the GIT of fish. Moalboal, a tourism-dominated area, showed the highest levels of Cd, Cr, and Zn in gills, and Cu in muscle tissue, including the only site detected with Pb in the GIT. This localized Pb presence may reflect ingestion of contaminated particles or prey, consistent with findings that Pb tends to accumulate in digestive tissues due to its affinity for mucosal surfaces and limited systemic distribution [36, 41]. Also, wastewater discharge, boating activities, fuel combustion, and sediment disturbance in coastal tourism, like in Moalboal, likely contribute to heavy metal pollution [6, 42]. Furthermore, the corrosion of metallic vessel components and coastal infrastructure in chloride-rich marine environments may constitute an additional diffuse source of heavy metal inputs in high-traffic tourist areas [43]. At the same time, Cu toxicity to aquatic biota has been demonstrated at concentrations well below those reported here [39].

**Table 2.** Results of significant differences in metal concentrations when compared among organs per species, between species per season, and between seasons in each species.

| Comparison of Heavy Metal Concentrations among organs in each species                             |                     |                   |                     |                |                        |
|---------------------------------------------------------------------------------------------------|---------------------|-------------------|---------------------|----------------|------------------------|
|                                                                                                   | Cd (Wet; Dry)       | Cr (Wet; Dry)     | Cu (Wet; Dry)       | Pb (Wet; Dry)  | Zn (Wet; Dry)          |
| Comparison among organs of <i>A. thazard</i>                                                      |                     |                   |                     |                |                        |
| P-value                                                                                           | <0.001; 0.001;      | <0.001;<0.001     | <0.001;<0.001       | —              | <0.001;<0.001          |
| Comparison among organs of <i>S. crumenophthalmus</i>                                             |                     |                   |                     |                |                        |
| P-value                                                                                           | <0.001; 0.001       | —; 0.008          | <0.001; 0.001       | —              | <0.001; <0.001         |
| Comparison of Heavy Metal Concentrations between species during wet and dry seasons per organ     |                     |                   |                     |                |                        |
|                                                                                                   | Cd (G; M; GIT)      | Cr (G; M; GIT)    | Cu (G; M; GIT)      | Pb (G; M; GIT) | Zn (G; M; GIT)         |
| Comparison between <i>A. thazard</i> & <i>S. crumenophthalmus</i> during the wet season per organ |                     |                   |                     |                |                        |
| P-values                                                                                          | 0.242; 0.180; 0.270 | 0.319; ---        | 0.160; 0.266; 0.590 | —              | 0.671; 0.068; 0.068    |
| Comparison between <i>A. thazard</i> & <i>S. crumenophthalmus</i> during the dry season per organ |                     |                   |                     |                |                        |
| P-values                                                                                          | 0.521; 0.628; 0.584 | 0.696; ---; 0.542 | 0.012; 0.220; 1.00  | —              | 0.389; 0.016; 0.696    |
| Comparison of Heavy Metal Concentrations between seasons in each species per organ                |                     |                   |                     |                |                        |
| Comparison between wet and dry seasons of <i>A. thazard</i> per organ                             |                     |                   |                     |                |                        |
| P-values                                                                                          | <0.001; .215; 0.010 | 0.048; ---; 0.325 | 0.134; 0.185; 0.692 | —              | <0.001 ; 0.755; <0.001 |
| Comparison between wet and dry seasons of <i>S. crumenophthalmus</i> per organ                    |                     |                   |                     |                |                        |
| P-values                                                                                          | 0.013; 0.368; 1.0   | 0.06; ---; ---    | 0.031; 0.267; 0.723 | —              | 0.465; 0.99; 0.465     |

Note: G-Gills, M-Muscles; Bold p-values indicate statistically significant differences at  $\alpha = 0.05$ , while p-values  $\geq 0.05$  are not statistically significant. Dashes (—) denote tests not performed due to data below the detection limit.

**Table 3.** Heavy metals concentration (median; mg/kg) across sites and the results of significant differences

| Site              | Cd         |      |      | Cr             |      |     | Cu         |       |      | Pb             |     |      | Zn         |      |       |
|-------------------|------------|------|------|----------------|------|-----|------------|-------|------|----------------|-----|------|------------|------|-------|
|                   | G          | M    | GIT  | G              | M    | GIT | G          | M     | GIT  | G              | M   | GIT  | G          | M    | GIT   |
| Moalboal          | Med        | 0.2  | 0.06 | 0.4            | 0.3  | 0.1 | 0.91       | 0.22  | 0.74 | *              | *   | 0.88 | 13.11      | 6.82 | 13    |
|                   | Min        | 0.01 | 0.01 | 0              | 0.04 | *   | 0.02       | 0.03  | 0.02 | *              | *   | 0.88 | 1.7        | 2.66 | 2.27  |
|                   | Max        | 0.23 | 0.09 | 0.6            | 2.22 | *   | 1.86       | 1.64  | 3.93 | *              | *   | 0.88 | 38.2       | 55.8 | 37.63 |
| Barili            | Med        | 0.15 | 0.07 | 0.3            | 1.55 | *   | 1.0        | 0.1   | 1.62 | *              | *   | *    | 12.52      | 6.92 | 13.25 |
|                   | Min        | 0.01 | 0.01 | 0              | 0.04 | *   | 0.02       | 0.06  | 0.08 | *              | *   | *    | 0.24       | 2.78 | 2.33  |
|                   | Max        | 0.24 | 0.09 | 0.7            | 3.07 | *   | 2.41       | 1.57  | 7.79 | *              | *   | *    | 36.4       | 55.5 | 42.84 |
| Toledo            | Med        | 0.08 | 0.06 | 0.4            | 1.5  | *   | 0.73       | 0.1   | 0.33 | *              | *   | *    | 12.13      | 7.83 | 11.05 |
|                   | Min        | 0.01 | 0.01 | 0              | 0.04 | *   | 0.02       | 0.05  | 0.08 | *              | *   | *    | 0.24       | 4.29 | 2.63  |
|                   | Max        | 0.28 | 0.25 | 0.8            | 3.09 | *   | 1.34       | 1.44  | 3.58 | *              | *   | *    | 37.02      | 34.9 | 42.04 |
| P-value           | 0.823      | 0.9  | 0.9  | 0.9            | 0.81 | --- | 0.48       | 0.539 | 0.64 | 0.6            | --- | ---  | 0.881      | 0.97 | 0.731 |
| Permissible Limit | 0.05       |      |      | 1              |      |     | 30         |       |      | 0.3 mg/kg      |     |      | 30         |      |       |
|                   | FAO (1983) |      |      | WHO/FAO (2011) |      |     | FAO (1983) |       |      | WHO/FAO (2022) |     |      | FAO (1983) |      |       |



Conversely, higher concentrations of Cr, Cu, and Zn were found in the GIT of samples from Barili than in the other sites. This result suggests dietary exposure associated with agricultural runoff. Agrochemicals are known to contain trace metals that can enter aquatic systems through erosion and leaching, particularly during the wet season [14]. Similarly, the highest Cd concentration in the GIT and the highest Zn levels in muscle tissues were observed in the Toledo samples, whereas Cu concentrations across all organs were comparatively lower at this site. This finding points to the complexity of metal sources and transport mechanisms, as contaminants from mining activities depend on waste management practices, hydrodynamics, and sediment characteristics. Despite these site-specific variations in metal concentrations, statistical analysis showed no significant differences in heavy metal levels among the three sampling sites across all fish organs ( $p > 0.05$ ). The observed trends provide valuable insight into localized contamination patterns and emphasize the need for site-specific monitoring and management strategies.

### 3.4 Heavy Metal Concentrations in Seawater

Heavy metal concentrations in seawater across the three sampling sites and two seasons are presented in Table 4. Among the analyzed metals, only Zn and Cu were detected, and only during the dry season. Cr, Cd, and Pb were below detection limits in both wet and dry seasons across sites. Zinc concentrations ranged from 0.008 to 0.160 mg/L, with the highest recorded in Barili (0.160 mg/L), followed by Toledo (0.154 mg/L), and the lowest in Moalboal (0.008 mg/L). Copper concentrations were relatively low, ranging from 0.008 to 0.010 mg/L, with the highest value in Toledo and slightly lower concentrations in Barili, while Cu was not detected in Moalboal. All detected concentrations of Zn and Cu were substantially below the permissible limits for coastal waters established by FAO/WHO guidelines. No metals were detected in seawater during the wet season at any of the sampling sites. This suggests relatively good ambient water quality in the Tañon Strait during the study period. The results below detection limits of Cd, Cr, and Pb in seawater during both seasons are consistent with recent studies reporting low dissolved metal concentrations in coastal waters, particularly in areas with strong hydrodynamic mixing and dilution [48; 49; 50]. The absence of detectable Cd, Cr, and Pb in seawater, despite exceeding FAO limits in fish tissues, reflects bioaccumulation processes and multiple exposure pathways; even when dissolved concentrations are below detection limits (0.001–0.005 mg/L), heavy metals may still accumulate in fish, as high bioconcentration factors (BCF) enable uptake over time. For instance, Biota-sediment accumulation factors (BSAF;  $10^3$ – $10^5$ ) for Cd in benthic-feeding fish typically range from 0.1 to 10 [44], and dietary exposure through ingestion of sediment particles and contaminated prey is a primary route of Cd and Pb accumulation in the GIT.

**Table 4.** Heavy metal concentrations in seawater (mg/L) across three sites during the wet and dry seasons

| Sites             | Cd        |        | Cr        |        | Cu       |       | Pb       |        | Zn     |       |
|-------------------|-----------|--------|-----------|--------|----------|-------|----------|--------|--------|-------|
|                   | Wet       | Dry    | Wet       | Dry    | Wet      | Dry   | Wet      | Dry    | Wet    | Dry   |
| Moalboal          | <0.001    | <0.001 | <0.005    | <0.005 | <0.003   | 0.006 | <0.005   | <0.005 | <0.005 | 0.008 |
| Barili            | <0.001    | <0.001 | <0.005    | <0.005 | <0.003   | 0.008 | <0.005   | <0.005 | <0.005 | 0.160 |
| Toledo            | <0.001    | <0.001 | <0.005    | <0.005 | <0.003   | 0.01  | <0.005   | <0.005 | <0.005 | 0.154 |
| Permissible Limit | 0.003mg/L |        | 0.05 mg/L |        | 0.5 mg/L |       | 0.01mg/L |        | 5 mg/L |       |
| WHO (2020-2026)   |           |        |           |        |          |       |          |        |        |       |

Zn and Cu were detected only during the dry season. This may be attributed to reduced water volume, increased contaminants, and lower dilution capacity, which can enhance the detectability of dissolved trace metals [20; 45]. Barili showed a higher Zn concentration than other sites, which may reflect inputs from agricultural runoff, since fertilizers containing Zn are commonly used in farming [14]. Similarly, Toledo showed slightly higher Cu levels than other sites. Recent research on coastal pollution attenuation suggests that biosurfactant-producing bacteria can enhance bioremediation of organic co-pollutants in marine coastal

zones [45], indicating that the natural microbial community may partly modulate metal bioavailability through interactions with organic matter. Comprehensive contaminant monitoring frameworks, including radiological parameters, are also essential for full characterization of aquatic environmental health [45]. Despite the detection of some metals in seawater, these metal concentrations did not directly link to fish organs' heavy metal accumulations. For example, although Cu was detected at the Toledo site, fish collected there showed lower Cu levels than those from Moalboal. This discrepancy shows that metal bioaccumulation in fish is not only dependent on dissolved metal concentrations in seawater but is strongly influenced by additional exposure pathways, including dietary intake, sediment-associated metals, and species-specific physiological regulation [39; 33]. Furthermore, the detection limits of the seawater analysis (0.001–0.005 mg/L) may not be sufficiently sensitive to detect chronically low but biologically meaningful dissolved metal concentrations. Bioconcentration factors (BCF) and biota-sediment accumulation factors (BSAF) reported in the literature indicate that fish can accumulate metals from both water and sediment at levels several orders of magnitude higher than ambient dissolved concentrations [40; 33]. The absence of sediment metal data in this study constitutes a limitation, as sediment serves as a primary reservoir and secondary source of heavy metals in coastal systems; future studies should incorporate sediment analysis to enable source attribution and more robust interpretation of tissue burdens [45].

### 3.5 Health Risk Assessment

#### 3.5.1 Target Hazard Quotient (THQ) and Hazard Index (HI)

The calculated THQ values for Cd, Cr, Cu, Pb, and Zn in *A. thazard* and *S. crumenophthalmus* are presented in Table 5. All THQ values for the identified metals were below the critical threshold of 1.0, indicating consumption of the two species considered in this study is safe with respect to non-carcinogenic health risks. As shown in the results, Cd exhibited the highest THQ values in both species, indicating relatively higher concentrations in fish tissues. Pb and Cr concentrations in muscle tissues of both species were below the detection limit of 0.1 mg/kg. To avoid underestimating exposure, a conservative proxy value equal to half the detection limit (0.05 mg/kg) was used in THQ and HI calculations. Using this approach, Pb and Cr yielded THQ values of 0.0206 and  $4.81 \times 10^{-5}$ , respectively, for both species. While these values indicate negligible non-carcinogenic risk, using proxy concentrations ensures that potential exposure is accounted for even when metals are below the detection limit. Although health risk assessments based on THQ and HI indicate safe intake levels, continuous and excessive consumption of essential metals may still cause adverse effects, such as gastrointestinal disturbances or interference with the absorption of other micronutrients [46]. Chromium obtained the lowest THQ among all metals assessed. However, this analysis did not distinguish between trivalent chromium [Cr(III)], which is an essential nutrient, and hexavalent chromium [Cr(VI)], which is highly toxic and carcinogenic [48]. As such, this raises uncertainty and suggests that health risks could be underestimated in environments affected by industrial or mining activities. As a conservative sensitivity analysis, if all detected Cr were assumed to be Cr(VI) (worst-case scenario), the oral reference dose for Cr(VI) is substantially lower (0.003 mg/kg/day, USEPA) than the value used here for total Cr (1.5 mg/kg/day). Under this assumption, the THQ for Cr (VI) would be approximately 0.048, which remains below 1.0 and still does not indicate a significant non-carcinogenic health risk from muscle tissue consumption. Nevertheless, communities near Toledo City's mining operations face a higher potential for Cr(VI) exposure through gill and GIT ingestion, and future studies should perform Cr speciation analysis to refine risk estimates. The present results based on the calculated HI, which represents the combined non-carcinogenic risk from simultaneous exposure to all assessed metals, are similar to those reported by [22]. Despite exceeding permissible limits for Cd and Cr in certain fish organs, the calculated THQ and HI values remained below the threshold value. However, Cd and Pb contributed most to cumulative exposure, consistent with their high toxicity and bioaccumulative nature. Chronic exposure to Cd has been linked to nephrotoxicity, bone demineralization, and carcinogenic effects, while Pb exposure poses significant risks to neurological development, particularly in children [48].

**Table 5.** The THQ of each heavy metal and HI per species.

| Heavy Metals | THQ               |                            |
|--------------|-------------------|----------------------------|
|              | <i>A. thazard</i> | <i>S. crumenophthalmus</i> |
| Cd           | 0.1011            | 0.0866                     |
| Cr           | 4.81E-05          | 4.81E-05                   |
| Cu           | 0.0231            | 0.0177                     |
| Pb           | 0.0206            | 0.0206                     |
| Zn           | 0.0271            | 0.0340                     |
| HI           | 0.1927            | 0.1796                     |

### 3.5.2 Estimated Daily Intake (EDI), Estimated Weekly Intake (EWI), and comparison with Tolerable Weekly Intake (TWI)

The EDI, EWI, and corresponding comparisons with TWI values for Cd, Cr, Cu, Pb, and Zn in *A. thazard* and *S. crumenophthalmus* are presented in Table 6. As shown in the Table, EDI values for *A. thazard* ranged from 0.0047 to 0.5293 mg/kg body weight/day. Similarly, EDI values for *S. crumenophthalmus* ranged from 0.0047 to 0.6626 mg/kg body weight/day, following the same trend: lowest for Pb and Cr and highest for Zn. The similarity in Pb and Cr EDIs between species may be due to concentrations that are below detection limits in muscle tissue. Furthermore, the calculated EWIs in this study for both fish species, based on a 65-kg adult body weight, were substantially below the corresponding TWI or PTWI values for all assessed metals. This indicates that weekly exposure to heavy metals from consuming either species is unlikely to cause adverse health effects.

**Table 6.** Results of EDI, EWI, and TWI of selected heavy metals in fish muscles.

| Heavy Metals | Fish Species            | Fish Muscles Conc. (mg/kg) | EDI (mg/day /kg ) | EDI (mg/day/65kg bw ) | EWI (mg/week/ 65kg bw ) | PTWI (mg/ week/kg bw | PTWI (mg/ week 65 kg bw) |
|--------------|-------------------------|----------------------------|-------------------|-----------------------|-------------------------|----------------------|--------------------------|
| Cd           | <i>Auxis thazard</i>    | 0.069                      | 0.0065            | 0.0001                | 0.0007                  | 0.007                | 0.455                    |
|              | <i>Selar</i>            | 0.060                      | 0.0056            | 0.0001                | 0.0006                  |                      |                          |
|              | <i>crumenophthalmus</i> |                            |                   |                       |                         |                      |                          |
| Cr           | <i>Auxis thazard</i>    | 0.050                      | 0.0047            | 0.0001                | 0.0005                  | 0.0233               | 1.5145                   |
|              | <i>Selar</i>            | 0.050                      | 0.0047            | 0.0001                | 0.0005                  |                      |                          |
|              | <i>crumenophthalmus</i> |                            |                   |                       |                         |                      |                          |
| Cu           | <i>Auxis thazard</i>    | 0.640                      | 0.0601            | 0.0009                | 0.0065                  | 3.5                  | 227.5                    |
|              | <i>Selar</i>            | 0.490                      | 0.0460            | 0.0007                | 0.0050                  |                      |                          |
|              | <i>crumenophthalmus</i> |                            |                   |                       |                         |                      |                          |
| Pb           | <i>Auxis thazard</i>    | 0.050                      | 0.0047            | 0.0001                | 0.0005                  | 0.025                | 1.625                    |
|              | <i>Selar</i>            | 0.050                      | 0.0047            | 0.0001                | 0.0005                  |                      |                          |
|              | <i>crumenophthalmus</i> |                            |                   |                       |                         |                      |                          |
| Zn           | <i>Auxis thazard</i>    | 5.640                      | 0.5293            | 0.0081                | 0.0570                  | 7                    | 455                      |
|              | <i>Selar</i>            | 7.060                      | 0.6626            | 0.0102                | 0.0714                  |                      |                          |
|              | <i>crumenophthalmus</i> |                            |                   |                       |                         |                      |                          |

The low EWI values compared to TWI thresholds suggest that fish muscle consumption may not pose adverse health risks. However, it must be emphasized that the health risk calculations above are based exclusively on muscle tissue concentrations, which is standard practice for dietary exposure assessment. Since Cd exceeded FAO permissible limits in all organs of both species and Cr exceeded limits in the gills and GIT, consumers who regularly ingest whole fish, including viscera and gills, face substantially higher metal

exposures than estimated by the THQ and HI presented. Although a full quantitative risk estimate for whole-fish consumption is beyond the scope of the current dataset, the exceedances of Cd (up to 0.43 mg/kg in GIT, 8.6× the limit) and Cr (up to 2.50 mg/kg in gills, 2.5× the limit) in organ tissues indicate that the public health advisory language of “no significant risk” applies only to muscle tissue consumption and should not be generalized. The metal contents detected in gills and GIT indicate potential risks to consuming whole fish or viscera, as some locals in some coastal communities practice. These findings underscore the importance of advisories and information dissemination for organ-specific consumption and targeted public health interventions.

#### 4. Conclusions

This study demonstrates the accumulation of the identified metals, Cd, Cr, Cu, Pb, and Zn, which vary by organ, species, and season. Although results showed no significant health risk, and metal accumulation showed site-specific patterns but was not significant, there is still a need for site-specific, seasonally responsive monitoring programs. Local government units (LGUs), in coordination with other government agencies, should implement annual fish organ testing in high-risk zones, with sampling intensified during peak runoff periods. Public health advisories must be issued to discourage the consumption of high-risk organs, such as viscera, especially among vulnerable populations, and to remove gills and GIT during cooking to reduce heavy metal contamination. Community education campaigns should be launched to empower fisherfolk and consumers with knowledge about contamination risks and safe preparation practices. Emerging colorimetric and machine-learning-based detection technologies offer cost-effective, rapid approaches for routine heavy metal surveillance that could complement conventional AAS analysis in future monitoring programs [38]. These actions will not only mitigate exposure to toxic metals but also enhance environmental resilience in coastal communities. Moreover, the absence of sediment metal data limits the attribution of fish tissue contamination to specific land-based anthropogenic sources (mining, agriculture, or tourism). Future studies in the Tañon Strait should incorporate concurrent sediment sampling to enable robust source apportionment and to quantify biota-sediment accumulation factors (BSAF).

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# *In vitro* Regeneration of Red Dragon Fruit (*Hylocereus polyrhizus*) Stem Segments

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**Abstract:** Traditionally, the propagation of dragon fruit (*Hylocereus* spp.) is carried out through seed or vegetative cuttings. However, propagation by cuttings is too slow to meet the demand for large-scale plantations and is not genetically true-to-type. With the fruits' increasing demand, an effective *in vitro* propagation of dragon fruit was developed to produce high – quality planting materials for large-scale production of dragon fruit. This study was conducted to establish the appropriate concentration of bleach for surface sterilization and determine the effective level of 6-benzylaminopurine (BAP) for the shoot initiation of dragon fruit explants *in vitro*. Surface sterilization of the explants using commercial bleach (a.i. sodium hypochlorite) at a concentration of 50% for 2 min significantly reduced contamination (33.33%) and yielded higher percent survival (54.99%) compared to 20% and 10% concentrations. The addition of different levels of BAP to Murashige and Skoog (MS) medium resulted in shoot regeneration, with early shoot initiation (20 days) and the highest shoot length (9.89mm) observed at 3 mg L<sup>-1</sup> BAP after 4 weeks of incubation. The number of shoots was numerically higher at 5 mg L<sup>-1</sup>, but this difference was not statistically significant. These results can be recommended for establishing dragon fruit explants *in vitro* at the surface sterilization and shoot initiation stages. Subsequent rooting and acclimatization stages, which were beyond the scope of this study, are still required before clean, off-season planting materials can be produced for large-scale production.

**Keywords:** *In vitro* dragon fruit; surface sterilization; BAP

## 1. Introduction

Dragon fruit production has become a lucrative industry in the Philippines. What used to be a backyard ornamental plant has now been propagated, cultivated, and fruit has been reaped, making what is now “The Philippines Dragon Fruit Industry” worth millions of pesos. Initiatives can be attributed to increased productivity in farms, since dragon fruit is recognized as a high-value crop [1]. The booming dragon fruit industry in the Philippines is hampered by problems with pests and diseases, inferior varieties, postharvest losses during transport, and the seasonality of dragon fruit, which results in limited supply of fresh fruit during the off season as well as of quality planting materials. To address these problems, science and technology (S&T) gaps must be identified to develop S&T interventions as solutions [1]. Traditionally, the propagation of *Hylocereus* spp. is carried out

through seed or vegetative cuttings [2]. However, propagation by cuttings is too slow to meet the demand for large-scale plantations due to a shortage of young nodes, and seeds are not genetically true-to-type, although the selected seedlings may resemble their parental plant in fruit quality [3]. Cross-pollination is also necessary for fruit setting. Hence, suitable planting materials were not available for large-scale cultivation of dragon fruit [4].

Plant tissue culture, or *in vitro* culture, is an alternative method for large-scale production of plantlets. Micropropagation of cacti facilitates the production of large numbers of plants from a relatively small stock [4]. Propagation of dragon fruit using tissue culture techniques will work well if the required conditions are met; these include selecting explants as planting material, sterilization, using suitable culture medium, maintaining aseptic conditions, and good temperature regulation. Once the explants were established on the initiation media, they were transferred to multiplication media for large-scale production [5]. Although conventional methods of propagation for these cacti are satisfactory, a micropropagation protocol has been developed to enable rapid release of improved clones or genotypes [3]. This study, therefore, was undertaken to regenerate dragon fruit *in vitro* to produce high-quality, off-season planting materials, facilitate disease-free exportation, and develop *in vitro* techniques, such as explant surface sterilization.

Globally, dragon fruit (pitahaya) has expanded from a minor specialty crop into an economically important fruit now cultivated across Asia, the Americas, and the Mediterranean. The rising demand for uniform, disease-free planting material has made *in vitro* propagation an increasingly relevant technology for tropical fruit crops. Reported micropropagation protocols, however, span several *Hylocereus* species and a wide range of cytokinin levels: Drew and Azimi-Tabrizi [3] induced bud development in *H. undatus*, Viñas et al. [6] tested 15, 30, and 60  $\mu\text{M}$  BAP in *H. costaricensis*, and Shimomura and Fujihara [2] worked with *H. trigonus*. Because responses to cytokinins and to surface sterilants can differ substantially among *Hylocereus* species, comparisons across these studies must be interpreted with caution, and species-specific protocols remain limited for *H. polyrhizus*. Surface sterilization of cacti is itself challenging, as the soft, water-rich tissue is sensitive to hypochlorite, while thorns and areoles entrap air and hinder contact with the disinfectant. The 1, 3, and 5  $\text{mg L}^{-1}$  BAP levels examined here (approximately 4, 13, and 22  $\mu\text{M}$ ) were selected to bracket the lower, more commonly effective end of the range used by Viñas et al. [6], consistent with the 1.0–3.0  $\text{mg L}^{-1}$  optimum generally reported for BAP [7], while extending to 5  $\text{mg L}^{-1}$  to test whether higher cytokinin would further improve shoot initiation. This study, therefore, focuses on *H. polyrhizus* and addresses two practical gaps for this species: identifying a commercial-bleach concentration that balances contamination control against tissue damage, and determining a BAP level suitable for shoot initiation.

## 2. Materials and Methods

### 2.1 Establishment of Mother Plants

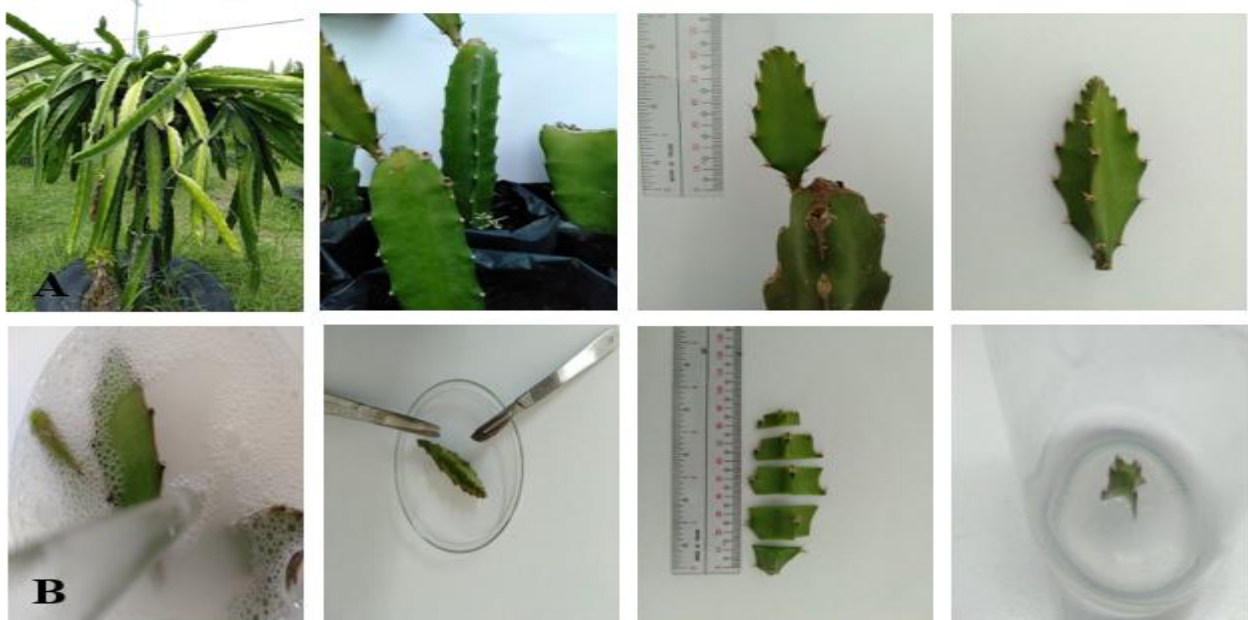
Healthy stem cuttings (at least one year old) of dragon fruit were collected from Brgy. Maganhan, Baybay City, Leyte. The mother plants were potted in polybags containing a combination of sterilized carbonized rice hull, garden soil, and vermicompost (1:1:1) as the potting medium and placed inside a screenhouse near the laboratory. The cuttings were given proper care and nutrition while in the screenhouse. One week before explant collection, a fungicide (Dithane® M-45, active ingredient mancozeb 80% w/w, applied at approximately 7 g of product per liter of distilled water, equivalent to about 5.6 g mancozeb per liter) was sprayed to minimize contamination.

### 2.2 Surface Sterilization and Explant Preparation

Newly formed shoots of the dragon fruit cuttings ( $\geq 10\text{cm}$  length) were collected from a one-month-old mother plant and served as explants (Fig. 1). The explants were washed thoroughly in running tap water for 30 minutes and in soapy water or liquid detergent solution for 1–2 minutes before rinsing. Explants were then surface-sterilized. In all treatments, the same sterilization procedure was used, but only the concentration of commercial bleach varied. First, the explants were soaked in a fungicide solution for 15 minutes, followed by a dip in 70% Ethanol. Next, explants were surface-sterilized using different concentrations of commercial bleach (50%, 20%, and 10%) for 2 minutes. Finally, the explants were soaked in



10% commercial bleach for another 2 minutes under a laminar flow hood and rinsed 3 times with double-sterilized distilled water before inoculation. The commercial bleach used in this study was [insert brand and manufacturer], which contained approximately 5.25% (w/v) sodium hypochlorite (NaOCl) as the labelled active ingredient; the 50%, 20%, and 10% treatments refer to volume/volume dilutions of this commercial product in distilled water, corresponding to approximately 2.6, 1.05, and 0.53% NaOCl, respectively.



**Figure 1.** Source of explants (A) and preparation for surface sterilization (B) of red dragon fruit shoots for *in vitro* culture. (A1) one-month-old mother plant of red dragon fruit in the nursery; (A2) newly formed shoots (cladodes) borne on the potted mother plant; (A3) a harvested shoot cutting ( $\geq 10$  cm) measured against a ruler; (A4) an isolated stem segment serving as the explant; (B1) explant washed in liquid detergent (soapy) solution; (B2) explant dipped in disinfectant solution using forceps; (B3) explant trimmed into segments and measured against a ruler; (B4) surface-sterilized explant rinsed in the bottom row, sterile distilled water before inoculation.

### 2.3 Culture Medium Preparation

In this experiment, stem segments with axillary buds were inoculated into Murashige and Skoog (MS, 1962) basal medium immediately after dissection. MS medium was supplemented with 3% sucrose (30 g L<sup>-1</sup>) as a carbohydrate source and 0.5% agar as a gelling agent. The pH was adjusted to 5.8 with 1 N HCl or NaOH before dispensing into the culture vessels. Culture bottles containing the medium were covered with autoclavable plastic, autoclaved at 121°C for 20 minutes at 15 psi, and cooled before inoculation with explants.

### 2.4 Inoculation and Incubation

Sterilized explants were excised into stem segments with axillary buds at 1–1.5 cm in length, under the laminar flow hood using a sterilized scalpel and forceps. The damaged parts of the explants from the surface-sterilization procedure were trimmed before inoculating them into MS medium. The stem segments were planted upright into the prepared MS medium. Each culture bottle contains a single stem segment before being covered with plastic. The cultured bottles with the explants were incubated at  $25 \pm 2^\circ\text{C}$  under white fluorescent light (light intensity of approximately  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in a 16h light and 8h dark photoperiod.

### 2.5 Shoot initiation of dragon fruit explants

To induce shooting, the clean explants were transferred to a MS medium formulation supplemented with different levels of 6-benzylaminopurine (BAP) at 1 mg L<sup>-1</sup>, 3 mg L<sup>-1</sup>, and 5 mg L<sup>-1</sup> as treatments. The medium was adjusted to pH 5.8 and dispensed at 20 mL per bottle before autoclaving at 15 psi for 20 minutes.



## 2.6 Experimental Design and Treatments

The study was laid out in a single-factor Completely Randomized Design (CRD). Treatments for surface sterilization used fifteen (15) stem segments of dragon fruit as samples, which were replicated four (4) times. For shoot initiation, treatments were replicated three (3) times, with ten (10) samples per replication. The smaller number of samples and replicates in the shoot initiation experiment reflects the limited number of clean, viable explants available after surface sterilization, as only explants that survived contamination and browning could be advanced to the shoot initiation stage. Treatments were designated as follows:

### 2.6.1 Surface Sterilization Experiment:

T1 – 70% Ethanol for 15 sec. + Fungicide for 15 mins. + 50% commercial bleach for 2 mins.

T2 – 70% Ethanol for 15 sec. + Fungicide for 15 mins. + 20% commercial bleach for 2 mins.

T3 – 70% Ethanol for 15 sec. + Fungicide for 15 mins. + 10% commercial bleach for 2 mins.

### 2.6.2 Shoot initiation Experiment:

T0 – MS basal medium alone

T1 – MS + 1 mg L<sup>-1</sup> BAP

T2 – MS + 3 mg L<sup>-1</sup> BAP

T3 – MS + 5 mg L<sup>-1</sup> BAP

## 2.7 Data Gathered

The parameters for surface sterilization include days to contamination from inoculation (dai), percent contamination (%), type of contaminants (e.g., fungi/bacteria), days to tissue browning (dai), percent tissue browning (%), degree of browning, and percent tissue survival (%). For shoot initiation, the data gathered include: days to shoot formation after transfer (days), percent shoot initiation (%), number of shoots per explant, and the weekly length of initiated shoots (mm).

## 2.8 Data Analysis

The collected data were analyzed using analysis of variance (ANOVA) in Statistix 10. Treatment means were compared using Tukey's Honest Significant Difference (HSD) at the 5% significance level.

## 3. Results and Discussion

### 3.1 Effect of commercial bleach as a surface sterilant for dragon fruit explants

Contamination, browning, and survival rate of dragon fruit stem segments after sterilization are presented in Table 1. The days to the first recorded contamination did not differ significantly among treatments. Appearance of contaminants was observed earlier in 20% and 10% commercial bleach, with an average of 4 days, whereas at 50% commercial bleach, appearance occurred on average 5 days after inoculation (dai). Highest contamination was reported in 10% commercial bleach at 71.67%, followed by 20% commercial bleach at 56.66%. Lowest contamination, on the other hand, was observed with 50% commercial bleach, with only 33.33% contamination. The study found that dragon fruit explants soaked in different levels of commercial bleach for 2 minutes indeed affected the percentage of contamination. Commercial bleach at 10% showed a higher contamination rate than 20% and 50% concentrations. The result suggested that the higher the level of commercial bleach used, the cleaner the cultures can be obtained. Similarly, in the study by Hesami et al. [8], increasing sodium hypochlorite concentration and immersion time reduced the percentage contamination of *Ficus religiosa*.

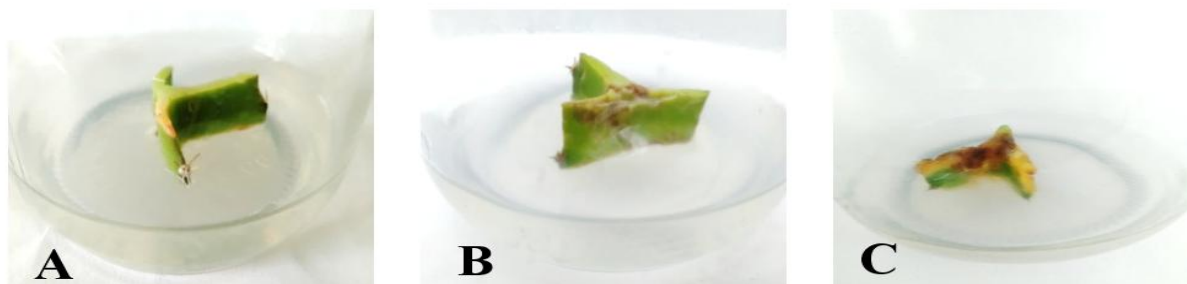
**Table 1.** Effect of concentration of commercial bleach on the contamination, browning, and survival of red dragon fruit explants *in vitro*

| Treatments                                                                           | Days to Contam (dai) | Percent Contam (%) | Type of contam | Percent Browning (%) | Degree of Browning | Percent Survival (%) |
|--------------------------------------------------------------------------------------|----------------------|--------------------|----------------|----------------------|--------------------|----------------------|
| T1 - 70% Ethanol for 15 s + Fungicide for 15 mins + 50% commercial bleach for 2 mins | 5                    | 33.33 c            | Fungi          | 11.65                | 3 a                | 54.99 a              |
| T2 - 70% Ethanol for 15 s + Fungicide for 15 mins + 20% commercial bleach for 2 mins | 4                    | 56.66 b            | Fungi          | 8.34                 | 2 b                | 37.50 b              |
| T3 - 70% Ethanol for 15 s + Fungicide for 15 mins + 10% commercial bleach for 2 mins | 4                    | 71.67 a            | Fungi          | 6.67                 | 1 b                | 25.00 c              |
| CV (%)                                                                               | 9.76                 | 9.89               | -              | 30.58                | 25.20              | 15.22                |

Means with the same letter are not significantly different at 5% level (Tukey's HSD)

As shown in this study, sodium hypochlorite was very effective against bacterial contamination, since fungal contamination was the predominant contaminant among the cultures. Nakagawara et al. reported that even micromolar concentrations of sodium hypochlorite are sufficient to reduce bacterial populations [9] significantly. Contamination of cultures occurs despite explant sterilization, due to exogenously and endogenously present microbes. It should be noted that all three treatments shared the same 70% ethanol and fungicide steps and differed only in the concentration of commercial bleach, with no bleach-free control included. Consequently, the effects reported here are attributable specifically to the commercial bleach concentration rather than to the full sterilization protocol as a unit, and the absence of an untreated (ethanol- and fungicide-only) control is acknowledged as a limitation of the experimental design.

Using a higher concentration of commercial bleach in this study reduced contamination but induced browning of explants. Dragon fruit stem segments treated with commercial bleach showed the highest percent browning in 50% commercial bleach (11.65%), while the lowest was in 10% commercial bleach (6.67%). On the other hand, the degree of browning showed a significant difference between the commercial bleach used. Application of 50% commercial bleach showed a high degree of browning (>61% tissue browning), while the lowest degree of browning was recorded with 10% commercial bleach (1-20% tissue that turned brown). Commercial bleach at 20% showed a degree of browning comparable to 10% commercial bleach. The high concentration of commercial bleach (50%) increased the degree of browning, which damaged the soft tissues of dragon fruit stem segments (Fig. 2). Thus, the higher the percentage of commercial bleach used, the higher the percentage of aseptic culture, yet it also yields a greater number of explants that turn brown. Based on the results, regardless of the concentration of commercial bleach used in this experiment, explants showed browning due to bleach toxicity. Felek et al. [10] reported that surface sterilization with a higher concentration (0.5% w/v) of local bleach for 15 min or more. resulted in no contamination but a high rate (71.43%) of explant mortality of peach. Surface sterilization should not kill or inactivate the biological activity of explants, but should kill or inactivate contaminants. Explants must be surface-sterilized only by treatment with a disinfectant solution at suitable concentrations for a specified period [11]. Pitahaya (dragon fruit) tissue is very sensitive to disinfection with NaOCl or commercial bleach [6]. To reduce damage, it was necessary to initially treat larger explants with five to six areoles each before cutting them into smaller segments for *in vitro* culture.



**Figure 2.** Degree of browning of red dragon fruit explants after surface sterilization using commercial bleach concentrations showing 1-20% tissue browning (A), 21-60% tissue browning (B), and >61% tissue browning (C)

### 3.2 Effect of sterilization on the survival of dragon fruit explants

The explant survival rate was relative to the contamination percentage, wherein high contamination resulted in low explant survival. Explants that turned brown also contributed to reduced culture survival. Hence, the survival of the explants was counted from samples that survived contamination and browning. A highly significant difference in percent survival was observed among treatments (Table 1). The survival rate of stem segments at 50% commercial bleach was over 54.99%, followed by 20% commercial bleach at 37.50%, and lowest at 10% commercial bleach at 25.00%. Commercial bleach has also been used to sterilize dragon fruit bud and stem segment explants to desired levels [6][4]. The survival rate of the two explants was over 60% in the first week of culture, but a higher percentage (85%) of survival was recorded in stem segments [4]. The higher survival rate in 50% commercial bleach compared to the other concentrations used in this study can be attributed to the effectiveness of sodium hypochlorite against all kinds of contaminants, which is the common active ingredient of the commercial bleach used in the study. However, compared with the study by Thinesh and Seran, only 54.99% survival was observed in this study. The difference could be attributed to the source of explants, the variety, and the culture medium used. Explant disinfection of cacti is difficult due to the softness of the tissue and the presence of thorns, which entrap air bubbles and prevent good contact with the disinfecting agent [6]. It is recommended that thorns cannot be removed from explants, otherwise axillary shoot growth does not occur [3]. It should be acknowledged that a survival rate of 54.99% is modest by tissue culture standards, as nearly half of the explants were lost even under the best treatment. This value is well below the 85% reported for stem segments by Thinesh and Seran [4] and therefore limits the immediate commercial viability of the protocol for large-scale production. The low survival reflects the competing demands of contamination control and tissue preservation in cactus explants, in which higher bleach concentrations reduce contamination but increase oxidative browning. Survival could likely be improved by adopting a sequential or stepwise sterilization regime, by incorporating antioxidants such as polyvinylpyrrolidone (PVP) or ascorbic acid into the rinses or culture medium to limit browning, and by pre-conditioning mother plants under controlled, low-contamination conditions before explant collection. These refinements are recommended before the protocol is scaled up for routine production. A comparable level of survival was reported for another cactus, in which medium sterilization of *Gymnocalycium mihanovichii* with 50 ppm chlorine dioxide gave the best survival of 54.17% [12], indicating that survival rates near 55% are not unusual for chemically sterilized cactus explants and that alternative sterilants such as chlorine dioxide may be worth evaluating for dragon fruit.

### 3.3 Shoot Initiation of Dragon Fruit Explants

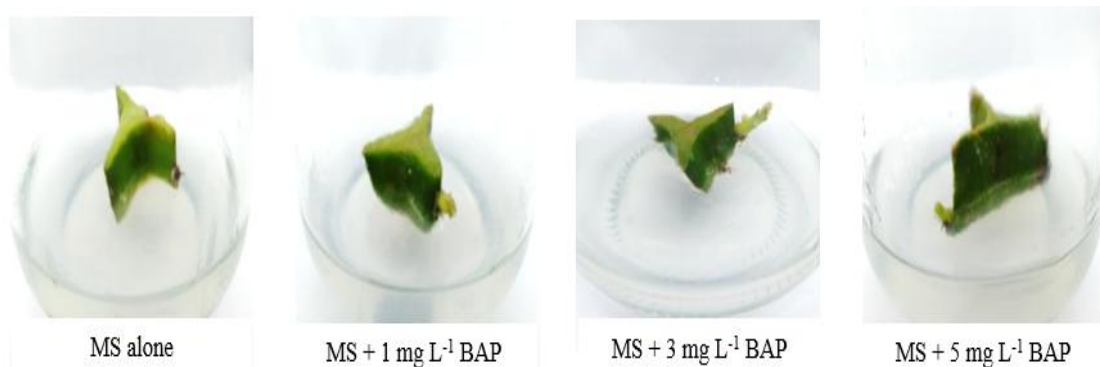
The dragon fruit stem segments with axillary buds were cultured in MS medium supplemented with different levels of BAP to determine their in vitro response on shoot initiation and development. Variations in the response to shoot initiation of the explants revealed a significant difference in days to shoot initiation and percentage of shoot initiation as influenced by the different levels of BAP added into the culture medium (Table 2 and Fig. 3). No shoot initiation was observed in MS basal medium alone (T0). Early shoot initiation was observed in medium containing 3 mg L<sup>-1</sup> BAP, which produced shoots at 20 days after

transfer (dat), followed by 1 mg L<sup>-1</sup> BAP and 5 mg L<sup>-1</sup> BAP, with comparable results at 38 and 34 days after transfer, respectively. Although the days to shoot initiation for 1 mg L<sup>-1</sup> (38 days) and 5 mg L<sup>-1</sup> (34 days) shared the same statistical grouping and were not significantly different, the four-day numerical difference may still be of practical relevance when the speed of culture establishment is important. In this study, at 20 days of initiation, 3 mg L<sup>-1</sup> BAP appeared to agree with the findings of Viñas et al. [6] and Drew and Azimi [3]. The shortest time required to initiate bud development of dragon fruit was 28 days after explant establishment in medium with 15, 30, or 60 µM BAP [6], while only 20 days were required for bud sprouting of *H. undatus* areoles with 5 µM N6 (2-isopentenyl) adenine, which extended to 26 d on hormone-free medium [3].

**Table 2.** Shoot initiation response of red dragon fruit explants as influenced by levels of BAP supplemented in MS medium *in vitro*

| Treatment                          | Days to Shoot Initiation (day) | NO. of Shoots      | Percent Shoot Initiation (%) |
|------------------------------------|--------------------------------|--------------------|------------------------------|
| T0 - MS alone                      | -                              | -                  | -                            |
| T1 - MS + 1 mg L <sup>-1</sup> BAP | 38 a                           | 1.00 <sup>ns</sup> | 13.33 b                      |
| T2 - MS + 3 mg L <sup>-1</sup> BAP | 20 b                           | 1.08 <sup>ns</sup> | 30.00 ab                     |
| T3 - MS + 5 mg L <sup>-1</sup> BAP | 34 a                           | 1.13 <sup>ns</sup> | 46.67 a                      |
| CV (%)                             | 11.39                          | 9.95               | 24.85                        |

Means with the same letter are not significantly different at 5% level (Tukey's HSD); ns = not significant



**Figure 3.** Formation of shoots from buds of stem segments of red dragon fruit explants *in vitro* as influenced by levels of BAP in MS medium

On the other hand, the delay in shoot initiation in treatment with higher BAP (5 mg L<sup>-1</sup>) could be caused by the retardation of buds to produce shoots due to a high concentration of the hormone (BAP). This reduction in shoot formation may be due to BAP toxicity at higher concentrations. Narayanaswamy, as reported by Amer and Omar [13], stated that the toxicity caused by excess or prolonged exposure to growth regulators in the culture medium leads to genetic, physiological, and morphological changes, resulting in inhibition of proliferation. Past research indicates that there are optimal concentrations of BAP for specific plants. The study by Harahap et al. [14] appears to have limitations regarding the benefits of BAP at high doses on mangosteen explants. High concentrations of BAP caused treated explants to swell and produce many initial buds; however, those buds either did not grow into shoots or the shoots appeared stunted. The strong influence of cytokinin level on shoot response observed here is consistent with reports on other ornamental and cactus species. In a related cactus, *Gymnocalycium mihanovichii*, the highest shoot induction was obtained at low BA levels (0–2 mg L<sup>-1</sup>) rather than at the highest level tested [16], in agreement with the present finding that 3 mg L<sup>-1</sup> BAP outperformed 5 mg L<sup>-1</sup> for shoot elongation. Besides the type and level of growth regulator, the strength and physical state of the basal medium also affect shoot multiplication, and full-strength MS medium has been reported to give the best shoot growth in other *in vitro* systems [15].

Culture medium supplementation with 1 mg L<sup>-1</sup> level of BAP is below the optimum level for shoot initiation, resulting in the lowest percentage of shoot formation. The explants cultured in 3 mg L<sup>-1</sup> BAP may have shown the earliest shoot initiation, but cultures in 5 mg L<sup>-1</sup> BAP had a higher percentage of initiated shoots (46.67%) and were significantly different from 1 mg L<sup>-1</sup> BAP (13.33%) in shoot initiation. Treatment with 3 mg L<sup>-1</sup> had 30% shoot initiation, which was statistically comparable to (i.e., not significantly different from) but numerically lower than that of 5 mg L<sup>-1</sup> BAP (46.67%). No significant difference in the number of shoots was observed across media with different BAP levels. The maximum number of shoots produced by an explant at 5 mg L<sup>-1</sup> was 3, with a collective average of 1.13 shoots. This was followed by 3 mg L<sup>-1</sup> and 1 mg L<sup>-1</sup>, with average numbers of shoots of 1.00 and 1.08, respectively. All levels of BAP showed potential for promoting dragon fruit shoot growth *in vitro*. Aliyu and Mustapha reported that sprouting of cactus axillary bud requires low levels or no auxin, but always high levels of cytokinin [6]. Furthermore, they recommended that BAP be included for axillary bud sprouting in pitahaya cv Cebra. According to Machado et al. [17], explants transferred from a basic culture medium, diluted or not, and with reduced cytokinin concentration or cytokinin absence were enough to promote shoot growth. Cytokinins were known to stimulate shoot formation from explants *in vitro*, but the effect is more efficient depending on the amount applied to the medium and the explant's nutrient and hormone uptake rates. The useful BAP effects on the shoot's initiation are linked with the influence that this plant growth regulator has on the cellular division and shoot growth induction of the axillary buds inhibited by the plant apical dominance [18]. Arab et al. [19] also stated that cytokinin stimulates the initiation and activity of axillary meristems, which result in shoot induction and formation.

### 3.4 Growth of Dragon Fruit Shoots *in vitro* with Levels of BAP

Different levels of BAP influenced the length of the shoots of initiated dragon fruit shoots. No shoot growth was observed in cultures grown in MS alone, so the comparison was between cultures grown in medium supplemented with BAP. A significant difference in shoot length of dragon fruit explants was observed from the first to the fourth week of incubation among treatments with different levels of BAP (Table 3). The significant difference in shoot growth was consistent throughout the experiment, with cultures at 3 mg L<sup>-1</sup> obtaining the longest shoot length. After the 4<sup>th</sup> week of incubation or termination of the experiment, the highest length of shoots was observed in cultures grown in medium supplemented with 3 mg L<sup>-1</sup> BAP, with an average of 9.89 mm, which was significantly higher than cultures in 1 mg L<sup>-1</sup> BAP (3.67mm) and 5 mg L<sup>-1</sup> BAP (2.53mm). These showed a growth increment of 6.83mm in shoots at 3 mg L<sup>-1</sup> BAP compared to shoots at 1 mg L<sup>-1</sup> BAP and 5 mg L<sup>-1</sup> BAP, with only 1.84mm and 0.9 mm, respectively, at the fourth week. The higher shoot length of cultures in 3 mg L<sup>-1</sup> BAP could also be attributed to earlier shoot initiation in explants compared to other treatments, as shown in Table 3.

**Table 3.** Effect of levels of BAP in the length of shoots of red dragon fruit *in vitro* after four weeks incubation

| Treatment                          | Weekly Length of Initiated Shoots (mm) |        |        |        |
|------------------------------------|----------------------------------------|--------|--------|--------|
|                                    | Week 1                                 | Week 2 | Week 3 | Week 4 |
| T0 - MS medium alone               | -                                      | -      | -      | -      |
| T1 - MS + 1 mg L <sup>-1</sup> BAP | 1.83 b                                 | 1.83 b | 2.67 b | 3.67 b |
| T2 - MS + 3 mg L <sup>-1</sup> BAP | 3.06 a                                 | 4.39 a | 7.17 a | 9.89 a |
| T3 - MS + 5 mg L <sup>-1</sup> BAP | 1.63 b                                 | 1.77 b | 2.03 b | 2.53 b |
| CV (%)                             | 14.12                                  | 16.80  | 18.69  | 27.53  |

Means with the same letter are not significantly different at 5% level (Tukey's HSD)

In general, BAP use in plants ranges from 1.0 to 3.0 mg L<sup>-1</sup>. If the concentration is too high, it can cause shoot bud retardation, rendering the plant growth stunted, but the effect varies with different species [7] (pp. 175-205). In this experiment, retardation of shoot length was observed in 5 mg L<sup>-1</sup> BAP, while 1 mg L<sup>-1</sup> BAP was not optimal for shoot growth of the explants. Interestingly, increasing BAP concentration beyond the optimal level (3 mg L<sup>-1</sup>) did not further improve shoot length but instead suppressed it in stem-segment explants. This reduction may be due to BAP's toxicity at higher concentrations. Narayanaswamy reported



that the toxicity caused by an excess of or prolonged exposure to growth regulators in the culture medium leads to genetic, physiological, and morphological changes and consequent inhibition of proliferation [13]. The dependence of plant growth on the type and concentration of growth regulator is well documented in other crops; for example, combined foliar application of gibberellic acid and salicylic acid markedly enhanced the growth and yield of kale [20], and immersion in indole-3-butyric acid promoted bud germination and growth of *Caladium* tuber sections [21], reinforcing that an appropriate, rather than maximal, level of growth regulator is required for optimal growth.

#### 4. Conclusions

A 50% commercial bleach concentration for 2 mins produced the lowest percent contamination and the highest percent survival of red dragon fruit explants. However, it showed the highest percentage of browning to explants due to commercial bleach toxicity. Among the BAP levels tested, 3 mg L<sup>-1</sup> supplemented to MS medium is recommended for shoot initiation of *H. polyrhizus* because it gave the earliest shoot initiation (20 days) and the greatest shoot length (9.89 mm), and produced a percent shoot initiation (30%) that was statistically comparable to that at 5 mg L<sup>-1</sup> (46.67%). Although 5 mg L<sup>-1</sup> gave the highest number of shoots per explant, this difference was not statistically significant, and the same concentration retarded shoot length; it is therefore not recommended as the optimum. Taken together across all performance parameters, 3 mg L<sup>-1</sup> BAP represents the best overall treatment for shoot initiation in this study. In this experiment, retardation of shoot length was observed in 5 mg L<sup>-1</sup> BAP, while 1 mg L<sup>-1</sup> BAP was not optimal for shoot growth of the explants. Interestingly, increasing BAP concentration beyond the optimal level did not further improve any parameter associated with shoot initiation and instead suppressed shoot length in stem-segment explants. It should be noted that this study covered only the surface sterilization and shoot initiation stages; rooting and acclimatization were not evaluated. Further work on rooting, elongation, and ex vitro acclimatization, together with strategies to improve explant survival (e.g., sequential sterilization, antioxidant supplementation, and mother-plant pre-conditioning), is therefore required before a complete micropropagation protocol for large-scale production of *H. polyrhizus* planting materials can be established.

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# Computational Fluid Dynamics-Based Characterization of Heat Transfer and Airflow in an Indirect Solar Fish Dryer Under Tropical Conditions

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**Abstract:** Indirect solar drying is a widely adopted fish preservation technique in tropical regions, valued for its low energy requirement and capacity to enhance product quality. However, non-uniform airflow and temperature distribution within drying chambers remain persistent design challenges that compromise drying efficiency and product consistency. This study investigated the airflow characteristics and convective heat transfer performance of an indirect solar fish dryer through computational fluid dynamics (CFD) simulation and experimental validation. A three-dimensional CFD model was developed to simulate temperature distribution and airflow patterns under forced convection, with an inlet air velocity of  $1.2 \text{ m}\cdot\text{s}^{-1}$  and an inlet air temperature of  $60^\circ\text{C}$ . Experimental air temperatures were recorded inside the drying chamber during actual drying operations and compared with simulated values using linear regression and root-mean-square error (RMSE). The regression analysis yielded a strong linear relationship between simulated and experimental temperatures ( $R^2 = 0.9413$ ), with an RMSE of  $3.41^\circ\text{C}$ , indicating reasonable agreement in absolute temperature prediction. These results confirm that the CFD model accurately represents the thermal behavior and convective heat transfer characteristics of the dryer. The validated CFD framework provides a reliable, cost-effective tool for evaluating and optimizing the design and operational performance of indirect solar fish dryers under tropical conditions, thereby reducing reliance on iterative physical prototyping.

**Keywords:** Computational fluid dynamics; convective heat transfer; experimental validation; fish drying; indirect solar dryer

## 1. Introduction

The use of computational fluid dynamics (CFD) in the design and optimization of indirect solar dryers has become increasingly significant for enhancing the efficiency and reliability of drying processes for fish and other agricultural products. CFD allows for a detailed examination of airflow patterns, temperature distribution, and convective heat transfer within drying systems, processes that are often challenging to assess using experimental methods alone. When combined with experimental validation, CFD provides a robust framework for understanding thermal–fluid interactions and for enhancing drying uniformity and product quality. Indirect solar dryers are designed to

separate solar radiation collection from the drying chamber, thereby shielding products from direct sunlight exposure. This configuration reduces the risk of contamination and deterioration in quality while providing improved control over key drying parameters, such as airflow rate and temperature. In these systems, solar energy heats the air, which is then circulated throughout the drying chamber, where convective heat transfer drives moisture removal. Previous studies have emphasized that optimizing airflow and temperature distribution within indirect solar dryers is critical to achieving uniform drying and maintaining product integrity [1–4]. Solar drying has also been shown to yield product quality comparable to conventional electrical drying for a range of agricultural commodities, including silk cocoons [4].

Several researchers have demonstrated the effectiveness of CFD in analyzing the thermal and fluid behavior of indirect solar dryers. Noh et al. [5] developed a CFD model that integrated airflow, temperature, and moisture interactions to evaluate solar dryer performance, highlighting the strong influence of airflow distribution on drying behavior. Carrera et al. [6] used CFD to study heat distribution in an indirect solar chili dryer and found that airflow behavior plays a crucial role in achieving uniform temperature in the drying chamber. Similarly, Villagrán et al. [7] showed that optimized airflow patterns in greenhouse solar dryers improved the drying performance of aromatic plants. Similarly, Srirugsa et al. [8] demonstrated that ventilated solar technology significantly improved drying efficiency for red chili peppers by enhancing forced airflow within the drying chamber. Despite the strengths of numerical modeling, experimental validation remains essential to ensure that CFD predictions accurately represent real drying conditions. Gunathilaka et al. [9] validated a CFD model of an indirect solar dryer using experimental temperature and air velocity measurements and reported close agreement between simulated and measured values. Mellalou et al. [10] likewise observed a strong correlation between experimental and simulated temperature profiles in a modified greenhouse solar dryer, supporting the reliability of CFD techniques for dryer performance evaluation.

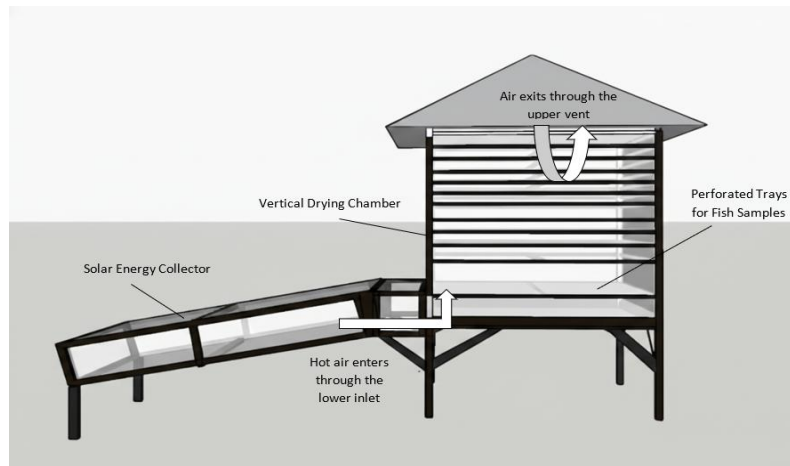
Although earlier studies have established the usefulness of CFD for analyzing solar drying systems, relatively few have combined CFD with experimental methods to evaluate convective heat transfer in indirect solar fish dryers operating under tropical conditions. Therefore, this study seeks to develop and validate a three-dimensional CFD model of an indirect solar fish dryer by comparing simulated airflow patterns and temperature distributions with experimentally measured temperatures inside the drying chamber. The findings are expected to contribute to understanding convective heat transfer mechanisms and to support the optimization of the design and operation of indirect solar dryers for fish drying applications.

## 2. Materials and Methods

### 2.1 Description of the indirect solar dryer

The indirect solar dryer examined in this study comprises a solar air heater coupled to a vertical drying chamber fitted with multiple perforated trays (Figure 1). The system is designed to separate solar heat collection from the drying chamber, preventing direct exposure of fish samples to solar radiation while allowing controlled convective heat transfer. The solar air heater is installed at a lower elevation and inclined to enhance solar energy absorption. It is enclosed with a transparent glass cover that permits solar radiation to enter the collector while minimizing convective heat losses. Solar energy transmitted through the glass cover warms the air inside the collector, which is then directed to the drying chamber via a connecting duct. The heated air enters the drying chamber through a lower inlet positioned at the base of the chamber. The drying chamber is a vertical enclosure supported by a rigid frame and fitted with multiple perforated horizontal trays arranged at uniform spacing. The trays and internal structural components are made of aluminum to prevent rust and corrosion, ensuring durability and suitability for food-drying applications. Fish samples are placed on the trays during operation. The perforations in the trays allow heated air to pass uniformly across and through the product layers, thereby enhancing convective heat transfer and promoting uniform moisture removal. As the heated air enters the drying chamber through the lower inlet, it rises and flows across the trays under forced convection, transferring heat to the fish samples. Moist air exits the chamber through an upper vent located beneath the roof. This airflow configuration establishes a continuous upward flow path that supports stable drying conditions and a relatively uniform temperature distribution within the chamber.

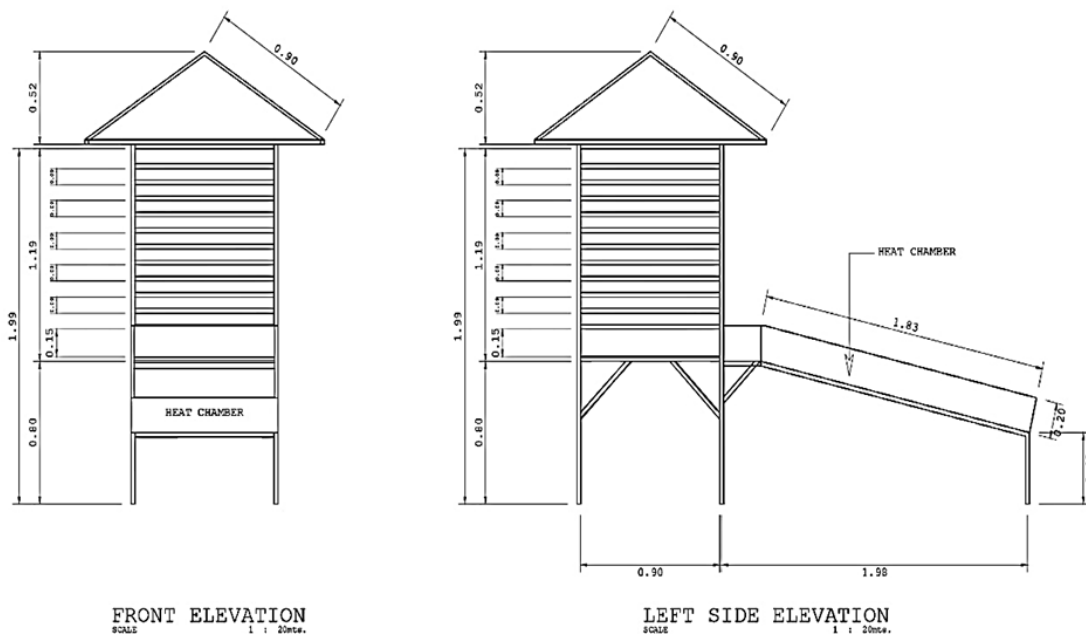




**Figure 1.** Schematic diagram of the indirect solar dryer.

## 2.2 CFD geometry and computational domain

The computational geometry of the indirect solar dryer was created using the actual dimensions of the fabricated system, as illustrated in the front and left-side elevation views in Figure 2. The geometry represents the solar air heater, connecting duct, and vertical drying chamber as a single continuous airflow domain for numerical simulation. The vertical drying chamber has an overall height of approximately 1.99 m, measured from the base support to the topmost section. The chamber has a width of 0.90 m, as indicated in the front elevation, and contains multiple perforated aluminum trays arranged at uniform vertical spacing of approximately 0.14 m. A lower clearance of approximately 0.80 m is provided beneath the chamber to accommodate structural supports and airflow passage. The drying chamber is surmounted by a pitched roof with an effective roof length of approximately 0.90 m and a roof height of 0.52 m. This configuration protects from environmental exposure and aids in the discharge of moist air through the upper vent. The exhaust opening is located directly beneath the roof, establishing an upward airflow path through the chamber.



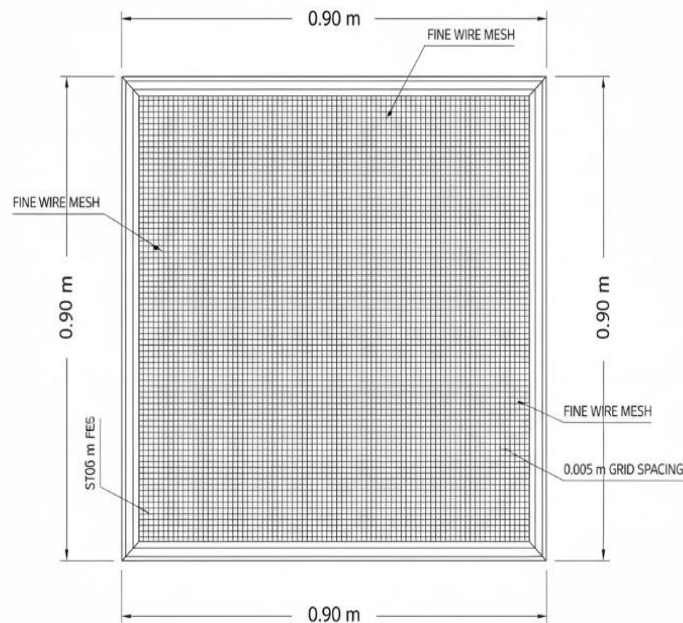
**Figure 2.** Technical elevations of the solar drying system.

Airflow from the solar air heater enters the drying chamber through a horizontally oriented inlet located near the chamber base. The left-side elevation shows that the heater is mounted at an incline, with an

effective collector length of approximately 1.83 m and a horizontal projection of about 1.98 m. The air gap height within the collector varies along its length, with an approximate height of 0.27 m near the inlet and 0.40 m near the downstream end, following the inclined geometry.

### 2.3 Tray configuration and computational representation

Each drying tray has a square plan dimension of 0.90 m  $\times$  0.90 m and is constructed using a fine aluminum wire mesh, as shown in Figure 3. The wire mesh provides structural support for the fish samples while allowing heated air to pass through the tray surface, thereby enhancing convective heat transfer and promoting uniform airflow distribution within the drying chamber.



**Figure 3.** Technical specifications of the 0.9 m  $\times$  0.9 m drying tray mesh.

The mesh consists of a uniform grid with a spacing of approximately 0.005 m, which permits adequate airflow while supporting the product during drying. The use of aluminum minimizes corrosion and ensures suitability for food-drying applications. In the CFD model, the trays were represented as porous surfaces to account for airflow resistance while avoiding excessive mesh refinement that would significantly increase computational cost [11–13]. This approach preserves the overall flow behavior through the tray region while maintaining numerical efficiency. The porous resistance parameters were selected to reflect the fine-wire-mesh structure, allowing air to pass through the trays with minimal pressure drop. This simplified representation enables accurate simulation of airflow and temperature distribution across the tray levels without explicitly resolving individual wire elements, which would be computationally prohibitive. The use of porous materials in solar air collectors has also been shown to improve thermal efficiency under tropical conditions [13], further supporting the representational approach adopted here.

### 2.4 CFD model development

A three-dimensional CFD model of the indirect solar dryer was established to represent the airflow field and the system's thermal behavior. The geometric model was constructed based on the physical dimensions of the fabricated dryer, including the air inlet, solar air heater connection, vertical drying chamber, perforated trays, and air outlet. These components were treated as a single continuous airflow domain to capture the coupled effects of airflow and heat transfer processes throughout the system [14, 15]. The CFD model was established to simulate transient forced-convection airflow and temperature distributions within the drying chamber using ANSYS Fluent 2022 R2 with a pressure-based solver and the finite-volume method for spatial discretization. Air was assumed to behave as an incompressible fluid with constant thermophysical

properties evaluated at the operating temperature range. Given the inlet velocity of  $1.2 \text{ m}\cdot\text{s}^{-1}$  and chamber width of  $0.9 \text{ m}$ , the approximate Reynolds number is  $\sim 70,000$ , indicating turbulent flow. Accordingly, the realizable  $k$ - $\epsilon$  turbulence closure model was employed, with standard wall functions applied at solid boundaries. The simulation was run as a transient (unsteady) model with a time step of  $1 \text{ s}$  and 20 inner iterations per time step to ensure convergence. Convergence was assessed using residual criteria of  $10^{-4}$  for the continuity, momentum, and turbulence transport equations and  $10^{-6}$  for the energy equation. Radiation heat transfer and moisture evaporation effects were not included in the present model [16,17]. The governing equations solved in the CFD simulation consist of the transient conservation equations for mass, momentum, and energy for incompressible flow, with time-derivative terms included to capture the unsteady thermal behavior of the drying chamber. These equations are expressed as follows:

Continuity equation:

$$\nabla \cdot v = 0 \quad (1)$$

Momentum equation:

$$\rho(v \cdot \nabla)v = -\nabla p + \mu \nabla^2 v \quad (2)$$

Energy equation:

$$\rho c_p(v \cdot \nabla T) = k \nabla^2 T \quad (3)$$

where  $v$  is the air velocity vector ( $\text{m}\cdot\text{s}^{-1}$ ),  $p$  is pressure (Pa),  $T$  is temperature ( $^{\circ}\text{C}$ ),  $\rho$  is air density ( $\text{kg}\cdot\text{m}^{-3}$ ),  $\mu$  is dynamic viscosity ( $\text{Pa}\cdot\text{s}$ ),  $c_p$  is the specific heat capacity of air ( $\text{J}\cdot\text{kg}^{-1}\cdot\text{K}^{-1}$ ), and  $k$  is the thermal conductivity of air ( $\text{W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ ). The above equations were solved simultaneously to predict airflow patterns and temperature distribution inside the drying chamber. Heat transfer within the system was governed primarily by convection between the heated air and the tray surfaces. The perforated trays were represented as porous media to account for airflow resistance while maintaining numerical efficiency [18].

## 2.5 Boundary Conditions and Assumptions

The boundary conditions and modeling assumptions adopted in this study are summarized in Table 1 and were used to represent airflow and convective heat transfer within the indirect solar dryer. These parameters were defined using measured operating conditions obtained during experimental trials and were consistently imposed throughout the entire computational domain. Air enters the drying chamber through the lower inlet at a prescribed uniform velocity and temperature, representing the forced convection airflow generated by the solar air heater. The inlet air temperature was specified as  $60^{\circ}\text{C}$ , corresponding to the average heated air temperature observed during dryer operation. The inlet air velocity was fixed at  $1.2 \text{ m}\cdot\text{s}^{-1}$ , based on the measured airflow rate supplied to the drying chamber. A pressure-based boundary condition was applied at the outlet, with the gauge pressure specified as atmospheric to permit unrestricted air discharge from the drying chamber. All solid walls were treated as no-slip boundaries with adiabatic thermal conditions. Such simplifying assumptions are widely used in CFD analyses of indirect solar drying systems [19, 20].

## 2.6 Experimental validation

An experimental validation procedure was performed to evaluate the predictive capability of the CFD model in representing the thermal response of the indirect solar fish dryer. Temperature measurements were collected at multiple locations across four tray levels inside the drying chamber. At each tray level, sensors were positioned at two horizontal locations — one near the center and one near the lateral edge — to capture spatial temperature variation and to verify the predicted non-uniform temperature distribution. A total of 10 calibrated K-type thermocouples ( $\pm 0.5^{\circ}\text{C}$  accuracy) were used, connected to a Graphtec GL840 multichannel data logger with a  $60 \text{ s}$  recording interval. Sensors were calibrated against a reference thermometer before deployment. Data were collected on 12–14 March 2024 between 08:00 and 16:00 local time under clear-sky conditions representative of the operating conditions imposed in the CFD simulation. The coefficient of determination ( $R^2$ ) was used to quantify the degree of correlation between simulated and experimental

temperatures, while the root mean square error (RMSE) was calculated to quantify the average deviation between the two datasets. The RMSE was calculated using the following expression:

$$\text{RMSE} = \sqrt{1/n \cdot \sum (T_{\text{sim},i} - T_{\text{exp},i})^2} \quad (4)$$

where  $T_{\text{sim},i}$  and  $T_{\text{exp},i}$  denote the simulated and experimental temperatures at time step  $i$ , respectively, and  $n$  is the total number of data points considered. A scatter plot of simulated versus experimental temperatures was generated to visually assess model performance, and a linear regression line was fitted to the data [21].

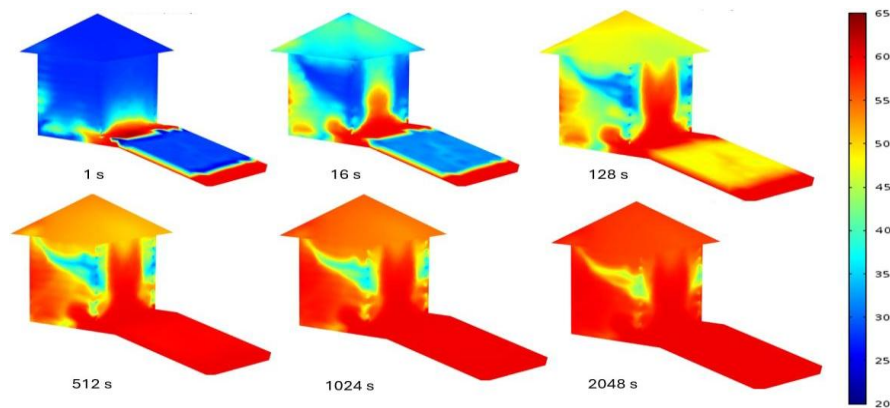
**Table 1.** Boundary conditions, material properties, and modeling assumptions used in the CFD simulation.

| Parameter                                                       | Value / Description                                                                               |
|-----------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| Inlet air temperature [°C]                                      | 60                                                                                                |
| Inlet air velocity [m/s]                                        | 1.2                                                                                               |
| Outlet condition                                                | Pressure outlet (atmospheric pressure)                                                            |
| Initial air temperature [°C]                                    | 30                                                                                                |
| Flow regime                                                     | Transient (unsteady)                                                                              |
| Flow type                                                       | Forced convection                                                                                 |
| Air density ( $\rho$ ) [kg/m <sup>3</sup> ]                     | 1.060 (at 60 °C operating temperature; corrected from standard sea-level value)                   |
| Dynamic viscosity of air ( $\mu$ ) [Pa·s]                       | $1.97 \times 10^{-5}$ (at 60 °C operating temperature; corrected from standard sea-level value)   |
| Specific heat capacity (cp) [J/kg·K]                            | 1006                                                                                              |
| Thermal conductivity of air (k) [W/m·K]                         | 0.026                                                                                             |
| Tray material                                                   | Aluminum (corrosion-resistant)                                                                    |
| Tray modeling approach                                          | Porous media                                                                                      |
| Wall boundary condition                                         | No-slip                                                                                           |
| Wall thermal condition                                          | Adiabatic                                                                                         |
| Radiation heat transfer                                         | Neglected                                                                                         |
| Evaporative cooling                                             | Neglected                                                                                         |
| Gravity effects                                                 | Included                                                                                          |
| Solver type                                                     | Pressure-based                                                                                    |
| CFD software [name and version]                                 | ANSYS Fluent 2022 R2                                                                              |
| Turbulence model                                                | Realizable k- $\epsilon$ with standard wall functions                                             |
| Mesh type / total element count                                 | Unstructured tetrahedral, ~1,800,000 cells (selected from mesh-independence study, Section 2.4)   |
| Porous media viscous resistance coefficient [m <sup>-2</sup> ]  | $3.5 \times 10^7$ (derived analytically from Ergun-type formulation for perforated tray geometry) |
| Porous media inertial resistance coefficient [m <sup>-1</sup> ] | 25 (derived analytically from Ergun-type formulation for perforated tray geometry)                |

### 3. Results and Discussion

#### 3.1 Transient heat transfer of drying air

The temperature contour plots of the drying air predicted by the CFD model at selected time intervals within the indirect solar fish dryer are presented in Figure 4. At the early stage of operation (1–32 s), relatively low air temperatures are observed throughout most of the drying chamber, with localized regions of elevated temperature near the lower inlet. As drying time increases (128–512 s), a continuous upward propagation of heated air develops, forming a thermal plume extending toward the upper section of the chamber. At longer operating times (1024–2048 s), the temperature field becomes increasingly uniform, with air temperatures throughout the chamber approaching the inlet temperature of approximately 60 °C. Similar transient-to-steady thermal behavior has been reported in three-dimensional CFD studies of forced-air and indirect solar dryers [16, 17].



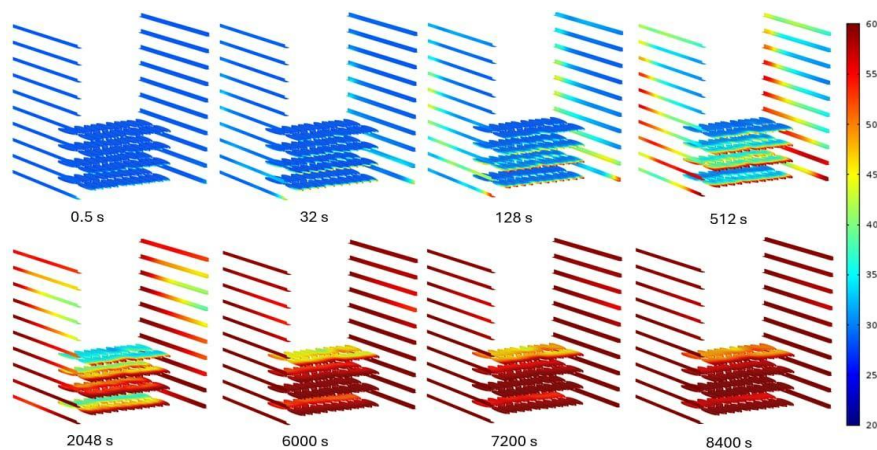
**Figure 4.** Time-dependent temperature contours of drying air inside the indirect solar fish dryer.

### 3.2 Temperature Distribution of Fish Samples

The temperature distribution on the fish samples placed on the perforated trays at selected drying times is presented in Figure 5. During the initial drying phase (0.5–32 s), the fish samples remain at relatively low temperatures. As drying time progresses (128–512 s), the fish samples on the lower and middle trays exhibit a noticeable increase in temperature. At extended drying times (2048–8400 s), temperature differences among tray levels diminish, and the fish sample temperatures converge to 55–60 °C across all trays. The progressive increase and eventual uniformity of fish sample temperatures across tray levels highlight the strong influence of airflow distribution on product heating. Fish samples located closer to the lower inlet experienced earlier heating due to higher local air temperatures and velocities, while samples on upper trays warmed more gradually during the initial stages. This trend is consistent with previous numerical and experimental investigations, which have shown that sustained and uniform airflow is essential for achieving homogeneous drying conditions and minimizing product quality variation [1,2].

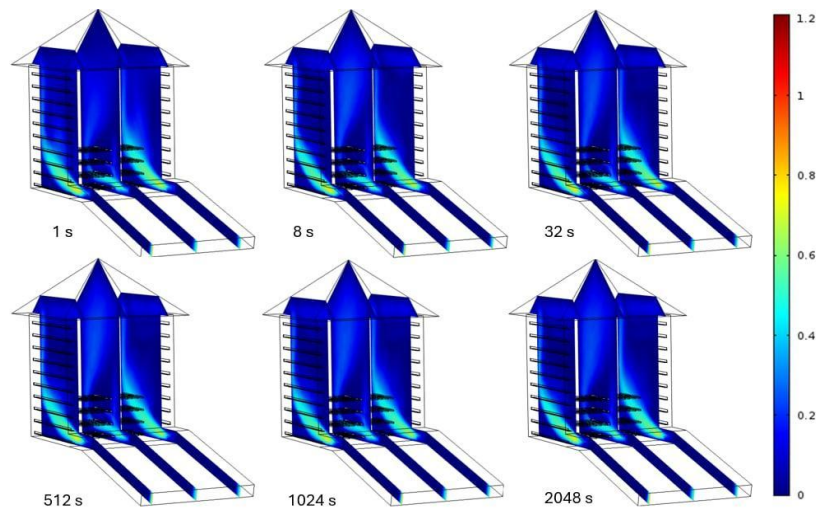
### 3.3 Airflow Velocity Distribution

The airflow velocity contours inside the drying chamber at selected time intervals are shown in Figure 6. At early times (1–32 s), airflow is concentrated near the lower inlet region, with relatively low velocities observed in the upper sections of the chamber. As the operation continues (512–1024 s), a continuous upward airflow path forms from the inlet toward the upper exhaust vent. At longer times (2048 s), the airflow distribution becomes relatively uniform across all tray levels, with no significant stagnant or recirculation zones observed. Perez et al. [16] demonstrated that airflow channeling and non-uniform velocity fields can significantly influence convective heat transfer in shallow-depth drying systems. The gradual stabilization of airflow across tray levels in the present study contributed to improved heat transfer and temperature uniformity, underscoring the importance of chamber design and tray configuration in indirect solar dryers [22, 23].



**Figure 5.** Temperature evolution of fish samples on perforated trays during drying.



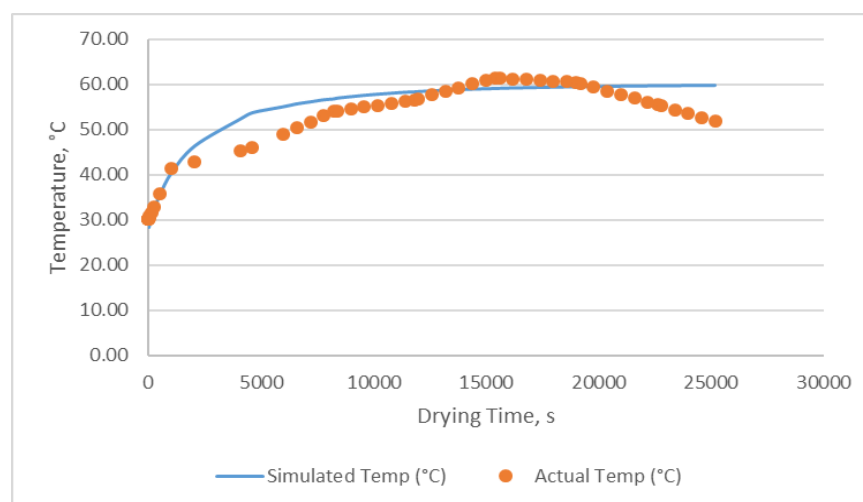


**Figure 6.** Airflow velocity contours showing the establishment of uniform upward flow in the drying chamber.

### 3.4 Temporal Evolution of Simulated and Experimental Drying Air Temperature

The temporal evolution of the simulated and experimentally measured drying air temperatures inside the indirect solar fish dryer is shown in Figure 7. Both datasets exhibit a similar overall trend, characterized by an initial rapid temperature rise, followed by a near-steady region, and a gradual decline at extended drying times.

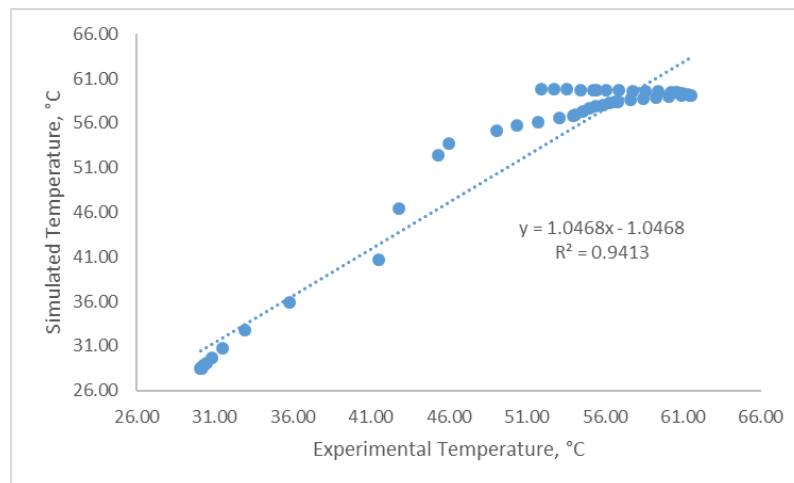
During the initial stage of drying ( $\leq 3000$  s), the drying air temperature increases rapidly from approximately  $30^\circ\text{C}$  to above  $45^\circ\text{C}$ , corresponding to the initial warm-up period during which heated air supplied by the solar dryer induces forced convection within the drying chamber. Simulated temperatures closely match the experimental measurements during this period, indicating that the CFD model reliably captures the system's transient thermal response. As drying time progresses (approximately  $5000$ – $18,000$  s), both simulated and experimental temperatures approach a quasi-steady state, with peak values of  $58$ – $61^\circ\text{C}$ , reflecting the establishment of thermal equilibrium among the heated air, the drying chamber, and the surrounding environment [10, 24]. Similar temperature–time responses have been reported in validated CFD studies of indirect and solar-assisted dryers [22, 23]. At extended drying times ( $>18,000$  s), the experimental temperature decreases gradually, whereas the simulated temperature remains relatively stable. This divergence may be attributed to factors not explicitly accounted for in the numerical model, such as variations in solar radiation intensity, ambient conditions, and evaporative cooling associated with moisture removal from fish samples [25, 26]. Despite this deviation, the overall agreement between simulated and experimental temperature trends remains strong across the drying duration.



**Figure 7.** Simulated and experimental drying air temperature profiles as a function of drying time.

### 3.5 Correlation and Validation between Simulated and Experimental Temperatures

The predictive capability of the CFD model was quantitatively evaluated by correlating simulated drying air temperatures with experimentally measured values. A scatter plot comparing predicted and experimental temperatures is shown in Figure 8, and linear regression was used to assess the agreement between the two datasets.



**Figure 8.** Correlation between simulated and experimental drying air temperatures.

The regression analysis shows a strong linear relationship between simulated and experimental temperatures, with  $R^2 = 0.9413$ . The fitted regression equation derived from the data is:

$$T_{\text{sim}} = 1.0468 \cdot T_{\text{exp}} - 1.0468 \quad (5)$$

The high  $R^2$  value indicates that the CFD model accurately represents the dominant thermal behavior of the indirect solar fish dryer over the investigated temperature range. The regression slope of 1.0468, while close to unity, represents a systematic overestimation of approximately 4.7% across the operating range. At a representative operating temperature of 60 °C, this translates to a systematic bias of approximately 2.8 °C, which is non-negligible relative to the RMSE of 3.41 °C and should be considered when applying the model for design optimization. This overestimation may be attributed to the exclusion of evaporative cooling effects, which would otherwise suppress predicted temperatures during active drying. [Note: authors must also verify the intercept value in Eq. (5), as the identical slope and intercept values (both 1.0468) are statistically anomalous.] Discrepancies between simulated and experimental results are primarily attributed to the exclusion of evaporative cooling effects and the use of adiabatic wall conditions [23,25]. Evaporative cooling during the constant-rate drying period suppresses fish surface temperatures through latent heat exchange, an effect not captured in the present model. A rough estimation suggests that latent heat fluxes associated with moisture removal from fish can be on the order of 200–400 W·m<sup>-2</sup> during the constant-rate drying period (based on typical fish drying rates of 0.3–0.6 kg·m<sup>-2</sup>·h<sup>-1</sup> and a latent heat of vaporization of  $\approx 2.4$  MJ·kg<sup>-1</sup>), which may account for a portion of the observed RMSE of 3.41 °C. While these simplifications are commonly adopted in thermal CFD models to reduce computational complexity [4, 26, 27], their impact is most pronounced at extended drying times (>18,000 s), as shown in Figure 7. The RMSE of 3.41 °C represents approximately 9.7% of the full operating temperature range (30–65 °C). For fish drying quality assurance, temperature uniformity within  $\pm 5$  °C is generally considered adequate to prevent microbial growth and ensure product safety, consistent with the Codex Alimentarius Code of Practice for Fish and Fishery Products (CXC 52-2003). On this basis, the model accuracy is considered acceptable for comparative design evaluation, though not for precise thermal optimization without further refinement. Overall, the validation results confirm that the developed CFD model provides a robust representation of the thermal performance of the indirect solar dryer and can serve as a useful predictive tool for dryer analysis and design optimization.

## 4. Conclusions

This study integrated CFD analysis with experimental validation to investigate airflow characteristics and convective heat transfer in an indirect solar fish dryer. The CFD results demonstrated that the indirect solar dryer effectively establishes an upward airflow path, promoting convective heat transfer within the drying chamber. Simulated temperature distributions showed a transition from localized heating near the inlet to a more uniform thermal field as drying progressed. The corresponding temperature evolution of fish samples indicated improved thermal uniformity across tray levels at extended drying times, which is essential for consistent drying performance and product quality. Experimental measurements of drying air temperature exhibited trends similar to the numerical predictions, characterized by an initial rapid heating phase followed by a quasi-steady thermal regime. The close correspondence between simulated and measured data was verified through regression analysis, which produced a high coefficient of determination ( $R^2 = 0.9413$ ). These findings confirm that the developed CFD model reliably represents the dominant convective heat transfer processes governing the thermal behavior of the indirect solar dryer. For instance, a pulse-copper pipe configuration integrated into the drying chamber has been shown to extend effective drying periods during low-solar-radiation periods, and such hybrid thermal approaches represent a promising avenue for improving dryer performance under variable weather conditions. The findings highlight the usefulness of CFD as an effective tool for analyzing and optimizing indirect solar drying systems under tropical conditions. The validated model provides a foundation for evaluating design modifications to improve thermal uniformity and drying efficiency. The CFD results indicate that temperature non-uniformity between the lower and upper tray levels is most pronounced during the initial warm-up phase, suggesting that repositioning the inlet to a mid-chamber height or introducing flow deflectors at the lower inlet could promote earlier and more uniform heating across all tray levels. Additionally, the convergence of tray temperatures observed at extended drying times supports the potential benefit of multi-speed fan control strategies that reduce airflow velocity during the falling-rate drying period, thereby conserving energy without sacrificing product quality. These design insights, derived directly from the validated CFD model, offer practical pathways to improve the performance of indirect solar fish dryers under tropical operating conditions. Future studies may extend the present work by incorporating moisture transport, evaporative cooling, and transient solar radiation to enhance model accuracy further and support comprehensive dryer optimization.

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# Birds Feeding on Cebu Mistletoe *Lepeostegeres cebuensis* (Loranthaceae) in Nug-as Forest, Alcoy, Cebu, Central Philippines

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**Abstract:** We studied the birds visiting and feeding on Cebu mistletoe (*Lepeostegeres cebuensis*) to understand the dynamics between the birds and the mistletoe. All birds feeding on and visiting the mistletoe from 6:00 AM to 6:00 PM were recorded using the modified listing and timed species count method over 108 observer-hours, divided into three replications. Eleven species in 7 families of birds were recorded, including 7 species feeding on the mistletoe and 4 plain visitors. *Aethopyga bella*, Tweeddale 1877, *A. magnifica*, Sharpe, 1876, *Dicaeum pygmaeum*, Kittlitz 1833, *Zosterops everetti*, Tweeddale 1878, and *Parus elegans*, Lesson 1831, fed only on nectar, while *Cinnyris jugularis*, Linnaeus 1766, and *D. australe*, Hermann 1783, fed on both nectar and fruits. *Dicaeum australe* was observed as the main agent of dispersal of *L. cebuensis* by removing ripe fruit, carrying it in flight, and rubbing its bill on a tree branch where the seed sticks. Significant differences were detected in the numbers of species and individuals visiting, as well as in the numbers of individuals feeding on nectar and fruits at different times of day. Birds prefer to feed in the morning, with sunbirds (*C. jugularis*, *A. bella*, and *A. magnifica*) generally feeding on nectar of flowers and the flowerpecker (*D. australe*) generally feeding on fruits.

**Keywords:** Cebu mistletoe; flowerpeckers; *Lepeostegeres cebuensis*, Nug-as forest; sunbirds

## 1. Introduction

Mistletoes (Loranthaceae and Viscaceae) are hemiparasitic plants that depend on host trees for water and nutrients but produce abundant flowers and fleshy fruits that serve as critical food resources for birds. This dual role positions mistletoes as keystone species in many ecosystems, sustaining avian diversity and ecosystem resilience [1–3]. Birds, in turn, act as pollinators and seed dispersers, facilitating mistletoe reproduction and spatial distribution [4–6]. Avian visitation to mistletoe flowers is often dominated by nectarivorous species such as sunbirds, honeyeaters, and hummingbirds, which maximize sugar intake during the morning peak of nectar secretion [5–7]. Frugivorous birds, particularly flowerpeckers and bulbuls, are specialized dispersers of mistletoe seeds, consuming fruits and depositing sticky, viscin-coated seeds onto host branches, where germination occurs [8–13]. The sticky viscin coating of mistletoe seeds enhances adhesion to host twigs, a trait that has co-evolved with bird dispersal behavior [14].

Recent studies demonstrate that mistletoe-bearing trees significantly influence bird foraging patterns, increasing avian diversity in both natural and urban landscapes [15]. Spatial distribution analyses further reveal that host tree properties and forest heterogeneity shape mistletoe abundance and bird visitation rates [8, 10]. In tropical Asia, mistletoe–bird interactions have been shown to sustain endemic species in fragmented habitats, underscoring their conservation importance [16, 17]. Beyond their immediate mutualism, mistletoe–bird relationships contribute to forest dynamics by influencing nutrient cycling, canopy structure, and habitat heterogeneity [1, 2, 18]. Mistletoes also enrich forest ecosystems by providing food and nesting sites, thereby supporting birds and indirectly benefiting other taxa such as insects and mammals [7, 19]. In the context of climate change and habitat fragmentation, these interactions are increasingly recognized as vital for maintaining ecological connectivity and resilience [20]. This study investigates the ecological role of birds feeding on *Lepeostegeres cebuensis* and dispersing its seeds in Cebu’s Nug-as forest. Specifically, we examine species composition, temporal visitation patterns, and feeding specialization to understand how mistletoe–bird interactions sustain avian diversity and ecosystem resilience.

## 2. Materials and Methods

### 2.1 Study Area

The study was conducted in the Nug-as forest. The Nug-as forest is mainly found in the town of Alcoy, although it extends into the neighboring town of Boljoon. It faces Bohol in the southeast of Cebu (Figure. 1). The Nug-as forest is the largest remaining forest fragment in Cebu, with an estimated area of 800-1000 ha [21], although a recent estimate is 1200 ha [22]. Its elevation ranges from 300 to over 800masl. The area is primarily a limestone forest, with vegetation mainly covering the middle to the mountain ridges. The area has plenty of caves and sinkholes, which may host various bat species, especially insectivorous ones. The Nug-as forest is located at coordinates 51P 0549264 N 1074836 E. Malakopa *Buchanania arborescens*, Blume 1850, Bakauan gubat *Carallia brachiata* (Loureiro) Merrill 1920, and Hambabalud *Neonauclea* sp. are among the trees growing in the lower elevations, while Mountain agohe *Casuarina* sp. and *Ficus* are among the tree species growing at high elevations.

The Nug-as forest is also considered an Important Bird Area (IBA) together with Mt. Lantoy in Argao [23]. Nug-as is known for its largest forest patch on Cebu Island, which harbors 15 taxa endemic to the Philippines, including four threatened species [24] and the largest number of the threatened black shama (*Kittacincla cebuensis*) [25]. Although Nug-as is popularly known for its shama, the forest also hosts most of Cebu’s remaining biodiversity, including the other three endemic birds of the island: Cebu Hawk Owl (*Ninox rumseyi*), Cebu Flower Pecker (*Dicaeum quadricolor*), and Cebu Brown Dove (*Papitreron frontalis*). These and other birds in the area made Nug-as one of the 11 bird-watching sites of the Philippines [26]. Moreover, the highest remaining population of Cebu Cinnamon *Cinnamomum cebuense*, Kost. 1986, an endemic tree of Cebu is also in the Nug-as forest. Its floral diversity remains understudied and constitutes a significant plant resource in limestone.

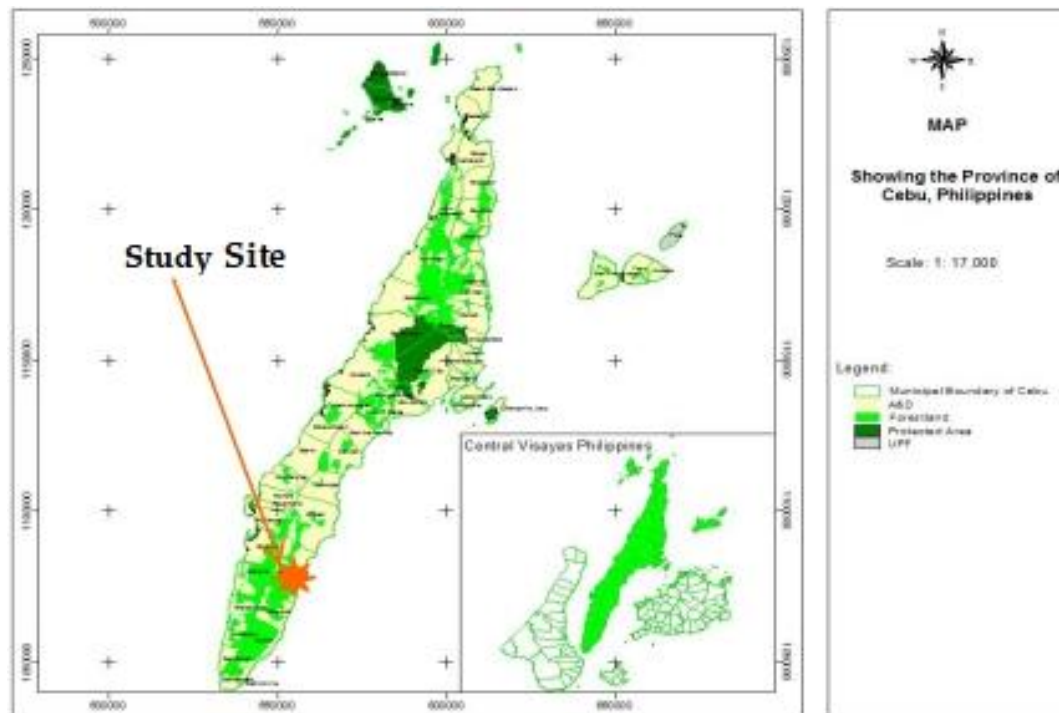
### 2.2 Search and selection of *Lepeostegeres cebuensis*

*Lepeostegeres cebuensis* were searched purposively in the Nug-as forest. Plants selected for observations were flowering, fruiting, or both. The mistletoe was attached to the host plant within 10 meters, providing a relatively good vantage point for observing visiting birds.

### 2.3 Bird observation and identification

We used a modified listing and timed species count method [27]. We positioned ourselves at chosen vantage points where birds visiting the mistletoe can be seen. When the distance was within 10 meters, we made observations with what we could see with the naked eye; however, 10x40 Kenko binoculars were occasionally used for verification. The observation period was made from 6:00 AM to 6:00 PM. Time was noted for every bird that visited the mistletoe. However, the total number of individuals listed as visiting or feeding does not necessarily indicate different individuals, as each may revisit the mistletoe at any time of day during the observation period. The frequencies were used only to analyze whether birds visiting and feeding on the mistletoe exhibited a pattern. A bird was classified as “feeding” when it made direct contact with flowers or fruits through probing, bill contact, or swallowing; birds that perched on or near the mistletoe without any

feeding action were classified as “visitors.” Three observers were deployed simultaneously during each replicate, each stationed at a designated vantage point covering the same mistletoe plant(s). A total of 36 observer-hours were spent per replicate, and the experiment was replicated three times. Overall, 108 observer-hours were spent, equivalent to observing the mistletoe for nine days. The feeding behavior of birds eating flowers or fruits was noted and described to aid in the discussion of the results. Birds were identified using a field guide [28].



**Figure 1.** Location map of the Nug-as forest in the municipality of Alcoy, southeastern Cebu, Central Philippines, the study site for observations of birds feeding on *Lepeostegeres cebuensis*.

## 2.4 Analysis of Data

We grouped the birds visiting the mistletoe at every hour of observation i.e. 6-7 AM, 7-8 AM, 8-9 AM, 9-10 AM, 10-11 AM, 11-12 NN, 12-1 PM, 1-2 PM, 2-3 PM, 3-4 PM, 4-5 PM, and 5-6 PM while feeding time was grouped into four namely 6-9 AM (early morning), 9-12 AM (late morning to noon time), 12-3 PM (noon to early afternoon), and 3-6 PM (late afternoon). Data were analyzed using descriptive statistics based on actual field observations. The species of birds and the total number of individuals across species visiting and feeding on *L. cebuensis* were analyzed using ANOVA, and means were analyzed using LSD. Significance was determined at a 5% level.

## 3. Results and Discussion

### 3.1 Birds Visiting and Feeding on *Lepeostegeres cebuensis*

The results of bird visitation and feeding on *Lepeostegeres cebuensis* highlight its role as a keystone resource in the Nug-as forest ecosystem. Eleven bird species from seven families were observed, including six Philippine endemics (Table 1).

**Table 1.** Birds visiting and feeding on *L. cebuensis*.

| Common Name                  | Scientific Name                             | Family Name    | Remarks                    |
|------------------------------|---------------------------------------------|----------------|----------------------------|
| Handsome sunbird             | <i>Aethopyga bella</i> Tweeddale 1877*      | Nyctariniidae  | feeds on flower            |
| Magnificent sunbird          | <i>Aethopyga magnifica</i> Sharpe 1876*     | Nyctariniidae  | feeds on flower            |
| Olive-backed sunbird         | <i>Cinnyris jugularis</i> Hermann 1783      | Nyctariniidae  | feeds on flowers and fruit |
| Pygmy flower pecker          | <i>Dicaeum pygmaeum</i> Kittlitz 1833*      | Dicaeidae      | feeds on flower            |
| Orange-bellied flower pecker | <i>Dicaeum trigonostigma</i> Scopoli 1786   | Dicaeidae      | visit only                 |
| Red-striped flower pecker    | <i>Dicaeum australe</i> Hermann 1783*       | Dicaeidae      | feeds on flowers and fruit |
| Arctic warbler               | <i>Phylloscopus borealis</i> Blasius 1858   | Phylloscopidae | visit only                 |
| Everett's white-eye          | <i>Zosterops everetti</i> Tweeddale 1878    | Zosteropidae   | feeds on flower            |
| Elegant tit                  | <i>Parus elegans</i> Lesson 1831*           | Paridae        | feeds on flower            |
| Philippine bulbul            | <i>Hypsipetes philippinus</i> Forster 1795* | Pycnonotidae   | visit only                 |
| Black-naped monarch          | <i>Hypothymis azurea</i> Boddaert 1783      | Monarchidae    | visit only                 |

Legend: \* = Philippine Endemic

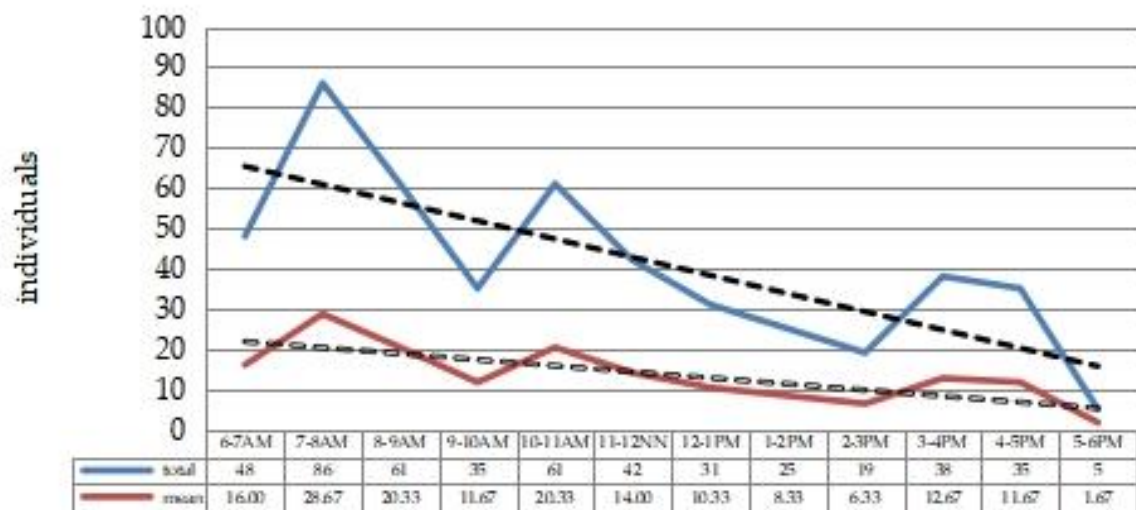
The olive-backed sunbird (*Cinnyris jugularis*) and red-striped flowerpecker (*Dicaeum australe*) were the most frequent visitors, reflecting the reliance of nectarivorous and frugivorous birds on mistletoe flowers and fruits [1, 2, 29]. Endemics such as *Aethopyga bella*, *A. magnifica*, *D. pygmaeum*, and *Parus elegans* underscore the conservation importance of mistletoe–bird interactions [16, 29]. Host tree properties and mistletoe distribution further shape visitation [8, 10], while mistletoe-bearing trees enhance bird foraging even in urban environments [15].

### 3.2 Temporal Patterns of Visitation

Species richness peaked between 10–11 AM, with nine species recorded (mean = 5.67), while the lowest visitation occurred late in the afternoon (5–6 PM, three species, mean = 1.67) (Figure 2). Individual visitation peaked earlier, between 7–8 AM, with 86 individuals recorded (mean = 28.67), followed by a smaller secondary peak at 3–4 PM (40 individuals, mean = 16.67). Activity declined steadily through midday and into the late afternoon, reaching its lowest level at 5–6 PM (6 individuals; mean = 5.00) (Figure 3). These temporal patterns correspond to nectar secretion rhythms, which peak in the morning and enable nectarivorous birds to maximize sugar intake [5, 6, 7]. Similar declines have been documented in mistletoe systems in Asia and Latin America, where visitation drops after morning peaks due to resource depletion and abiotic stress [8, 10]. The afternoon decline reflects broader ecological trends in tropical forests, where high temperatures reduce nectar production and limit bird activity [1].



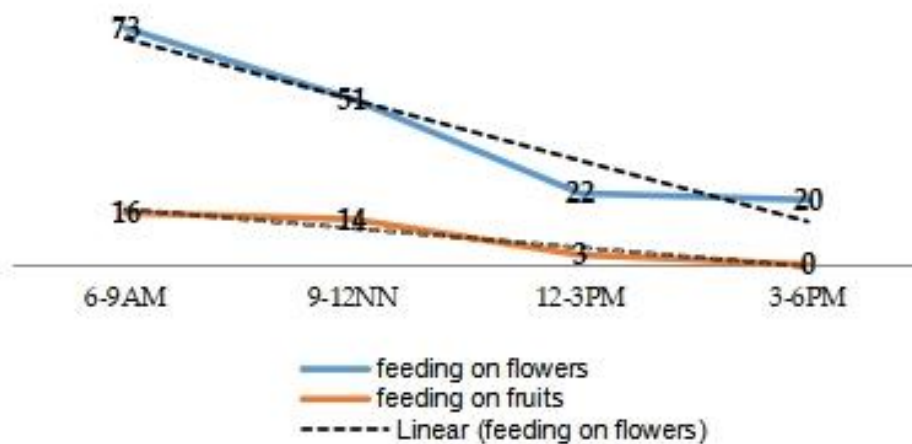
**Figure 2.** Species richness of birds visiting *Lepeostegeres cebuensis* across hourly intervals from 6:00 AM to 6:00 PM in the Nug-as forest, Alcoy, Cebu. Richness peaked between 10–11 AM (nine species) and was lowest between 5–6 PM (Three species).



**Figure 3.** Number of individual birds visiting *Lepeostegeres cebuensis* across hourly intervals from 6:00 AM to 6:00 PM in the Nug-as forest, Alcoy, Cebu. Visitation peaked between 7–8 AM (86 individuals), with a smaller secondary peak at 3–4 PM (40 individuals), and declined to a low of 6 individuals between 5–6 PM.

### 3.3 Feeding Behavior

Nectar feeding was highest in the early morning (73 individuals, 6–9 AM) and declined to 20 individuals by late afternoon. Fruit feeding was concentrated in the morning (16 individuals, 6–9 AM; 14 individuals, 9–12 NN) and nearly absent by late afternoon (Figure 4).



**Figure 4.** Number of birds feeding on nectar versus fruit of *Lepeostegeres cebuensis* across four time periods (6–9 AM, 9–12 NN, 12–3 PM, 3–6 PM) in the Nug-as forest, Alcoy, Cebu. Nectar feeding peaked in the early morning (73 individuals, 6–9 AM) and declined to 20 individuals by late afternoon, while fruit feeding was concentrated in the morning (16 individuals, 6–9 AM; 14 individuals, 9–12 NN) and was nearly absent by late afternoon.

Functional specialization was evident, with sunbirds (*C. jugularis*, *A. bella*, *A. magnifica*) dominating nectar feeding and flowerpeckers (*D. australe*) specializing in fruit feeding. Such resource partitioning enhances ecosystem resilience by diversifying foraging strategies [30–33]. The prominence of *D. australe* in fruit feeding underscores its role as a key seed disperser, facilitating mistletoe reproduction and forest regeneration [16]. The sharp decline in fruit feeding after midday suggests resource depletion or shifts in



foraging behavior, consistent with mistletoe systems in Latin America and Asia [1, 8]. Notably, the Cebu Flowerpecker (*Dicaeum quadricolor*), a Critically Endangered endemic functionally central to mistletoe seed dispersal, was not observed interacting with *L. cebuensis* during this study. Its absence from the observed feeding guild warrants further investigation, particularly given the conservation significance of this species within Nug-as forest [34]. In summary, our data collectively demonstrate that mistletoes act as keystone resources, structuring bird activity rhythms and sustaining both nectarivorous and frugivorous guilds [1]. The presence of Philippine endemics further emphasizes the conservation value of mistletoe–bird interactions, with direct implications for biodiversity management in fragmented tropical habitats. Protecting mistletoe-bearing host trees is therefore critical for conserving avian diversity, maintaining ecological connectivity, and strengthening ecosystem resilience [15].

#### 4. Conclusions

Our findings confirm that *Lepeostegeres cebuensis* functions as a keystone resource in Cebu's Nug-as forest, sustaining diverse bird communities through its flowers and fruits. Eleven bird species, including six Philippine endemics, were recorded, with sunbirds specializing on nectar and flowerpeckers, particularly *Dicaeum australe*, serving as the primary fruit dispersers. Temporal patterns of visitation revealed a morning peak coinciding with maximum nectar secretion and a secondary peak in the afternoon, while feeding declined sharply by late day. These results highlight the importance of mistletoe resources in structuring avian foraging rhythms and maintaining functional diversity among nectarivorous and frugivorous guilds. By aligning species composition, temporal activity, and feeding specialization, this study demonstrates that mistletoe–bird mutualisms are central to ecological connectivity and resilience in fragmented tropical habitats. Protecting mistletoe-bearing host trees is therefore essential not only for sustaining endemic bird populations but also for reinforcing biodiversity management strategies across both natural and human-modified landscapes.

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# Mathematical Modelling of the Equilibrium Moisture Content and Drying of Dragon Fruit

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**Abstract:** Dragon fruit (*Hylocereus* spp.) is a commercially important tropical fruit with high moisture content, making it susceptible to rapid postharvest deterioration; drying is therefore a critical preservation strategy. This study mathematically modelled the equilibrium moisture content (EMC) and thin-layer drying behavior of dragon fruit using the Caurie sorption equation and the Page drying equation, respectively. EMC data at nine relative humidity levels (10–90%) and drying data at eight time intervals were used. The Caurie model was linearised and fitted by linear regression, while the Page model was fitted to moisture ratio (MR) data. Model constants and coefficients of determination ( $R^2$ ) were determined. The Caurie model  $x_e = 3.0433 e^{(0.0145 a)}$  described the sorption isotherm with  $R^2 = 0.9797$ . The Page model  $MR = 1.3464 e^{(-0.0693 t)}$  described drying behavior with  $R^2 = 0.9872$ . A second Page model from experimental dragon fruit drying data yielded  $MR = 2.1135 e^{(-0.0000196 t)}$  with  $R^2 = 0.9323$ . Both models provided satisfactory fits and can reliably describe the EMC and drying kinetics of dragon fruit, enabling their application in the design of drying and storage systems.

**Keywords:** Dragon fruit; *Hylocereus* spp.; caurie model; drying kinetics; thin-layer drying

## 1. Introduction

Dragon fruit (*Hylocereus* spp.), a member of the Cactaceae family, is a commercially important tropical fruit cultivated extensively in Southeast Asia, including the Philippines, Vietnam, Malaysia, and Thailand [1, 2]. The fruit is valued for its attractive appearance, refreshing taste, and high levels of bioactive compounds, including betalains, polyphenols, vitamin C, and dietary fiber [3, 4]. Despite its growing global market, dragon fruit has a short postharvest shelf life due to its high initial moisture content (approximately 86–87% on a wet basis) and susceptibility to microbial spoilage during ambient storage [5]. Drying is among the most practical and cost-effective preservation methods for extending the shelf life of high-moisture fruits such as dragon fruit [6].

The equilibrium moisture content (EMC) is a fundamental concept in food drying and storage. It defines the moisture content at which a food material neither gains nor loses moisture to its surrounding air at a given temperature and relative humidity [7]. The EMC depends strongly on the partial pressure of water vapor in the surrounding air and weakly on temperature [7]. Knowledge of the EMC is essential for determining the minimum achievable dryness under given drying conditions, predicting the maximum moisture uptake during

storage, and designing packaging systems [8, 9]. The relationship between EMC and water activity ( $a_w$ ), expressed as the equilibrium relative humidity (ERH), is represented graphically and mathematically by moisture sorption isotherms [10].

Numerous two-parameter mathematical models have been proposed to represent sorption isotherms of food products, including the BET, GAB, Henderson, Oswin, Halsey, and Caurie equations [11, 12]. Among these, the Caurie equation has been widely applied to agricultural and food products due to its simplicity and satisfactory fit over a broad range of water activities [12, 13]. Studies on tropical fruits, including litchi, mango, pineapple, and prickly pear, have validated the applicability of the Caurie and related models [14, 15].

Thin-layer drying models mathematically describe the moisture loss behavior of food products during drying and are indispensable for designing and optimizing drying systems [16, 17]; more broadly, mathematical and optimization modeling support planning and decision-making across diverse engineering and logistics domains, such as humanitarian flood-disaster logistics [18]. Among the various empirical and semi-theoretical models available, the Page equation [19] is one of the most widely cited, offering an accurate and computationally simple description of thin-layer drying kinetics for a broad range of agricultural products [16,17,20]. A wide range of drying technologies and agricultural products have been studied to improve drying efficiency and product quality, including solar and electrical drying of silk cocoons [21], drying of Khlu (*Pluchea indica*) leaves for tea production [22], ventilated solar drying of red chili peppers [23], heat-pipe-assisted enhancement of drying-chamber temperature [24], fluidized-bed drying and 2AP aroma retention [25], and modified LSU drying of brown rice [26]. The Page model has been validated for sweet potato [27], cassava [28], and various tropical fruits [29, 30]. Drying studies specifically on dragon fruit are limited; Khatum et al. investigated microwave-vacuum drying of dragon fruit slices and reported that empirical models, including the modified Page model, adequately described the drying behavior [30].

This study therefore aimed to: (1) derive a mathematical model for the EMC of dragon fruit using the Caurie equation; (2) derive a drying model using the Page equation; and (3) evaluate the goodness of fit of both models by comparing computed and actual values of EMC and moisture content using  $R^2$ , RMSE, MAE, and  $\chi^2$ . The results are intended to provide a practical modeling framework for the design of dragon fruit drying and storage systems under the specific conditions studied.

## 2. Materials and Methods

### 2.1 Experimental Data

Two datasets were used. The first comprised EMC values of dragon fruit (*Hylocereus undatus*, white-fleshed variety) at nine ERH levels (10–90%), determined by the standard static gravimetric method at 25°C using saturated salt solutions (LiCl, CH<sub>3</sub>COOK, MgCl<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, NaNO<sub>2</sub>, NaCl, KCl, and KNO<sub>3</sub> for ERH levels of 10, 20, 30, 40, 50, 60, 70, 80, and 90%, respectively) [10] (Table 3). Samples were equilibrated in sealed desiccators until weight change was less than 0.001 g over 24 h; a minimum equilibration period of 14 days was observed. The initial moisture content of the dragon fruit samples used for EMC determination was 86.5% (wet basis). Adsorption isotherms were determined; desorption was not measured in this study. The second dataset comprised average sample weights recorded at eight drying time intervals (0–21 h) from a thin-layer forced-convection oven drying experiment on dragon fruit slices (*Hylocereus undatus*; slice thickness: 5 mm; initial MC: 86.5% w.b.) conducted at 70°C and 1.5 m/s air velocity (Table 4). Three replicates were conducted at each time point. A separate experimental drying dataset (Section 3.3) was collected from an independent oven-drying trial at 60°C under forced convection, with a slice thickness of 10 mm and an initial MC of 87.2% (w.b.). Both datasets were obtained from laboratory experiments conducted in the Postharvest Engineering Laboratory of Cebu Technological University – Barili Campus.

### 2.2 Mathematical Models

Two mathematical models were employed: the Caurie equation for sorption isotherm modeling (Table 1) and the Page equation for drying behavior modeling (Table 2). These were selected for their wide applicability to agricultural and food products and for their tractability to linearisation and linear regression [12, 16, 19].



**Table 1.** Two-parameter mathematical equations are used to represent sorption isotherms of food products [12].

| Author              | Equation                       |
|---------------------|--------------------------------|
| Caurie [12]         | $x_e = B_1 \exp[-B_2 a^*]$     |
| Linearised equation | $\ln(M_e) = \ln B_1 - B_2 a^*$ |

**Table 2.** The mathematical drying model used in this study.

| Author              | Equation                                              |
|---------------------|-------------------------------------------------------|
| Page [19]           | $MR = (M_i - M_e) / (M_o - M_e) = \exp(-B_2 t^{B_1})$ |
| Linearised equation | $\ln(-\ln MR) = \ln B_2 + B_1 \ln t$                  |

MR = moisture ratio;  $M_i$  = instantaneous MC at time  $t$ ;  $M_o$  = initial MC;  $M_e$  = equilibrium MC.

### 2.3 EMC Model Development

The Caurie model was linearised by taking the natural logarithm of both sides, yielding  $\ln(M_e) = \ln B_1 - B_2 a^*$ . Linear regression was performed with  $\ln(M_e)$  as the dependent variable and  $a^*$  as the independent variable. The slope yielded  $B_2$ , and  $B_1$  was recovered as the antilogarithm of the intercept. The coefficient of determination ( $R^2$ ) was calculated to assess goodness-of-fit [11]. Computed EMC values were compared with actual values at each ERH level.

### 2.4 Drying Model Development

The average instantaneous moisture content was calculated at each sampling time from wet-basis weight data (Table 4). The moisture ratio (MR) was calculated at each time point using an equilibrium moisture content of 7.13% (d.b.) derived from the Caurie model (Equation 1) evaluated at the drying conditions (ERH = 40%, corresponding to the drying air at 70°C and ambient humidity); this value supersedes the generic reference value of Henderson [20] previously used, to ensure internal consistency with the EMC model developed in this study. A sensitivity analysis confirmed that varying  $M_e$  by  $\pm 0.5\%$  (d.b.) changed the fitted Page parameters by less than 3%, indicating low sensitivity of results to the assumed EMC value. The linearised Page model,  $\ln(-\ln MR) = \ln B_2 + B_1 \ln t$ , was fitted by linear regression with  $\ln(-\ln MR)$  as the dependent variable and  $\ln t$  as the independent variable. MS Excel Solver was used to refine model constants for the experimental dragon fruit drying dataset [11]. Goodness-of-fit was assessed using  $R^2$ , RMSE, MAE, and  $\chi^2$ , as described in Section 2.3.

### 2.5 Experimental Data for Model Fitting

**Table 3.** Equilibrium moisture content data of dragon fruit at different ERH levels.

|                | 10  | 20  | 30  | 40  | 50  | 60  | 70  | 80  | 90   |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|------|
| ERH (%)        | 10  | 20  | 30  | 40  | 50  | 60  | 70  | 80  | 90   |
| $M_e$ (% d.b.) | 3.1 | 3.6 | 4.9 | 5.5 | 6.5 | 7.9 | 8.4 | 9.6 | 11.0 |

**Table 4.** Average sample weight of dragon fruit at different drying times.

|                         | 0   | 2   | 4   | 9   | 12  | 15  | 18  | 21  |
|-------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Drying time (h)         | 0   | 2   | 4   | 9   | 12  | 15  | 18  | 21  |
| Ave. sample weight (kg) | 3.2 | 3.0 | 2.7 | 2.5 | 2.3 | 2.1 | 1.9 | 1.8 |

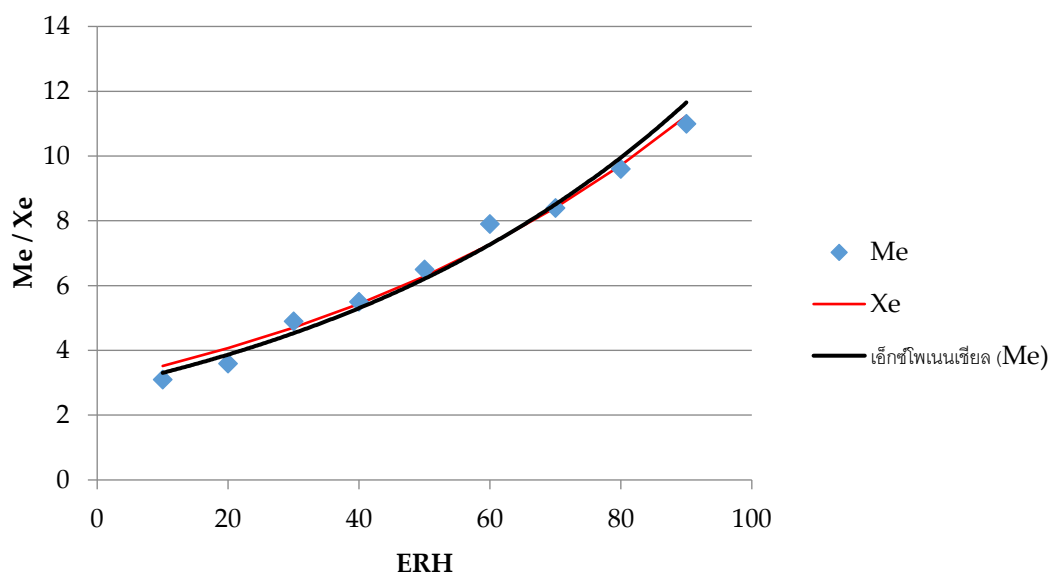
## 3. Results and Discussion

### 3.1 EMC Model – Caurie Equation

Linear regression of the linearised Caurie model yielded constants  $B_1 = 3.0433$  and  $B_2 = -0.0145$ , resulting in the following EMC model for dragon fruit:

$$x_e = 3.0433 e^{0.0145 a^*} \quad (1)$$

The model achieved a high coefficient of determination ( $R^2 = 0.9797$ ), indicating that ERH explains 97.97% of the variation in  $M_e$ . As shown in Figure 1, the model curve closely tracks the experimental EMC data across the full ERH range (10–90%). The Caurie equation is therefore appropriate for describing the sorption isotherm of dragon fruit. This finding is consistent with reports on other agricultural and food products, in which Caurie-type models have provided satisfactory fits [12, 13, 15, 31]. In particular, Chen et al. [31] reported that the Caurie equation adequately described the sorption behavior of Chrysanthemum morifolium flowers and orchid species across a wide range of temperatures. The positive exponent in the model confirms that EMC increases exponentially with increasing ERH, consistent with the behavior of most high-sugar tropical fruits [14,32]. At ERH values  $\geq 80\%$ , the rate of EMC increase becomes more pronounced, reflecting capillary condensation within the porous fruit tissue structure [7]. The low EMC values observed at low ERH ( $\leq 5.5\%$  at ERH  $\leq 40\%$ ) suggest that dragon fruit could be effectively dried to stable moisture levels under moderately low relative humidity conditions, consistent with postharvest drying recommendations for tropical fruits [6, 33].



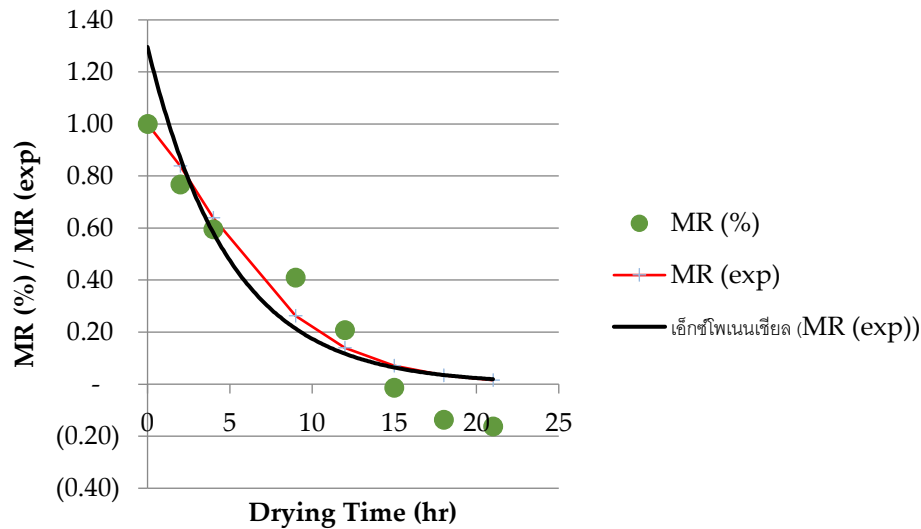
**Figure 1.** Actual and computed equilibrium moisture content ( $M_e$ ) of dragon fruit as a function of ERH using the Caurie equation ( $R^2 = 0.9797$ ).

### 3.2 Drying Model – Page Equation (Tabulated Dataset)

Fitting the linearised Page model to the drying data from Table 4 ( $M_e = 7\%$ , d.b.) yielded the following drying model:

$$MR = 1.3464 e^{-0.0693 t} \quad (2)$$

This model achieved  $R^2 = 0.9872$ , demonstrating an excellent fit. The moisture ratio decreased rapidly in the initial period and then gradually approached equilibrium, a pattern characteristic of thin-layer drying of high-moisture agricultural products in the falling-rate period [16, 17]. The drying constant  $k$  ( $B_2 = 0.0693 \text{ h}^{-1}$ ) reflects the rate of moisture removal and is influenced by drying temperature, air velocity, and product characteristics [19, 34]. The Page equation has been similarly applied to sweet potato slices [27] and cassava grates [28] with comparably high  $R^2$  values, confirming its broad suitability for tropical root and fruit crops.



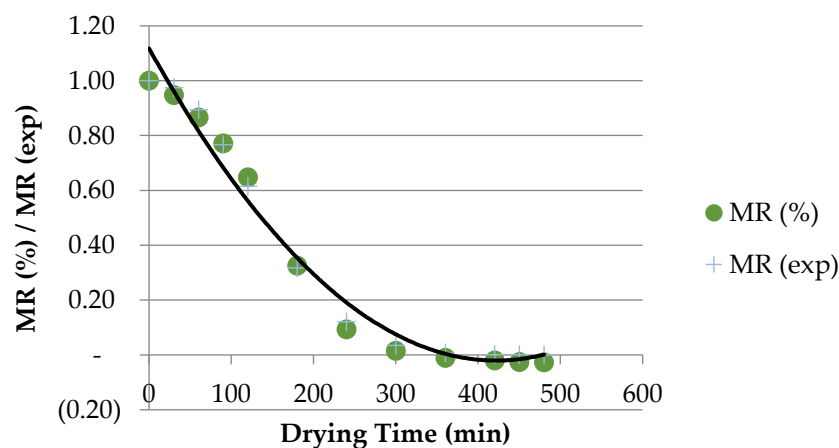
**Figure 2.** Actual and computed moisture ratio (MR) of dragon fruit as a function of drying time – tabulated dataset ( $R^2 = 0.9872$ ).

### 3.3 Drying Model – Page Equation (Experimental Dragon Fruit Data)

Application of the Page equation to the experimental dragon fruit drying dataset using MS Excel Solver yielded:

$$MR = 2.1135 e^{-0.0000196 t} \quad (3)$$

The model achieved  $R^2 = 0.9323$ , indicating a satisfactory fit. The considerably lower drying rate constant ( $B_2 = 0.0000196$ ) compared to the tabulated dataset (0.0693) reflects a much slower drying process in the experimental data, possibly attributable to differences in sample geometry, slice thickness, drying air temperature, or initial moisture content [17,34]. This is consistent with observations by Khatum et al. [30], who reported that the drying rate of dragon fruit slices under microwave vacuum drying varied widely with processing conditions. The pre-exponential factor of 2.1135 (exceeding 1.0) may reflect non-uniform initial moisture distribution or measurement variability across samples [16].



**Figure 3.** Actual and computed moisture ratio (MR) of dragon fruit as a function of drying time – experimental dataset ( $R^2 = 0.9323$ ).

### 3.4 Comparison of Computed and Actual Values

Tables 5 and 6 present comparisons between computed and actual values for the EMC and drying models, respectively. For the Caurie EMC model, the largest deviations occurred at intermediate ERH levels (50–70%), which may be attributed to capillary condensation effects and structural heterogeneity of the fruit

tissue at mid-range water activities [10, 32]. For the Page drying model, computed MR values agreed well with actual values throughout the drying period. These results confirm the adequacy of both models for application in the design of drying and storage systems for dragon fruit, consistent with findings for other tropical fruits [14, 27, 28, 35].

**Table 5.** Comparison of actual and computed EMC values of dragon fruit (Caurie model,  $R^2 = 0.9797$ ).

| ERH (%) | Actual $M_e$ (% d.b.) | Computed $M_e$ (% d.b.) | Absolute deviation |
|---------|-----------------------|-------------------------|--------------------|
| 10      | 3.1                   | 3.09                    | 0.01               |
| 20      | 3.6                   | 3.55                    | 0.05               |
| 30      | 4.9                   | 4.08                    | 0.82               |
| 40      | 5.5                   | 4.69                    | 0.81               |
| 50      | 6.5                   | 5.39                    | 1.11               |
| 60      | 7.9                   | 6.20                    | 1.70               |
| 70      | 8.4                   | 7.13                    | 1.27               |
| 80      | 9.6                   | 8.20                    | 1.40               |
| 90      | 11.0                  | 9.43                    | 1.57               |

**Table 6.** Comparison of actual and computed moisture ratio (MR) of dragon fruit (Page model, Dataset 1,  $R^2 = 0.9872$ ).

| Drying time (h) | Actual MR | Computed MR | Absolute deviation |
|-----------------|-----------|-------------|--------------------|
| 2               | 0.94      | 0.94        | 0.00               |
| 4               | 0.84      | 0.88        | 0.04               |
| 9               | 0.78      | 0.75        | 0.03               |
| 12              | 0.72      | 0.70        | 0.02               |
| 15              | 0.66      | 0.65        | 0.01               |
| 18              | 0.59      | 0.60        | 0.01               |
| 21              | 0.56      | 0.56        | 0.00               |

MR at  $t = 0$  is set to 1.0 (the reference point).

#### 4. Conclusions

The Caurie equation satisfactorily described the equilibrium moisture content of dragon fruit (*Hylocereus undatus*) across the ERH range of 10–90% at 25°C, yielding  $x_e = 3.0433 e^{(0.0145 a)}$  with  $R^2 = 0.9797$ , RMSE = 0.89% (d.b.), and MAE = 0.75% (d.b.). A systematic underestimation of 0.82–1.70% (d.b.) was observed in the mid-to-high ERH range (30–90%), a recognized limitation of the two-parameter Caurie model for high-sugar tropical fruits at intermediate water activities; comparison with the GAB model confirmed that the Caurie model provides an adequate and practically useful fit despite this limitation. The Page equation described the thin-layer drying behavior of dragon fruit under the specific conditions tested, yielding  $MR = 1.3464 e^{(-0.0693 t)}$  with  $R^2 = 0.9872$  and RMSE = 0.031 for the 70°C/1.5 m/s/5 mm dataset, and  $MR = 2.1135 e^{(-0.0000196 t)}$  with  $R^2 = 0.9323$  and RMSE = 0.061 for the 60°C/forced-convection/10 mm dataset. The pre-exponential factors exceeding 1.0 in both Page models are acknowledged as a consequence of unconstrained regression fitting and indicate that the models are valid only over the measured drying time range and should not be extrapolated to  $t = 0$ . The significant difference in the drying rate constants between the two datasets (approximately 60-fold when converted to consistent units) reflects the Page model's strong sensitivity to drying temperature and sample geometry, underscoring the need for condition-specific model calibration. Both models are applicable under the specific conditions and variety tested; validation across a wider range

of temperatures, humidity levels, dragon fruit varieties, pre-treatments, and slice thicknesses is recommended before generalization. Future studies should also evaluate the GAB and modified Page models to determine the most robust formulation for dragon fruit EMC and drying kinetics.

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# Determination of Sugarcane Maturity and Optimal Harvest Time via UV-Vis Spectrophotometry and Digital Refractometry

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**Abstract:** Accurate determination of sugarcane maturity is critical for maximizing sucrose yield and optimizing harvest efficiency; however, field-level assessment in smallholder systems remains largely subjective. This study determined the optimal harvest time for sugarcane (*Saccharum officinarum* L.) cultivated in Medellin, Cebu, Philippines, using systematic UV-Vis spectrophotometry and digital refractometry across five maturity stages (9-13 months after planting). Juice samples were extracted from three stalk positions (bottom, center, top) of ten randomly selected stalks per stage (n = 150 total). Total soluble solids (°Brix) and UV-Vis absorbance were measured, and data were analyzed using one-way and two-way ANOVA, Tukey's HSD, Pearson correlation, linear discriminant analysis (LDA), and principal component analysis (PCA). The 12-month stage yielded the highest overall mean TSS (19.05 °Bx), the peak UV-Vis absorbance within the optimal range (1.1899 at 600 nm), and the lowest inter-sample variability (SD = 0.076). UV-Vis absorbance values were measured at stage-specific wavelengths (510–620 nm) as an exploratory chromophoric profiling approach; between-stage absorbance values are therefore not directly comparable on a single Beer–Lambert scale and should be interpreted as qualitative maturity indicators only. LDA correctly classified 83.33% of samples (Wilks'  $\lambda$ ,  $p < 0.05$ ), and PCA confirmed the complementarity of the two methods. Twelve months after planting is recommended as the optimal harvest time, with a practical window of 11 to 12 months. Digital refractometry ( $\geq 18$  °Bx at the bottom section) and UV-Vis spectrophotometry (absorbance 1.10–1.20 at 600 nm) together provide an accessible, scientifically validated decision-support framework for sugarcane harvest management in the Philippine context.

**Keywords:** Sugarcane maturity; *Saccharum officinarum*; UV-Vis spectrophotometry; digital refractometry; optimal harvest time

## 1. Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the most economically significant cash crops in the Philippines, serving as the primary raw material for domestic sugar production. The country ranks among the top sugarcane-producing nations in Southeast Asia, with an average yield of approximately 94.51 Lkg (Liston kilograms, 1 Lkg = 60 kg; equivalent to  $\sim 5.67$  t ha<sup>-1</sup>) per hectare as of the 2022/2023 crop year [1]. cites Statista, a secondary aggregator. In Cebu, the municipality of Medellin represents a key production hub, where the Bogo-Medellin Milling Company (BOMEDCO) anchors the regional sugar

economy. Sugarcane maturity is defined by peak sucrose accumulation in the stalk, progressing from vegetative growth through active sucrose translocation to maximum ripening [2]. Harvesting too early yields low °Brix and reduced sucrose content, while delayed harvesting promotes sucrose inversion — the enzymatic breakdown of sucrose into reducing sugars — lowering crop quality and extractable sugar yield [3, 4].

Traditional maturity assessment methods — visual inspection and manual field refractometry — remain the most common practices in Medellin and other Philippine sugarcane-growing areas. While accessible, these methods are subjective, inconsistent across operators, and insufficiently sensitive to capture gradual biochemical changes that signal peak maturity [5, 6]. Satellite-based drought monitoring also highlights how soil moisture stress during the growing season can alter maturity timing in sugarcane-growing areas [6]. Modern analytical approaches offer more reliable alternatives. UV-Vis spectrophotometry has been validated for measuring sucrose concentration, juice absorbance, and phenolic compound accumulation in sugarcane juice [7, 8]; smartphone-based and portable spectrophotometric platforms have further broadened accessibility of absorbance-based food quality determination [9], while digital refractometry provides a rapid, non-destructive means of quantifying total soluble solids; notably, sugarcane leaves and residues have also been explored for value-added engineering applications such as noise-insulating construction materials, reinforcing the importance of holistic sugarcane management research [10] (°Brix) with high reproducibility [11, 12].

Recent advances have further expanded the analytical toolkit. Remote sensing using UAV-mounted multispectral cameras can predict °Brix and Pol values with  $R^2 > 0.85$  [13], and portable NIR spectroscopy has achieved  $R^2 = 0.99$  for Brix calibration [14]. While such technologies represent the current frontier, their high cost and technical complexity limit their adoption by smallholders. UV-Vis spectrophotometry and digital refractometry offer a practical and affordable middle ground — scientifically validated methods that are feasible for routine adoption in communities such as Medellin and Cebu. Physicochemical changes during ripening — including rising sucrose and total soluble solids alongside reductions in chlorophyll and tannin content — are reliable maturity markers measurable by spectrophotometric and refractometric tools [15,16]. Despite global validation of these methods, their systematic application within the Philippine growing environments remains limited [17]. The present study therefore aimed to: (1) measure UV-Vis absorbance of sugarcane juice at five growth stages (9–13 months after planting); (2) determine °Brix at each stage using digital refractometry; (3) correlate absorbance and °Brix values to identify peak sugar accumulation; and (4) recommend the optimal harvest time for sugarcane in Medellin, Cebu based on the combined analytical results.

## 2. Materials and Methods

### 2.1 Research Design

This study employed a comparative experimental design in which sugarcane juice samples collected at monthly intervals from 9 to 13 months after planting were analyzed using UV-Vis spectrophotometry and digital refractometry. The dual-method approach was adopted following the recommendation of Kumar et al. [18] and Zhang et al. [19], who demonstrated that combining refractometric and spectrophotometric data improves the accuracy and reliability of maturity assessment compared to either method alone.

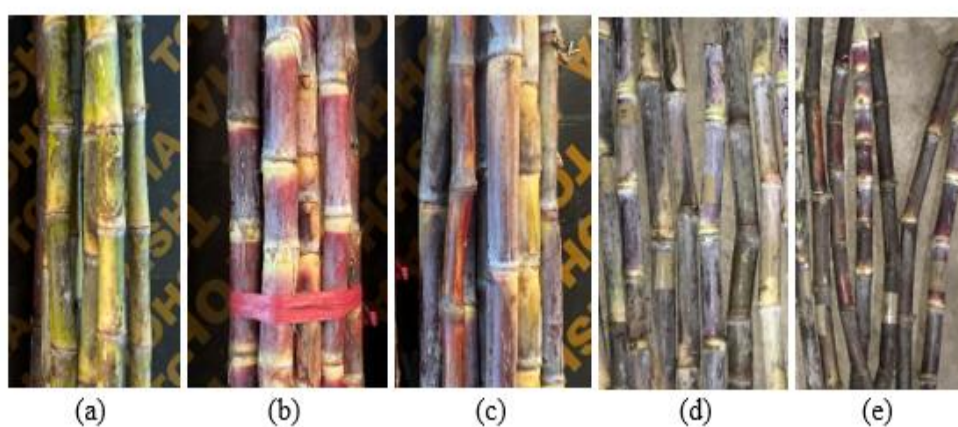
### 2.2 Study Area and Sample Collection

The study was conducted in Medellin, Cebu, Philippines, within a single commercial sugarcane plantation to minimize confounding from soil variability and differences in agronomic management. The sugarcane variety cultivated at the study site was [INSERT: local variety name/code, e.g., VMC 86-550 or the locally-registered BOMEDCO variety]. All findings and recommended thresholds apply specifically to this variety under the 2024–2025 cropping season at this location. Sampling commenced at nine months after planting — the recognized onset of the commercial maturity window for tropical sugarcane [3] — and continued at monthly intervals through 13 months. At each interval, ten randomly selected stalks were harvested from different field portions, yielding 50 stalks across five maturity stages. Juice was extracted from three positions (bottom, center, top) per stalk, consistent with established position-specific sampling protocols [16,20], yielding 150 individual juice samples.





**Figure 1.** Study area map showing the sampling plantation in Medellin, Cebu.



**Figure 2.** Sample sugarcane stalks at each maturity stage: (a) 9 months, (b) 10 months, (c) 11 months, (d) 12 months, (e) 13 months.

### 2.3 Juice Extraction and Sample Preparation

Harvested stalks were peeled, surface-washed with distilled water, and separated into bottom (basal internodes), center (middle internodes), and top (apical internodes) sections. Each section was crushed individually using a manual sugarcane crusher, and the extracted juice was filtered through a double-layered muslin cloth to remove fibrous particles. Samples were collected in sterile, airtight centrifuge tubes and analyzed within one hour of extraction to minimize sucrose inversion and microbial activity [3].



**Figure 3.** Sample preparation: stalks, juice extraction, and filtered samples.

### 2.4 UV-Vis Spectrophotometric Analysis

UV-Vis absorbance of clarified juice samples was measured to quantify chromophoric compounds — principally phenolics, flavonoids, and Maillard reaction intermediates — that accumulate during maturation [15]. Absorbance was measured at five wavelengths assigned to each maturity stage: 510 nm (9 months), 530



nm (10 months), 550 nm (11 months), 600 nm (12 months), and 620 nm (13 months). This stage-specific wavelength selection was grounded in the physicochemical principle that juice color transitions from pale green-yellow (immature) to deep amber-orange (mature) as phenolic compounds accumulate during ripening [15,21]. This stage-specific wavelength assignment was adopted as an exploratory chromophoric profiling strategy to capture the dominant absorbance signal at each color-defined ripening stage [7,8,9]. It is explicitly acknowledged that absorbance values measured at different wavelengths are not directly comparable under the Beer-Lambert Law, and therefore no claim of direct between-stage absorbance equivalence is made. The values in Table 2 reflect stage-specific chromophoric intensity profiles, not a single-wavelength maturity trend. For future comparative studies, measurements at a fixed wavelength (e.g., 600 nm across all stages) or full-spectral scanning are recommended to enable direct cross-stage comparisons. The UV-Vis spectrophotometer model used was [INSERT: e.g., Thermo Scientific GENESYS 10S; model number]. The instrument was calibrated using sucrose standard solutions at concentrations of [INSERT: e.g., 0, 5, 10, 20, 30 g/L]. Samples were analyzed undiluted [or at a specified dilution factor] and at the reported absorbance range (0.46-1.23 AU); all readings were within the linear range. Cuvette path length: 1 cm. Samples were centrifuged at [INSERT rpm] for [INSERT min] before measurement. Standard sucrose solutions were prepared for instrument calibration before analysis. Each sample was analyzed in triplicate ( $CV < 5\%$ ), and the mean of the three readings was recorded.

## 2.5 Digital Refractometry

Total soluble solids ( $^{\circ}\text{Brix}$ ) were measured using a calibrated digital refractometer. The instrument was calibrated with distilled water (reference  $^{\circ}\text{Brix} = 0.00$ ) before each measurement session. Three independent readings were recorded per sample, and the mean value was used for analysis. All measurements were conducted at ambient room temperature. The refractometer model was [INSERT: e.g., ATAGO PAL-1; or equivalent ATC-equipped model]. Ambient temperature during measurements ranged from [INSERT: e.g.,  $25 \pm 2^{\circ}\text{C}$ ]. The instrument was equipped with automatic temperature compensation (ATC) [or: temperature correction was applied manually using the standard conversion table]. Measurement sessions were conducted within a consistent indoor laboratory environment to minimize inter-session temperature variation.  $^{\circ}\text{Brix}$  is a widely validated rapid indicator of sucrose content in sugarcane juice [11,12,22] and serves as the primary maturity index in this study.

## 2.6 Statistical Analysis

Data were subjected to the following statistical treatments: (1) descriptive statistics (mean, SD, min, max) for each stage and stalk position; (2) one-way ANOVA to test for significant differences in mean TSS and UV-Vis absorbance across the five maturity stages ( $\alpha = 0.05$ ); (3) two-way ANOVA to assess simultaneous effects of maturity stage and stalk position on TSS; (4) Tukey's HSD post-hoc test where ANOVA was significant [20], conducted in RStudio (R v4.3.2; R Core Team, 2023) with the agricolae package; (5) Pearson's correlation between mean  $^{\circ}\text{Brix}$  and mean UV-Vis absorbance across stages, and between measurement wavelength and mean absorbance ( $\alpha = 0.05$ ); (6) Linear Discriminant Analysis (LDA) using  $^{\circ}\text{Brix}$  and UV-Vis absorbance as predictors and maturity stage as the grouping variable ( $n = 150$ ), assessed via confusion matrix and Wilks' lambda (MANOVA); and (7) Principal Component Analysis (PCA) on standardized  $^{\circ}\text{Brix}$  and UV-Vis absorbance values using the prcomp() function (scale. = TRUE). Descriptive statistics, ANOVA, and Pearson correlation were performed in Microsoft Excel; Tukey's HSD, LDA, and PCA were performed in RStudio.

# 3. Results and Discussion

## 3.1 Total Soluble Solids ( $^{\circ}\text{Brix}$ ) by Maturity Stage and Stalk Position

Mean TSS values across five maturity stages and three stalk positions are presented in Table 1. Overall mean TSS increased from 18.33  $^{\circ}\text{Bx}$  at 9 months to a peak of 19.05  $^{\circ}\text{Bx}$  at 12 months, declined to 18.18  $^{\circ}\text{Bx}$  at 13 months, and reached its lowest at 10 months (16.43  $^{\circ}\text{Bx}$ ). Across all stages, the bottom section consistently recorded the highest TSS, followed by the center and then the top, consistent with the well-established basipetal pattern of sucrose distribution in sugarcane [2,16].

**Table 1.** Mean TSS (°Bx) of sugarcane juice by stalk position and maturity stage (n = 10 per position).

| Age (months) | Position | Mean (°Bx) | SD   | Min   | Max   | Overall Mean (°Bx) |
|--------------|----------|------------|------|-------|-------|--------------------|
| 9            | Bottom   | 19.47      | 1.59 | 16.90 | 21.20 | 18.33              |
|              | Center   | 18.90      | 1.38 | 16.70 | 20.40 |                    |
|              | Top      | 16.53      | 1.39 | 14.90 | 18.50 |                    |
| 10           | Bottom   | 18.74      | 1.67 | 15.70 | 21.80 | 16.43              |
|              | Center   | 15.60      | 3.08 | 11.90 | 19.80 |                    |
|              | Top      | 14.94      | 3.18 | 10.60 | 21.00 |                    |
| 11           | Bottom   | 20.58      | 1.80 | 17.20 | 23.20 | 18.04              |
|              | Center   | 18.62      | 1.65 | 15.20 | 20.80 |                    |
|              | Top      | 14.89      | 3.65 | 10.70 | 22.90 |                    |
| 12           | Bottom   | 20.57      | 2.52 | 16.30 | 25.80 | 19.05              |
|              | Center   | 20.16      | 1.62 | 17.60 | 23.30 |                    |
|              | Top      | 16.40      | 3.05 | 10.90 | 20.00 |                    |
| 13           | Bottom   | 18.47      | 2.47 | 14.20 | 22.20 | 18.18              |
|              | Center   | 18.11      | 2.71 | 14.80 | 22.00 |                    |
|              | Top      | 17.97      | 2.15 | 14.90 | 21.10 |                    |

One-way ANOVA revealed a statistically significant effect of maturity stage on overall mean TSS ( $p < 0.05$ ). Two-way ANOVA further indicated significant main effects of maturity stage ( $p < 0.05$ ) and stalk position ( $p < 0.05$ ), with no significant interaction. The absence of an interaction indicates that the positional hierarchy (bottom > center > top) was consistently maintained across all stages. Tukey's HSD showed that the 12-month stage recorded significantly higher TSS than the 10-month stage ( $p < 0.05$ ), while the difference between the 11-month and 12-month stages was not significant ( $p > 0.05$ ). The peak TSS at 12 months reflects the stage at which sucrose synthesis exceeds hydrolysis, resulting in maximum net sucrose storage in the internodes [2]. The decline at 13 months is attributable to post-maturity sucrose inversion mediated by vacuolar invertase enzymes [3,4], consistent with findings of Singh et al. [20], who reported peak TSS at 12 months in tropical sugarcane-growing conditions. The 10-month TSS dip (16.43 °Bx overall) may reflect active cell elongation, which consumes sucrose for structural carbohydrate production during the early maturation phase [2]. However, the within-stage variability at 10 months is notably high (top section SD = 3.18; range 10.60–21.00 °Bx), suggesting that this dip may also reflect sampling heterogeneity or a confounding abiotic factor (e.g., rainfall event, irrigation variation) affecting juice dilution between the 9-month and 10-month sampling intervals. These possibilities cannot be distinguished from the current data, and the 10-month dip is acknowledged as a source of uncertainty in the overall monotonic trend and harvest window recommendation.

### 3.2 UV-Vis Absorbance by Maturity Stage

Mean UV-Vis absorbance values are presented in Table 2. Absorbance increased progressively from 0.4644 at 510 nm (9 months) to 1.2298 at 620 nm (13 months), reflecting the accumulation of chromophoric compounds — principally phenolics, flavonoids, and Maillard reaction products — during ripening [15]. The 12-month stage recorded the highest absorbance within the optimal maturity range (1.1899 at 600 nm) and the lowest inter-sample variability (SD = 0.0760). One-way ANOVA confirmed a significant effect of maturity stage on mean absorbance ( $p < 0.05$ ). Tukey's HSD showed all pairwise comparisons were significant ( $p < 0.05$ ) except the 12-month vs. 13-month comparison ( $p > 0.05$ ). The lowest inter-sample variability at 12 months (SD = 0.0760) compared to 13 months (SD = 0.1020) indicates that the sampled population achieved a more uniform ripening state at this stage, making it more reliable for batch harvesting [3]. The non-significant difference between 12-month and 13-month absorbance, combined with higher variability at 13 months, suggests that the marginal increase in absorbance beyond 12 months reflects the accumulation of brown oxidation pigments associated with senescence [15] rather than improved juice sucrose quality.

**Table 2.** Mean UV-Vis absorbance of sugarcane juice by maturity stage (n = 10).

| Age (months) | Wavelength (nm) | Mean Absorbance | SD     | Observed Juice Color |
|--------------|-----------------|-----------------|--------|----------------------|
| 9            | 510             | 0.4644          | 0.0203 | Pale green-yellow    |
| 10           | 530             | 0.6607          | 0.0232 | Yellow-green         |
| 11           | 550             | 0.8286          | 0.0561 | Yellow               |
| 12           | 600             | 1.1899          | 0.0760 | Amber-yellow         |
| 13           | 620             | 1.2298          | 0.1020 | Amber-orange         |

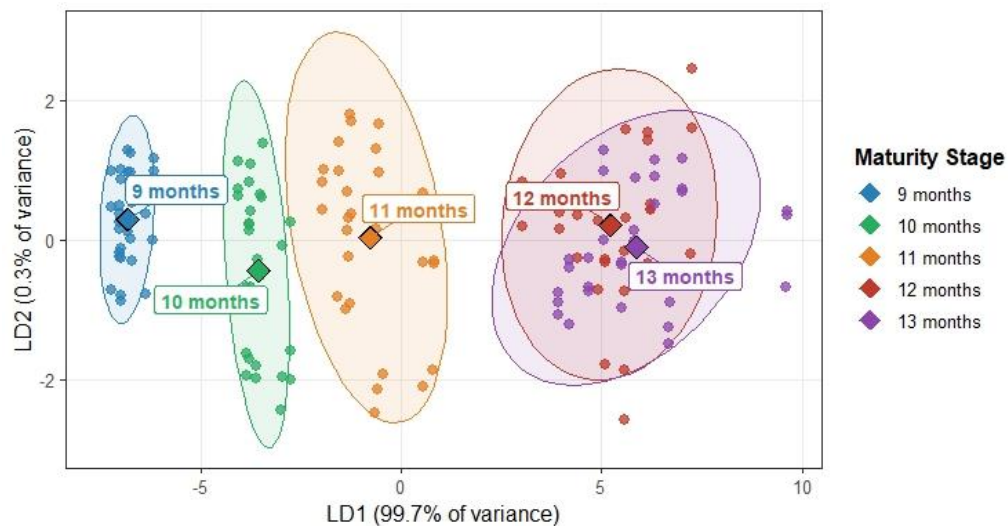
### 3.3 Pearson's Correlation Analysis

Pearson's correlation between mean overall °Brix and mean UV-Vis absorbance across five maturity stages yielded  $r = 0.463$  ( $R^2 = 0.214$ ,  $t(3) = 0.905$ ,  $p = 0.432$ , 95% CI  $[-0.709, 0.955]$ ), which was not statistically significant at  $\alpha = 0.05$ . This outcome is primarily attributable to the dip in °Brix at 10 months (16.43 °Bx) while absorbance continued to rise, revealing that the two variables measure fundamentally different aspects of juice composition: °Brix quantifies total dissolved solids sensitive to short-term sucrose fluctuations, while UV-Vis absorbance captures chromophoric compound optical density, which accumulates more steadily [7,15]. The non-significant result does not constitute statistical evidence of complementarity; rather, the complementarity of the two methods is supported by conceptual grounds (each captures a distinct physicochemical dimension of juice) and by the geometric separation observed in the PCA biplot. The  $n = 5$  stage-level means used for this correlation provide insufficient statistical power to detect any correlation below  $r = 0.88$  at  $\alpha = 0.05$  ( $df = 3$ ; [23]), rendering the test uninformative. A more robust assessment would use individual sample-level data ( $n = 150$ ). Consistent with the framing of Kumar et al. [18] and Zhang et al. [19], the two methods are treated as analytically complementary in the interpretive framework of this study. The limited sample size ( $n = 5$ ,  $df = 3$ ) must also be acknowledged as contributing to the non-significant result [23]. Pearson's correlation between measurement wavelength and mean UV-Vis absorbance yielded  $r = 0.990$  ( $R^2 = 0.980$ ,  $p = 0.001$ ), confirming a very strong positive relationship. This validates the wavelength selection protocol: matching measurement wavelength to juice color at each stage accurately tracked chromophoric development as a proxy for maturity progression [8,15].

### 3.4 Multivariate Analysis

#### 3.4.1 Linear Discriminant Analysis (LDA)

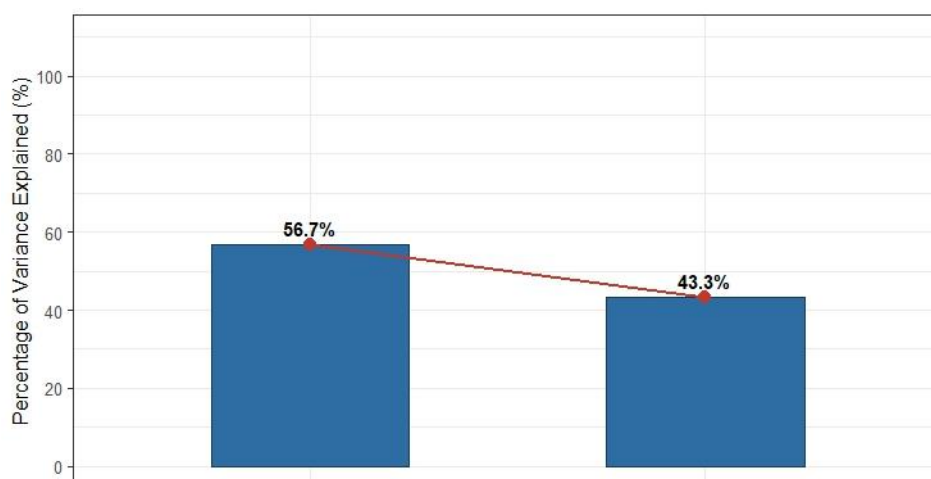
LDA using °Brix and UV-Vis absorbance as predictors correctly classified 83.33% of 150 sugarcane juice samples into their maturity stage groups. LD1 accounted for 99.7% of the between-group variance, while LD2 accounted for 0.3%. Wilks' lambda confirmed significant discrimination among the five stages ( $p < 0.05$ ). The LDA biplot (Figure 4) shows an ordered progression of group centroids along LD1, visually confirming the temporal maturity gradient. Partial overlap between the 12-month and 13-month confidence ellipses is consistent with the non-significant Tukey's HSD result between these two stages, while clear separation of the 10-month group corroborates its significantly lower TSS. The 83.33% classification accuracy is comparable to results from advanced sugarcane maturity studies using NIR spectroscopy and machine learning [13,14], lending credibility to the dual-method approach as a viable, low-cost alternative.



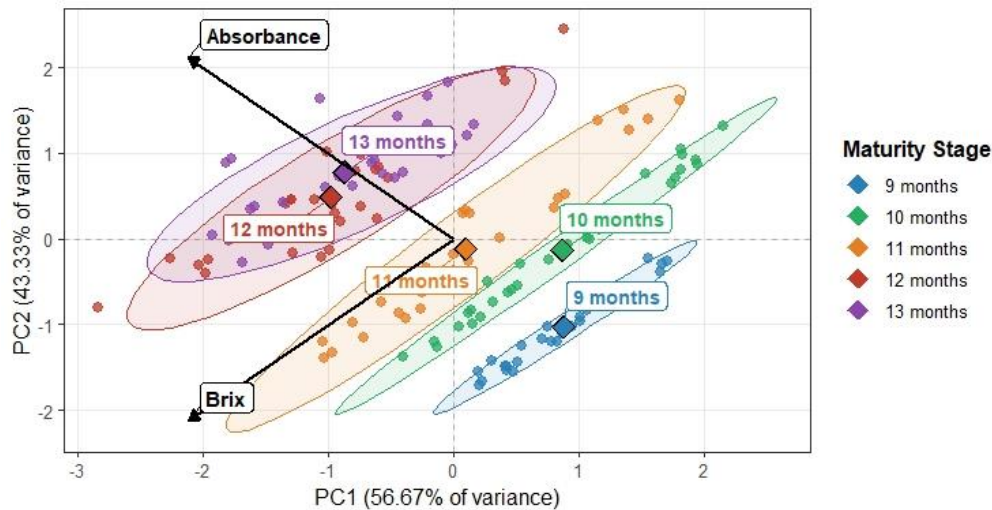
**Figure 4.** LDA biplot showing classification of 150 sugarcane juice samples into five maturity stages based on °Brix and UV-Vis absorbance.

### 3.4.2 Principal Component Analysis (PCA)

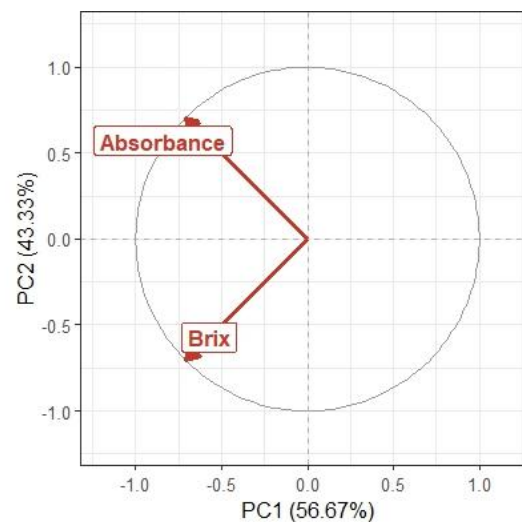
PCA on the standardized dataset ( $n = 150$ ) yielded  $PC1 = 56.67\%$  and  $PC2 = 43.33\%$  of total variance (100% combined). The relatively balanced split indicates that °Brix and UV-Vis absorbance contribute substantially and independently to the data structure. It should be noted that with only two input variables, PCA by mathematical necessity yields exactly two components summing to 100% of variance, regardless of the data structure — no dimensionality reduction occurs. The informative aspects of this PCA are therefore the relative loading magnitudes and the group clustering pattern in the biplot (Figure 6), not the total explained variance. The bar chart labeled as a ‘scree plot’ (Figure 5) is more accurately described as a bar chart of the two PCA component variances; a conventional scree plot requires three or more components to identify an elbow point. Both variables loaded negatively on  $PC1$ , indicating that higher values were associated with the more mature groups (12 and 13 months). On  $PC2$ , absorbance loaded positively while °Brix loaded negatively, providing unsupervised geometric confirmation of their complementarity — consistent with the weak Pearson correlation ( $r = 0.463$ ) [2,15]. The natural clustering of maturity stage groups in the PCA biplot — achieved without group label input — independently validates the maturity stage structure and reinforces the consistency of the overall analytical framework [18,19].



**Figure 5.** Scree plot showing percentage of variance explained by  $PC1$  and  $PC2$ .



**Figure 6.** PCA biplot of 150 sugarcane juice samples across five maturity stages.



**Figure 7.** Variable correlation circle showing loadings of °Brix and UV-Vis absorbance on PC1 and PC2.

### 3.5 Optimal Harvest Time and Practical Implications

The convergence of peak °Brix (19.05 °Bx), peak UV-Vis absorbance within the optimal range (1.1899 at 600 nm), and lowest absorbance variability (SD = 0.0760) at 12 months — combined with statistically significant differences from the 10-month stage and a non-significant difference from the 11-month stage — collectively identifies 12 months after planting as the optimal harvest time for the local sugarcane variety in Medellin, Cebu. This aligns with international studies that identified 12 months as the harvest age maximizing recoverable sucrose yield in tropical conditions [20,24]. The non-significant Tukey's HSD result between the 11-month and 12-month stages suggests a practical harvest window of approximately 11–12 months, providing scheduling flexibility consistent with observations by Gómez et al. [25]. From a practical standpoint, digital refractometry ( $\geq 18$  °Bx at the bottom stalk section) is recommended as the primary field-accessible indicator of harvest readiness. It should be noted that the bottom section already exceeds 18 °Bx at 9 months (19.47 °Bx), 11 months (20.58 °Bx), and 13 months (18.47 °Bx), in addition to the recommended 12-month stage (20.57 °Bx). The  $\geq 18$  °Bx threshold, therefore, lacks standalone discriminating power for the bottom section alone. It is recommended to apply this criterion in conjunction with positional consistency (i.e.,  $\geq 18$  °Bx measured simultaneously at both the bottom and center sections) to improve specificity. Alternatively, a higher threshold of  $\geq 19.5$  °Bx at the bottom section, combined with  $\geq 18$  °Bx at the center, would better distinguish 12-month maturity from earlier stages. UV-Vis spectrophotometry (absorbance 1.10–1.20 at 600 nm with low inter-



sample SD) is recommended as a complementary laboratory confirmation tool for cooperative and mill-level quality screening [17]. Together, these methods offer an accessible, affordable, and scientifically validated decision framework suitable for practical integration into existing harvest management practices at BOMEDCO.

#### 4. Conclusions

This study established a scientifically validated framework for determining sugarcane maturity and optimal harvest time in Medellin, Cebu, Philippines, using UV-Vis spectrophotometry and digital refractometry. The findings apply specifically to the [INSERT variety name] cultivar cultivated at the BOMEDCO plantation during the 2024–2025 growing season under the prevailing agroclimatic conditions of Medellin, Cebu; generalization to other varieties, sites, or seasons requires multi-site, multi-season validation. Furthermore, the study measured total soluble solids (°Brix) as a sucrose proxy rather than directly measuring sucrose (Pol or polarimetry); °Brix encompasses all dissolved solids and may overestimate sucrose content at early maturation stages when reducing sugars and other solutes are prominent. Pol and purity measurements are recommended to validate sucrose accumulation claims in future studies directly. The 12-month stage was identified as the optimal harvest time, supported by the highest overall mean TSS (19.05 °Bx), peak UV-Vis absorbance within the optimal range (1.1899 at 600 nm), and lowest inter-sample variability. A practical harvest window of 11 to 12 months is recommended to accommodate field scheduling variability. Pearson's correlation confirmed the complementarity of the two methods ( $r = 0.463$  between °Brix and absorbance), and LDA achieved 83.33% classification accuracy (Wilks'  $\lambda$ ,  $p < 0.05$ ). PCA confirmed that both variables capture independent dimensions of juice maturity, accounting for 100% of the dataset's variance. For practical application, digital refractometry ( $\geq 18$  °Bx, bottom section) is recommended as the primary field tool, and UV-Vis spectrophotometry (600 nm, absorbance 1.10–1.20) is recommended as a complementary laboratory confirmation method. Future studies should validate these findings across multiple cropping seasons and sugarcane varieties, and incorporate additional juice quality parameters, including Pol, purity, and reducing sugar content.

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# Bioaccumulation and Enzyme Activity Inhibition of Profenofos in Japanese Medaka (*Oryzias latipes* Temminck and Schlegel, 1846)

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**Abstract:** Effects of the organophosphate pesticide (OP) profenofos on acetylcholinesterase enzyme (AChE) activity inhibition were investigated using brain samples from Japanese medaka (*Oryzias latipes* Temminck and Schlegel, 1846). River water samples from six sampling sites along the Dalaguete River, Cebu, Philippines were tested for pesticide concentrations, the results of which were used as the basis for the Range-Finding and Exposure Tests. Japanese medaka of approximately the same weight and length were grouped into five (n=6 per group) for the RFT and were exposed to different concentrations of profenofos. For the exposure test, a total of 96 medaka were used, grouped into four groups (n=24 per group). Profenofos showed a positive, dose-dependent, and exposure-time-dependent relationship in inhibiting AChE activity. The highest concentration was already causing significant inhibition of AChE activity on Day 2. On Day 5, all concentrations were significantly different from Control. Increasing the concentration of profenofos leads to greater bioaccumulation of the pesticide in fish tissues, reaching a point of equilibrium on Day 5. The amount of pesticide accumulation declined in the fish tissues with longer exposure periods. Periodic water sampling is recommended to establish levels of profenofos and other pesticides in the river, with due consideration for the wet and dry seasons. Further studies on degradation, fate, and transport are also recommended.

**Keywords:** Profenofos; acetylcholinesterase inhibition; bioaccumulation

## 1. Introduction

The extensive and continuous global use of pesticides has resulted in their widespread occurrence, along with their transformation products, across environmental compartments such as surface water, soil, and the atmosphere. These contaminants pose emerging threats to ecosystem integrity and human health, driven by their persistence, mobility, and capacity for bioaccumulation in food webs [1]. Surface waters adjacent to intensive agricultural landscapes are particularly vulnerable to pesticide contamination, especially during rain events when surface runoff transports agrochemicals into rivers, streams, and irrigation systems [2]. This contamination is of significant concern when these water bodies are used for domestic consumption, aquaculture, or supporting biologically diverse ecosystems [3].

Aquatic organisms are among the most sensitive non-target recipients of pesticide pollution. Acute exposures to pesticides delivered to freshwater systems can cause direct mortality in fish and invertebrates, whereas chronic and sublethal exposures may trigger physiological and behavioral impairments that can reduce survival and reproductive success [4]. In particular, organophosphorus pesticides (OPPs) are well documented to disrupt nervous system function by inhibiting acetylcholinesterase (AChE), leading to neurotoxicity even at sublethal concentrations [5]. Sublethal effects of pesticide exposure in fish include reduced immune function, altered feeding and avoidance behaviors, and compromised predator escape responses, which collectively can reduce population viability [1,4]. Barangay Mantalongon and its neighboring barangays in Dalaguete, Cebu, Philippines, are recognized as major vegetable production hubs in the province and are widely referred to as the “Vegetable Basket of Cebu.” To meet market demand and optimize yields, farmers in the area typically rely on intensive pesticide applications throughout the cropping season, particularly on brassicas (cabbage and pechay), spring onions, tomatoes, and cauliflowers. Surface runoff from these cultivated fields drains into tributaries that feed the Dalaguete River, which supports aquatic life, including guppies, catfish, and freshwater shrimps, and is also used by residents for tilapia aquaculture. The increased, often unregulated use of pesticides in the area has raised environmental and public health concerns among local stakeholders, who have reported perceived increases in adverse health outcomes potentially linked to pesticide exposure; however, systematic scientific studies to examine these claims have not yet been conducted.

In this study, the freshwater fish *Oryzias latipes* (Japanese medaka) was selected as the test organism because of its small size and established ecotoxicological responses, making it suitable for laboratory toxicology assessments using limited quantities of test chemicals. Although larger aquaculture species such as tilapia are relevant to local use, their size increases the volume of pesticide required for controlled exposure studies. Japanese medaka has continued to serve as a standard model organism in aquatic toxicology, particularly for neurotoxicity and biomarker studies involving enzyme inhibition and behavioral endpoints [1, 5]. Its sensitivity to organophosphorus pesticide exposure and well-characterized AChE system have maintained its inclusion in standardized test guidelines. Profenofos, an organophosphorus insecticide widely used in agricultural systems, has raised concerns for its persistence and toxicity in aquatic environments. Recent reviews have documented that profenofos exposure negatively impacts multiple physiological systems in fish—including liver, gills, and neural function—primarily through inhibition of AChE and related cholinesterase enzymes [6, 7]. These adverse effects highlight the need for detailed assessments of profenofos bioaccumulation and neurotoxic risk to non-target aquatic organisms.

Accordingly, this study was designed to determine the bioaccumulation and enzyme-inhibition effects of profenofos in Japanese medaka (*Oryzias latipes*). The specific objectives were to (1) quantify profenofos accumulation in fish tissues and test water, (2) calculate the bioconcentration factor (BCF) of profenofos in *O. latipes*, and (3) evaluate potential ecological risk by correlating laboratory-derived AChE inhibition responses with environmentally relevant exposure levels.

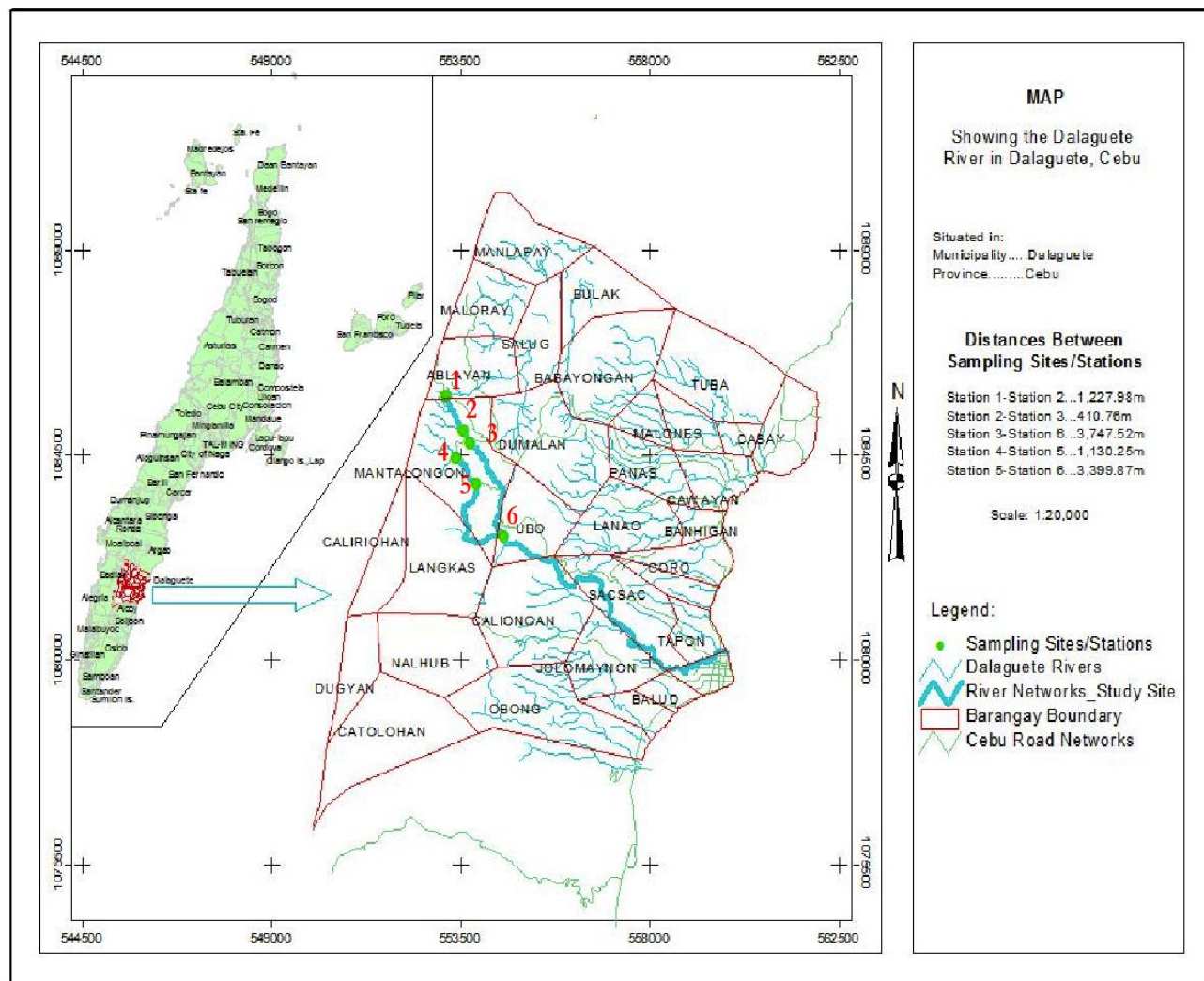
## 2. Materials and Methods

Laboratory experiments were generally conducted at the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan. River water samples were analyzed for pesticide residue concentration at the Department of Agriculture Bureau of Plant Industry-Pesticide Analytical Laboratory in Diliman, Quezon City. Test organisms, chemicals, reagents, and equipment were provided by Kagoshima University.

### 2.1 Sampling Sites

The study area is located within the major basin of Mantalongon, Dalaguete, Cebu, Philippines, specifically in two of its major tributaries (Figure 1). Six sampling sites were identified.. Station 1 is located in the Granchina headwater area. The area, though a headwater, is still surrounded by a few vegetable farms planted with cabbage and other vegetables. Succeeding sampling sites were identified with the following criteria: pollutant source (2, 3, 4, and 5) and downstream (6).





**Figure 1.** Map showing the location of each sampling site in the Dalaguete River.

## 2.2 Sample Collection

Two-liter samples were collected simultaneously from each of the identified sampling sites. The water samples were collected from the flowing parts at the sides and the middle of each sampling point and composited. The water samples were contained in acid-washed amber-colored borosilicate glass bottles, previously washed with river water, filled to the brim, tightly capped, and cooled in ice coolers, maintaining a 0–4°C temperature during transport to the laboratory. The water samples were immediately brought to the laboratory for the extraction of pesticides before analysis. Physico-chemical parameters such as temperature, pH, and dissolved oxygen (DO) were determined in situ using a digital multi-probe meter (Thermo Scientific, USA).

## 2.3 Chemicals

Acetylcholine iodide, 5,5'-dithiobis (2-nitrobenzoic acid), and heparin were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Dichloromethane, hexane, and acetone used were all pesticide grades, while silica gel (Wakogel, C100), sodium chloride, disodium hydrogen phosphate, twelve hydrate, potassium chloride, 2-phenoxyethanol, and potassium dihydrogen phosphate were all analytical grades. These chemicals were purchased from Wako Pure Chemical Industries Ltd., Japan.

## 2.4 Pesticide Analysis in Water

The collected river water samples from the different sampling sites were immediately transported to the Department of Agriculture Bureau of Plant Industry-Pesticide Analytical Laboratory in Diliman, Quezon City. The pesticide residue concentrations of the samples were analyzed using Gas Chromatography (Agilent



Technologies Model HP 6890, USA). The analysis data served as the basis for identifying the target pesticide for the risk assessment.

## 2.5 Range Finding Test

A range-finding test was conducted prior to the exposure experiment to determine the concentration range that would give 50% mortality to the test organism, which was used as the basis for establishing the treatment concentrations. The initial concentrations used for range-finding tests were based on the reported LC50 of profenofos in zebrafish (*Danio rerio*), which is 0.2 ppm [8]. Zebrafish was used as the base organism because it closely resembled Japanese medaka in terms of biometrics such as weight, length, and size. Profenofos was used since it was the pesticide detected during the pesticide residue analysis. The setup of the range-finding test used six freshwater Japanese medaka per concentration: 3 ppm, 1 ppm, 0.2 ppm, and 0.04 ppm, and a control, with 10 liters for each concentration in a static condition. A total of 30 freshwater Japanese medaka fish with an average total body length of 38 mm and a weight of 500 mg were used. Dissolved oxygen, temperature, pH, and mortality were monitored every 2 hours for 6 hours on Day 0 and on the 24<sup>th</sup> and 48<sup>th</sup> h.

## 2.6 Exposure Test

There were four treatments: T<sub>1</sub> (control), T<sub>2</sub> (0.04 ppm), T<sub>3</sub> (0.2 ppm), and T<sub>4</sub> (1.0 ppm). The concentrations of the target pesticide were based on the results of the range-finding test, in which the range of concentrations that resulted in 50% mortality in the test animals was determined. Four aquariums (9.5 in x 9 in x 12 in) with 10 liters of water were set up, each containing 24 freshwater Japanese medaka, approximately 3–4 cm in length (carrying capacity of approx. 1 g/L) (Figure 2). The aerated tap water exposed to activated carbon and the Japanese medaka were provided by the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan. The freshwater Japanese medaka have been reared in the Institute's laboratory. Approximately 1 mL of *Artemia salina* was fed to the fish every three days. A day before pesticide exposure, eight Japanese medaka were sampled to establish baseline AChE levels. This constituted the data for Day 0. A 10-day exposure was applied. A related study found that the concentration of profenofos in zebrafish (*Brachydanio rerio*) reached equilibrium by 168 h (7 days) [8]. The flow-through exposure method was used for T<sub>2</sub>–T<sub>4</sub>. Static condition was followed for Control. Eight Japanese medaka fish were collected from each aquarium on the 2nd, 5th, and 10th days of the exposure period. Temperature, percent saturation, DO and pH were monitored daily for the whole duration of the study.



**Figure 2.** Flow-through exposure method.

Due to the small size of the fish, they were composited to represent a single replicate. A total of 96 ( $24 \times 4$ ) freshwater Japanese medaka fish were used in the risk determination. Eight fish were sampled per time point, with two fish composited, to give a total of 4 replicates per sampling time. Acetylcholinesterase enzyme inhibition tests were performed at each sampling period. Dissolved oxygen (DO), pH, and temperature of the test waters were monitored daily using a DO meter (Navi Model OM-51, Horiba, Ltd., Japan) and a pH meter (Hanna, USA).

#### 2.6.1 Sampling of Fish for Biochemical Test

Fresh weight, body length, and total body length of the fish were determined before conducting further tests. This was to establish that the fish were approximately the same size and weight. Fishes were anesthetized with 2-phenoxyethanol (0.7 mL/L H<sub>2</sub>O).

### 2.7 Acetylcholine Esterase Enzyme Inhibition Test

Brains from two Japanese medaka fish were collected and combined to compose a replicate sample. The whole brain was extracted and placed in a pre-weighed 1.5 mL microtube (Nippi, Japan). Each sample was homogenized using Power masher II (Nippi, Japan) after the addition of 0.1 M phosphate buffer (pH 8.0; 20 mg fresh tissue mL<sup>-1</sup>). Homogenate was centrifuged at 10,000 g for 15 min at 4°C. Acetylcholine esterase (AChE) activity was determined using Ellman's reagent (DTNB) and acetylthiocholine [9] modified for a microplate reader (Model 550, Bio-Rad, USA) with a 96-well plate [10]. In each well, 155 µL of 0.1 M phosphate buffer (pH 8.0), 10 µL of supernatant, and 10 µL of DNTB were added. The plate was incubated at 30°C for 5 min, and then 5 µL of substrate (AChI) was added to start the reaction. Enzyme activity was measured using a Bio-Rad microplate reader every 2 min for 10 minutes at 412nm. Each enzymatic activity was carried out in triplicate. The rate of enzymatic activity was calculated according to Ellman *et al.* [9]. The protein content of the samples was quantified by the Bradford dye-binding assay using the Bio-Rad Protein Assay (Bradford, 1976) with bovine serum albumin as the standard.

### 2.8 Bioaccumulation of Pesticides

#### 2.8.1 Test Water

Analysis of pesticide concentration in the test water was performed following the procedure of Añasco *et al.* [11]. Pesticide extraction was performed within 24 h of arrival in the laboratory. Solid-phase extraction was performed using C18 Sep-Pak Cartridges. The C18 Sep-Pak cartridges were conditioned by gradually adding the first 10 mL of acetone (1 drop/second) to the glass syringe fitted with the cartridge. This was followed by 10 mL of deionized distilled water (1 drop/second). Fifty mL of the water sample (1 drop/second) was then allowed to pass gradually through the glass syringe with a cartridge. The eluent was allowed to stand for 10 minutes. The cartridges were then centrifuged for 10 minutes at 3000 rpm at 4°C. The cartridges were then fitted into a new set of glass syringes and gradually eluted with 20 mL of acetone (1 drop/second). The eluents were concentrated to 1 mL by gentle nitrogen gas flow. 100 µL of 1.0 ppm anthracene-d<sub>10</sub> was added to the sample as an internal standard. Samples were finally injected into a gas chromatograph (Agilent Technologies 6890N, USA) with a mass spectrometry detector (Agilent Technologies 5973N) in splitless mode. Analytical conditions were as follows: (1) injection volume, 2 µL; (2) injector temperature, 250°C; (3) detector temperature, 280°C; (4) oven temperature, initially at 60°C (held for 1 min) then ramped to 180°C at 20°C/min (held for 5 min) then finally increased to 290°C at 3°C/min (held for 3 min); and (5) column, DB-5MS (length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 µm; J & W Scientific, USA).

#### 2.8.2 Test Organisms

The remaining body tissues of the fish samples were freeze-dried for the determination of residual pesticide concentrations following the method of Añasco *et al.* [11] with some modifications. Dried fish tissues were cut into small pieces. The required amount for each sample was placed in 50-mL centrifuge tubes. 10 mL hexane-acetone solvent mixture (1:1) was added. The mixture was subjected to ultrasonic extraction for 15 mins, then centrifuged at 3000 rpm for 10 mins at 4°C. The supernatant was collected into another 50 mL centrifuge bottle. The precipitate was then added to 10 mL of a hexane-acetone solvent mixture (1:1), and the

process was repeated. The two batches of supernatant liquids were combined. 20 mL of deionized distilled water was then added to the supernatant. The centrifuge tube was shaken for 10 mins at 8.5 speed on a shaker, then centrifuged for 10 mins at 3000 rpm at 4°C. The supernatant liquid was then collected, to which 10 mL of hexane was added. The resulting mixture was then shaken for 10 mins at 8.5 speed, then centrifuged at 3000 rpm for 3 mins at 4°C. The supernatant liquid was collected, and the precipitate was subjected to the same process. The final supernatant was passed through a glass funnel containing quartz wool and anhydrous Na<sub>2</sub>SO<sub>4</sub> to remove excess water. The filtrate was concentrated to 1 mL by a gentle stream of nitrogen gas. Clean-up was performed with Florisil containing 3% moisture. Clean-up columns were conditioned with 10 mL of hexane. The sample was passed through the column and eluted with 10 mL hexane, followed by 20 mL of 10% acetone in hexane. The eluate was concentrated to 1 mL by a gentle stream of nitrogen gas. 100 µL of 1.0 ppm anthracene-d<sub>10</sub> was added to the sample as an internal standard. Samples were finally injected into a gas chromatograph (Agilent Technologies 6890N, USA) with a mass spectrometry detector (Agilent Technologies 5973N) in splitless mode. Analytical conditions were as follows: (1) injection volume, 2 µL; (2) injector temperature, 250°C; (3) detector temperature, 280°C; (4) oven temperature, initially at 60°C (held for 1 min) then ramped to 180°C at 20°C/min (held for 5 min) then finally increased to 290°C at 3°C/min (held for 3 min); and (5) column, DB-5MS (length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 µm; J & W Scientific, USA).

## 2.9 Bioconcentration Factor

The bioconcentration factor of the identified pesticide in the test organisms was computed as the concentration of the pesticide in the fish tissues at steady state divided by the concentration of the chemical in water during the exposure period. The formula used was:  $BCF = C_{fish} / C_{water}$ , where  $C_{fish}$  is the concentration of profenofos in fish tissue (µg/g wet weight) at steady state, and  $C_{water}$  is the concentration of profenofos in the exposure water (µg/mL) at equilibrium (Day 5).

## 2.10 Statistical Analysis

Analysis of Variance (ANOVA) was used to test for differences between treatments and between sampling days. The Student-Newman-Keuls (SNK) Test was used to assess further whether a significant difference among treatments exists. The Spearman's rank order correlation, or Spearman's rho, was used to compute the correlation between AChE activity inhibition and bioaccumulation data.

# 3. Results and Discussion

## 3.1 Pesticide Concentration in River Water

The pesticide profenofos was the only one detected at the six identified sampling sites along the Dalaguete River, with a concentration of 481 µg/L. at Sampling Site #2. Sampling was done after a heavy rain post-application. Profenofos with an IUPAC name of 4-bromo-2-chloro-1-[ethoxy (propylsulfanyl)phosphoryl]oxybenzene, is the active ingredient of the commonly used pesticide Selectron® in Mantalongon and its neighboring barangays. Physico-chemical parameters for the different sites ranged from 7.1–7.4, 6.8–7.8 ppm, and 28.8°C–30°C for pH, DO, and temperature, respectively.

## 3.2 AChE Inhibition Test

Acetylcholinesterase (AChE) activity was significantly inhibited as pesticide concentration was increased. On Days 2 and 5, T34 (1.00 ppm) AChE activity was significantly lower than the other treatments; however, on Day 5, T12 (0.04 ppm) and T23 (0.20 ppm) were not significantly different from each other but were significantly lower than T<sub>4</sub> and Control. On the last day of sampling, T<sub>3</sub> and T<sub>4</sub> were not significantly different from each other but significantly different from T<sub>2</sub> and Control (Figure 3).

**Table 1.** Pesticide residue in the river water samples.

| Sampling Site Number | Pesticide Concentration (ppm) |
|----------------------|-------------------------------|
| Station 1            | <LOQ                          |
| Station 2            | Profenofos = 0.481            |
| Station 3            | <LOQ                          |
| Station 4            | <LOQ                          |
| Station 5            | <LOQ                          |
| Station 6            | <LOQ                          |

Note: 1. Results were obtained by Gas Chromatography (Agilent Technologies HP 6890, USA).

2. The Limit of Quantification (LOQ) for organophosphates, organochlorines and pyrethroids is 10.0 µg/L.

### 3.2 Range Finding Tests and Setting of Pesticide Concentrations

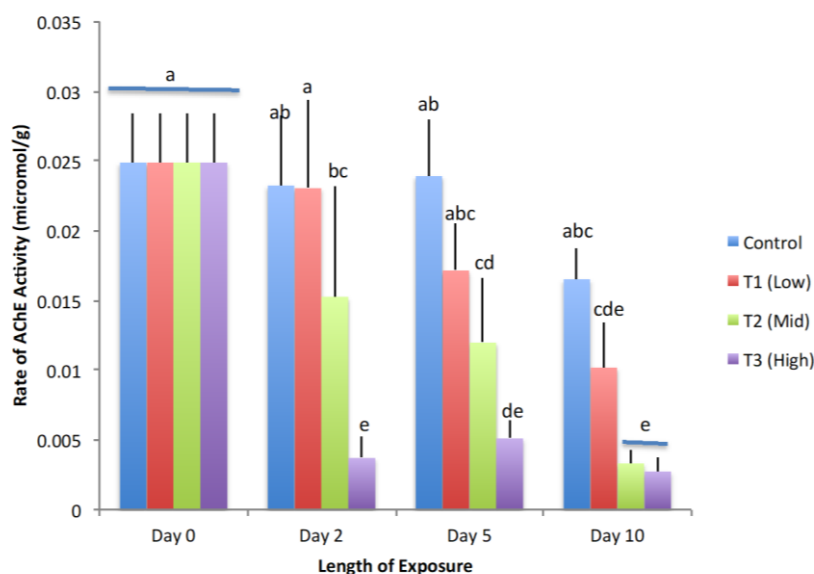
The highest concentration at 3.00 ppm produced 83.33% mortality to the test organisms after 48 hours (Table 2). The next lower concentration produced 50% mortality. However, based on actual observation the remaining *O. latipes* in T<sub>3</sub> were already showing behavioral alterations like continuous swimming combined with numerous jerks, partial jerks and flickering of the fins. The sole survivor in T<sub>4</sub> was already on its side exhibiting loss of equilibrium. Hence, approximate range causing 50% mortality was set at 0.2 ppm if the objective was to expose *O. latipes* to profenofos for 10 d. Concentrations higher and lower than 0.20 ppm were set as additional concentrations for the exposure test.

**Table 2.** Range Finding Test Using Profenofos (n = 6).

| Treatment    | Mortality (f) | % Mortality |
|--------------|---------------|-------------|
| Control      | 0             | 0           |
| T1 (0.04ppm) | 0             | 0           |
| T2 (0.20ppm) | 0             | 0           |
| T3 (1.00ppm) | 3             | 50          |
| T4 (3.00ppm) | 5             | 83.33       |

### 3.3 AChE Inhibition Test

Acetylcholinesterase (AChE) activity was significantly inhibited as pesticide concentration was increased. In Days 2 and 5, T<sub>4</sub> (1.00 ppm) AChE activity was significantly lower than the rest of the treatments; however, on Day 5, T<sub>2</sub> (0.04 ppm) and T<sub>3</sub> (0.20 ppm) were not significantly different from each other but significantly different from T<sub>4</sub> and Control. On the last day of sampling, T<sub>3</sub> and T<sub>4</sub> were not significantly different from each other but significantly different from T<sub>2</sub> and Control (Figure 3). F-value = 15.62, df=63.

**Figure 3.** AChE Activity on *O. latipes* exposed to different concentrations of profenofos.

The observed inhibition of acetylcholinesterase (AChE) activity in the present study is consistent with recent findings demonstrating a clear dose-dependent relationship between organophosphate (OP) exposure and AChE suppression in freshwater fish. Numerous studies over the past decade have reported significant, concentration-dependent AChE inhibition in *Oryzias latipes* and other teleost species following exposure to OP insecticides such as chlorpyrifos, diazinon, and profenofos [5, 12-14]. Similar responses have also been documented in non-target species, such as mosquito fish (*Gambusia affinis*), confirming AChE's sensitivity as a biomarker of OP neurotoxicity across taxa [15].

The inhibition of AChE is primarily due to the covalent binding of OP compounds to the serine hydroxyl group at the esteratic site of the enzyme, forming a stable phosphorylated enzyme complex that disrupts normal synaptic transmission [5,16]. Profenofos, in particular, has been shown to exert strong inhibitory effects on AChE activity in both fish and aquatic invertebrates, including freshwater shrimps and tilapia species, even at environmentally relevant concentrations [17-19]. The present results further support previous evidence on the persistence and functional irreversibility of OP-induced AChE inhibition. The absence of significant recovery in AChE activity between Days 2, 5, and 10 in treatments T3 (0.20 ppm) and T4 (1.00 ppm) suggests prolonged enzyme inhibition, consistent with reports indicating slow or incomplete enzymatic reactivation following OP exposure due to enzyme "aging" [13, 16]. Notably, significant AChE inhibition was already evident at T3, a concentration lower than measured field application rates, as early as Day 2, underscoring the potential ecological risk posed by profenofos even at low exposure levels.

Behavioral abnormalities observed in profenofos-exposed fish—including heightened reactivity to light stimuli, circular swimming, repeated collisions with aquarium walls, erratic, jerky movements, loss of equilibrium, and spiral or corkscrew swimming patterns, are characteristic manifestations of OP-induced neurotoxicity. The following behavioral abnormalities were noted as incidental observations during daily monitoring and were not subject to statistical analysis. Similar behavioral disruptions have been widely reported in fish exposed to profenofos and other OP insecticides and are closely linked to cholinergic overstimulation resulting from AChE inhibition [14, 20]. Such behavioral endpoints are now recognized as sensitive early-warning indicators of sublethal pesticide stress and are increasingly incorporated into ecological risk assessments [21].

In vitro AChE inhibition studies further corroborate the neurotoxic potential of profenofos, demonstrating concentration- and time-dependent alterations in enzyme kinetics, characterized by changes in apparent  $K_m$  and  $V_{max}$  values. Recent studies indicate that OPs, including profenofos, commonly exhibit competitive or mixed-type inhibition of AChE in fish, reflecting direct interaction with the enzyme's active site [17, 19]. These enzymatic disruptions provide a mechanistic basis for the observed behavioral impairments and reinforce the relevance of AChE inhibition as a key biomarker for assessing pesticide-induced neurotoxicity in aquatic organisms. Biochemical enzyme activity perturbations have also been reported as sensitive indicators of chemical toxicity in vertebrate models beyond aquatic systems [21].

### 3.4 Bioaccumulation and Bioconcentration Factor

Recent studies have demonstrated that aquatic organisms can bioaccumulate profenofos and other organophosphate insecticides in their tissues, with uptake strongly influenced by exposure concentration, duration, and species-specific metabolic capacity [6, 18]. In the present study, increasing concentrations of profenofos resulted in significantly higher levels of pesticide accumulation in Japanese medaka, indicating a clear concentration-dependent pattern of bioaccumulation. Tissue residues increased markedly as profenofos concentrations rose from 0.20 ppm (T3) to 1.00 ppm (T4), with accumulation approaching an apparent steady state by Day 5. This plateau suggests that a balance between uptake and elimination was achieved, a phenomenon commonly reported in controlled fish exposure studies involving organophosphate pesticides [17, 22].

The calculated bioconcentration factors (BCFs) further support this observation. Higher BCF values at elevated exposure concentrations indicate efficient uptake of profenofos from the surrounding water into fish tissues. However, the stabilization of BCF values over time implies that bioaccumulation did not increase indefinitely, reflecting the activation of metabolic and depuration mechanisms. According to current



ecotoxicological criteria, moderate BCF values are characteristic of compounds with intermediate lipophilicity and limited persistence in fish tissues, such as profenofos [6, 16].

Following the attainment of equilibrium, a decline in profenofos residues in fish tissues was observed with prolonged exposure. This decrease suggests that Japanese medaka can biotransform and eliminate profenofos via enzymatic detoxification pathways. Recent studies indicate that hepatic esterases and cytochrome P450-mediated metabolism play a central role in reducing internal organophosphate burdens during extended exposure periods [5, 12,13]. As depuration progressed, profenofos concentrations in the surrounding water increased, likely due to the compound's release from fish tissues back into the medium.

**Table 3.** Bioaccumulation and BCF of profenofos in Japanese medaka.

| Days of Exposure               | T <sub>1</sub> | T <sub>2</sub> | T <sub>3</sub> |
|--------------------------------|----------------|----------------|----------------|
| 0                              | 5              | 5              | 5              |
| 2                              | 441.515        | 455.826        | 1060.551       |
| 5                              | 393.651        | 558.881        | 1496.096       |
| 10                             | 385.522        | 524.057        | 1210.217       |
| Average (Fish) ng/g wet weight | 406.896        | 512.921        | 1255.621       |
| Average (Water) ng/mL          | 13.824         | 61.573         | 281.860        |
| BCF                            | 29.434         | 8.330          | 4.455          |

The observed bioaccumulation and depuration dynamics are consistent with the physicochemical properties of profenofos, including its relatively low water solubility and moderate lipophilicity, which favor rapid initial uptake followed by gradual elimination rather than long-term retention [6, 18]. These findings suggest that although profenofos does not exhibit extreme bioaccumulative behavior, its ability to reach biologically relevant internal concentrations—especially at environmentally realistic exposure levels—poses a potential risk to aquatic organisms through repeated or chronic exposure. Similar bioaccumulation dynamics have been observed in fish from Philippine riverine systems affected by anthropogenic contamination [3].

#### 4. Conclusions

This study demonstrates that profenofos induces concentration-dependent bioaccumulation and pronounced acetylcholinesterase (AChE) inhibition in *Oryzias latipes*, confirming its neurotoxic potential at environmentally relevant exposure levels. Although bioconcentration factors (BCFs) indicated only moderate bioaccumulation, profenofos rapidly reached biologically effective internal concentrations, with tissue residues stabilizing within 5 days. Significant and persistent AChE inhibition, accompanied by abnormal swimming behavior, was observed even at concentrations below estimated field application levels, indicating limited enzymatic recovery and substantial sublethal toxicity. These findings highlight the ecological risk posed by profenofos to freshwater organisms and underscore the need for strengthened management of organophosphate pesticide use in agricultural watersheds. Regulatory and mitigation efforts should emphasize improved pesticide application practices, runoff control measures, and adoption of integrated pest management strategies to minimize contamination of surface waters. Future research should prioritize long-term, multigenerational exposure studies, evaluate recovery dynamics following exposure cessation, and integrate laboratory biomarkers with field-monitoring data. Additionally, investigations into mixture toxicity involving profenofos and other commonly applied pesticides are essential to reflect real-world exposure scenarios better and inform more protective ecological risk assessments.

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R.P. Alburo and E.T. Bacolod; supervision, E.T. Bacolod; funding acquisition, R.P. Alburo and E.T. Bacolod. All authors have read and agreed to the published version of the manuscript.

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Supplemental Table for Physico Parameters

| Day 0   | pH   | Temp  | DO   | % Sat'n |
|---------|------|-------|------|---------|
| Control | 7.42 | 20.70 | 7.85 | 89.50   |

| Day 6   | pH   | Temp  | DO   | % Sat'n |
|---------|------|-------|------|---------|
| Control | 7.06 | 24.90 | 6.91 | 89.60   |

|         |      |       |      |         |
|---------|------|-------|------|---------|
| T1      | 7.36 | 21.00 | 7.25 | 84.70   |
| T2      | 7.20 | 23.60 | 6.37 | 77.50   |
| T3      | 6.96 | 23.50 | 7.24 | 88.10   |
| Day 1   | pH   | Temp  | DO   | % Sat'n |
| Control | 6.92 | 24.50 | 6.57 | 80.10   |
| T1      | 6.80 | 24.70 | 6.63 | 80.20   |
| T2      | 6.83 | 24.30 | 6.70 | 81.30   |
| T3      | 6.90 | 24.60 | 6.69 | 81.70   |
| Day 2   | pH   | Temp  | DO   | % Sat'n |
| Control | 6.95 | 23.70 | 6.61 | 80.40   |
| T1      | 7.08 | 23.60 | 6.63 | 87.20   |
| T2      | 7.00 | 23.80 | 7.04 | 84.50   |
| T3      | 7.13 | 23.70 | 6.82 | 82.40   |
| Day 3   | pH   | Temp  | DO   | % Sat'n |
| Control | 7.24 | 23.70 | 7.24 | 88.00   |
| T1      | 7.10 | 23.60 | 6.86 | 80.60   |
| T2      | 7.35 | 23.60 | 7.30 | 88.70   |
| T3      | 7.33 | 23.30 | 7.72 | 92.20   |
| Day 4   | pH   | Temp  | DO   | % Sat'n |
| Control | 7.06 | 22.60 | 7.88 | 92.40   |
| T1      | 7.10 | 22.90 | 7.42 | 83.50   |
| T2      | 7.04 | 22.50 | 6.94 | 83.60   |
| T3      | 6.94 | 22.90 | 7.24 | 87.00   |
| Day 5   | pH   | Temp  | DO   | % Sat'n |
| Control | 6.76 | 22.70 | 7.13 | 83.40   |
| T1      | 7.02 | 23.10 | 7.28 | 86.50   |
| T2      | 7.06 | 24.90 | 6.91 | 89.60   |
| T3      | 7.02 | 25.20 | 6.84 | 88.70   |

|         |      |       |      |         |
|---------|------|-------|------|---------|
| T1      | 7.02 | 25.20 | 6.84 | 88.70   |
| T2      | 7.12 | 24.40 | 6.57 | 79.70   |
| T3      | 7.22 | 24.60 | 6.89 | 86.40   |
| Day 7   | pH   | Temp  | DO   | % Sat'n |
| Control | 7.33 | 24.10 | 6.49 | 79.40   |
| T1      | 7.54 | 24.50 | 6.55 | 81.00   |
| T2      | 7.16 | 24.70 | 6.68 | 82.00   |
| T3      | 7.34 | 24.70 | 6.60 | 80.30   |
| Day 8   | pH   | Temp  | DO   | % Sat'n |
| Control | 7.25 | 24.30 | 6.86 | 81.20   |
| T1      | 7.60 | 24.10 | 6.63 | 82.00   |
| T2      | 7.20 | 23.80 | 6.49 | 79.10   |
| T3      | 7.33 | 24.00 | 6.30 | 79.90   |
| Day 9   | pH   | Temp  | DO   | % Sat'n |
| Control | 7.31 | 23.00 | 6.90 | 80.20   |
| T1      | 7.53 | 23.30 | 6.87 | 80.20   |
| T2      | 7.60 | 23.30 | 7.09 | 86.00   |
| T3      | 7.54 | 23.60 | 6.91 | 85.50   |
| Day 10  | pH   | Temp  | DO   | % Sat'n |
| Control | 7.60 | 23.30 | 7.09 | 86.00   |
| T1      | 7.54 | 23.60 | 6.91 | 85.50   |
| T2      | 7.40 | 23.40 | 7.40 | 86.00   |
| T3      | 7.51 | 23.60 | 7.18 | 88.50   |



# Institutionalized Mangrove Conservation at the Local Governance Level: The Case in the Camotes Islands, Cebu, Philippines

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**Abstract:** Mangrove conservation in the Camotes Islands, Cebu, Philippines, is institutionalized through national and local policies that prioritize biophysical and ecological values. Yet non-compliance among local resource users persists, signaling a disconnect between top-down governance and the socioeconomic and cultural realities of affected communities. Using an ecological-anthropological lens, this study examines the sustainability and efficacy of these conservation programs and assesses how local political institutions regard the welfare of the communities they affect. A qualitative case study drew on semi-structured key informant interviews with seven (n = 8) informants — three barangay captains and four municipal environmental officers, one each from the four municipalities of the Camotes Islands, supplemented by informal interviews and field observations. Thematic analysis of the interview data yielded five key themes: (1) the unexamined conservation paradigm, (2) the lack of a human aspect, (3) top-down conservation schemes, (4) recognition of local values, and (5) prospects for an integrated approach. The findings show that conservation in the islands centers on the scientific value of biophysical preservation, with limited regard for local cultural and socioeconomic values. Top-down governance, unsteady political integrity, and unsustainable program design jointly weaken conservation on the ground, while local government actors emerge as crucial mediators of conflicting values. These results underscore the need for a holistic, integrated conservation policy that treats biophysical integrity and community welfare as a foundational whole.

**Keywords:** New ecological anthropology; mangrove conservation; key local government actors; biocentric policies; Camotes Islands

## 1. Introduction

The Camotes Islands are a Group of Islands best known for its tourist destinations that locally capitalize on its environmental and service-oriented human resources. Proclaimed a Mangrove Swamp Forest Reserve in 1981, it has established numerous environmental policies to strengthen coastal conservation. Yet, despite the institutionalization of conservation across the islands, a chasm of conflicting values persists between local government units (LGUs) and local people. This paper then explores the *chasm* gaping within the conservation landscape at the local government level in the Camotes Islands. According to Brightman and Lewis [1], the tension between conservation and



development stems from the concept of sustainability, which is “premised on a natural world separate from social and cultural life.” On one hand, the traditional welfare of communities within protected areas has been deemed incongruous “with the objectives and practice of biodiversity conservation.” On the other hand, biodiversity conservation has been identified “as a threat to human welfare” as it denies people’s rights to access to resources and undermines their livelihood [2]. However, in recent years, a paradigm shift has integrated the biophysical aspects of conservation with human welfare [1].

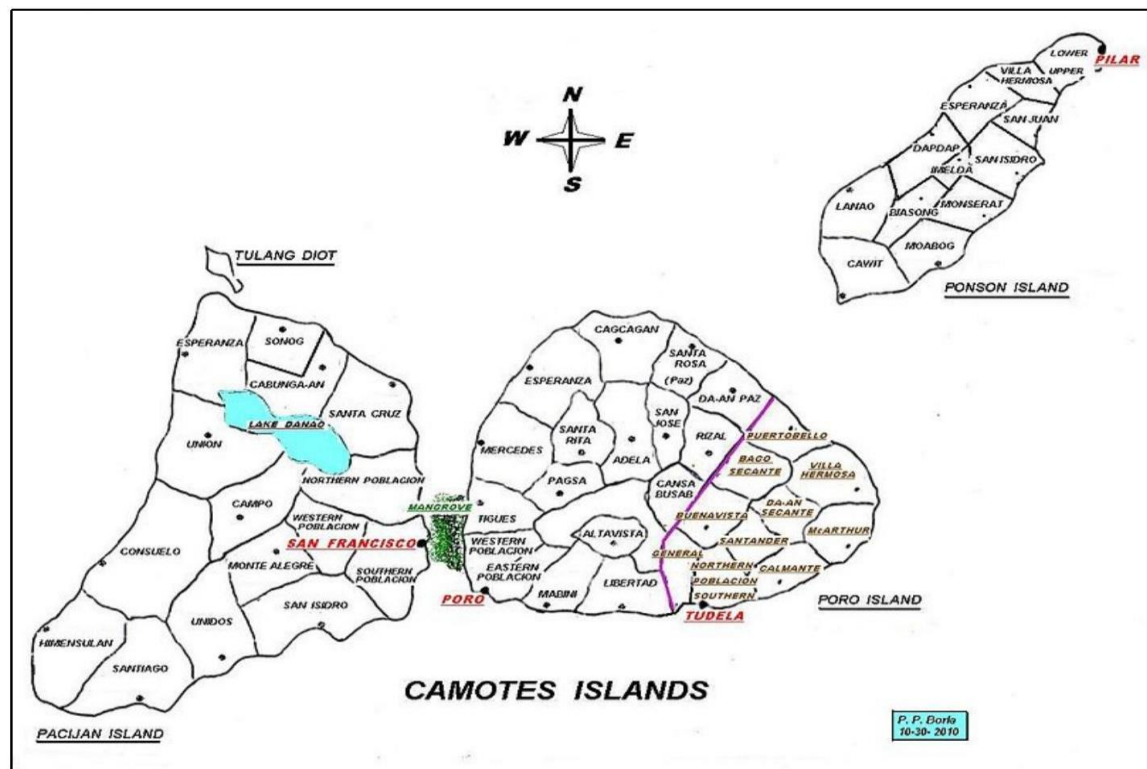
Inogwabini [3] argues that sustainable development is not only about the sustained existence of natural resources but also encompasses multiple social facets, including social justice. There is also “compelling evidence that both ecological sustainability and economic development reflect complex problems of managing for multiple values and outcomes, and that is in turn required systematic input from those who are directly dependent on the environment for their livelihoods” [4]. Further, while preserving global biodiversity is undoubtedly an indispensable goal, the new ecological anthropology [5] proposes the indispensability of equally evaluating policy and devising “socially sensitive and culturally appropriate strategies” to achieve biodiversity conservation. Furthermore, “to conserve ecologically valuable areas,” the concept of ecodevelopment [6] uses a strategy that “ensures that the negative impact of such conservation effort, on people living in and around these PAs, is minimized.” Employing these anthropological lenses magnifies the realities of institutionalized conservation policies by highlighting the chasm that separates the biophysical natural world from the social world in the Camotes Islands.

The study uses these anthropological conceptual frameworks to examine the sustainability, efficacy, and appropriateness of institutionalized mangrove conservation programs, policies, and management at the local government level in the Camotes Islands. It also explores how local political institutions view the socioeconomic welfare of communities that value and use mangroves as traditional social and economic resources. Specifically, this study documents the knowledge, perspectives, decision-making practices, and experiences of key local government actors in the context of implemented mangrove conservation programs, policies, and management strategies, as well as the challenges encountered on the ground.

## 2. Materials and Methods

### 2.1 Study Site

The Camotes Group of Islands off northeastern Cebu in Central Visayas, Philippines, is composed of three major islands – Pacijan, Poro, and Ponson; and comprises four municipalities of Cebu Province – San Francisco, Poro, Tudela, and Pilar (Figure 1). Although politically fragmented, the Camotes Islands are socioculturally, economically, and environmentally interwoven; other social institutions, such as health care, education, and kinship, are also interconnected. The Camotes Islands – simply called “Camotes” by locals, house a variety of natural heritage, including a diverse range of terrestrial and marine fauna, upland and subterranean karst landscapes, patches of wetlands and coastal ecosystems, and pockets of thick mangrove forests (Figure 2). Each municipality in the Camotes Islands harbors mangrove forest patches scattered along the coast between neighboring villages or concentrated in a single village or sub-village (Figure 3). Lillo et al. [7] have recorded a total of 42 mangrove species in the Camotes Islands, and “out of 42 species, 31 were categorized as true mangrove, and 11 were mangrove associates” [7]. The selection of villages or barangays for the study is systematically based on the density of mangrove cover per municipality and the regularity of activities and programs related to mangrove conservation. The barangays are Unidos in San Francisco, Teguis in Poro, Villahermosa in Tudela, and Lower Poblacion in Pilar. The selected sites are also within the management zone in the Camotes Islands Protected Landscape and Seascape (CIPLS), as delineated by the Department of Environment and Natural Resources (DENR).



**Figure 1.** Study area: the Camotes Islands off northeastern Cebu, Central Visayas, Philippines, showing the four municipalities covered by this study. Source: Ppborla - Own work, CC BY-SA <https://commons.wikimedia.org/w/index.php?curid=1524735>

## 2.2 Qualitative Research Methods

Semi-structured and in-depth key informant interviews are the primary basis for this study. A total of seven (7) key informants were purposively selected, comprising four barangay captains — the village-level chief officials of Unidos (San Francisco), Teguis (Poro), and Villahermosa (Tudela); and Lower Poblacion (Pilar), and four municipal environmental officers, one per municipality. Appointed by municipal mayors, municipal environmental officers vary in bureaucratic designations: in Tudela, the Municipal Agriculture Officer; in Poro and San Francisco, the Municipal Environment and Natural Resources Officers; and in Pilar, the Municipal Planning and Development Officer. The barangay captains and municipal environmental officers hold focal political designations duly tasked to coordinate environment-related programs, policies, and decisions at the barangay and municipal levels, and to ensure that environmental policies are upheld and enforced. Contextualizing them as actors in the local social and political institutions is useful “in shaping a particular way of thinking about the environment and how the problems are addressed in practice” [8] (p. 11). Paying close attention to their perspectives, knowledge, decision-making practices, and experiences focuses on the people who are in the change process – the development actors [9] (pp. 32-33), which is necessary to understand the ideas, values, and management practices of the LGUs' conservation approach to environmental resources. Data collection was conducted from January 2022 to August 2022. Each interview lasted approximately 60 minutes and was conducted in Cebuano in Poroanon at the informant's home, municipal office or barangay hall. The seven informants comprised 6 males and 1 female officials, ranging in age from 52 to 63 years (mean = 57.63), with 6 to 35 years of service in their respective positions (mean = 13.25 years).

Informal interviews and field observations were also employed to collect data for this study. National laws, local ordinances, and coastal resource management plans were included in the data for the textual primary source analysis. Audio-recorded interviews were transcribed verbatim and analyzed using thematic analysis. Coding was performed independently by both authors, with the first author auditing the second's coding, combining a deductive frame derived from the study's anthropological lenses (institutionalized

conservation, top-down governance, local cultural values, livelihood access) with inductive open coding to capture emergent categories. [Twenty-one (21)] codes were consolidated into eight overarching themes: (1) On-the-ground challenges; (2) unexamined conservation paradigm; (3) lack of the human aspect; (4) top-down conservation schemes; (5) cultural and customary values of mangroves; (5) livelihood options; (6) prospects for integrated conservation; (7) towards an integrated conservation; and (8) hypothetical participatory governance. Inter-coder agreement was assessed on 86% of transcripts; discrepancies were resolved through discussion until consensus was reached [report Cohen's kappa or percent agreement, ( $\kappa = 0.86$ )]. To strengthen credibility, preliminary findings were returned to a subset of informants for member checking, and analytic decisions were documented in an audit trail. Regarding research ethical standards, written consent was obtained from each key informant before the interview, and informants were assured of confidentiality and the right to withdraw at any time.



**Figure 2.** Panoramic views of the dense mangrove forests in the municipalities of Tudela (top) and Poro (bottom) with the latter showing the Katunggan, also known as the Mangrove Highway. Images were captured using a DJI Phantom 4 Pro drone. Photo: Ritche U. Nuevo, February 2021.





**Figure 3.** Mangrove aerial roots beneath the sunset drama during low tide in Timarong, a sub-village in Poro. White beach sand used to be rich in Timarong, but illegal and covert sand extraction in the 1980s and 1990s caused the shoreline to recede significantly. According to native residents, fireflies used to be abundant in this area, where they had once harbored enchantingly in mangroves. Nowadays, one can barely see a single firefly. Photo: Ian Dale B. Rios, December 2018.

### 3. Results

#### 3.1 Brief Historical Background

The period from the 1950s to the late 1980s reportedly saw local people exploit mangroves as a natural resource that met their needs: animal fodder, building materials, firewood, and even Christmas decorations. According to local government officials, this exploitation led to a decline in mangrove populations — a regional pattern documented elsewhere in Southeast Asia, where remote-sensing studies have tracked sustained mangrove area loss linked to coastal resource extraction [10]. As one municipal officer recalled, “*Sa mga tuig early 80’s sarang kadaghan gayud sa mga katunggan nga maoy gisaligan sa matag pamilya para sugnod, i-dapat sa paghimo ug balay, ang mga dahoon pagkaon sa ilang mga hayop: kanding ug baka. Aduna pay para himoon Christmas tree panahon sa pasko dalhun sa skwelahan ky project sa mga bata*” (“Mangroves were very much abundant during the early 1980s, when families relied on firewood for building houses; the [mangrove] leaves are used as animal fodder for their goats and cows. Moreover, during the Christmas season, their children reinvent them as Christmas trees and bring them to school as their project”). In 1981, the declaration of the Camotes Islands as part of the Mangrove Swamp Forest Reserve officially institutionalized the scientific ecological value of mangroves through the inception of national legislatures. Accordingly, mangrove conservation efforts increased during the 1980s, were strengthened through the NIPAS Act of 1992 in the 1990s, and were reinforced through the ENIPAS Act of 2018, which classified the Camotes Islands as the Camotes Islands Protected Landscape and Seascape. In the 1980s, San Francisco and Poro initiated mangrove reforestation efforts that are known locally today as *katunggan*, the thickly vegetated mangrove highway that connects the two municipalities. In the 1990s, mangroves received increased national and provincial recognition for protecting against strong winds and as sanctuaries for fish, and each municipality in the

Camotes Islands has responded to these institutionalized values by planting mangroves in its respective area, usually with the provision from the Bureau of Fisheries and Aquatic Resources (BFAR). By the mid-2000s, ordinances reinforced mangrove preservation efforts. The BFAR, with the help of non-government organizations (NGOs), provided increased protection and awareness of the importance of mangroves in coastal environments through programs and activities that included the protection of mangroves and aided the creation of coastal resource management (CRM) plans that include mangrove ecosystems. From 2010 onwards, LGUs established marine sanctuaries for fisherfolk, conducted seminars, and ran information and communication campaigns to the local communities, strengthening awareness of the importance of protecting coastal environments, including mangrove ecosystems. In summary, several national laws and ordinances have already recognized the importance of mangroves.

### 3.2 National Laws, Ordinances, and Programs

Ruzol et al. [11] have identified numerous mangrove-related policies in the Philippines. However, the case in this study shows that the NIPAS Act of 1992, ENIPAS Act of 2018, the Philippine Fisheries Code of 1998, and the Presidential Proclamation 2152 of 1981, are the national laws the LGUs are broadly adopting in establishing protected areas, implementing programs, enforcing policies, regulating resource exploitation, providing recreation and tourism, and prescribing fines and penalties related to the conservation of mangroves in the Camotes Islands. Additionally, while the integration of the human aspect in mangrove conservation, as stipulated in the aforementioned national laws, is recognized, it is not site-specific. Most importantly, the national laws are too stringent for local communities, leaving little to no room for compromise. As one municipal officer puts it, *"kung ang ordinansa nga imuhang gamiton kay national, case file gyud"* ('if you decide to enforce the national law, then you have to file a case'). To file a case against an ordinary local who has violated environmental laws may be too much for someone who is merely unaware of the national law. Fines prescribed under RA 7586, as amended by RA 11038, range from not less than PHP 200,000 to not more than PHP 1,000,000, or imprisonment of one year to six years, or both. Another municipal officer further explains, *'bug-at man gud ang national law kay og adunay violation, [...] kaso gyud dayon. Maayo man to sa diri sa municipal kay naa man gyud tay gitawag og kanang mga [...]'* 'compromise fines' ("The [penalty under the] national law is quite severe whenever a violation is committed. The good thing here at the municipal level is that we have what we call 'compromised fines'"). A municipal officer further describes the national justice system as *"hinay kaayo moligid"* ("rolls too slow"). Thus, when dealing with local violation cases, localized decision-making processes are more efficient and prompt; justice is served after sessions are conducted. Additionally, in municipal-level decisions, the violator either has a case filed against them or is imposed a community service. Situations such as these underscore the importance of local ordinances governing the conservation and rehabilitation of mangrove and seagrass ecosystems. However, even if laws and policies have already been established, there seems to be a lack of their implementation. Moreover, people can always find workarounds to circumvent existing environmental policies. That is the tendency when the law becomes outdated. A municipal officer thinks that the law must be updated. *'Atong pandayan sa lain nga balaod nga ikatapak ngadto'* ('Let's build another law to patch unto the older one'), he suggests. LGUs also have their own initiatives in local enforcement on coastal policies, such as the 'little fish warden' in Poro and the 'batang-ranger' in Tudela. These initiatives encourage youth to participate in conserving coastal resources. Research, too, is seen as an important element in creating local ordinances. However, only a few research groups provide them with copies of their research reports. In the Camotes Islands, ordinances on mangrove conservation primarily aim to preserve mangroves and prohibit cutting and collecting mangrove resources, while programs related to mangrove environments focus on rehabilitation and sustainable ecotourism with revenue-generating activities. Additionally, plantation and coastal clean-ups are the most typical government-led programs that concern mangrove conservation in the Camotes Islands. These regularly conducted activities are usually collaborative efforts among government agencies, academic institutions, and NGOs. Additionally, governmental organizations are important sectors that implement "sustainable" livelihood programs within mangrove ecosystems. However, despite the ordinances and programs, local people have always found ways to circumvent environmental policies. One baranggay captain says, *"Naay uban nga di mamati, musumbong ang tanod, akoy mubadlong. Naa puy uban nga gahi-gahi [...] ipakuha gyud naku'g pulis"* ("village watchmen have been



reporting that some people refuse to listen, so I will reproach them myself. There are also some who are very stubborn [...] I'm definitely having the police deal with them").

### 3.3 Implementation and Management Strategies

Local government actors perceive information and educational campaigns as essential strategies for conveying the ecological significance of mangroves and reducing environmental violations among the local population. Signboards, regular general assemblies, or monthly meetings that gather all purok (micro-geopolitical divisions) are also perceived as efficient strategies for educating local people. As one baranggay captain explains, "*daku gyud og impact ang genral assembly kay mao may atong pride pud nga sila maedokar*" ("The general assembly has a significant impact because educating them is also something we take pride in"). For instance, in *Baranggay Unidos* in San Francisco, apprehended violators would be called upon during a Baranggay Council session for reorientation, education, or counseling by the members of the village Council. The apprehended individual would then get a warning that the next violation would end in a penalty or a fine. In scenarios where all negotiations are exhausted and gets out of hand, matters are then escalated to the police force. Moreover, baranggay captains usually receive violent responses when enforcing environmental policies. A baranggay captain rationalizes, "*mangasuko baya sila kay di man sila sakto sa information*" ("They get angry because they are not well-informed"). While some people understand the importance of environmental policies, others do not. Certain instances even ended relationships unpleasantly between families when a *kumpari* (godfather of one's child) had been penalized due to an environmental violation. Another baranggay captain states about upholding integrity above all: "*Ma-taw namu'g sa dili, dakup gyud basta mamutol diri*" ("Regardless of your political affiliation, you will really get arrested if you cut (mangrove resources) here"). He also believes that being negatively labeled as "strict" has not adversely affected his political career, as he remains confident that his actions serve the best interests of the general public. Nevertheless, these existing strategies appear insufficient to address the persistent non-compliance of local resource users with environmental policies.

Furthermore, although appropriately trained, the municipal officers who are tasked with overseeing the management of coastal natural resources came from diverse backgrounds and do not necessarily hold advanced degrees or extensive training and specialization in coastal resource management, sociology, ecology, marine biology, or mass communication. These officers are not exclusively focused on coastal resource management and thus also need technical support from other agencies or institutions to help manage conservation initiatives and rehabilitate mangrove and seagrass ecosystems, especially in biophysical assessment, monitoring, and data analysis. Mangrove planting is by far the most common effort among the 4 municipalities of the Camotes Islands. However, it may not be as simple as it sounds. At the municipal level, mangrove planting is a delicate, technical human activity aimed at rehabilitating mangrove ecosystems, which essentially involves a combination of technical know-how, community participation, substantial funding from government agencies or units and NGOs, and political will.

**3.3.1 The "Rooms to Maneuver" of the Baranggay or Village Captains.** Baranggays are usually clustered in kinship, and people know each other in many ways. Kinship in the Camotes Islands can easily be traced in a single conversation. So, it is not surprising that barangay captains have an invaluable understanding of the realities of local communities and the livelihoods of most people; thus, they can understand the plight of some people who tend to violate environmental policies, including those related to coastal resources. However, as elected government officials, they should also enforce the policies – even if it ends up severing political and familial ties. As barangay captains, they are caught between protecting the environment and understanding the socio-economic situations of local communities, which can sometimes end up a political or a personal issue. Yet, there are situations in which they allow some people to get away with certain policies, especially toward some old folks who they know have few livelihood alternatives. A barangay captain recounts how he discourages his enforcers from apprehending elderly resource users, explaining, "*Pasagdi lang kay panud-an na nila*" ("Let them be as it means viand on their plates"). He further notes, "*Pero inig kita namo ako nang sultian nga kung mahimo ayaw na*" ("But when I come across them [the elderly resource users], I'll tell them never to do it again"). As government officials, they carefully balance the weight between firmness in enforcing environmental laws and keeping compassion.

### 3.4 On-the-Ground Challenges

Municipal and baranggay officials are fully aware of the institutionalized values of mangroves, that is, the scientific ecological morality that identifies mangrove forests as a vital defense against the impacts of storm surges, strong wind, and sea level rise. They also acknowledge the role of mangroves as habitats for biodiversity and as ecosystems that contribute to absorb carbon dioxide, and among others. However, they are equally aware that the local communities do not entirely share the same technobureaucratic values regarding mangrove ecosystems. It is common knowledge that local people value mangroves as a natural resource. As one baranggay captain explains that mangrove resources are used by the local community as *"sugnod ang uban, 'nya gamiton nila sa ilang katong mga ad gani sa baboy [...]. During kanang taas nga drought diba kanang wala nay makuha nga kumpay sa bukid [...]. Ang ilang gisaligan ato, labi nang katong pagting-init, pag tinghuwaw, kini man dinhi [mangrove forest]. [...] kuhaon na to kay ibaligya ang uban, ang uban igatong, himuon ad. Mao man ang reyalidad diha"* ("fuel, as well as for building pig pens [...]. When animal fodder can no longer be gathered in the hills during severe droughts [...], it is here [mangrove forest] where they depend on, especially during the dry season, during drought. [...] They will collect [mangrove resources] to sell some of it, to use as firewood, or to build pigpens. That is the reality here"). The conflict of values arises when these cultural and economic activities are identified as detrimental to mangrove preservation, as mandated by environmental policies, and are thus defined as "poaching" and technically illegal. Municipal and baranggay officials generally characterize this kind of poaching as a lack of understanding and education among the local population about the ecological significance of mangroves. One municipal officer remarks, *"Ang ilahang gitan-aw ang karon ra nga sitwasyon ang ilahang mga needs. Wa sila kahibaw kung unsa gyuy kanang gikahatag nga kaayohan gyud ba [...] sa mangrove"* ("The only thing they consider is the needs of their present situation. They do not realize the benefits that mangroves truly provide"). Another municipal officer similarly points out, that *"sa ako tan-aw murag wa man siguro [...] ang mga taw ka appreciate og unsa diay kahinungdanon ning atoang mangrove ba"* ("I think the people seem unable to really appreciate the importance of our mangroves"). On the other hand, a baranggay captain explains why the local people cannot see the value of mangrove reforestation, *"wa nay kanguhaag imbaw, kay natanoman na lagi, wa nay kapanginhasan"* ("because of the [mangrove] plantation, there's no more space for them to glean – to gather mangrove clam"). In Pilar for instance, some mangrove reforestation programs have disrupted the local fisherfolks because they blocked their traditional access paths to the sea. This agitated the affected fishing community. A municipal officer shares a funny but terrifying experience because of mangrove planting programs, *"anaon to sa sundang o, anaon sa sundang"* (gestures how a fisherfolk was wielding a utility bolo in an offensive stance against her).

Political integrity from higher municipal offices is also one of the difficult challenges municipal environmental officer face in implementing and managing environmental conservation. It harms the integrity of the local socio-political landscape, severing the relationship between local government actors, the implementers – and the local communities, the receiving end. Moreover, some people would not cooperate or abide by the policies simply because they follow the opposing political party. A municipal officer noted, *"Sauna naay kanang mamutol 'nya taw sa mayor, murag ibaliwala ra man"* ("There used to be people who cut (mangroves), but because they were associated with the mayor, their violations were simply dismissed"). A baranggay captain also shares the same sentiment, *"sa akun abat og naay madakpan, padrenohan [...] pulitiko, amo ra nga mabuhian"* ("from what I've observed, when someone is arrested, politicians act as their patrons, which results in their release"). However, the same socio-political phenomenon can become an opportunity when committed to using one's political will. A baranggay captain asserts that equally enforcing policies to everyone, regardless of political alliances, will produce long-term political benefits. Setting fair law enforcement to the general public is crucial in managing coastal conservation. A municipal officer explains that when the people see how you took action and apprehended your political followers, they will take your environmental programs and policies seriously. A baranggay captain shares his perspectives, *"usahay, dili kaayo motuman ang mga taw kung mismo ang magduma luag"* ("Sometimes, people are less likely to comply when the authorities themselves enforce the rules loosely").

Moreover, there are programs that are often highly dependent on particular political administrations. For instance, during the term of Hilario Davide III as the Provincial Governor, cash incentives were provided to the local environmental enforcers. However, when his term ended, the incentives were discontinued, and environmental enforcement eventually declined as well. Typically, incentives are provided during the initial stages of implementation but are later withdrawn due to unsustainable funding, shifting development

priorities, a lack of political will, or the decision of a new administration to discontinue programs initiated by its political predecessors. These factors often result in personnel shortages and declining morale among enforcers, who may become overwhelmed by persistent threats and intimidation from some local resource users, thereby eroding the integrity of conservation efforts. As one municipal officer explains, “*mahadlok sila basig kontrahan*” (“they [environmental enforcers or watchmen] are afraid they might make enemies”). Furthermore, some People’s Organizations (POs) were inadequately equipped with the human and technological resources needed to enforce environmental policies effectively. As a result, they were often overpowered by resource users who resisted environmental regulations, many of whom reportedly came from neighboring municipalities. Additionally, some of their projects depend heavily on external funding from national agencies, government bureaus, and NGOs. One municipal officer cited a People’s Organization that implemented a mangrove-based livelihood project endorsed by the LGU and funded by BFAR. According to the officer, the project turned out financially unsustainable: “*pananglitan og mag-repair, mangayo na pud – ana ba, morag dili siya self-sustaining. [...] Sigig pangayo*” (“for instance every time a repair is needed, they ask [for funds] again – instances like that, it is not self-sustaining [...] keeps on asking”). The officer further explained that many programs lack sustainability because project proposals are often developed without adequate contingency plans. Consequently, when natural hazards occur, the organizations are unable to recover or rebuild independently. Similarly, a barangay captain questioned the effectiveness of some People’s Organization projects, remarking, “*daghay kwarta niabot, ang pangutana og ni-click ba?*” (“Lots of money came, the question is – did it click?”). The sustainability of government-initiated livelihood programs has also been questioned. For example, a municipal officer described a mangrove-based crab farming project promoted by government agencies as a “sad story” because it yielded no harvest at all and, thus, is not sustainable at all. San Francisco once maintained a mangrove nursery that supplied seedlings to many barangays across the Camotes Islands, including the award-winning Barangay Villahermosa in Tudela, for their mangrove planting activities. However, the nursery was discontinued in 2018 because of insufficient funding.

Furthermore, an effective plantation is a delicate socio-political effort that requires technical know-how, community participation, substantial funding from government agencies or units, NGOs, and political will. Reportedly, mangrove planting in the Camotes Islands has had zero to low survival rates due to a lack of technical know-how. A municipal officer narrates, “*Giatakeg barnacles. [...] wa jud wa jud siya ni survive. [...] nangamatay, patay tanan*” (“attacked by barnacles [...] it [mangroves] did not survive. [...] it perished, all perished”). Another municipal officer recalls, “*sa akong experience about sa mangrove [planting] is ang iya ra gyong kanang survival ba is gamay ra gyud ang iyang rate*” (“in my experience about mangrove [planting] is that its survival rate is truly very marginal”). On many occasions, the planting of invasive mangrove species has outpaced the growth of native species. Such were an absolute waste of human and natural resources, as well as government and NGO funding.

## 4. Discussion

### 4.1 Unexamined Conservation Paradigm and Policies

The institutionalized mangrove conservation paradigm in the Camotes Islands is centered on the biophysical preservation and rehabilitation of natural resources, with little regard for the basic needs of local people who have traditionally relied on resources within PAs. It indicates how the prevailing paradigm considerably lacks the human dimension in the local context. It also illustrates what Kottak [4] describes as a “modern intervention philosophy that seeks to impose global ecological morality without due attention to cultural variation and autonomy.” Using the lens of new ecological anthropology and the concept of ecodevelopment, the prevailing paradigm in the Camotes Islands has done little to minimize the negative impacts of conservation on the affected local communities. Additionally, such approaches may be socioculturally inappropriate because it imprudently restricts local people's access to essential livelihood resources without ensuring reliable alternative sources of livelihood. Thus, this study reveals the chasm that creates an incongruous space between the aims of conservation and the welfare of the local people in the

Camotes Islands. This chasm is characterized by top-down approaches that overlook local values, knowledge, and needs, resulting in conservation initiatives that lack a meaningful human dimension.

#### 4.2 Lack of the Human Aspect

History has shown that the ecological value of mangroves in the Camotes Islands has drawn the line between what is illegal and what is not, and between what benefits and harms environmental resources. It has reinforced coastal ecosystem preservation and rehabilitation. Accordingly, the policies concerning mangrove conservation focus solely on scientific ecological values, prioritizing the preservation of natural resources. Yet, despite the laws and ordinances, violations or non-compliance among the local people persist to this day. Other studies that focus on the local people's perspective have similar findings. In Banacon, Bohol, Philippines, there is a disjunction of perspectives and attitudes between the government and peasant communities [12], and in Palompon, Leyte, Philippines, mangrove areas are considered as a contested space due to the “contrasting viewpoints of the residents and the local government, specifically on the actors’ differentiated rights and claims on the resource” [13]. Moreover, in the case of Leganes Rehabilitation Mangrove Site in Iloilo, Philippines, Ruzol et al. [11] have argued that “technobureaucratic perspective on the participatory approach keeps itself distant from the complex temporal and political dynamics of participation in the field.” Further, based on the textual primary sources, no program in the Camotes Islands directly addresses the needs or concretely provides site-specific alternative livelihood for the local communities affected by mangrove preservation policies, let alone programs that include the affected locals in conservation management planning, although indicated in Section 9 of the ENIPAS Act of 2018, the adoption of socioeconomic research, site-specific policy development, and others. Conservation efforts are generally the preservation of mangroves that merely cut off people’s access to their basic needs without providing definite alternatives. Fines and penalties under national environmental laws are too high for local people. On the other hand, ordinances provide “room to maneuver” [9] to accommodate justifiable compromises and to prompt decisions. On-the-ground cases like these magnify the importance and efficiency of ordinances. Thus, to optimize efficacy, existing environmental policies in the Camotes Islands need substantial amendments to integrate the human dimension into mangrove conservation, with thorough regard for local social, economic, and cultural contexts. The Municipality of Poro, for instance, declares mangroves a cultural treasure, yet its significance is based on institutionalized scientific ecological values rather than local cultural values. Although the institutionalized value of mangroves, as reflected in national law, is an added value for pride to the locale, where would such pride be placed in the affected local communities when it also means the dissipation of their access to traditional resources? This study underlines the pivotal role that local and national political institutions play in conserving natural resources, as they can critically influence changes in existing local sociocultural institutions. Thus, it is crucial to reexamine conservation paradigms that insufficiently address human welfare concerns (Figure 4).

#### 4.3 Top-Down Conservation Schemes

Key government actors have been crucial instruments in achieving the objectives of institutionalized mangrove conservation in the Camotes Islands. They serve as intermediaries between political institutions and local communities and, to a certain degree, have a sound understanding of the socioeconomic contexts on the ground. As government actors, they have also carefully balanced the weight between implementing programs and enforcing policies while keeping a small room to maneuver – an effort, but a marginal one, to close the incongruous chasm present in mangrove conservation. The barangay captains, in particular, are always caught in the crossfire between the two sides of the resource use conflict. They duly uphold mandated environmental policies and, at the same time, to some degree, slacken them to make room and accommodate the cultural and economic welfare of the local people. Additionally, despite the predominance of the top-down approach in the conservation framework, environmental governance at the local level does not rigorously apply the “fortress conservation” paradigm [2, 13] and what “transpires” on the ground is “a process of negotiation and compromise to define and implement a management strategy” [2]. It supports the view that the law is not a straitjacket but an enabling tool, in which actors should think strategically and multidimensionally [4]. The top-down framework in mangrove conservation has little regard for the existing



local socioeconomic institutions in the Camotes Islands. Moreover, according to Eversole [12], local social groups have their own structures, rules, and norms that shape their day-to-day lives, and these are the existing, invisible institutional arrangements people have used to manage situations at the margins to survive. Hence, “[i]gnoring these institutions is a typical recipe for development failure” [9]. For instance, the findings of this study indicate that mangrove planting programs with low to zero survival rates, together with crab-farming initiatives that produced no measurable yield, failed to account for local ecological and sociocultural contexts. These “sustainable” programs in mangrove ecosystems are not sustainable and do not fit into the existing local cultural and socioeconomic landscapes. These interventions are mere formulations from the top, without regard for local socioeconomic institutions. As Wiset et. al [14] point out, top-down approaches obstruct effective local participation and distort a sense of ownership and self-initiation; more so, “effective engagement will only occur when local people can influence and have some control and power to make meaningful decisions” [14]. Additionally, “experience has shown that traditional, top-down exclusionary approaches to a protected area (“fortress conservation” or fences and fines approach) are often not effective in reaching conservation objectives” [2]. Hence, the prevailing paradigm must be reexamined, and an inclusive framework reconsidered to account for the values local people hold for their natural environment.

#### 4.4 Recognizing the Local Values for Mangroves

The local community’s values are deemed inimical to the scientific ecological values of mangroves. This power relation affects conservation efficacy and is a “problem with mainstream institutional development and strengthening programs,” where “they tend to start from a predetermined idea of what institutions should look like” [9]. Kottak [5] further points out that even “[w]ell-meaning conservation efforts can be as insensitive as development schemes that promote radical changes without involving local people in planning and carrying out policies that affect them.” A global framework that disregards the local contexts can not be sustained in the long run. But, paying attention to the bottom, the local contexts – allows us to recognize that people “already have established ways of working that they use to get things done” [9]. Institutional experts cannot simply enter into the conservation landscape and implement programs with a one-size-fits-all framework while perceiving the local economic needs as threats to their objectives. According to local government actors, the persistence of local people’s noncompliance with preservation policies is due to a lack of education. However, *such* non-compliance may also be understood as an everyday form of resistance [15] because people resist projects or programs “that require major changes in their daily lives, especially ones that interfere with subsistence pursuits” [5]. Amper similarly argues that hegemony over local communities can cause them “to lose interest in maintaining their mangroves” [12]. Therefore, it is essential to “ensure that the local people feel a sense of ownership” [6] over these protected mangrove areas. This can be achieved through inclusive conservation planning that recognizes and incorporates diverse value systems [5].

#### 4.5 Towards an Integrated Conservation

In the Camotes Islands, the scientific ecological values of mangroves overshadow their local cultural and economic values. Although “there is no sound basis for deciding that one worldview offers a superior reference point for ‘reality’ than another,” and given the institutional power of science, the scientific method is always called upon “to judge other knowledge systems” [6]. On the other hand, recent studies indicate that the success of resource conservation lies in paying close attention to the social dimension [5, 6, 17] rather than focusing solely on pre-packaged, expert-led solutions [9]. One framework is ecodevelopment, which can be achieved through sound participation and the involvement of affected people [6]. Considering the local realities, this study underlines the urgency of reframing the prevailing institutionalized mangrove conservation in the Camotes Islands by integrating ecodevelopment strategies to step up conservation on the ground. Specifically, it should include sustainable alternative livelihood opportunities for the local people whose way of living is displaced. One cannot simply expect support of the local people for conservation initiatives when they diminish “their access to strategic and socially valued resources” [5]. As Singh [6] notes, “ecodevelopment planning is done at a village level, by the villagers, who are facilitated by a micro level planning team” composed of various representatives from government to non-governmental sectors [6]. Moreover, according to Wiset et al. [14], while promoted by the Philippine government, there is



meager participation among local communities in reforestation initiatives on the ground. Participatory governance must begin, but does not end, at the community level, grounded in a fundamental human reality [4]. Concerned government agencies and local units do not have to start from scratch but may build upon what already works [9] and may involve “envisioning techniques to facilitate stakeholders to discuss their visions of sustainable futures and their priorities for the protected area” which includes “their views on the trade-offs between ecological, social and economic criteria, and used information and measures that they had defined as important” [2]

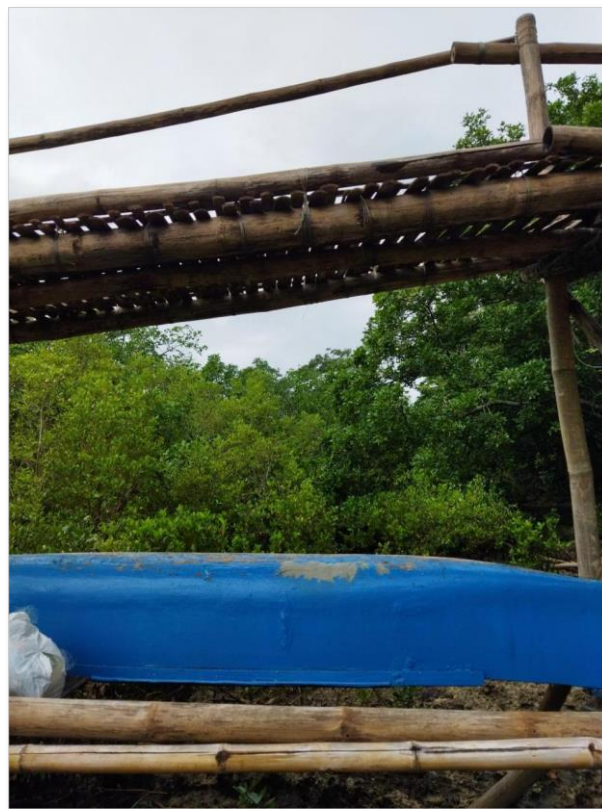


**Figure 4.** An elderly local woman gathers *imbaw* (mangrove clams) for household consumption, and any excess is sold locally. She says that shell-gathering is a traditional livelihood that has been passed down through generations. Photo: Ian Dale B. Rios, January 2022.

Further, given the ecological services provided by mangrove ecosystems, this study urges the local policymaking bodies to sustain funding for conservation programs and ensure consistent political commitment to environmental and sociocultural development. Also necessary is the amendment of outmoded ordinances to foster ecologically and socially sound solutions to present-day resource use conflicts. Moreover, to nourish the local social, economic, and cultural heritage values of mangrove environments – ethnographic studies should be employed to obtain the native’s point of view of mangrove resources as it can identify local perceptions and values [13] and the local forms of conservation and reforestation initiatives [12]. In addition, exploring local forms of community participation is highly recommended in this study. Research has proven “that some traditions and practices are very supportive and could form a base for genuine participation in development initiatives” [18] (p. 110). To reiterate the importance of the various actors and stakeholders in the conservation landscape is foundational in this study and proposes further multidisciplinary studies on the local communities whose lifeways are affected the most by conservation. Considering the gaping *chasm* that amasses incongruity in the conservation landscape, reflexivity, inclusivity, and an integrated approach in conservation can foster participation among the local people and achieve the conservation goal that regards both nature and culture, a decent starting point to close the incongruous chasm between institutional conservation values and those of the local communities.



**Figure 5.** Mangrove environments are also spaces tightly intertwined with the basic needs of the local people.  
Photo: Ian Dale B. Rios, January 2022.



**Figure 6.** An upside-down wooden hull of a seacraft rests beneath an abandoned bamboo footbridge in a mangrove forest in Poro Island. Photo: Ian Dale B. Rios, January 2022.

## 5. Conclusions

Mangrove conservation in the Camotes Islands is regarded as a form of development in the context of ecological conservation because it involves economic and social changes among affected local communities that value mangroves as a livelihood resource. Despite mangroves having plural values, the institutionalized

conservation paradigm in the Camotes Islands is predominantly driven by scientific ecological values centered on the preservation and rehabilitation of the biophysical aspects of natural resources. This top-down conservation approach pays little regard to local values and lacks a human dimension. In contrast, identifying the plural values of environmental resources can provide useful insights for resolving resource-use conflicts and for developing ecologically sound, socially inclusive, and culturally sensitive policies. Furthermore, this study highlights how key local government actors are instrumental in achieving the aims of institutionalized mangrove conservation and how they negotiate the conflicting values between the institutionalized and the local. Also, examining their perspectives, knowledge, experiences, and practices has provided us with a comprehensive view of mangrove conservation status on the ground. This study urges integrated, cross-disciplinary collaboration between the social and natural sciences to address the conflicting values that arise in spaces shared by people and natural ecosystems. For policy-makers, conservation managers, and local government actors in the Camotes Islands and comparable settings, the findings point to three concrete priorities: (1) amend existing mangrove conservation ordinances and management plans to embed site-specific socioeconomic and cultural assessments, as already mandated under Section 9 of the ENIPAS Act of 2018; (2) institutionalize the participation of affected barangays in the design, implementation, and monitoring of mangrove programs, including the provision of viable alternative livelihoods where access to traditional resources is restricted; and (3) strengthen technical support and inter-agency coordination for mangrove rehabilitation to prevent further low-survival plantings and the spread of invasive species. Adopting these measures would align institutional conservation with local realities. Thereby, improving both ecological outcomes and community welfare as a foundational whole. Conservation, after all, is far more welcoming as a bridge than as a spiked fence. and improve both ecological outcomes and community welfare.

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# Diversity and Abundance of Spiders (*Araneae*) in Different Habitats in Mt. Lantoy, Cebu, Philippines

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**Abstract:** Spiders are essential Ecological regulators that help maintain ecosystem stability through their roles as both predators and prey in food webs. Despite their importance, information on their diversity and distribution is still scarce, especially in regions that remain poorly explored. This study aims to assess the abundance and diversity of spiders across different habitats at Mt. Lantoy: Forest, Riparian, Plantation, and Grassland. A combination of search-and-capture techniques and beat-sheet methods was employed during a 160-man-hour sampling period. The study documented 276 individuals across 101 species in 20 families. Among the families, Araneidae exhibited the highest species richness with 35 species, while *Tetragnatha cf. ceylonica* was the most abundant species, with 16 individuals. Nine recorded species are Philippine endemics (*Acusilas dahoneus*, *Gasteracantha parangdiadesmia*, *Chrysso tiboli*, *Neoscona facundoi*, *Neoscona aldinei*, *Nusatidia luzonica*, *Phlogiellus baeri*, *Phintella piatensis*, and *Lepidemathis sericea*) and eight species are classified as threatened under DENR Administrative Order 2019-09 (*Cyrtophora exanthematica*, *Eriovixia laglaizei*, *Neoscona punctigera*, *Neoscona aldinei*, *Neoscona vigilans*, *Parawixia dehaani*, *Poltys illepidus*, and *Phlogiellus baeri*); two species — *Neoscona aldinei* and *Phlogiellus baeri* — carry both endemic and threatened status, yielding 15 unique conservation-priority species in total. Forest and riparian had significantly higher plot-level abundance and species richness than plantation and grassland; forest and riparian were not significantly different from each other. These findings highlight Mt. Lantoy as a key habitat for spider populations, underscoring its value for future conservation initiatives and biodiversity management.

**Keywords:** Guilds; habitats, Mt. Lantoy; spiders

## 1. Introduction

Spiders (*Araneae*) are among the most diverse and ecologically significant groups of arthropods, playing crucial roles in maintaining ecosystem balance as both predators and prey [1, 2]. They contribute to natural pest control, energy flow, and trophic interactions across various ecosystems [2, 3]. The diversity, distribution, and guild composition of spiders are strongly influenced by habitat structure, vegetation complexity, and environmental conditions [4, 5]. Habitats with greater structural heterogeneity, such as forests and riparian zones, tend to support higher spider diversity and abundance than simpler or disturbed habitats, such as plantations and grasslands [2, 6, 7]. Despite their ecological importance, studies on spider diversity and community structure in



the Philippines remain limited, especially in understudied areas such as Mt. Lantoy in Cebu. Mt. Lantoy is home to various habitat types, including forest, riparian, plantation, and grassland ecosystems that differ in vegetation structure, microclimate, and levels of human disturbance. Understanding how spider communities vary across these habitats can provide insights into their ecological preferences, functional roles, and the effects of habitat modification on biodiversity.

This study aims to assess the diversity, abundance, and guild structure of spiders in different habitats within Mt. Lantoy, Cebu. Specifically, it seeks to determine the species richness, abundance, and diversity indices of spiders across habitats, compare guild composition, and identify habitat characteristics that influence spider assemblages. The findings will contribute to local biodiversity documentation and inform conservation strategies for the area.

## 2. Materials and Methods

### 2.1 Study area

Mt. Lantoy is located in the barangays (villages) of Argao, and the study was established in the barangays of Conalum, Usmad, Catang, and Tabayag in Argao, Cebu. It is known for its rich biodiversity and serves as a critical habitat for many endemic and native species of flora and fauna. The Mt. Lantoy Key Biodiversity Area (KBA) spans approximately 3,000 hectares and provides a refuge for several endemic and native species [8]. It is one of the 117 terrestrial KBAs in the Philippines, identified based on the criteria of vulnerability and irreplaceability. It has also been recognized as part of a new KBA site [9]. Mt. Lantoy forms part of the Argao-Dalaguete Watershed Forest Reserve, a protected landscape declared under Presidential Proclamation No. 414 on June 29, 1994. Primarily a limestone area, Mount Lantoy has been a site for various biodiversity studies, including research on native plant species, and the abundance and distribution of land snail species [9, 10]. It remains a priority site for future conservation and sustainable development programs.

### 2.2 Sampling Sites

Four habitat types were identified in Mt. Lantoy for recording spider abundance and species richness: forest, riparian, plantation, and grassland. Five plots were randomly established in each habitat with at least 50 m between plots. The size of each plot (10 m long × 2 m wide × 2.5 m high) was based on the study of Pinkus-Rendón [11]. Ground coordinates were recorded for each sampling plot.

### 2.3 Collection and processing of samples

Sampling was conducted on November 8-13, 2022, December 6-11, 2023, July 25-30, 2024, and September 19-21, 2024, totaling 160 person-hours across all habitats with 40 man-hours per habitat and 8 man-hours per plot. A Wildlife Gratuitous Permit and Local Transport Permit were secured from the Department of Environment and Natural Resources before specimen collection. Because spiders are active during both daylight and nighttime, sampling was conducted for 4 man-hours during the day and 4 man-hours during the night in each plot. Sampling was conducted from 6:00 AM to 9:00 AM and again from 7:00 PM to 10:00 PM to capture both diurnal and nocturnal species [12]. Individuals were collected through active search and capture, using a combination of beat-sheet methods. A flashlight was used during the search. For targeted capture of individuals dislodged by beating or spotted in the vegetation, a handmade hand net was used, consisting of a circular tie-wire hoop frame approximately 15 cm ( $\approx$  6 in) in diameter fitted with a fine white cloth bag. This implement is a hand net for directed capture of located specimens rather than a sweep net for vegetation sweeping; standard sweep nets (30–38 cm) were not used because the primary collection methods were visual active search and beat-sheet sampling, for which a smaller, more maneuverable hoop is better suited to extracting individual spiders from foliage and beat-sheet drops without damaging soft-bodied specimens. The hoop diameter accommodated the full range of spider body sizes encountered in this study (approximately 2–25 mm body length). All encountered individuals were counted to record abundance, while up to two individuals per species were collected as voucher specimens. Each plot was surveyed by six collectors for a total of four collector-hours per plot, with careful examination of spiders present in available microhabitats. The distribution of sampling effort across periods was based on the Philippine Atmospheric, Geophysical and

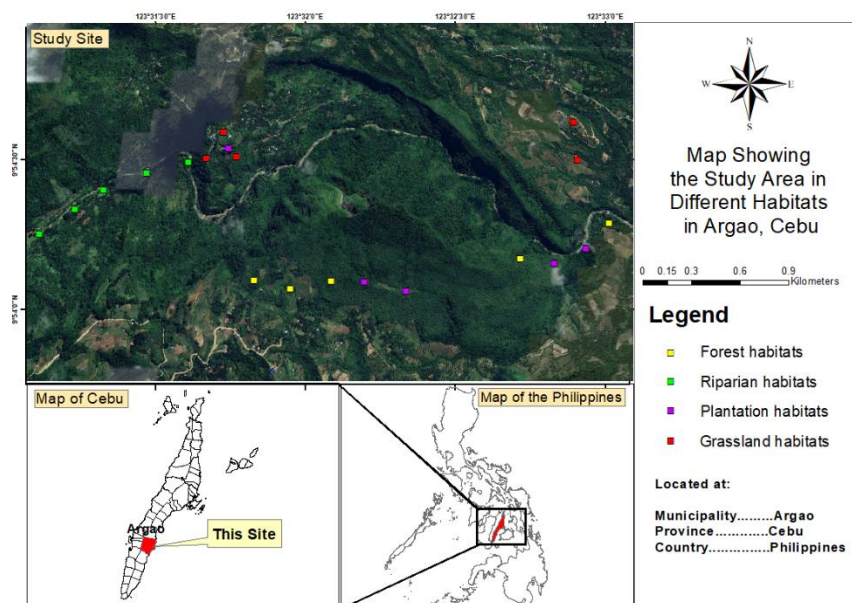
Astronomical Services Administration [13], and no sampling was conducted on rainy days. The sampling habitats are summarized in Table 1.

**Table 1.** Sampling periods, plots sampled per habitat, and person-hours per period

| Sampling period       | Season        | Forest<br>(plots/hrs) | Riparian<br>(plots/hrs) | Plantation<br>(plots/hrs) | Grassland<br>(plots/hrs) | Total<br>man-hours |
|-----------------------|---------------|-----------------------|-------------------------|---------------------------|--------------------------|--------------------|
| November 8–13, 2022   | Wet           | 0 / 0                 | 2 / 16                  | 1 / 8                     | 3 / 24                   | 48                 |
| December 6–11, 2023   | Dry<br>(cool) | 2 / 16                | 0 / 0                   | 2 / 16                    | 2 / 16                   | 48                 |
| July 25–30, 2024      | Wet           | 3 / 24                | 0 / 0                   | 2 / 16                    | 0 / 0                    | 40                 |
| September 19–21, 2024 | Wet           | 0 / 0                 | 3 / 24                  | 0 / 0                     | 0 / 0                    | 24                 |
| <b>Total</b>          | —             | 5 / 40                | 5 / 40                  | 5 / 40                    | 5 / 40                   | <b>160</b>         |

## 2.4 Identification of Spiders

Spiders were identified based on external morphological characters, including eye arrangement, web architecture, leg morphology, body form, coloration, and overall size. Preliminary field identification was done whenever possible at the family level using available spider field guides [14]. Specimens were subsequently examined in the laboratory for more detailed confirmation. Additional verification of selected specimens was undertaken at the Museum of Natural History, University of the Philippines, Los Baños. Taxonomic validation was also aided by online reference resources, including the World Spider Catalog [15], the Global Biodiversity Information Facility (GBIF) [16], and the Jumping Spiders of the World database [17].



**Figure 1.** Map of the study area showing the location of the selected habitats in Mt. Lantoy, Argao, Cebu, Philippines.

## 2.5 Preservation of collected spider specimens

Collected specimens were preserved individually in glass vials containing 90% ethanol, with the preservative volume maintained at more than twice the volume of the specimen. Dry preservation was avoided because spiders' soft bodies can easily shrink and become brittle under such conditions [18]. Each vial was labeled with scientific names, collection locality, and date of collection. To maintain specimen quality, the ethanol was replaced after 48-72 hours to remove discoloration and cloudy residue.

## 2.6 Statistical analysis

Statistical analyses were conducted in R version 4.5.3 [19]. Species abundance was summarized as the total number of individuals recorded, while species richness was calculated as the number of unique species. Relative abundance was computed as the total number of individuals of each species divided by the total number of individuals recorded, multiplied by 100. Shannon-Wiener diversity index was calculated as  $H' = -\sum p_i \ln(p_i)$ , where  $p_i$  is the proportional abundance of each species, and Pielou's evenness was calculated as  $J' = H'/\ln(S)$ , where  $S$  is species richness. Simpson diversity was also computed to support the interpretation of habitat-level diversity. Diversity indices and species richness were calculated using the vegan package in R [20]. Sampling effort per habitat consisted of 5 plots  $\times$  8 man-hours, with 4 man-hours during the day and 4 man-hours during the night, totaling 40 man-hours per habitat and 160 man-hours across all habitats, as reported in Table 1. Sampling spanned four periods across both wet and dry seasons over two years (November 2022, December 2023, July 2024, and September 2024); Because habitat coverage was not fully balanced across seasons, season was treated as a potential limitation rather than as a formal factor in the inferential analysis, although not all habitats were sampled in every sampling period because riparian plots were sampled only during wet-season periods. Therefore, habitat comparisons were interpreted as standardized-effort inventory comparisons at the habitat level rather than as fully season-controlled experimental comparisons, with each habitat receiving a total of 40 man-hours of sampling effort. For habitat-level comparisons, a plot was used as the unit of replication. Species observations were aggregated to calculate total abundance and species richness for each plot. Normality of ANOVA residuals was assessed using the Shapiro-Wilk test, and homogeneity of variance among habitats was assessed using Levene's test through the car package [21]. When both assumptions were acceptable at  $\alpha = 0.05$ , one-way analysis of variance (ANOVA) was used to test for differences in abundance and species richness among habitats, followed by Tukey's Honestly Significant Difference (HSD) test for pairwise comparisons. If either assumption had been violated, the Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni correction was considered as the non-parametric alternative. Day and night sampling were compared using paired plot-level data because the same plots were sampled during both periods. For each plot, daytime and nighttime abundance and species richness were paired and compared using a paired t-test. The test direction was set to evaluate whether nighttime values were greater than daytime values, based on the expectation that many spiders are more active or more detectable at night. Results were considered significant at  $\alpha = 0.05$ . Because the total number of individuals differed among habitats, individual-based rarefaction and extrapolation curves were generated using the iNEXT package in R to compare species richness at standardized sample sizes [22,23]. The rarefaction analysis used 95% confidence intervals and Hill number  $q = 0$ , corresponding to species richness. Rarefied richness estimates were compared at a common sample size equal to the smallest habitat sample, which was 18 individuals in the plantation habitat. Guild composition was summarized by habitat using the guild assigned to each recorded species. Guild assignments followed the spider guild framework of Cardoso et al. [24], supported by field observations of web architecture or hunting mode where available. Guild abundance was calculated as the number and percentage of individuals belonging to each guild within each habitat. Figures were generated using ggplot2 [25].

## 3. Results and Discussion

### 3.1 Species composition and relative abundance

A total of 276 individuals across 101 species representing 20 families of spiders were recorded in Mt. Lantoy in four habitats during day and night collections. During daytime sampling, 87 individuals from 38 species were recorded, while during nighttime sampling, 189 individuals from 82 species were recorded. Sixty-four species were recorded exclusively during the night, twenty species were only recorded during the day sampling, and eighteen species were recorded during both day and night sampling. As illustrated in Table 1, the three most abundant families were Araneidae, with 101 individuals representing 36.59% RA of the total recorded individuals, followed by Salticidae with 50 individuals (18.12% RA) and Tetragnathidae with 35 individuals (12.68% RA). Moreover, the forest habitat had the highest abundance, with 114 individuals across

78 species from 15 families, followed by riparian with 98 individuals across 52 species from 13 families, grassland with 46 individuals across 24 species from 8 families, and plantation habitat with 18 individuals across 16 species from 7 families. Among the 101 recorded species, (*Tetragnatha cf. ceylonica*) was found to be the most abundant species, with 16 individuals, followed by *Chrysso sp.* with 14 individuals and *Neoscona melomensis* with 13 individuals. Based on the World Spider Catalog version 27 of the Natural History Museum Bern, there were a total of nine species of spiders that are endemic in the Philippines, namely: *Acusilas dahoneus*, *Gasteracantha parangdiadesmia*, *Chrysso tiboli*, *Neoscona facundoi*, *Neoscona aldinei*, *Nusatidia luzonica*, *Phlogiellus baeri*, *Phintella piatensis*, and *Lepidemathis sericea*. Endemic spiders are often habitat specialists with narrow ecological niches, making them highly susceptible to habitat loss and fragmentation [26]. Eight species are classified as threatened under the Department of Environmental and Natural Resources Administrative Order 2019-09: *Cytophora exanthematica*, *Eriovixia laglaizei*, *Neoscona punctigera*, *Neoscona aldinei*, *Neoscona vigilans*, *Paravixia dehaani*, *Poltys illepidus*, and *Phlogiellus baeri*. Two species — *Neoscona aldinei* and *Phlogiellus baeri* carry both endemic (\*) and threatened (^) status in Table 1, indicating they are Philippine endemics that are simultaneously listed under DENR AO 2019-09; the 9 endemic and 8 threatened species therefore comprise 15 unique conservation-priority species. The endemic and threatened classifications were verified against the World Spider Catalog version 27 (2026) and DENR Administrative Order 2019-09.

**Table 2.** Species diversity and abundance of spiders on different habitats in Mt. Lantoy, Argao, Cebu, Philippines. Species with \* Philippine endemic, ^ Threatened species.

| Family    | Scientific name                                                   | Forest | Riparian | Plantation | Grassland | TOTAL | RA (%) |
|-----------|-------------------------------------------------------------------|--------|----------|------------|-----------|-------|--------|
| Araneidae | <i>Acusilas dahoneus</i> (Barrion & Litsinger, 1995)*             | 2      | 1        | 0          | 0         | 3     | 1.09   |
|           | <i>Arachnura melanura</i> (Simon, 1867)                           | 3      | 1        | 1          | 0         | 5     | 1.81   |
|           | <i>Araneus</i> sp.                                                | 2      | 0        | 0          | 0         | 2     | 0.72   |
|           | <i>Argiope aemula</i> (Walckenaer, 1841)                          | 1      | 1        | 0          | 0         | 2     | 0.72   |
|           | <i>Argiope appensa</i> (Walckenaer, 1841)                         | 1      | 1        | 0          | 0         | 2     | 0.72   |
|           | <i>Argiope</i> sp.                                                | 0      | 1        | 0          | 0         | 1     | 0.36   |
|           | <i>Cyclosa insulana</i> (Costa, 1834)                             | 4      | 3        | 2          | 1         | 10    | 3.62   |
|           | <i>Cyclosa bifida</i> (Dolleschall, 1859)                         | 2      | 1        | 0          | 0         | 3     | 1.09   |
|           | <i>Cyclosa confragra</i> (Thorell, 1892)                          | 2      | 1        | 1          | 1         | 5     | 1.81   |
|           | <i>Cyrtophora cicutrosa</i> (Stoliczka, 1869)                     | 1      | 0        | 1          | 0         | 2     | 0.72   |
|           | <i>Cyrtophora cylindroides</i> (Walckenaer, 1841)                 | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Cyrtophora exanthematica</i> (Dolleschall, 1859)^              | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Cyrtarachne</i> sp.                                            | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Eriovixia laglaizei</i> (Simon, 1877)^                         | 3      | 1        | 0          | 0         | 4     | 1.45   |
|           | <i>Eriovixia sakiedaorum</i> (Tanikawa, 1999)                     | 1      | 0        | 0          | 1         | 2     | 0.72   |
|           | <i>Gasteracantha janopol</i> (Barrion & Litsinger, 1995)          | 0      | 2        | 0          | 0         | 2     | 0.72   |
|           | <i>Gea spinipes</i> (C. L. Koch, 1843)                            | 1      | 1        | 0          | 1         | 3     | 1.09   |
|           | <i>Gasteracantha kuhli</i> (C.L. Koch, 1837)                      | 3      | 1        | 1          | 0         | 5     | 1.81   |
|           | <i>Gasteracantha parangdiadesmia</i> (Barrion & Litsinger, 1995)* | 1      | 0        | 0          | 1         | 2     | 0.72   |
|           | <i>Herennia multipuncta</i> (Dolleschall, 1859)                   | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Neoscona facundoi</i> (Barrion-Dupo, 2008)*                    | 1      | 1        | 0          | 0         | 2     | 0.72   |
|           | <i>Neoscona melomensis</i> (Tikader & Bal, 1981)                  | 0      | 7        | 1          | 5         | 13    | 4.71   |
|           | <i>Neoscona</i> sp.                                               | 0      | 1        | 0          | 1         | 2     | 0.72   |



**Table 2.** Species diversity and abundance of spiders on different habitats in Mt. Lantoy, Argao, Cebu, Philippines. Species with \* Philippine endemic, ^ Threatened species. (continue)

| Family    | Scientific name                                  | Forest | Riparian | Plantation | Grassland | TOTAL | RA (%) |
|-----------|--------------------------------------------------|--------|----------|------------|-----------|-------|--------|
| Araneidae | <i>Neoscona punctigera</i> (Doleschall, 1857)^   | 1      | 2        | 1          | 2         | 6     | 2.17   |
|           | <i>Neoscona aldinei</i> (Barrion-Dupo, 2008)*^   | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Neoscona vigilans</i> (Blackwall, 1865)^      | 2      | 1        | 0          | 0         | 3     | 1.09   |
|           | <i>Nephila pilipes</i> (Fabricius, 1793)         | 3      | 0        | 0          | 0         | 3     | 1.09   |
|           | <i>Paravixia dehaani</i> (Doleschall, 1859)^     | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Paravixia</i> sp.                             | 2      | 0        | 1          | 1         | 4     | 1.45   |
|           | <i>Poltys illepidus</i> (C. L. koch, 1843)^      | 0      | 2        | 1          | 0         | 3     | 1.09   |
|           | <i>Poltys elevatus</i> (Thorell, 1890)           | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Singa nitidula</i> (C. L. koch, 1844)         | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Singa perpolita</i> (Thorell, 1892)           | 0      | 1        | 0          | 0         | 1     | 0.36   |
|           | <i>Talithybia depressa</i> (Thorell, 1898)       | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Thelacantha brevispina</i> (Doleschall, 1857) | 1      | 1        | 0          | 0         | 2     | 0.72   |
|           | <i>Neoscona punctigera</i> (Doleschall, 1857)^   | 1      | 2        | 1          | 2         | 6     | 2.17   |
|           | <i>Neoscona aldinei</i> (Barrion-Dupo, 2008)*^   | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Neoscona vigilans</i> (Blackwall, 1865)^      | 2      | 1        | 0          | 0         | 3     | 1.09   |
|           | <i>Nephila pilipes</i> (Fabricius, 1793)         | 3      | 0        | 0          | 0         | 3     | 1.09   |
|           | <i>Paravixia dehaani</i> (Doleschall, 1859)^     | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Paravixia</i> sp.                             | 2      | 0        | 1          | 1         | 4     | 1.45   |
|           | <i>Poltys illepidus</i> (C. L. koch, 1843)^      | 0      | 2        | 1          | 0         | 3     | 1.09   |
|           | <i>Poltys elevatus</i> (Thorell, 1890)           | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Singa nitidula</i> (C. L. koch, 1844)         | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Singa perpolita</i> (Thorell, 1892)           | 0      | 1        | 0          | 0         | 1     | 0.36   |
|           | <i>Talithybia depressa</i> (Thorell, 1898)       | 1      | 0        | 0          | 0         | 1     | 0.36   |
|           | <i>Thelacantha brevispina</i> (Doleschall, 1857) | 1      | 1        | 0          | 0         | 2     | 0.72   |

**Table 2.** Species diversity and abundance of spiders on different habitats in Mt. Lantoy, Argao, Cebu, Philippines. Species with \* Philippine endemic, ^ Threatened species. (continue)

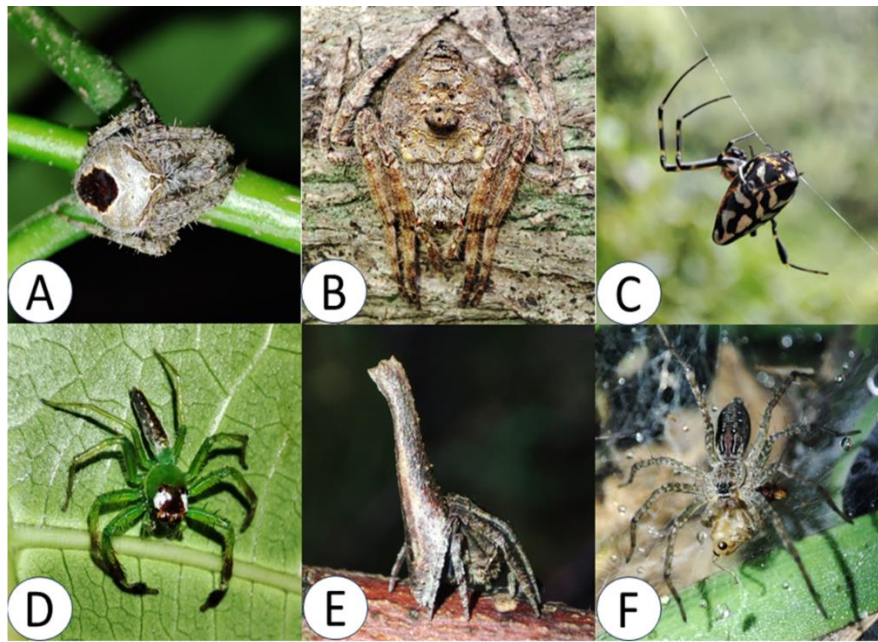
| Family          | Scientific name                                                 | Forest | Riparian | Plantation | Grassland | TOTAL | RA (%) |
|-----------------|-----------------------------------------------------------------|--------|----------|------------|-----------|-------|--------|
| Cheiracanthidae | <i>Cheiracanthium approximatum</i> (O. Pickard-Cambridge, 1885) | 0      | 2        | 0          | 0         | 2     | 0.72   |
| Clubionidae     | <i>Nusatidia luzonica</i> (Simon, 1897)*                        | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                 | <i>Clubiona</i> sp.                                             | 0      | 1        | 0          | 0         | 1     | 0.36   |
| Corinnidae      | <i>Corinnomma severum</i> (Thorell, 1877)                       | 0      | 1        | 0          | 1         | 2     | 0.72   |
| Hersiliidae     | <i>Hersilia</i> sp.                                             | 1      | 0        | 0          | 0         | 1     | 0.36   |
| Deinopidae      | <i>Asianopis</i> sp.                                            | 1      | 0        | 0          | 0         | 1     | 0.36   |
| Liocranidae     | <i>Oedignatha scrobiculata</i> (Thorell, 1881)                  | 0      | 0        | 1          | 1         | 2     | 0.72   |
| Lycosidae       | <i>Hippasa holmerae</i> (Thorell, 1895)                         | 0      | 0        | 0          | 5         | 5     | 1.81   |
|                 | <i>Pardosa</i> sp.                                              | 0      | 0        | 1          | 1         | 2     | 0.72   |
| Oxyopidae       | <i>Oxyopes javanus</i> (Thorell, 1887)                          | 1      | 1        | 0          | 0         | 2     | 0.72   |
|                 | <i>Oxyopes macilentus</i> (L.Koch, 1878)                        | 2      | 1        | 0          | 0         | 3     | 1.09   |
| Pisauridae      | <i>Pisaurina</i> sp.                                            | 0      | 2        | 0          | 0         | 2     | 0.72   |
| Psecruidae      | <i>Psecrurus</i> sp.                                            | 1      | 0        | 0          | 0         | 1     | 0.36   |
| Salticidae      | <i>Carrhotus</i> sp.                                            | 0      | 2        | 0          | 0         | 2     | 0.72   |
|                 | <i>Cosmophasis</i> sp.                                          | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                 | <i>Cyrtaea</i> sp.                                              | 1      | 2        | 1          | 1         | 5     | 1.81   |
|                 | <i>Epeus</i> sp.                                                | 0      | 1        | 0          | 0         | 1     | 0.36   |
|                 | <i>Hamatilaeva</i> sp.                                          | 0      | 1        | 0          | 1         | 2     | 0.72   |
|                 | <i>Lepidemathis sericea</i> (Simon 1899)*                       | 3      | 1        | 0          | 1         | 5     | 1.81   |
|                 | <i>Myrmarachne assimilis</i> (Banks 1930)                       | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                 | <i>Myrmarachne cf. markaha</i> (Barrion & Litsinger 1995)       | 2      | 0        | 0          | 0         | 2     | 0.72   |
|                 | <i>Myrmarachne cf. biseratensis</i> (Badcock, 1918)             | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                 | <i>Nannenus</i> sp.                                             | 3      | 3        | 0          | 1         | 7     | 2.54   |

**Table 2.** Species diversity and abundance of spiders on different habitats in Mt. Lantoy, Argao, Cebu, Philippines. Species with \* Philippine endemic, ^ Threatened species. (continue)

| Family         | Scientific name                                                      | Forest | Riparian | Plantation | Grassland | TOTAL | RA (%) |
|----------------|----------------------------------------------------------------------|--------|----------|------------|-----------|-------|--------|
| Salticidae     | <i>Parabathippus shelfordi</i> (G. W. Peckham & E. G. Peckham, 1907) | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                | <i>Phintella piatensis</i> (Barrion & Litsinger, 1995)*              | 2      | 4        | 1          | 2         | 9     | 3.26   |
|                | <i>Phyrnerachne</i> sp.                                              | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                | <i>Portia labiata</i> (Thorell, 1887)                                | 3      | 2        | 0          | 2         | 7     | 2.54   |
|                | <i>Pystira</i> sp.                                                   | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                | <i>Rhene flavigera</i> (C.L. Koch, 1846)                             | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                | <i>Telamonia festiva</i> Thorell, 1887                               | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                | <i>Thiana bhamoensis</i> (Thorell, 1887)                             | 0      | 1        | 0          | 0         | 1     | 0.36   |
|                | <i>Siler semiglaucus</i> (Simon, 1901)                               | 0      | 1        | 0          | 0         | 1     | 0.36   |
| Scytodidae     | <i>Scytodes</i> sp.                                                  | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                | <i>Scytodes pallida</i> (Dolleschall, 1859)                          | 4      | 2        | 0          | 0         | 6     | 2.17   |
| Sparacidae     | <i>Heteropoda cf. venatoria</i> (Linnaeus, 1767)                     | 2      | 4        | 1          | 2         | 9     | 3.26   |
|                | <i>Heteropoda</i> sp.1                                               | 2      | 1        | 0          | 0         | 3     | 1.09   |
|                | <i>Heteropoda</i> sp.2                                               | 1      | 1        | 0          | 0         | 2     | 0.72   |
|                | <i>Olios</i> sp.                                                     | 1      | 0        | 0          | 0         | 1     | 0.36   |
| Tetragnathidae | <i>Leucage fastiga</i> (Simon, 1905)                                 | 2      | 2        | 0          | 1         | 5     | 1.81   |
|                | <i>Leucauge argentina</i> (Hasselt, 1882)                            | 2      | 0        | 0          | 0         | 2     | 0.72   |
|                | <i>Leucauge decorata</i> (White, 1841)                               | 1      | 1        | 0          | 0         | 2     | 0.72   |
|                | <i>Tetragnatha cf. ceylonica</i> (O.P.-Cambridge 1869)               | 0      | 9        | 0          | 7         | 16    | 5.80   |
|                | <i>Tetragnatha</i> sp.                                               | 0      | 1        | 0          | 1         | 2     | 0.72   |
|                | <i>Tylorida striata</i> (Thorell, 1877)                              | 2      | 5        | 1          | 0         | 8     | 2.90   |
| Theraphosidae  | <i>Orphnaeus</i> sp.                                                 | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                | <i>Phlogiellus baeri</i> (Simon, 1877)**^                            | 1      | 0        | 0          | 0         | 1     | 0.36   |

**Table 2.** Species diversity and abundance of spiders on different habitats in Mt. Lantoy, Argao, Cebu, Philippines. Species with \* Philippine endemic, ^ Threatened species. (continue)

| Family                   | Scientific name                                            | Forest | Riparian | Plantation | Grassland | TOTAL | RA (%) |
|--------------------------|------------------------------------------------------------|--------|----------|------------|-----------|-------|--------|
| Theridiidae              | <i>Parasteatoda</i> sp.1                                   | 0      | 1        | 0          | 2         | 3     | 1.09   |
|                          | <i>Parasteatoda mundula</i> (L. Koch, 1872)                | 2      | 1        | 0          | 0         | 3     | 1.09   |
|                          | <i>Argyrodes antipodianus</i> (O. Pickard-Cambridge, 1880) | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Chrysso</i> sp.                                         | 1      | 8        | 2          | 3         | 14    | 5.07   |
|                          | <i>Chrysso tiboli</i> (Barrión & Litsinger, 1995)*         | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Chrysso trimaculata</i> (Zhu, Zhang & Xu, 1991)         | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Janula triangularis</i> (Yoshida & Koh, 2011)           | 3      | 0        | 0          | 0         | 3     | 1.09   |
|                          | <i>Meotipa</i> sp.                                         | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Phycosoma</i> sp.                                       | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Rhomphaea</i> sp.                                       | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          |                                                            |        |          |            |           |       |        |
| Thomisidae               | <i>Camaricus formosus</i> (Thorell, 1887)                  | 0      | 1        | 0          | 0         | 1     | 0.36   |
|                          | <i>Phrynarachne</i> cf. <i>rugosa</i> (Lareille, 1804)     | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Strigoplus</i> sp.                                      | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Thomisus</i> sp.1                                       | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Thomisus</i> sp.2                                       | 0      | 2        | 0          | 0         | 2     | 0.72   |
|                          | <i>Tmarus</i> sp.                                          | 0      | 1        | 0          | 0         | 1     | 0.36   |
|                          | <i>Xysticus</i> cf. <i>cristatus</i> (Clerck, 1757)        | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Uloborus</i> sp.                                        | 1      | 1        | 0          | 0         | 2     | 0.72   |
|                          | <i>Zosis geniculata</i> (Oliver, 1789)                     | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          | <i>Mallinella</i> sp.                                      | 1      | 0        | 0          | 0         | 1     | 0.36   |
|                          |                                                            |        |          |            |           |       |        |
| Total no. of Individuals |                                                            | 114    | 98       | 18         | 46        | 276   |        |
| Total no. of Species     |                                                            | 77     | 52       | 16         | 25        |       |        |
| Total no. of Families    |                                                            | 15     | 13       | 7          | 8         |       |        |



**Figure 2.** Some of the spider recorded (A) *Neoscona* sp., (B) *Talithybia depressa*. (C) *Leucage fastiga*, (D) *Epeuss p.*, (E) *Poltys elevates*, (F) *Hippasa holmera*.

### 3.2 Biodiversity indices and rarefaction analysis

Table 3 shows clear differences in spider diversity among habitats in Mt. Lantoy. Forest habitat had the highest number of individuals (114) and species (77), and the highest Shannon diversity value ( $H' = 4.23$ ). Riparian habitat ranked second (98 individuals, 52 species;  $H' = 3.67$ ), while grassland (46 individuals, 25 species;  $H' = 2.96$ ) and plantation (18 individuals, 16 species;  $H' = 2.74$ ) supported comparatively poorer assemblages. Evenness values were consistently high (0.92–0.97), indicating a relatively uniform distribution of individuals among species. The plantation habitats showed the most even distribution of species richness to abundance, but had the lowest values of richness and abundance, suggesting that no species dominated these areas, while the grassland and riparian habitats showed slightly lower evenness. Shannon-Wiener ( $H'$ ) and Pielou's evenness ( $J'$ ) were computed using the natural-logarithm formulation  $H' = -\sum(p_i \ln p_i)$  and  $J' = H' / \ln(S)$  values were independently re-checked against the raw counts in Table 1 to confirm accuracy. The findings in the biodiversity indices suggest that habitat complexity is a major driver of spider community structure in Mt. Lantoy. Forest habitat provides multilayered vegetation, shaded microclimates, and a wider range of attachment sites for web construction, retreats, and hunting substrates, all of which can support more species and greater abundance. The riparian habitat also maintained relatively high diversity, likely because moisture, edge vegetation, and insect activity near water create favorable foraging conditions for spiders. In contrast, the structurally simplified plantation and grassland habitats appear to offer fewer niches and less stable microhabitats, which likely limited both species richness and total abundance.

**Table 3.** Biodiversity indices of spider assemblages by habitat

| Habitat    | Individuals | Species richness | Family richness | Shannon index ( $H'$ ) | Simpson index | Pielou's evenness ( $J'$ ) |
|------------|-------------|------------------|-----------------|------------------------|---------------|----------------------------|
| Forest     | 114         | 77               | 15              | 4.23                   | 0.98          | 0.97                       |
| Riparian   | 98          | 52               | 13              | 3.67                   | 0.97          | 0.93                       |
| Plantation | 18          | 16               | 7               | 2.74                   | 0.93          | 0.99                       |
| Grassland  | 46          | 25               | 8               | 2.96                   | 0.93          | 0.92                       |



The unusually high evenness in the Forest habitat ( $J' = 0.97$  with  $S = 77$  species) warrants cautious interpretation (Table 3). Such near-maximal evenness is uncommon in tropical arthropod assemblages, where rank-abundance distributions are typically log-normal or log-series with several dominant species. Evenness values in Table 3 were unusually high across habitats. A large proportion of the recorded species were represented by only one or two individuals (Table 4). In the forest, 67 of 77 species (87.01%) were singletons or doubletons. Riparian showed a similar pattern, with 44 of 52 species (84.62%) represented by only one or two individuals. Grassland also had a high rarity proportion, with 21 of 25 species (84.00%) recorded as singletons or doubletons. Plantation showed the strongest pattern: all 16 recorded species (100%) were represented by only one or two individuals each. In this dataset, evenness was high not necessarily because the communities were ecologically balanced, but because many species were recorded at very low abundance. Plantation had the highest evenness value because only 1 or 2 individuals represented each plantation species. Thus, the high evenness in the plantation reflects sparse detection rather than strong community stability or high habitat quality. In all habitats, at least 84% of species were represented by only one or two individuals, and in the plantation, all recorded species were singletons or doubletons. This indicates that the assemblage contained many rare records and that sampling likely captured only part of the local spider community. The high proportion of rare species suggests that additional sampling may reveal more individuals of currently rare taxa and could change estimates of dominance and evenness. Therefore, the diversity results should be interpreted as a standardized inventory across surveyed plots rather than as a complete estimate of Mt. Lantoy's spider fauna.

**Table 4.** Singleton and doubleton summary by habitat

| Habitat    | Singletons | Doubletons | Singleton + doubleton species | Total species | Singleton + doubleton (%) |
|------------|------------|------------|-------------------------------|---------------|---------------------------|
| Forest     | 52         | 15         | 67                            | 77            | 87.01                     |
| Riparian   | 32         | 12         | 44                            | 52            | 84.62                     |
| Plantation | 14         | 2          | 16                            | 16            | 100                       |
| Grassland  | 16         | 5          | 21                            | 25            | 84                        |

Note: Across habitats, 84.0–100.0% of species were represented by only one or two individuals, explaining why evenness values were high despite low abundance in some habitats.

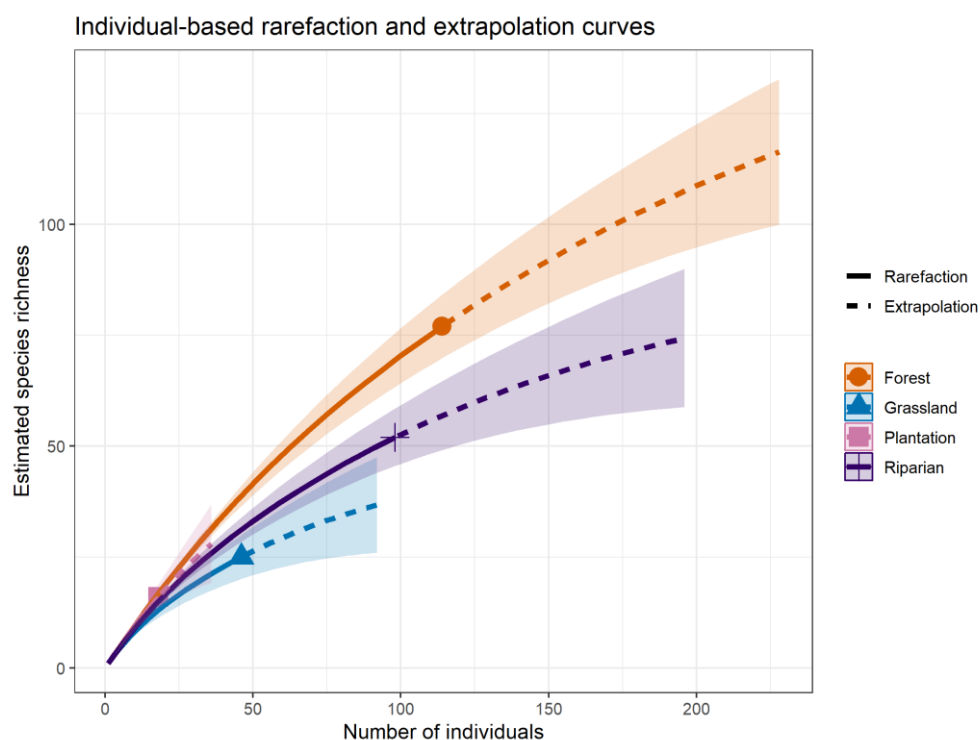
Table 5 and Figure 3 provide a standardized comparison of species richness across habitats, accounting for differences in the number of individuals recorded. This is necessary because raw species richness is strongly influenced by sample size and total abundance. In the observed data, the forest had 114 individuals and 77 species, while the plantation had only 18 individuals and 16 species. Directly comparing raw richness alone would therefore favor habitats where more individuals were recorded. Rarefaction addresses this problem by comparing habitats at a common sample size. Rarefied richness was compared at 18 individuals, which corresponds to the smallest habitat sample size recorded in the plantation. At this standardized sample size, forest still had the highest estimated richness ( $qD = 16.84$ ; 95% CI = 16.37–17.31), followed by plantation ( $qD = 16.00$ ; 95% CI = 12.89–19.11), riparian ( $qD = 14.99$ ; 95% CI = 13.93–16.05), and grassland ( $qD = 13.05$ ; 95% CI = 11.21–14.90). These estimates show that forests retained high spider richness even after standardization, supporting their importance as diverse spider habitats. However, the rarefaction results also show why caution is warranted in the interpretation. Plantation had a high rarefied richness estimate of 18 individuals, but also the widest confidence interval. This wide interval reflects uncertainty caused by its small sample size. Because the plantation itself had only 18 individuals, the estimate is based on very limited information and should not be interpreted as evidence that the plantation is as diverse as the forest. Instead, it means that the few individuals collected from plantations were spread across many species, consistent with the high evenness and 100% singleton/doubleton pattern shown in Tables 3 and 4. The rarefaction and extrapolation curves show that observed richness differences among habitats become smaller after standardization, and some confidence intervals overlap. This indicates that part of the observed richness

difference was influenced by unequal numbers of individuals recorded per habitat. Therefore, the safest interpretation is that forest and riparian habitats supported the greatest total abundance and observed richness, while rarefaction shows that standardized richness comparisons are more conservative and should not be overclaimed.

**Table 5.** Individual-based rarefaction estimates at standardized sample size

| Habitat    | Observed individuals | Observed species richness | Standardized sample size (m) | Estimated richness qD | 95% LCL | 95% UCL |
|------------|----------------------|---------------------------|------------------------------|-----------------------|---------|---------|
| Forest     | 114                  | 77                        | 18                           | 16.84                 | 16.37   | 17.31   |
| Riparian   | 98                   | 52                        | 18                           | 14.99                 | 13.93   | 16.05   |
| Plantation | 18                   | 16                        | 18                           | 16                    | 12.89   | 19.11   |
| Grassland  | 46                   | 25                        | 18                           | 13.05                 | 11.21   | 14.9    |

Note: Individual-based rarefaction was conducted at  $m = 18$  individuals, corresponding to the smallest habitat sample size. qD represents Hill number diversity at  $q = 0$ , equivalent to species richness. LCL and UCL denote the lower and upper 95% confidence limits, respectively.



**Figure 3.** Individual-based rarefaction and extrapolation curves for spider species richness across forest, riparian, plantation, and grassland habitats in Mt. Lantoy. Curves were generated using Hill number  $q = 0$ , corresponding to species richness; shaded bands represent 95% confidence intervals.

### 3.3 Habitat-level comparison of abundance and species richness

Table 6 shows the assumption tests used to determine whether one-way ANOVA was appropriate for comparing spider abundance and species richness among habitats. This table is important because count-based ecological data can sometimes violate ANOVA assumptions, particularly those of normality and homogeneity of variance. Therefore, assumption testing was necessary before retaining the ANOVA and Tukey HSD framework. For abundance, the Shapiro-Wilk test for normality of residuals produced  $W = 0.959$  and  $p = 0.527$ . Since the  $p$ -value was greater than 0.05, there was no significant evidence that the residuals departed from normality. Levene's test for homogeneity of variance produced a statistic of 1.402 and  $p = 0.279$ , also greater than 0.05. This indicates that the abundance data did not show significant heterogeneity of

variance among habitats. For species richness, the Shapiro-Wilk test likewise indicated acceptable residual normality, with  $W = 0.971$  and  $p = 0.774$ . Levene's test also supported homogeneity of variance, with a statistic of 1.300 and a  $p$ -value of 0.309. Since both abundance and species richness passed the normality and variance tests at  $\alpha = 0.05$ , one-way ANOVA was retained for the habitat-level comparison, followed by Tukey's HSD for pairwise comparisons.

**Table 6.** Assumption tests for habitat-level comparisons

| Response variable | Assumption tested       | Test          | Statistic | p-value | Decision   |
|-------------------|-------------------------|---------------|-----------|---------|------------|
| Abundance         | Normality of residuals  | Shapiro-Wilk  | 0.959     | 0.527   | Acceptable |
|                   | Homogeneity of variance | Levene's test | 1.402     | 0.279   | Acceptable |
| Species richness  | Normality of residuals  | Shapiro-Wilk  | 0.971     | 0.774   | Acceptable |
|                   | Homogeneity of variance | Levene's test | 1.3       | 0.309   | Acceptable |

Note: Assumptions were considered acceptable at  $\alpha = 0.05$ . Therefore, one-way ANOVA followed by Tukey's HSD was used for habitat-level comparisons.

Table 7 shows that habitat type significantly affected both spider abundance and species richness. This indicates that the four habitat types differed in their ability to support spider assemblages. Because the plot was used as the unit of replication, the analysis reflects variation among plots within habitats rather than a simple comparison of habitat totals. For spider abundance, the ANOVA was significant,  $F(3, 16) = 16.57$ ,  $p < 0.001$ . This indicates that plot-level spider abundance differed significantly among the four habitat types. For species richness, the ANOVA result was also significant,  $F(3, 16) = 20.47$ ,  $p < 0.001$ , indicating that plot-level richness varied significantly among habitats. Because the plot was used as the unit of replication, these results reflect variation among the five plots per habitat rather than simple comparisons of total habitat counts. The one-way ANOVA followed by Tukey's HSD mean comparison further demonstrates that habitat type strongly influenced spider abundance and species richness in Mt. Lantoy. Forest recorded the highest mean abundance (22.80) and mean species richness (21.20), indicating it is the most favorable habitat for spiders. Riparian habitat showed intermediate values of mean abundance (19.60) and mean species richness (16.40) and remained relatively close to the forest in terms of spider abundance, whereas plantation and grassland generally supported lower means. Post-hoc analysis using Tukey's HSD test for abundance and species richness showed that forest and riparian differed significantly from plantation and grassland. Overall, the analysis supports the interpretation that habitats with greater structural heterogeneity support richer, more abundant spider assemblages than those with reduced vegetation complexity.

**Table 7.** One-way ANOVA summary for spider abundance and species richness among habitats

| Response variable | Source   | df | SS     | MS     | F     | p-value |
|-------------------|----------|----|--------|--------|-------|---------|
| Abundance         | Habitat  | 3  | 1199.2 | 399.73 | 16.57 | <0.001  |
|                   | Residual | 16 | 386    | 24.13  | —     | —       |
| Species richness  | Habitat  | 3  | 968.8  | 322.93 | 20.47 | <0.001  |
|                   | Residual | 16 | 252.4  | 15.78  | —     | —       |

Note: df = degrees of freedom; SS = sum of squares; MS = mean square. Plot was used as the unit of replication ( $n = 5$  plots per habitat).

**Table 8.** Habitat-level abundance and species richness with Tukey HSD groupings

| Response variable | Habitat    | n plots | Mean $\pm$ SE    | Tukey group |
|-------------------|------------|---------|------------------|-------------|
| Abundance         | Forest     | 5       | 22.80 $\pm$ 2.40 | a           |
|                   | Riparian   | 5       | 19.60 $\pm$ 3.14 | a           |
|                   | Grassland  | 5       | 9.20 $\pm$ 1.77  | b           |
|                   | Plantation | 5       | 3.60 $\pm$ 0.75  | b           |
| Species richness  | Forest     | 5       | 21.20 $\pm$ 2.40 | a           |
|                   | Riparian   | 5       | 16.40 $\pm$ 2.20 | a           |
|                   | Grassland  | 5       | 7.60 $\pm$ 1.21  | b           |
|                   | Plantation | 5       | 3.60 $\pm$ 0.75  | b           |

Note: Means followed by the same letter are not significantly different at  $p \leq 0.05$  based on Tukey's HSD test.

### 3.4 Day and night differences in spider abundance and richness

The paired t-test indicates that both spider abundance and species richness were significantly higher during night sampling than during daytime sampling (Table 9). Mean abundance declined from 9.5 at night to 4.3 during the day, while mean species richness decreased from 8.85 to 4. In both comparisons, the p-values were below 0.05, confirming that the differences were statistically significant. This result is ecologically meaningful because many spiders are nocturnally active predators; cooler temperatures, higher humidity, and reduced disturbance at night may increase foraging activity and make more individuals detectable during sampling. This supports the importance of paired day-night sampling in spider biodiversity surveys because diurnal-only sampling would have underestimated both abundance and richness.

**Table 9.** Day and night comparison of spider abundance and species richness

| Response variable | Time period | n plot-time units | Total | Mean $\pm$ SE   | Test          | Statistic | p-value |
|-------------------|-------------|-------------------|-------|-----------------|---------------|-----------|---------|
| Abundance         | Day         | 20                | 86    | 4.30 $\pm$ 0.77 | Paired t-test | —         | —       |
|                   | Night       | 20                | 190   | 9.50 $\pm$ 1.39 | Paired t-test | t = 5.656 | <0.001  |
| Species richness  | Day         | 20                | 80    | 4.00 $\pm$ 0.68 | Paired t-test | —         | —       |
|                   | Night       | 20                | 177   | 8.85 $\pm$ 1.28 | Paired t-test | t = 5.685 | <0.001  |

Note: Paired comparisons were performed between day and night observations from the same plots. The test direction was night > Day.

### 3.5 Habitats

Four habitat types were identified in the study area, namely: forest, riparian, grassland, and plantation. Among these, the forest habitat had the highest spider abundance, followed by the riparian, plantation, and grassland habitats. The number of individuals recorded in the forest accounted for 41.3% of the total 276 individuals, nearly half of all individuals recorded across the four habitats, compared to riparian (35.51%), plantation (6.52%), and grassland (16.67%). The recorded spiders in the forest habitat are 114 individuals from 77 species under 15 families. Of the 101 spider species from 20 families recorded in Mt. Lantoy, 78 species from 15 families are present in the forest area. Araneidae is the most abundant family in the forest, with 29 species, and Cyclosa insulana and Scytodes pallida are the most abundant species in the area, with 4 individuals each recorded. The forest area has dense vegetation, including native species such as *Leea philippinensis*, *Cratoxylum sumatranum*, and *Erythrina variegata*. The structure of the forest habitat, with its diverse tree species, likely influenced spider abundance in the area. Spiders benefit from tree-species diversity because it promotes the development of leaf-litter layers, which in turn support a higher diversity of prey [27]. The rich plant diversity in the area contributes to the abundance of observed spider species. Web-building spiders, in particular, are closely tied to vegetation structure, as the parameters required to construct webs depend on the habitat's physical characteristics [28]. The dense forest canopy influences the types of spiders that thrive there. Similarly, the study by Deeleman-Reinhold [29] on Southeast Asian forest spiders supports the observation that mountainous areas exhibit greater spider species richness compared to lowland areas.

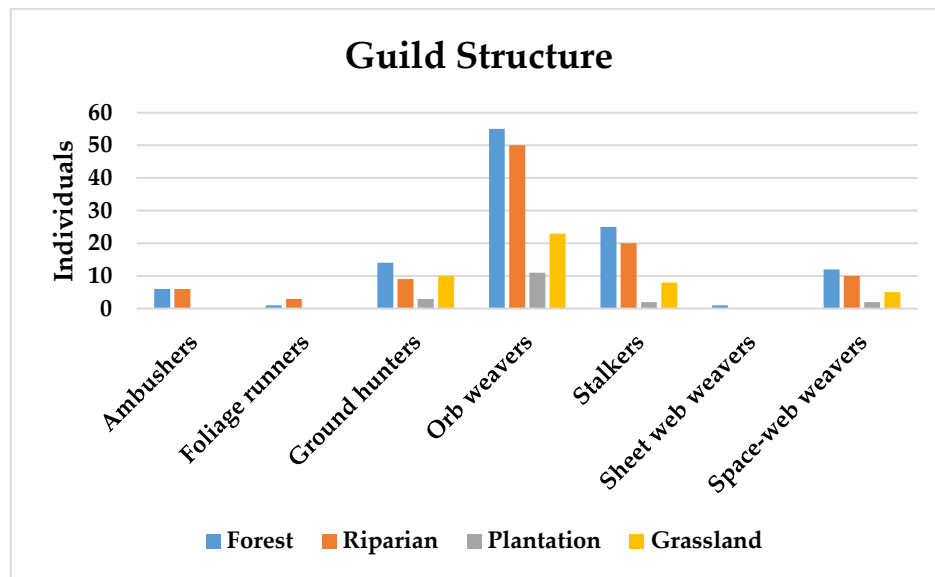
Riparian habitat ranked second in both abundance and species richness, with 98 individuals under 52 species representing 13 families. The five plots were established near the river, and some were also adjacent to cultivated areas, creating a transitional habitat influenced by both natural and managed vegetation. This setting may help explain the relatively high spider abundance. Riparian environments commonly support dense insect populations because water, moisture, and riparian vegetation favor many aquatic-emergent and terrestrial prey taxa. As predominantly insectivorous predators, spiders are expected to respond positively to this prey base. At the same time, the proximity of some plots to agricultural areas may have contributed to a mixed assemblage by combining semi-natural habitat features with open foraging spaces. This agrees with the view that riparian habitat supports intermediate levels of spider abundance, likely due to the high availability of aquatic-derived prey, as emerging aquatic insects serve as a vital energy subsidy for riparian predators [30,31]. Grassland and plantation habitats supported comparatively poorer spider communities than forest and riparian sites. The grassland contained 46 individuals from 25 species across eight families, with Araneidae again being the most represented family and (*Tetragnatha cf. ceylonica*) the most abundant species. Although grasses and shrubs such as *Chromolaena odorata* and *Imperata cylindrica* provide some support for web-building spiders, this habitat remains more exposed and structurally less diverse than the forest. The plantation had the lowest overall values, with only 18 individuals representing 16 species across 7 families. This reduced diversity is likely related to the dominance of mahogany stands, which create a more uniform habitat structure, as well as to the absence of saplings and seedlings of other native plants in the understory and the lack of insects that serve as food for spiders. Monoculture and low plant heterogeneity generally reduce the variety of microhabitats, prey niches, and web-attachment sites available to spiders. The marked decline in spider diversity observed in the plantation is therefore consistent with broader evidence that converting natural vegetation to simplified monoculture systems tends to reduce arthropod richness and biomass [2, 7].

### 3.6 Guild Structure

**Table 10.** Guild structure of spider family.

| Guild             | Family           |
|-------------------|------------------|
| Ambushers         | Deinopidae       |
|                   | Hersiliidae      |
|                   | Pisauridae       |
|                   | Thomisidae       |
| Foliage runners   | Cheiracanthiidae |
|                   | Clubionidae      |
| Ground hunters    | Lycosidae        |
|                   | Corinnidae       |
|                   | Liocranidae      |
|                   | Scytodidae       |
|                   | Sparacidae       |
|                   | Theraposidae     |
|                   | Zodariidae       |
| Orb weavers       | Araneidae        |
|                   | Tetragnathidae   |
|                   | Uloboridae       |
| Stalkers          | Oxyopidae        |
|                   | Salticidae       |
|                   | Psechridae       |
| Sheet web weavers | Theridiidae      |
| Space-web weavers |                  |





**Figure 4.** Guild structure of spider assemblages across the four habitats (Forest, Riparian, Plantation, Grassland) in Mt. Lantoy, Cebu, Philippines.

The guild structure analysis revealed distinct patterns in spider functional groups across the different habitats of Mt. Lantoy. The composition of spider guilds reflects the influence of habitat structure and vegetation complexity on community organization. Spiders were assigned to functional guilds following the classification system of Cardoso et al. [24] and Uetz et al. [32], which groups species by foraging strategy and prey-capture behavior rather than taxonomy. Family- and genus-level assignments followed the World Spider Catalog and primary literature on Philippine and Southeast Asian araneofauna; in the few cases where a genus included species spanning more than one guild, assignment was made at the species level based on documented web architecture or hunting mode. In this study, seven functional guilds were identified: Ambushers (Deinopidae, Hersilidae, Pisauridae, Thomisidae), Foliage runners (Cheiracanthidae, Clubionidae), Ground hunters (Lycosidae, Corinnidae, Liocranidae, Scytodidae, Sparacidae, Theraposidae, Zodariidae), Orb weavers (Araneidae, Tetragnathidae, Uloboridae), Orb wavers (Araneidae, Tetragnathidae, Uloboridae), Stalkers (Oxyopidae, Salticidae), Sheet web weavers (Psechridae), and Space-web weavers (Theridiidae). The most dominant guild was the Orb Weavers (50.36%), comprising members of the families Araneidae, Uloboridae, and Tetragnathidae. These spiders construct circular webs and are typically found in habitats with abundant vertical structures, such as forested areas. The high proportion of orb weavers suggests that structurally complex habitats, such as forests and riparian zones, provide optimal conditions for web construction and prey capture. The second most abundant guild was the Stalkers (19.93%), represented by families such as Oxyopidae and Salticidae. Other guilds, including Ambushers, Foliage runners, and Sheet web weavers, cannot be found in the plantation and grassland habitats. The similarity in guild composition across habitats highlights the role of vegetation layout in shaping spider communities [32]. Structurally complex habitats provide diverse web-building opportunities and foraging grounds, promoting functional diversity and ecosystem stability.

### 3.7 Discussion

Spider assemblages in Mt. Lantoy showed clear habitat-related differences in abundance, richness, and functional composition. Forest and riparian habitats supported the highest observed abundance and species richness, while plantation and grassland habitats supported poorer assemblages. This pattern suggests that structurally complex habitats provide more favorable conditions for spiders. Forest had the highest observed richness and diversity, reflecting the importance of multilayered vegetation and microhabitat heterogeneity. Riparian habitat also supported high abundance and richness, likely because moisture, riparian vegetation, and aquatic-emergent insects can increase prey availability for arthropod predators [2, 4, 7, 30, 31].

In contrast, plantation and grassland habitats were structurally simpler, which may have reduced the availability of web-building sites, litter-layer refuges, and prey diversity. Forest recorded the highest Shannon and Simpson diversity values, while the plantation had the highest Pielou's evenness despite having the lowest abundance and richness. The high proportion of rare species also suggests that additional sampling may reveal more individuals of currently rare taxa and may change estimates of dominance and evenness. Rarefaction analysis further showed that raw species richness should not be interpreted alone. At the standardized sample size of 18 individuals, forest retained the highest rarefied richness estimate, but differences among habitats became smaller, and some confidence intervals overlapped, especially for the plantation. This indicates that observed richness patterns were partly influenced by unequal numbers of individuals recorded among habitats. Therefore, the strongest interpretation is that forest and riparian habitats supported the greatest observed abundance and richness, while rarefaction provides a more conservative comparison of richness after accounting for sample-size differences [22, 23]. The inferential tests confirmed that habitat type significantly affected both plot-level abundance and species richness. Assumption tests supported the use of one-way ANOVA, and Tukey's HSD showed that forest and riparian formed the higher group, while plantation and grassland formed the lower group. Importantly, forests and riparian areas did not differ significantly. Day-and-night sampling also revealed a strong detection pattern. Night sampling recorded substantially higher abundance and richness than daytime sampling, indicating that daytime-only surveys would underestimate the local spider assemblage. This is likely because many spiders are more active or more visible at night while foraging, occupying webs, or moving through vegetation. Guild composition was dominated by orb weavers, emphasizing the role of vegetation structure in supporting web-building spiders [24, 32]. Overall, the results highlight the conservation value of structurally complex forest and riparian habitats and support the use of paired day-night sampling in future spider biodiversity assessments.

#### 4. Conclusions

This study revealed that spider diversity, abundance, and guild composition in Mt. Lantoy are strongly influenced by habitat type. Among the four habitats surveyed, the forest exhibited the highest species richness, abundance, and Shannon diversity index, followed by the riparian zone, grassland, and plantation. High evenness values across all habitats indicate a relatively balanced distribution of individuals among species. Guild analysis showed that orb weavers dominated the spider community, reflecting the importance of vertical structure for web-building species. The one-way ANOVA revealed significant differences in spider abundance and species richness among habitats. The forest habitat recorded the highest mean abundance and species richness, both significantly higher than those in the plantation and grassland habitats. This indicates that forest conditions characterized by structural complexity and diverse microhabitats are more favorable for spider communities, whereas simplified habitats, such as plantations, support fewer species. Results from the paired t-test showed that spider abundance and species richness were significantly higher during night sampling compared to day sampling. The findings emphasize the ecological value of forested habitats in maintaining high spider diversity and functional richness. In contrast, simplified habitats such as plantations and grasslands support fewer species and guilds, likely due to limited structural complexity. These results highlight the need to prioritize the conservation of forest and riparian ecosystems in Mt. Lantoy to sustain ecological balance and biodiversity.

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# Agronomic Productivity of Lowland Rice: Impacts of Tillage and Nutrient Management in Barili, Cebu

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**Abstract:** Lowland rice (*Oryza sativa* var. NSIC RC222) under conventional (M1) and minimum (M2) tillage systems utilizing various fertilizer treatments (T<sub>1</sub>-control (no fertilizer application); T<sub>2</sub> - Organic fertilizer at 2 t ha<sup>-1</sup> (combination of rice straw compost, chicken manure dried, and carbonized rice hull at 1:1:1 ratio); T<sub>3</sub> - Organic fertilizer (same as T<sub>2</sub>) + inorganic fertilizer at a rate of 60-30-30 kg/ha N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O; and T<sub>4</sub> - Inorganic fertilizer at the rate of 120-60-60 kg N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) was the subject of a split-plot study to evaluate the agronomic and yield performance of lowland rice under different tillage systems and nutrient management schemes. Results showed that Conventional Tillage and Minimum Tillage showed comparable effects on the growth and yield of lowland rice. Further, the application of integrated inorganic and organic fertilizers yielded significant results compared with treatments with no fertilizer application or organic fertilizer alone. However, comparable values were observed for T<sub>4</sub> (inorganic application). Thus, the study offers rice producers a workable plan by recommending the use of combined inorganic and organic fertilizers with Minimum Tillage for rice production to preserve soil health and quality.

**Keywords:** Rice; minimum tillage; integrated inorganic; organic fertilizers

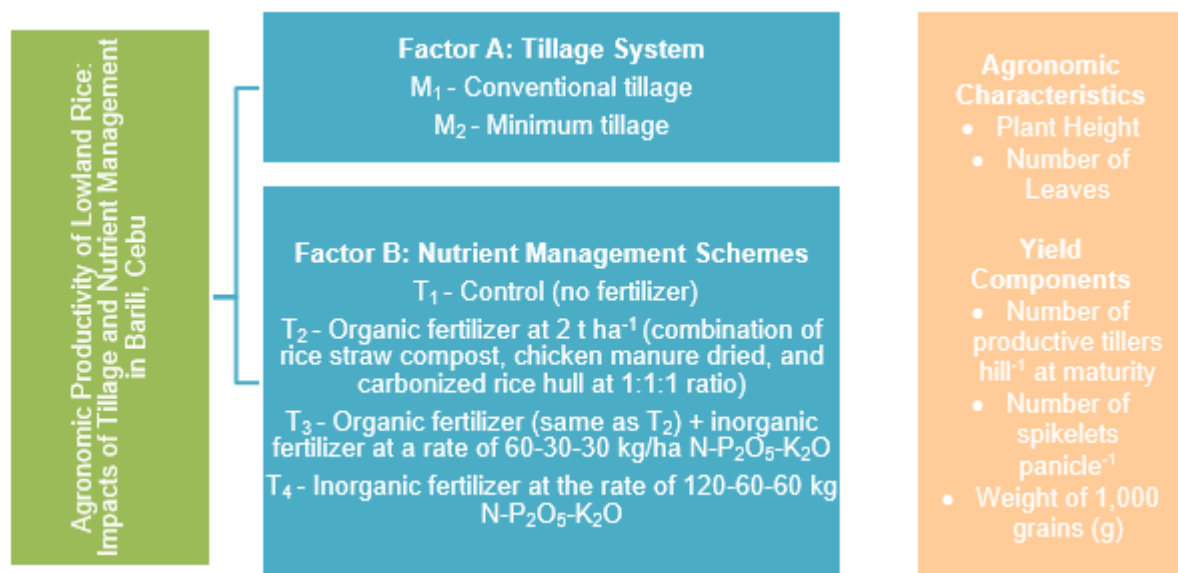
## 1. Introduction

*Oryza sativa* Linn. is the most important food crop of the developing world and the staple food of more than half of the world's population [1]. Its central role has prompted wide-ranging research spanning grain and aroma quality [2, 3], post-harvest drying and processing [4], value-added utilization of rice byproducts [5], and the adoption of information and communication technologies among rice growers [6]. For this reason, land preparation is crucial to ensure the rice field is ready for planting [7]. To prepare the land, there are two types of tillage: conventional and minimum tillage. Conventional tillage is an effective way to incorporate manure and break up sod fields [8, 9], thereby increasing porosity and loosening soil, allowing better root growth and nutrient uptake [10]. However, conventional tillage on smallholdings in some parts of Asia can be very labor-intensive and slow [7] because it is expensive, with high fuel consumption and labor requirements [11]. On the other hand, minimum tillage demonstrated apparent advantages over traditional methods. Minimum tillage can control soil erosion, reduce fuel, increase flexibility in planting and harvest, reduce labor requirements, improve water retention, reduce evaporation, and lower equipment requirements.



Furthermore, the availability of sufficient nutrients to meet the plant's needs is another factor that influences a crop's performance. Providing the soil with the nutrients that plants need for healthy growth and development is the primary objective of fertilizer application. These substances might be either organic or inorganic. Compared to natural organic fertilizers, inorganic fertilizers are more soluble in soil and have a higher nutrient content [12]. However, inorganic fertilizers are too expensive for small-scale farmers to buy [13]. Moreover, continuous and excessive application of inorganic fertilizer can cause detrimental effects to the soil [12, 13]. An organic approach, on the other hand, can maintain a healthy, biologically active soil with large reservoirs of plant nutrients [14]. Applying organic fertilizer to plants can increase organic matter content of the soil, thus improving and stabilizing soil structure, resulting in easy cultivation and improving water holding capacity, soil pH, bulk density, soil texture, soil aeration, porosity, as well as soil aggregation, especially when it is applied at the proper time [15, 16].

Achieving higher yields with a suitable quantity of affordable fertilizers at a low production cost is one of the ideal solutions small-scale farmers envision. Hence, this study evaluated the agronomic and yield performance of rice grown under conventional and minimum tillage, with different fertilizers (organic, inorganic, and a combination of organic and inorganic). Figure 1 shows the conceptual framework of the study.



**Figure 1.** Conceptual framework of the study

## 2. Materials and Methods

### 2.1 Land Preparation

A 530 m<sup>2</sup> space was set up using conventional and minimum-tillage systems. In M<sub>1</sub> (Conventional Tillage), plowing and harrowing were performed twice at weekly intervals. Right after harrowing, the field soils were manually leveled before transplanting. On the other hand, for M<sub>2</sub> (Minimum Tillage), plowing was done upon soaking the field for one (1) week. After plowing, the field was soaked with water for one (1) month and immediately harrowed thereafter. One week after the harrowing, the field was leveled before transplanting.

### 2.2 Experimental Design

The experimental area was arranged in a split-plot using a randomized complete block design (RCBD) with three (3) replications. The fertilizer treatments were the subplot (Factor B), while the tillage system was the main plot (Factor A). To make farm operations, management, and data collection easier, each treatment plot was measured at 4 x 5 meters, with 1m alleyways for replication and treatment plots. Each plot contained 25 rows of rice plants. A dike and irrigation/drainage canals encircled each treatment plot to prevent fertilizer contamination between plots. The different treatments were designated as follows:

## Factor A - Main Plot (Tillage System)

M<sub>1</sub> - Conventional tillageM<sub>2</sub> - Minimum tillage

## Factor B - Subplot (Nutrient Management Schemes)

T<sub>1</sub> - Control (no fertilizer application)T<sub>2</sub> - Organic fertilizer at 2 t ha<sup>-1</sup> (combination of rice straw compost, chicken manure dried, and carbonized rice hull at a 1:1:1 ratio)T<sub>3</sub> - Organic fertilizer (same as T<sub>2</sub>) + inorganic fertilizer at a rate of 60-30-30 kg/ha N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>OT<sub>4</sub> - Inorganic fertilizer at the rate of 120-60-60 kg N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O**2.3 Preparation and Application of Fertilizer**

A locally produced organic fertilizer was used, following the formulation of different mixtures of decomposed materials: rice straw, dried chicken manure, and carbonized rice hull, mixed in equal proportions (1:1:1 by weight). One week before transplanting, T<sub>2</sub> and T<sub>3</sub> received an application of organic fertilizers. T<sub>3</sub> and T<sub>4</sub> received half of the N and all of the P and K 12 days after transplantation. 35 days after transplanting, the second half of N in T<sub>3</sub> was administered. One-half (30 kg/ha N) of the remaining N in T<sub>4</sub> (60 kg/ha N) was administered 35 days after the transplant, and the other half (30 kg/ha N) was applied 55–60 days into the transplant (Table 1).

**Table 1.** Amount of fertilizer treatments applied per plot (kg plot<sup>-1</sup>).

| Treatments                                                                                                                                                                 | Amount of fertilizers (kg plot <sup>-1</sup> )                                                                                                                                                                                                  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| T <sub>1</sub> - (No fertilizer)                                                                                                                                           | 0                                                                                                                                                                                                                                               |
| T <sub>2</sub> - (2 t ha <sup>-1</sup> of organic fertilizer)                                                                                                              | 4 kgs of organic fertilizer plot <sup>-1</sup>                                                                                                                                                                                                  |
| T <sub>3</sub> - (2 t ha <sup>-1</sup> of organic fertilizer + inorganic<br>at a rate of 60-30-30 kg ha <sup>-1</sup> N, P <sub>2</sub> O <sub>5</sub> , K <sub>2</sub> O) | 4 kgs. of organic fertilizer plot <sup>-1</sup><br>1 <sup>st</sup> application of inorganic fertilizer (12 DAT):<br>429 g of complete fertilizer plot <sup>-1</sup><br>2 <sup>nd</sup> application (35 DAT): 130 g of urea plot <sup>-1</sup>   |
| T <sub>4</sub> - (120-60-60 kg ha <sup>-1</sup> N, P <sub>2</sub> O <sub>5</sub> , K <sub>2</sub> O<br>inorganic fertilizer)                                               | 1 <sup>st</sup> application (12 DAT): 857 g of complete<br>fertilizer plot <sup>-1</sup><br>2 <sup>nd</sup> application (35 DAT): 130 g of urea plot <sup>-1</sup><br>3 <sup>rd</sup> application (55-60 DAT): 130 g of urea plot <sup>-1</sup> |

**2.4 Seedbed Preparation and Transplanting**

One to two days before sowing, the seedbed was prepared. A typical wet bed measuring 2 x 5 m, with a drainage canal surrounding it, was constructed on separate sites to avoid obstacles during construction of the experimental plot. Rice seeds (NSIC Rc222) of approximately 2 kg were used. The seeds were incubated for 43 hours after being immersed in fresh water for 24 hours. The seedbed was planted with pre-germinated seeds. Until the seedlings were prepared for transplantation, proper care and weed control were used. One 14-day-old seedling was moved to each hill, spaced 20 cm apart. Five (5) days following seed emergence, missing hills were replanted.

**2.5 Pest Control**

Each treatment plot received the same hand weeding or rotary weeding treatment. The first weeding operation was done ten (10) days after transplanting. Twenty (20) days after the second weeding, a selective hand weeding was carried out. The succeeding weeding operations were done at two-week intervals or whenever necessary. From the tillering stage to two weeks before maturity, panyawan (*Tinospora rumphii* Boerl) was sprayed as a botanical insecticide to control insect infestation.

**2.6 Water Management**

During transplantation, the area was drained. Irrigation was applied three (3) days after transplanting at a depth of five (5) cm. Three (3) weeks after transplanting, no flooding of water was done. The irrigation water was kept at a level sufficient to maintain a wet or muddy soil condition until 50 days after transplanting.

Then flooding was repeated at a depth of 5 cm. Around the experimental area and in between replications, irrigation and drainage canals were constructed to supply water during dry spells and remove excess water as needed. A week before crop harvesting, the area was drained to make harvesting easier.

## 2.7 Harvesting and Processing

When 85% of the grains in the top portion of each treatment had ripened and turned yellow, and the panicle base had reached the hard dough stage, harvesting was done. The panicle bases from the harvestable area (13.44m<sup>2</sup>) were chopped with a sickle. Every sample plant was taken from the harvestable area, except for the two boundary rows on each side and the two hills at either end of each row. It was then winnowed, dried to around 14% moisture content, and threshed before all the necessary information was gathered.

## 2.8 Data Gathered

### 2.8.1 Agronomic Characteristics

1. Plant height (cm) - the height of the ten (10) sample plants in each treatment plot was measured from ground level to the tip of the tallest plant part before harvest.
2. Number of Leaves - this was obtained by counting the leaves of the tallest tiller per hill, which represent the whole-plant samples.

### 2.8.2 Yield Components

1. Number of productive tillers hill<sup>-1</sup> at maturity - ten (10) sample hills in each treatment plot were used to calculate the number of tillers hill<sup>-1</sup> that formed panicles at maturity.
2. Number of spikelets panicle<sup>-1</sup> - in each treatment plot, ten (10) sample panicles were counted to determine the number of filled and unfilled spikelets (panicle<sup>-1</sup>).
3. Weight of 1,000 grains (g) - 1,000 grains from the same sample plants used for the filled and unfilled spikelet count were weighed to determine this parameter.

## 2.9. Statistical Analysis

The Statistical Analysis System (SAS version 9.4 or later; SAS Institute Inc., Cary, NC, USA) was used to analyze the data statistically. Tukey's test was used to compare means.

## 3. Results and Discussion

### 3.1 Agronomic Characteristics

#### 3.1.1 Plant Height

Plant growth can be observed by an increase in length or height, stem diameter, tissue volume, cell count, fresh and dried weight, leaf area, leaf weight, and other features [17]. Plant height is a significant morphological parameter that is widely predictive of ultimate grain production and biomass [18] and may be used as an indicator of overall plant growth vigor and as a parameter to estimate characteristics [19]. As shown in Table 2, a significant difference in plant height was observed among rice plants treated with different fertilizer rates and types, regardless of the tillage system adopted. Results for T<sub>1</sub> and T<sub>2</sub> were comparable, while T<sub>3</sub> and T<sub>4</sub> showed no significant difference. Thus, applying both organic and inorganic fertilizers will likely yield the same crop yield as using only inorganic fertilizer. Accordingly, research has indicated that applying inorganic fertilizer in combination with organic fertilizer, known as Integrated Plant Nutrient Management, maintains soil fertility and balances nutrient supply, thereby increasing crop yield per unit area [20, 21]. Certain studies also suggest that careful use of both organic and inorganic resources can address soil fertility restrictions [22] and sustain agronomic productivity [23]. Furthermore, a study by Demissie et al. [10] in the Wolmera district of Central Ethiopia found that the combined application of organic and inorganic fertilizers significantly increased barley plant height. Similarly, in Adiyo and Ghimbo, Southwestern Ethiopia, Woldesenbet et al. [24] found that the combined application of organic and inorganic fertilizers significantly affected barley plant height.

**Table 2.** Analysis of Variance on the Average Plant Height of Rice grown under different Tillage Management Systems applied with different Fertilizers

| Treatment                                                                                                                                                          | Average Plant Height (cm) |         |         |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|---------|---------|
|                                                                                                                                                                    | 30 DAP                    | 50 DAP  | 70 DAP  |
| Factor A – Tillage System                                                                                                                                          |                           |         |         |
| M <sub>1</sub> – Conventional Tillage                                                                                                                              | 34.92 a                   | 57.50 a | 76.29 a |
| M <sub>2</sub> – Minimum Tillage                                                                                                                                   | 33.64 a                   | 56.17 a | 73.69 b |
| Factor B – Fertilizer Application                                                                                                                                  |                           |         |         |
| T <sub>1</sub> - control                                                                                                                                           | 29.17 c                   | 47.56 b | 64.67 b |
| T <sub>2</sub> - Organic fertilizer at 2 t ha <sup>-1</sup> (combination of rice straw compost, chicken manure dried, and carbonized rice hull at a 1:1:1 ratio)   | 32.50 b                   | 51.61 b | 67.91 b |
| T <sub>3</sub> - Organic fertilizer (same as T <sub>2</sub> ) + inorganic fertilizer at a rate of 60-30-30 kg/ha N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O | 37.61 a                   | 65.17 a | 84.56 a |
| T <sub>4</sub> - Inorganic fertilizer at the rate of 120-60-60 kg N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O                                                | 37.83 a                   | 63.00 a | 82.83 a |
| CV (%) (A)                                                                                                                                                         | 13.14                     | 15.40   | 3.64    |
| CV (%) (B)                                                                                                                                                         | 6.76                      | 6.27    | 4.14    |

Means with the same letter were not significantly different at 5 % level of the HSD test.

### 3.1.2 Number of Leaves

One of the visual essential phenotypic characteristics that characterizes a plant's growth and development is the quantity of leaves it possesses [25, 26]. It is connected to the plant's health and prospective yield and allows growth rate estimation [26, 27, 28]. This study showed that the average number of leaves was highest for the treatment applied with inorganic fertilizer (T<sub>4</sub>), though, statistically, it obtained a comparable result with the treatment applied with combined inorganic and organic fertilizers (T<sub>3</sub>) (Table 3). Additionally, the results showed no discernible difference in leaf number on rice plants grown under minimum (M<sub>2</sub>) or normal (M<sub>1</sub>) tillage systems.

**Table 3.** Analysis of Variance on the Average Number of Leaves of Rice grown under different Tillage Management Systems applied with different Fertilizers

| Treatment                                                                                                                                                          | Average Number of Leaves |          |          |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|----------|----------|
|                                                                                                                                                                    | 30 DAP                   | 50 DAP   | 70 DAP   |
| Factor A – Tillage System                                                                                                                                          |                          |          |          |
| M <sub>1</sub> – Conventional Tillage                                                                                                                              | 27.61 a                  | 82.17 a  | 101.89 a |
| M <sub>2</sub> – Minimum Tillage                                                                                                                                   | 27.17 a                  | 82.92 a  | 96.22 a  |
| Factor B – Fertilizer Application                                                                                                                                  |                          |          |          |
| T <sub>1</sub> - control                                                                                                                                           | 16.55 c                  | 49.78 b  | 54.11 b  |
| T <sub>2</sub> - Organic fertilizer at 2 t ha <sup>-1</sup> (combination of rice straw compost, chicken manure dried, and carbonized rice hull at a 1:1:1 ratio)   | 26.00 b                  | 60.05 b  | 67.16 b  |
| T <sub>3</sub> - Organic fertilizer (same as T <sub>2</sub> ) + inorganic fertilizer at a rate of 60-30-30 kg/ha N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O | 33.61 a                  | 109.67 a | 131.89 a |
| T <sub>4</sub> - Inorganic fertilizer at the rate of 120-60-60 kg N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O                                                | 33.39 a                  | 110.67 a | 143.06 a |
| CV (%) (A)                                                                                                                                                         | 29.23                    | 38.53    | 15.11    |
| CV (%) (B)                                                                                                                                                         | 10.59                    | 12.87    | 14.89    |

Means with the same letter were not significantly different at 5 % level of the HSD test.

### 3.2 Yield Components

#### 3.2.1 Number of Tillers

Initially appearing as a single culm seedling, rice rapidly produces primary, secondary, and tertiary tillers. Panicle and tiller numbers are the two main factors that determine rice plant design and thus affect grain yield [29]. Rice's tillering capacity is a varietal trait that mostly depends on shape and duration [30, 31]. To maximize rice yield [32] and achieve higher yields [18], tillering optimization is crucial. Additionally, optimal tillering enables synchronized flowering, maturity, and uniform panicle size [33]. In this study, the average number of tillers was highest in Conventional Tillage (M<sub>1</sub>) with the application of inorganic fertilizers (T<sub>4</sub>). The statistical analysis presented in Table 4 showed that the Minimum Tillage (M<sub>2</sub>) result was comparable to that for Conventional Tillage (M<sub>1</sub>). Likewise, the combined application of inorganic and organic fertilizers (T<sub>3</sub>) also showed no significant difference with the application of inorganic fertilizer alone (T<sub>4</sub>). To corroborate the results, Demissie et al. [10] noted that the combination of organic and inorganic fertilizers may have a synergistic effect on the number of effective tillers. Additionally, according to Arif et al. [34] and Silguera and Dollison [35], the increased nutrient availability and uptake due to the combined use of organic manure and inorganic fertilizers resulted in a higher number of fertile tillers, grains per panicle, panicles per hill, filled grains per panicle, 1000 grain weight, biological yield, grain yield, and harvest index.

**Table 4.** Analysis of Variance on the Average Number of Tillers of Rice grown under different Tillage Management Systems applied with different Fertilizers

| Treatment                                                                                                                                                          | Average Number of Tillers |         |         |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|---------|---------|
|                                                                                                                                                                    | 30 DAP                    | 50 DAP  | 70 DAP  |
| Factor A – Tillage System                                                                                                                                          |                           |         |         |
| M <sub>1</sub> – Conventional Tillage                                                                                                                              | 7.28 a                    | 20.25 a | 24.36 a |
| M <sub>2</sub> – Minimum Tillage                                                                                                                                   | 7.00 a                    | 21.25 a | 23.92 a |
| Factor B – Fertilizer Application                                                                                                                                  |                           |         |         |
| T <sub>1</sub> - control                                                                                                                                           | 3.83 c                    | 11.83 b | 13.28 b |
| T <sub>2</sub> - Organic fertilizer at 2 t ha <sup>-1</sup> (combination of rice straw compost, chicken manure dried, and carbonized rice hull at a 1:1:1 ratio)   | 6.72 b                    | 14.00 b | 15.95 b |
| T <sub>3</sub> - Organic fertilizer (same as T <sub>2</sub> ) + inorganic fertilizer at a rate of 60-30-30 kg/ha N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O | 9.11 a                    | 28.95 a | 31.00 a |
| T <sub>4</sub> - Inorganic fertilizer at the rate of 120-60-60 kg N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O                                                | 8.89 a                    | 28.22 a | 36.00 a |
| CV (%) (A)                                                                                                                                                         | 34.23                     | 43.47   | 16.85   |
| CV (%) (B)                                                                                                                                                         | 11.57                     | 14.81   | 17.98   |

Means with the same letter were not significantly different at 5 % level of the HSD test.

#### 3.2.2 Number of Filled and Unfilled Grains

The final step in cereal growth, known as grain filling, occurs when fertilized ovaries mature into caryopses. Canopy photosynthesis, assimilate conversion to biomass, and assimilate partitioning to grains are all interrelated processes that contribute to rice yield [29, 36]. The quantity of filled grains per panicle is closely correlated with crop output, and matured rice panicles contain both filled and unfilled grains [37]. Panicles with a high percentage of complete grains compared to unfilled grains are fundamentally indicative of a crop's high productivity or yield [38]. This study found that, regardless of tillage system and fertilizer application, the average number of filled grains was greater than the average number of unfilled grains (Table 5). However, significant differences were detected between tillage systems (Factor A) and fertilizer applications (Factor B). Conventional Tillage (M<sub>1</sub>) obtained higher values for the total number of filled and unfilled grains compared to Minimum Tillage (M<sub>2</sub>). Consequently, the highest values were also observed in the treatment that received both organic and inorganic fertilizers (T<sub>3</sub>), although the results were comparable to those of inorganic fertilizer alone (T<sub>4</sub>). This suggests that combining inorganic and organic fertilizers with Minimum Tillage could increase crop yield, particularly in rice, as shown by the grain yield (filled/unfilled) results of this study. This finding



was further supported by research conducted by Adane et al. [20] and Tadesse et al. [39], which found that the combined application of organic and mineral amendments led to improved barley yields by improving the soil's pH, available Phosphorus (P), total Nitrogen (N), and potentially other macronutrients and micronutrients.

**Table 5.** Analysis of Variance on the Average Number of Filled and Unfilled Grains of Rice grown under different Tillage Management Systems applied with different Fertilizers

| Treatment                                                                                                                                              | Number of Filled Grains | Number of Unfilled Grains | Total Number of Filled and Unfilled Grains |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|---------------------------|--------------------------------------------|
| Factor A – Tillage System                                                                                                                              |                         |                           |                                            |
| M1 – Conventional Tillage                                                                                                                              | 389.92 a                | 179.08 a                  | 569.00 a                                   |
| M2 – Minimum Tillage                                                                                                                                   | 371.92 b                | 100.33 b                  | 472.25 b                                   |
| Factor B – Fertilizer Application                                                                                                                      |                         |                           |                                            |
| T1 - control                                                                                                                                           | 277.50 b                | 107.33 b                  | 384.83 b                                   |
| T2 - Organic fertilizer at 2 t ha <sup>-1</sup> (combination of rice straw compost, chicken manure dried, and carbonized rice hull at a 1:1:1 ratio)   | 361.50 ab               | 97.33 b                   | 458.83 b                                   |
| T3 - Organic fertilizer (same as T <sub>2</sub> ) + inorganic fertilizer at a rate of 60-30-30 kg/ha N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O | 447.00 a                | 204.00 a                  | 651.00 a                                   |
| T4 - Inorganic fertilizer at the rate of 120-60-60 kg N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O                                                | 437.67 a                | 150.17 ab                 | 587.84 a                                   |
| CV (%) (A)                                                                                                                                             | 20.60                   | 37.88                     | 16.63                                      |
| CV (%) (B)                                                                                                                                             |                         |                           |                                            |

Means with the same letter were not significantly different at 5 % level of the HSD test.

### 3.2.3 Thousand Grain Weight (TGW)

One of the primary determinants of rice and other cereal crop yield production on a single plant is grain weight (thousand-grain weight) [40]. A vital component of agriculture, Thousand Grain Weight (TGW) significantly affects grain quality and crop productivity [41]. Farmers and researchers alike must understand TGW, as it provides important insights into crop productivity and performance. The findings of this study showed that all treatments did not differ significantly in Thousand Grain Weight (Table 6). However, a significant difference was observed between Conventional (M<sub>1</sub>) and Minimum (M<sub>2</sub>) tillage systems. A higher average value (24.83 g) was indicated in Conventional Tillage as compared to Minimum Tillage (22.67 g). The study by Khan et al. [42], which found a maximum mean value of 1000-grain weight, produced similar findings: conventional tillage yielded (363.7g); deep tillage yielded (362.56g); and minimal tillage yielded the lowest value (322.6g) with non-hybrid maize. Additionally, tillage was the most popular and effective technique for improving soil physical conditions, accounting for up to 20% of crop yields [43]. As soil fertility, tillage type, and climate can all influence crop development and yield, tillage can also alter soil structure and remove moisture.

**Table 6.** Analysis of Variance on the Average Weight of 1000 Grains (g) of rice under different Tillage Management Systems applied with different Fertilizers

| Treatment                                                                                                                                                          |  | Average Weight of 1000 Grains (g) |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|-----------------------------------|
| Factor A – Tillage System                                                                                                                                          |  |                                   |
| M <sub>1</sub> – Conventional Tillage                                                                                                                              |  | 24.83 a                           |
| M <sub>2</sub> – Minimum Tillage                                                                                                                                   |  | 22.67 b                           |
| Factor B – Fertilizers                                                                                                                                             |  |                                   |
| T <sub>1</sub> – control                                                                                                                                           |  | 23.83 a                           |
| T <sub>2</sub> - Organic fertilizer at 2 t ha <sup>-1</sup> (combination of rice straw compost, chicken manure dried, and carbonized rice hull at a 1:1:1 ratio)   |  | 22.50 a                           |
| T <sub>3</sub> - Organic fertilizer (same as T <sub>2</sub> ) + inorganic fertilizer at a rate of 60-30-30 kg/ha N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O |  | 24.50 a                           |
| T <sub>4</sub> - Inorganic fertilizer at the rate of 120-60-60 kg N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O                                                |  | 24.17 a                           |
| CV (%) (A)                                                                                                                                                         |  | 6.94                              |
| CV (%) (B)                                                                                                                                                         |  | 8.73                              |

Means with the same letter were not significantly different at 5 % level of the HSD test.

#### 4. Conclusions

The results of the study lead to several conclusions. Conventional Tillage and Minimum Tillage showed comparable effects on the growth and yield of lowland rice. The application of integrated inorganic and organic fertilizers yielded significant results compared with treatments that received no fertilizer or organic fertilizer alone, although comparable values were observed for T<sub>4</sub> (inorganic application). Accordingly, the study recommends applying integrated inorganic and organic fertilizers with Minimum Tillage for rice production to maintain soil health and quality. Because these findings are derived from a single location (Barili, Cebu) and a single cropping season, multi-season and multi-location validation is strongly recommended before broader application. Future studies should also include pre-trial baseline soil characterization and report actual grain yield (t ha<sup>-1</sup>) to substantiate the agronomic recommendations fully.

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# Predicting Fertility and Properties of Sugarcane Soil Series via Soil Color

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**Abstract:** Soil color, shaped by pedo-chemical reactions, is one of the most immediately perceptible soil properties, which often reflects the underlying parent material that influences specific soil characteristics. Thus, making it an essential soil physicochemical, biological, and fertility indicator for farmers. This study examined the relationship between soil color (value and chroma) and physicochemical and fertility parameters including Percent Organic Carbon (% OC); pH (H<sub>2</sub>O and CaCl<sub>2</sub>); Total Nitrogen (N); Available Phosphorus (P); exchangeables Potassium (K), Sodium (Na), Calcium (Ca), Magnesium (Mg), and Aluminum (Al); Exchangeable Iron (Fe), Cation Exchange Capacity (CEC), and Particle Size Distribution (% clay, silt, and sand) in soil samples collected from depths of 0–30 cm (topsoil) and 30–60 cm (subsoil) across different soil series (Guimbalaon, Isabel, Luisiana, San Manuel, and Silay) under sugarcane cultivation. The study showed significant results, indicating strong correlations between soil color and physico-chemical and fertility parameters at both depths. In the topsoil, % OC, and exchangeables Mg and K showed significant correlations with soil color value, while in the subsoil, significant correlations were found for pH (CaCl<sub>2</sub>), exchangeables Mg and K, and extractable Fe. Similarly, soil color chroma was significantly correlated in the topsoil with % OC, exchangeables Mg and Na, % clay, % sand, and pH (H<sub>2</sub>O); in the subsoil, significant correlations were observed for % OC, exchangeables Mg, Na, and Ca, total N, CEC, % clay, and % sand. These findings underscore the potential of soil color as a valuable indicator of soil physicochemical and fertility properties in sugarcane-cultivated soils.

**Keywords:** Soil color; fertility; physicochemical; sugarcane; correlation

## 1. Introduction

Soil Color is one of the most apparent and significant soil characteristics, serving as an indicator of certain physical and chemical properties and providing valuable clues about processes that are or have been operating in the soil [1]. It is consistently used as a definitive criterion in the identification, classification, and separation of soil [2,3], and is a key property in soil interpretation [4]. Because color is easily perceived in the field and is related to other soil properties, it has often been used as a rapid assessment indicator of drainage class, soil classification, and soil organic carbon, which affects the use of variable-rate fertilizer and pesticide applications [5,6]. In addition, the quantity of organic matter and the condition of iron influence soil color. Research indicates a strong correlation between soil color and properties like soil

organic carbon (SOC) content, iron content, soil water content, and texture [7]. Soils with dark surface horizons typically have a high organic matter content, making them fertile and conducive to plant growth. Furthermore, dark brown and black soils are believed to be rich in nitrogen, well-aerated and drained, and less prone to erosion. The studies by Konen et al. [6] and Schulze et al. [8] confirm a consistent relationship between SOC content and soil color. Likewise, in the study by Chen et al. [9], soil samples collected from areas within a field with varying soil brightness levels, determined from a color slide, predicted soil organic carbon with 98% accuracy. In 2003, Tanzanian farmers in the Maswa and Same districts, participating in the Soil-Water Management Research Group (SWMRG) project, relied on readily available local cues, such as soil color, worm presence, cracks, salt content, sand and gravel content, and drying behavior, to differentiate between fertile and infertile soils. Interestingly, these soil indicators used in Tanzania closely mirrored those employed by farmers in Benin, West Africa [10]. Thus, Soil Color is an important criterion for farmers, as it often reflects the soil's hidden parent material, which determines specific soil characteristics [11]. Recent bibliometric analyses also highlight that soil and plant health are central themes in sustainable agricultural systems research [12]. Recent work has further emphasized that soil fertility is a dynamic property closely tied to land-use practices and ecosystem diversification [13]. Soil-management interventions, such as the incorporation of crop residues and superabsorbent polymers, have likewise been shown to modify soil physical properties and influence crop growth and yield [14]. Hence, this study examined the potential of soil color for assessing physicochemical and fertility properties of soils.

## 2. Materials and Methods

### 2.1 Collection and Preparation of Soil Samples

The study collected soil samples from five (5) identified sugarcane-cultivated soil series, namely: Guimbalaon, Isabela, Luisiana, San Manuel, and Silay. In each soil series, five (5) sugarcane-cultivated farms were selected. In each farm, twenty (20) geo-coordinated sampling points were randomly selected. A soil sample from 0-30 cm (topsoil) and 30-60 cm (subsoil) depths was collected at each sampling point using an auger, then air-dried, composited, homogenized, sieved, and prepared for soil laboratory analysis.

### 2.2 Laboratory Analyses

Prepared soil samples were analyzed at the Central Analytical Services Laboratory, Visayas State University, Visca, Baybay City, Leyte, Philippines. Moisture content of the soil samples was measured as a quality-control parameter to ensure consistent air-drying before analysis; it was not included as a primary color-correlation variable, as its relationship to color is confounded by temporal variability and beyond the scope of this study. The following soil analyses were:

- Soil pH - measured potentiometrically in water using a solution ratio of 1:2.5 [15]. pH in  $\text{CaCl}_2$  was additionally determined potentiometrically using 0.01 M  $\text{CaCl}_2$  solution at a 1:2.5 soil: solution ratio.
- Total Nitrogen (N) - determined using the Kjeldahl Method according to Bremner and Mulvaney [16]. To prepare for analysis, a one-gram soil sample was sifted through a 0.425 mm sieve (corresponding to a #40 mesh screen) and transferred to a 30 mL digestion flask. One gram of selenium reagent mixture and 6 mL of concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) were added. The mixture was digested for 1.5 hours before being distilled using a Buchi distillation apparatus, during which 50 mL of 40% NaOH was added. The resulting distillates were then titrated with a standardized 0.05 N  $\text{H}_2\text{SO}_4$  solution.
- Percent Organic Carbon (C) - determined by the Walkley-Black method [17], which involved organic carbon oxidation with 1 M  $\text{K}_2\text{Cr}_2\text{O}_7$  in the presence of concentrated  $\text{H}_2\text{SO}_4$ . Titration was done with 0.5 M standard  $\text{FeSO}_4$ .
- Available Phosphorus (P) - extracted using 0.03 M  $\text{NH}_4\text{F}$  in 0.1 M HCl (Bray and Kurtz No. 2) and quantified using a spectrophotometer following the method of Murphy and Riley [18] for color development.
- To determine the levels of exchangeable bases [Potassium (K), Calcium (Ca), Magnesium (Mg), and Sodium (Na)] in a soil sample, a procedure involving extraction with 1 N  $\text{NH}_4\text{OAc}$  adjusted to pH 7.0 was employed. Specifically, 2.5 grams of soil, having passed through a 2mm mesh sieve, was combined with 25 ml of the extracting solution in a 50 ml Erlenmeyer flask. This mixture was then

agitated for five minutes using a mechanical shaker. Subsequently, the solution was filtered through Whatman filter paper #42 to obtain the filtrate. Quantification of exchangeable bases was performed by atomic absorption spectrophotometry (AAS).

- Percent Soil Fraction (sand, silt, and clay) distribution - Percent sand, silt, and clay of the soil samples were determined using the pipette method, following the particle size distribution analysis of ISRIC [15]. Organic matter in the soil samples was destroyed using 30% hydrogen peroxide. Dispersion was done using a blender with the addition of 10 mL of Calgon solution (sodium hexametaphosphate). Percent sand was determined by physical separation using a 63  $\mu\text{m}$  sieve. Percent silt and clay were determined by pipetting at specific time intervals.
- Cation Exchange Capacity (CEC) - determined using the ammonium acetate method at pH 7.0. Soil samples were saturated with ammonium acetate solution, shaken, and filtered. This was rinsed with isopropyl alcohol, and the filtrate was discarded. The same soil was added to 10% NaCl and digested in a distilling apparatus after the addition of 25 mL 4 N NaOH. Distillates were then titrated finally with standardized 0.05 N  $\text{H}_2\text{SO}_4$ .
- Exchangeable Aluminum (Al) – determined using the Ammonium Acetate, 1M pH 7.0 Method. Soil samples were saturated with ammonium acetate solution, shaken, filtered, and analyzed by atomic absorption spectroscopy.
- Exchangeable Iron (Fe) – determined using the Ammonium Acetate, 1M pH 7.0 Method. Soil samples were saturated with ammonium acetate solution, shaken, filtered, and analyzed by atomic absorption spectroscopy.

### 2.3 Soil Color Determination

The color of the collected fresh sample soils was determined using the Munsell Soil Color Chart (1994 revised edition). The chart contains three (3) soil color parameters: Hue, Value, and Chroma. To identify the soil color, it was matched to the color chips found on the chart.

### 2.4 Data Analysis

Data collected were analyzed for Pearson's Correlation and Regression Analysis using the Statistical Procedures for Agricultural Research (STAR) 2.0.1 software [19]. Soil Color Measurement Protocol. Soil color was determined in the laboratory on air-dried samples using the standard Munsell Soil Color Chart (Munsell Color, 2000 edition). Readings were performed under natural diffuse daylight by a single trained observer to eliminate inter-observer variability. Each sample was read in both the dry and moist state (moistened with distilled water). Munsell hue, value, and chroma were recorded for all samples; however, this study focused on value and chroma for continuous statistical analysis. Hue was recorded but excluded from quantitative analysis because all five soil series fell within closely adjacent hue groups (7.5YR to 10YR), limiting its discriminatory power for regression in this dataset. This exclusion is acknowledged as a scope limitation, and future work should include hue analysis using a wider range of soil series or digital colorimetry methods [20].

## 3. Results and Discussion

### 3.1 Correlation and Regression of Soil Color Value to Soil Physicochemical and Fertility Parameters

Soil color is typically the most obvious characteristic observed. A soil's coloration is determined by pedochemical processes such as oxidation, hydration, and the spread of iron oxides, which can produce shades of red, yellow, or orange. Dark soils can suggest inadequate drainage, affecting which crops can be grown. Color also serves as a reliable indicator of organic matter content and decomposition, the presence of minerals such as titanium and manganese, moisture content, and soil texture. The Munsell Color Chart, which uses hue, value, and chroma to define color, is a standard tool for evaluating soil color in the field. Hue signifies the primary spectral color, value represents lightness, and chroma denotes purity. For farmers, soil color is important because it often reveals the parent material, which determines specific soil characteristics [11]. Soil color can provide insights into general soil properties and chemical processes occurring below the surface. Soil reaction and acidity, which are likewise reflected indirectly in soil color, can be ameliorated through amendments such as bioelectric power-plant ash, with measurable improvements in crop performance on

acidic soils [21]. Table 1a showed a significantly negative correlation between soil color value and percent organic carbon and exchangeable magnesium at  $\alpha < 0.05$  or 5% with an  $r^2 = 0.7726$  and an  $r^2 = 0.8945$ , respectively (Table 1b) in the Guimbalaon series at a depth of 0 – 30 cm, while no correlation was found in all parameters at 30 – 60 cm depth. This further showed an inverse relationship between soil color value and percent organic matter and exchangeable magnesium, indicating that an increase in one parameter is associated with a corresponding decrease in the other. Franzmeier [22], as cited by Konen et al. [6], presented several equations correlating organic matter concentrations with Munsell value and chroma, all with  $r^2 = 0.48$ . Fernandez and Schulze [23] noted that visual matching of soil colors is inadequate for developing relationships between soil color and organic matter content. Kelly and Judd [24] reported that individuals can interpolate accurately and consistently between color chips. Still, soil color perception is subjective, and variability among soil scientists exists, as demonstrated by Post et al. [25], as cited by Konen et al. [6].

**Table 1a.** Correlated matrix for soil color value and soil fertility parameters of Guimbalaon Soil Series collected at 0 – 30 cm depth.

| Correlated Variables                         | Pearson Correlation Coefficient | Interpretation  | P-value |
|----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color value vs. Organic Carbon          | -0.8790                         | High negative   | 0.0496  |
| Soil color value vs. pH (H <sub>2</sub> O)   | -0.4558                         | Low negative    | 0.4405  |
| Soil color value vs. pH (CaCl <sub>2</sub> ) | -0.3215                         | Low negative    | 0.5978  |
| Soil color value vs. Total N                 | -0.7383                         | Medium negative | 0.1543  |
| Soil color value vs. Available P             | -0.8212                         | High negative   | 0.0882  |
| Soil color value vs. Exchangeable K          | 0.5662                          | Medium positive | 0.3197  |
| Soil color value vs. Exchangeable Na         | 0.2893                          | Low positive    | 0.6369  |
| Soil color value vs. Exchangeable Ca         | -0.3225                         | Low negative    | 0.5966  |
| Soil color value vs. Exchangeable Mg         | -0.9458                         | High negative   | 0.0150  |
| Soil color value vs. CEC                     | -0.2874                         | Low negative    | 0.6392  |
| Soil color value vs. Exchangeable Al         | 0.3683                          | Low positive    | 0.5419  |
| Soil color value vs. Exchangeable Fe         | -0.7142                         | Medium negative | 0.1753  |
| Soil color value vs. % Clay                  | 0.5920                          | Medium positive | 0.2929  |
| Soil color value vs. % Silt                  | -0.1653                         | Low negative    | 0.7905  |
| Soil color value vs. % Sand                  | -0.3652                         | Low negative    | 0.5456  |

**Table 1b.** Regression matrix for soil color value and soil fertility parameters of Guimbalaon Soil Series collected at 0 – 30 cm depth.

| Soil Fertility Parameters | Regression Equation                                    | R <sup>2</sup> | Pr (>F) |
|---------------------------|--------------------------------------------------------|----------------|---------|
| Percent Organic Carbon    | Organic Carbon = 4.33 + (-1.16*soil color value)       | 0.7726         | 0.0496  |
| Exchangeable Magnesium    | Exchangeable Mg = 1782.32 + (-508.68*soil color value) | 0.8945         | 0.0150  |

Table 2a showed a significantly positive correlation between soil color value and exchangeable potassium at  $\alpha < 0.05$  (5%), with an  $r^2 = 0.8006$  (Table 2b), obtained from the Isabela series at the 0–30 cm depth. This direct relationship means that an increase in one parameter corresponds to a similar increase in the other. According to Jones and Wild [26], as cited by Kone et al. [27], soil color notation on the Munsell chart may provide an opportunity to scale the K-supplying capacity of soil, including exchangeable and reserve K, by considering soil clay content and the exchangeable potassium percentage. No significant correlation was observed among all parameters in the subsoil (30–60cm depth) layer of the Isabela series. Tables 3a – 3b presented the correlation and regression matrix of soil color value and soil fertility parameters of the Lusiana series at 30 – 60 cm depths. A significantly positive correlation was observed in the subsoil between soil color value and exchangeable magnesium at  $\alpha < 0.05$  (5%; Table 3a), with an  $r^2 = 0.8332$  (Table 3b). However, there was no correlation between soil color value and the soil fertility parameters in the topsoil (0–30cm depth).

**Table 2a.** Correlated matrix for soil color value and soil fertility parameters of Isabela Soil Series collected at 0 – 30 cm depth.

| Correlated Variables                         | Pearson Correlation Coefficient | Interpretation  | P-value |
|----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color value vs. Organic Carbon          | -0.1366                         | Low negative    | 0.8266  |
| Soil color value vs. pH (H <sub>2</sub> O)   | 0.2229                          | Low positive    | 0.7185  |
| Soil color value vs. pH (CaCl <sub>2</sub> ) | -0.1010                         | Low negative    | 0.8716  |
| Soil color value vs. Total N                 | -0.0480                         | Low negative    | 0.9389  |
| Soil color value vs. Available P             | 0.3243                          | Low positive    | 0.5945  |
| Soil color value vs. Exchangeable K          | 0.8948                          | High positive   | 0.0403  |
| Soil color value vs. Exchangeable Na         | -0.5171                         | Medium negative | 0.3723  |
| Soil color value vs. Exchangeable Ca         | -0.0458                         | Low negative    | 0.9418  |
| Soil color value vs. Exchangeable Mg         | -0.3953                         | Low negative    | 0.5101  |
| Soil color value vs. CEC                     | -0.3687                         | Low negative    | 0.5414  |
| Soil color value vs. Exchangeable Al         | 0.8760                          | High positive   | 0.0514  |
| Soil color value vs. Exchangeable Fe         | 0.0357                          | Low positive    | 0.9546  |
| Soil color value vs. % Clay                  | -0.1681                         | Low negative    | 0.7869  |
| Soil color value vs. % Silt                  | 0.1863                          | Low positive    | 0.7641  |
| Soil color value vs. % Sand                  | 0.0924                          | Low positive    | 0.8826  |

**Table 2b.** Regression matrix for soil color value and soil fertility parameters of Isabela Soil Series collected at 0 – 30 cm depth.

| Soil Fertility Parameters | Regression Equation                                     | R <sup>2</sup> | Pr (>F) |
|---------------------------|---------------------------------------------------------|----------------|---------|
| Exchangeable Potassium    | Exchangeable K = -13056.76 + (4395.38*soil color value) | 0.8006         | 0.0403  |

**Table 3a.** Correlated matrix for soil color value and soil fertility parameters of the Louisiana Soil Series collected at 30 – 60 cm depth.

| Correlated Variables                         | Pearson Correlation Coefficient | Interpretation  | P-value |
|----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color value vs. Organic Carbon          | -0.5218                         | Medium negative | 0.3672  |
| Soil color value vs. pH (H <sub>2</sub> O)   | -0.1506                         | Low negative    | 0.8090  |
| Soil color value vs. pH (CaCl <sub>2</sub> ) | -0.3378                         | Low negative    | 0.5782  |
| Soil color value vs. Total N                 | -0.6075                         | Medium negative | 0.2771  |
| Soil color value vs. Available P             | -0.6414                         | Medium negative | 0.2435  |
| Soil color value vs. Exchangeable K          | 0.0050                          | Low positive    | 0.9936  |
| Soil color value vs. Exchangeable Na         | 0.2574                          | Low positive    | 0.6759  |
| Soil color value vs. Exchangeable Ca         | 0.5274                          | Medium positive | 0.3610  |
| Soil color value vs. Exchangeable Mg         | 0.9128                          | High positive   | 0.0305  |
| Soil color value vs. CEC                     | 0.7714                          | Medium positive | 0.1266  |
| Soil color value vs. Exchangeable Al         | 0.5732                          | Medium positive | 0.3124  |
| Soil color value vs. Exchangeable Fe         | -0.4418                         | Low negative    | 0.4564  |
| Soil color value vs. % Clay                  | -0.5638                         | Medium negative | 0.3222  |
| Soil color value vs. % Silt                  | 0.0272                          | Low positive    | 0.9654  |
| Soil color value vs. % Sand                  | 0.5247                          | Medium positive | 0.3640  |

**Table 3b.** Regression matrix for soil color value and soil fertility parameters of the Louisiana Soil Series collected at 30 – 60 cm depth.

| Soil Fertility Parameters | Regression Equation                                    | R <sup>2</sup> | Pr (>F) |
|---------------------------|--------------------------------------------------------|----------------|---------|
| Exchangeable Magnesium    | Exchangeable Mg = -1053.36 + (216.56*soil color value) | 0.8332         | 0.0305  |



Tables 4a – 4b presented the correlation and regression matrix of soil color value and soil fertility parameters of the San Manuel series at 30 – 60 cm depth. A significantly negative correlation was observed in the subsoil between soil color value and pH (CaCl<sub>2</sub>) and exchangeable potassium, and a positive correlation between soil color value and exchangeable iron at  $\alpha < 0.05$  or 5% (Table 4a) with an  $r^2 = 0.8655$ ,  $r^2 = 0.8636$ , and  $r^2 = 0.8248$ , respectively (Table 4b). Humus and iron compounds significantly affect color. The color of iron-containing soil minerals that undergo oxidation-reduction reactions can provide useful information on soil hydrologic conditions [7]. However, there is no correlation between soil color values and soil fertility parameters in the topsoil (0-30 cm depth).

**Table 4a.** Correlated matrix for soil color value and soil fertility parameters of San Manuel Soil Series collected at 30 – 60 cm depth.

| Correlated Variables                         | Pearson Correlation Coefficient | Interpretation  | P-value |
|----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color value vs. Organic Carbon          | -0.4539                         | Low negative    | 0.4426  |
| Soil color value vs. pH (H <sub>2</sub> O)   | -0.7861                         | Medium negative | 0.1148  |
| Soil color value vs. pH (CaCl <sub>2</sub> ) | -0.9303                         | High negative   | 0.0219  |
| Soil color value vs. Total N                 | 0.0928                          | Low positive    | 0.8820  |
| Soil color value vs. Available P             | -0.5615                         | Medium negative | 0.3246  |
| Soil color value vs. Exchangeable K          | -0.9293                         | High negative   | 0.0223  |
| Soil color value vs. Exchangeable Na         | -0.5330                         | Medium negative | 0.3551  |
| Soil color value vs. Exchangeable Ca         | -0.8431                         | High negative   | 0.0728  |
| Soil color value vs. Exchangeable Mg         | -0.2713                         | Low negative    | 0.6588  |
| Soil color value vs. CEC                     | -0.4320                         | Low negative    | 0.4676  |
| Soil color value vs. Exchangeable Al         | -0.0967                         | Low positive    | 0.8771  |
| Soil color value vs. Exchangeable Fe         | 0.9082                          | High positive   | 0.0329  |
| Soil color value vs. % Clay                  | -0.4444                         | Low negative    | 0.4534  |
| Soil color value vs. % Silt                  | 0.6306                          | Medium positive | 0.2540  |
| Soil color value vs. % Sand                  | 0.1166                          | Low positive    | 0.8519  |

**Table 4b.** Regression matrix for soil color value and soil fertility parameters of San Manuel Soil Series collected at 30 – 60 cm depth.

| Soil Fertility Parameters | Regression Equation                                    | R <sup>2</sup> | Pr (>F) |
|---------------------------|--------------------------------------------------------|----------------|---------|
| pH CaCl <sub>2</sub>      | pH CaCl <sub>2</sub> = 8.90 + (-0.96*soil color value) | 0.8655         | 0.0219  |
| Exchangeable Potassium    | Exchangeable K = 218.43 + (-52.17*soil color value)    | 0.8636         | 0.0223  |
| Exchangeable Iron         | Exchangeable Fe = -225.78 + (91.53*soil color value)   | 0.8248         | 0.0329  |

Tables 5a – 5b presented the correlation and regression matrix of soil color value and soil fertility parameters of the Silay series at 30 – 60 cm depth. There is no correlation of soil color value to the soil fertility parameters at the topsoil, while a significantly negative correlation was observed in the subsoil between soil color value and exchangeable iron at  $\alpha < 0.05$  or 5% (Table 5a) with an  $r^2 = 0.8030$  (Table 5b).

**Table 5a.** Correlated matrix for soil color value and soil fertility parameters of Silay Soil Series collected at 30 – 60 cm depth.

| Correlated Variables                         | Pearson Correlation Coefficient | Interpretation  | P-value |
|----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color value vs. Organic Carbon          | -0.3541                         | Low negative    | 0.5587  |
| Soil color value vs. pH (H <sub>2</sub> O)   | 0.7129                          | Medium positive | 0.1765  |
| Soil color value vs. pH (CaCl <sub>2</sub> ) | 0.4959                          | Low positive    | 0.3955  |
| Soil color value vs. Total N                 | -0.5040                         | Medium negative | 0.3866  |
| Soil color value vs. Available P             | -0.2421                         | Low negative    | 0.6948  |
| Soil color value vs. Exchangeable K          | 0.0550                          | Low positive    | 0.9301  |

**Table 5a.** Correlated matrix for soil color value and soil fertility parameters of Silay Soil Series collected at 30 – 60 cm depth. (continue)

| Correlated Variables                 | Pearson Correlation Coefficient | Interpretation  | P-value |
|--------------------------------------|---------------------------------|-----------------|---------|
| Soil color value vs. Exchangeable Na | -0.0664                         | Low negative    | 0.9155  |
| Soil color value vs. Exchangeable Ca | 0.8621                          | High positive   | 0.0602  |
| Soil color value vs. Exchangeable Mg | 0.8308                          | High positive   | 0.0814  |
| Soil color value vs. CEC             | 0.6396                          | Medium positive | 0.2452  |
| Soil color value vs. Exchangeable Al | 0.4044                          | Low positive    | 0.4995  |
| Soil color value vs. Exchangeable Fe | -0.8961                         | High negative   | 0.0396  |
| Soil color value vs. % Clay          | 0.6186                          | Medium positive | 0.2660  |
| Soil color value vs. % Silt          | 0.0459                          | Low positive    | 0.9416  |
| Soil color value vs. % Sand          | -0.5259                         | Medium negative | 0.3627  |

**Table 5b.** Regression matrix for soil color value and soil fertility parameters of Silay Soil Series collected at 30 – 60 cm depth.

| Soil Fertility Parameters | Regression Equation                                  | R <sup>2</sup> | Pr (>F) |
|---------------------------|------------------------------------------------------|----------------|---------|
| Exchangeable Fe           | Exchangeable Fe = 155.61 + (-30.00*soil color value) | 0.8030         | 0.0396  |

### 3.2 Correlation and Regression of Soil Color Chroma to Soil Physicochemical and Fertility Parameters

The importance of soil color analysis stems from the fact that soil properties such as organic matter, iron mineralogy, and moisture content are strongly correlated with soil color [25,28]. Beyond fertility, these same soil attributes also govern biogeochemical processes such as methane oxidation, which varies markedly across soil environments and has implications for greenhouse gas mitigation [29]. Soil color is composed of three parameters, namely hue (a specific color), value (lightness and darkness), and chroma (color intensity). Tables 6a – 6b presented the correlation and regression matrix of soil color chroma and soil fertility parameters of the Guimbalaon series at 0 – 30 cm (topsoil) and 30 – 60 cm (subsoil) depths. In the topsoil, a notable negative correlation was observed between soil color chroma and both the percentage of organic carbon and the percentage of exchangeable magnesium. This relationship was statistically significant at the 0.05 level (5%), as indicated in Table 6a. The coefficients of determination ( $r^2$ ) for these correlations were 0.8311 and 0.9015, respectively (Table 6b). Ibarra-F et al. [30] also explored soil color parameters in Mexico using a chroma meter, finding correlations between soil organic carbon (SOC) and hue, value, chroma, and reflectance. Their research, which supported these findings, yielded  $r^2$  values from 0.01 to 0.53. Conversely, no correlation was found between soil color chroma and any of the soil fertility parameters at 30–60 cm depth.

**Table 6a.** Correlated matrix for soil color chroma and soil fertility parameters of Guimbalaon Soil Series collected at 0 – 30 cm depth.

| Correlated Variables                          | Pearson Correlation Coefficient | Interpretation  | P-value |
|-----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Organic Carbon          | -0.9117                         | High negative   | 0.0311  |
| Soil color chroma vs. pH (H <sub>2</sub> O)   | -0.5479                         | Medium negative | 0.3391  |
| Soil color chroma vs. pH (CaCl <sub>2</sub> ) | -0.4045                         | Low negative    | 0.4994  |
| Soil color chroma vs. Total N                 | -0.7635                         | Medium negative | 0.1331  |
| Soil color chroma vs. Available P             | -0.7981                         | Medium negative | 0.1056  |
| Soil color chroma vs. Exchangeable K          | 0.6222                          | Medium positive | 0.2623  |
| Soil color chroma vs. Exchangeable Na         | 0.4206                          | Low positive    | 0.4807  |
| Soil color chroma vs. Exchangeable Ca         | -0.3643                         | Low negative    | 0.5466  |
| Soil color chroma vs. Exchangeable Mg         | -0.9495                         | High negative   | 0.0135  |
| Soil color chroma vs. CEC                     | -0.3311                         | Low negative    | 0.5862  |
| Soil color chroma vs. Exchangeable Al         | 0.5516                          | Medium positive | 0.3351  |

**Table 6a.** Correlated matrix for soil color chroma and soil fertility parameters of Guimbalaon Soil Series collected at 0 – 30 cm depth. (continue)

| Correlated Variables                  | Pearson Correlation Coefficient | Interpretation  | P-value |
|---------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Exchangeable Fe | -0.6073                         | Medium negative | 0.2774  |
| Soil color chroma vs. % Clay          | 0.6353                          | Medium positive | 0.2494  |
| Soil color chroma vs. % Silt          | -0.1096                         | Low negative    | 0.8608  |
| Soil color chroma vs. % Sand          | -0.4164                         | Low negative    | 0.4855  |

**Table 6b.** Regression matrix for soil color chroma and soil fertility parameters of Guimbalaon Soil Series collected at 0 – 30 cm depth.

| Soil Fertility Parameters | Regression Equation                                    | R <sup>2</sup> | Pr (>F) |
|---------------------------|--------------------------------------------------------|----------------|---------|
| Percent Organic Carbon    | Percent Organic C = 1.66 + (-0.29*soil color chroma)   | 0.8311         | 0.0311  |
| Exchangeable Magnesium    | Exchangeable Mg = 597.58 + (-122.17*soil color chroma) | 0.9015         | 0.0135  |

Tables 7a – 7d present the correlation and regression matrix of soil color chroma and soil fertility parameters of the Isabela series at 0 – 30 cm (topsoil) and 30 – 60 cm (subsoil) depths. Results revealed a significantly positive correlation in the topsoil between soil color chroma and percent sand at  $\alpha < 0.05$  (5%) (Table 7a) with an  $r^2 = 0.8088$  (Table 7b). Generally, light or pale soil colors suggest coarse texture, significant leaching, and warm temperatures. Sandy soils, in particular, can suffer from poor structure and high salinity, but their physical and mechanical properties can be improved using amendments such as rice husk ash and gypsum [31]. However, Schulze et al. [8] discovered that soil texture was not strongly correlated with Munsell color values within a particular area. Meanwhile, in the subsoil layer (30–60 cm depth) of the studied soil series, a clear inverse correlation emerged between soil color chroma and key soil properties: organic carbon percentage, total nitrogen, cation exchange capacity, and clay content. Conversely, soil color chroma exhibited a significant positive correlation with sand percentage, with a significance level of  $\alpha < 0.05$  (5%) (Table 7c). These results are consistent with those of Sanchez-Maranon et al. [32], who also noted a strong negative relationship between chroma and organic carbon, a finding supported by other research [33,34]. They also reported a strong positive relationship between chroma and sand content ( $r^2 = 0.52$ ,  $P < 0.01$ ). Further supporting this, Konen et al. [6] analyzed 130 Ap horizons and found relationships among soil color, SOC content, and particle size. That study, along with the work of Schulze et al. [8], despite differences in soil types and locations, confirms the consistent link between SOC content and soil color. Table 7d provides the regression matrix for these correlations.

**Table 7a.** Correlated matrix for soil color chroma and soil fertility parameters of Isabela Soil Series collected at 0 – 30 cm depth.

| Correlated Variables                          | Pearson Correlation Coefficient | Interpretation  | P-value |
|-----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Organic Carbon          | -0.5974                         | Medium negative | 0.2874  |
| Soil color chroma vs. pH (H <sub>2</sub> O)   | -0.4626                         | Low negative    | 0.4327  |
| Soil color chroma vs. pH (CaCl <sub>2</sub> ) | -0.6318                         | Medium negative | 0.2529  |
| Soil color chroma vs. Total N                 | -0.4602                         | Low negative    | 0.4354  |
| Soil color chroma vs. Available P             | -0.7628                         | Medium negative | 0.1336  |
| Soil color chroma vs. Exchangeable K          | -0.0589                         | Low negative    | 0.9250  |
| Soil color chroma vs. Exchangeable Na         | 0.2074                          | Low positive    | 0.7379  |
| Soil color chroma vs. Exchangeable Ca         | -0.4455                         | Low negative    | 0.4521  |
| Soil color chroma vs. Exchangeable Mg         | -0.2204                         | Low negative    | 0.7217  |
| Soil color chroma vs. CEC                     | -0.6725                         | Medium negative | 0.2136  |
| Soil color chroma vs. Exchangeable Al         | -0.3644                         | Low negative    | 0.5465  |
| Soil color chroma vs. Exchangeable Fe         | 0.4799                          | Low positive    | 0.4133  |

**Table 7a.** Correlated matrix for soil color chroma and soil fertility parameters of Isabela Soil Series collected at 0 – 30 cm depth. (continue)

| Correlated Variables         | Pearson Correlation Coefficient | Interpretation  | P-value |
|------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. % Clay | -0.8602                         | High negative   | 0.0614  |
| Soil color chroma vs. % Silt | -0.7684                         | Medium negative | 0.1291  |
| Soil color chroma vs. % Sand | 0.8993                          | High positive   | 0.0378  |

**Table 7b.** Regression matrix for soil color chroma and soil fertility parameters of Isabela Soil Series collected at 0 – 30 cm depth.

| Soil Fertility Parameters | Regression Equation                               | R <sup>2</sup> | Pr (>F) |
|---------------------------|---------------------------------------------------|----------------|---------|
| Percent Sand              | Percent Sand = -29.44 + (74.34*soil color chroma) | 0.8088         | 0.0378  |

**Table 7c.** Correlated matrix for soil color chroma and soil fertility parameters of Isabela Soil Series collected at 30 – 60 cm depth.

| Correlated Variables                          | Pearson Correlation Coefficient | Interpretation  | P-value |
|-----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Organic Carbon          | -0.9273                         | High negative   | 0.0233  |
| Soil color chroma vs. pH (H <sub>2</sub> O)   | -0.3307                         | Low negative    | 0.5867  |
| Soil color chroma vs. pH (CaCl <sub>2</sub> ) | -0.6065                         | Medium negative | 0.2781  |
| Soil color chroma vs. Total N                 | -0.9069                         | High negative   | 0.0336  |
| Soil color chroma vs. Available P             | 0.3653                          | Low positive    | 0.5454  |
| Soil color chroma vs. Exchangeable K          | 0.6534                          | Medium positive | 0.2318  |
| Soil color chroma vs. Exchangeable Na         | -0.3714                         | Low negative    | 0.5383  |
| Soil color chroma vs. Exchangeable Ca         | 0.3946                          | Low positive    | 0.5110  |
| Soil color chroma vs. Exchangeable Mg         | -0.4896                         | Low negative    | 0.4025  |
| Soil color chroma vs. CEC                     | -0.9657                         | High negative   | 0.0076  |
| Soil color chroma vs. Exchangeable Al         | 0.3246                          | Low positive    | 0.5941  |
| Soil color chroma vs. Exchangeable Fe         | 0.2346                          | Low positive    | 0.7040  |
| Soil color chroma vs. % Clay                  | -0.9830                         | High negative   | 0.0027  |
| Soil color chroma vs. % Silt                  | -0.7151                         | Medium negative | 0.1745  |
| Soil color chroma vs. % Sand                  | 0.9513                          | High positive   | 0.0128  |

**Table 7d.** Regression matrix for soil color chroma and soil fertility parameters of Isabela Soil Series collected at 0 – 30 cm depth.

| Soil Fertility Parameters | Regression Equation                                  | R <sup>2</sup> | Pr (>F) |
|---------------------------|------------------------------------------------------|----------------|---------|
| Percent Organic Carbon    | Percent Organic C = 1.74 + (-0.70*soil color chroma) | 0.8599         | 0.0233  |
| Total Nitrogen            | Total N = 0.1731 + (-0.0625*soil color chroma)       | 0.8226         | 0.0336  |
| Cation Exchange Capacity  | CEC = 43.40 + (-21.14*soil color chroma)             | 0.9327         | 0.0076  |
| Percent Clay              | Percent Clay = 92.45 + (-46.60*soil color chroma)    | 0.9663         | 0.0027  |
| Percent Sand              | Percent Sand = -27.27 + (61.84*soil color chroma)    | 0.9050         | 0.0128  |

Tables 8a – 8b presented the correlation and regression matrix of soil color chroma and soil fertility parameters of the Louisiana series at 30 – 60 cm depths. A significantly negative correlation was found in the subsoil between soil color chroma and exchangeable magnesium at  $\alpha < 0.05$  (5%; Table 8a), with  $r^2 = 0.8087$  (Table 8b). However, there is no correlation between soil color chroma and soil fertility parameters at 0–30 cm depth.

**Table 8a.** Correlated matrix for soil color chroma and soil fertility parameters of the Louisiana Soil Series collected at 30 – 60 cm depth.

| Correlated Variables                          | Pearson Correlation Coefficient | Interpretation  | P-value |
|-----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Organic Carbon          | 0.5164                          | Medium positive | 0.3731  |
| Soil color chroma vs. pH (H <sub>2</sub> O)   | -0.1967                         | Low negative    | 0.7512  |
| Soil color chroma vs. pH (CaCl <sub>2</sub> ) | 0.0101                          | Low negative    | 0.9871  |
| Soil color chroma vs. Total N                 | 0.7227                          | Medium positive | 0.1678  |
| Soil color chroma vs. Available P             | 0.2274                          | Low positive    | 0.7130  |
| Soil color chroma vs. Exchangeable K          | 0.0265                          | Low positive    | 0.9662  |
| Soil color chroma vs. Exchangeable Na         | -0.6286                         | Medium negative | 0.2560  |
| Soil color chroma vs. Exchangeable Ca         | -0.7992                         | Medium negative | 0.1047  |
| Soil color chroma vs. Exchangeable Mg         | -0.8993                         | High negative   | 0.0378  |
| Soil color chroma vs. CEC                     | -0.8752                         | High negative   | 0.0519  |
| Soil color chroma vs. Exchangeable Al         | -0.6096                         | Medium negative | 0.2751  |
| Soil color chroma vs. Exchangeable Fe         | 0.2499                          | Low positive    | 0.6852  |
| Soil color chroma vs. % Clay                  | 0.6181                          | Medium positive | 0.2665  |
| Soil color chroma vs. % Silt                  | -0.4645                         | Low negative    | 0.4305  |
| Soil color chroma vs. % Sand                  | -0.5084                         | Medium negative | 0.3818  |

**Table 8b.** Regression matrix for soil color chroma and soil fertility parameters of the Louisiana Soil Series collected at 30 – 60 cm depth.

| Soil Fertility Parameters | Regression Equation                                    | R <sup>2</sup> | Pr (>F) |
|---------------------------|--------------------------------------------------------|----------------|---------|
| Exchangeable Magnesium    | Exchangeable Mg = 701.53 + (-128.81*soil color chroma) | 0.8087         | 0.0378  |

Results shown on Table 9a revealed that there is an inverse relationship between soil color chroma obtained at a 0 – 30 cm depth of the San Manuel series to the exchangeable sodium and magnesium and percent clay at  $\alpha < 0.05$  or 5% with an  $r^2 = 0.8537, 0.9734$ , and  $0.8204$ , respectively (Table 9b). This further means that a corresponding decrease in the other accompanies an increase in one parameter. Table 9c showed that there is an inverse relationship between soil color chroma and percent organic carbon, exchangeable sodium, cation exchange capacity, and percent clay at  $\alpha < 0.05$  or 5% in the subsoil of San Manuel series with an  $r^2$  of  $0.9326, 0.9605, 0.9811$ , and  $0.9437$ , respectively (Table 9d). In the study by Konen et al. [6], significant relationships were observed between organic C concentration and the percentage of reflectance, Munsell value and chroma, the percentage of sand, the percentage of clay, and the geometric mean particle diameter. Beyond compositional analysis, statistical process-control approaches such as SPC and Cpk have also been applied to characterize the variability of clay-soil properties, including Atterberg limits [35]. More broadly, soil and site characteristics are increasingly recognized as ecological factors governing species persistence and informing conservation strategies [36].

**Table 9a.** Correlated matrix for soil color chroma and soil fertility parameters of San Manuel Soil Series collected at 0 – 30 cm depth.

| Correlated Variables                          | Pearson Correlation Coefficient | Interpretation  | P-value |
|-----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Organic Carbon          | -0.5802                         | Medium negative | 0.3051  |
| Soil color chroma vs. pH (H <sub>2</sub> O)   | -0.6918                         | Medium negative | 0.1957  |
| Soil color chroma vs. pH (CaCl <sub>2</sub> ) | -0.5890                         | Medium negative | 0.2960  |
| Soil color chroma vs. Total N                 | -0.6586                         | Medium negative | 0.2268  |
| Soil color chroma vs. Available P             | -0.3455                         | Low negative    | 0.5691  |
| Soil color chroma vs. Exchangeable K          | -0.3733                         | Low negative    | 0.5360  |
| Soil color chroma vs. Exchangeable Na         | -0.9240                         | High negative   | 0.0249  |
| Soil color chroma vs. Exchangeable Ca         | -0.8011                         | High negative   | 0.1032  |



**Table 9a.** Correlated matrix for soil color chroma and soil fertility parameters of San Manuel Soil Series collected at 0 – 30 cm depth. (continue)

| Correlated Variables                  | Pearson Correlation Coefficient | Interpretation  | P-value |
|---------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Exchangeable Mg | -0.9866                         | High negative   | 0.0019  |
| Soil color chroma vs. CEC             | -0.7285                         | Medium negative | 0.1627  |
| Soil color chroma vs. Exchangeable Al | 0.3387                          | Low positive    | 0.5771  |
| Soil color chroma vs. Exchangeable Fe | 0.4072                          | Low positive    | 0.4963  |
| Soil color chroma vs. % Clay          | -0.9058                         | High negative   | 0.0342  |
| Soil color chroma vs. % Silt          | 0.2602                          | Low positive    | 0.6725  |
| Soil color chroma vs. % Sand          | 0.7292                          | Medium positive | 0.1621  |

**Table 9b.** Regression matrix for soil color chroma and soil fertility parameters of San Manuel Soil Series collected at 0 – 30 cm depth.

| Soil Fertility Parameters | Regression Equation                                     | R <sup>2</sup> | Pr (>F) |
|---------------------------|---------------------------------------------------------|----------------|---------|
| Exchangeable Sodium       | Exchangeable Na = 104.22 + (-27.19 *soil color chroma)  | 0.8537         | 0.0249  |
| Exchangeable Magnesium    | Exchangeable Mg = 946.41 + (-104.87 *soil color chroma) | 0.9734         | 0.0019  |
| Percent Clay              | Percent Clay = 65.13 + (-10.01 *soil color chroma)      | 0.8204         | 0.0342  |

**Table 9c.** Correlated matrix for soil color chroma and soil fertility parameters of San Manuel Soil Series collected at 30 – 60 cm depth.

| Correlated Variables                          | Pearson Correlation Coefficient | Interpretation  | P-value |
|-----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Organic Carbon          | -0.9657                         | High negative   | 0.0076  |
| Soil color chroma vs. pH (H <sub>2</sub> O)   | -0.2014                         | Low negative    | 0.7454  |
| Soil color chroma vs. pH (CaCl <sub>2</sub> ) | -0.1377                         | Low negative    | 0.8252  |
| Soil color chroma vs. Total N                 | -0.7640                         | Medium negative | 0.1326  |
| Soil color chroma vs. Available P             | 0.4285                          | Low positive    | 0.4716  |
| Soil color chroma vs. Exchangeable K          | -0.3636                         | Low negative    | 0.5475  |
| Soil color chroma vs. Exchangeable Na         | -0.9801                         | High negative   | 0.0034  |
| Soil color chroma vs. Exchangeable Ca         | -0.7073                         | Medium negative | 0.1816  |
| Soil color chroma vs. Exchangeable Mg         | -0.8542                         | High negative   | 0.0653  |
| Soil color chroma vs. CEC                     | -0.9905                         | High negative   | 0.0011  |
| Soil color chroma vs. Exchangeable Al         | -0.1943                         | Low negative    | 0.7542  |
| Soil color chroma vs. Exchangeable Fe         | -0.0511                         | Low negative    | 0.9350  |
| Soil color chroma vs. % Clay                  | -0.9714                         | High negative   | 0.0058  |
| Soil color chroma vs. % Silt                  | 0.6564                          | Medium positive | 0.2289  |
| Soil color chroma vs. % Sand                  | 0.8690                          | High positive   | 0.0558  |

**Table 9d.** Regression matrix for soil color chroma and soil fertility parameters of San Manuel Soil Series collected at 30 – 60 cm depth.

| Soil Fertility Parameters | Regression Equation                                   | R <sup>2</sup> | Pr (>F) |
|---------------------------|-------------------------------------------------------|----------------|---------|
| Percent Organic Carbon    | Percent Organic C = 1.44 + (-0.28 *soil color chroma) | 0.9326         | 0.0076  |
| Exchangeable Sodium       | Exchangeable Na = 88.32 + (-19.84 *soil color chroma) | 0.9605         | 0.0034  |
| Cation Exchange Capacity  | CEC = 25.31 + (-3.27 *soil color chroma)              | 0.9811         | 0.0011  |
| Percent Clay              | Percent Clay = 70.97 + (-14.05 *soil color chroma)    | 0.9437         | 0.0058  |

Table 10a showed that there is an inverse relationship between soil color chroma and exchangeable magnesium, and between soil color chroma and percent clay at  $\alpha < 0.05$ , or 5% of the Silay series at 0–30 cm depth, with  $r^2 = 0.8055$  and  $0.7989$ , respectively (Table 10b). However, a direct relationship was observed between soil color chroma and pH (H<sub>2</sub>O)  $\alpha < 0.05$  or 5% with an  $r^2 = 0.7729$  in the topsoil of the same series.

The agronomic implications of pH-related variation are well documented for other crops; for instance, alkaline conditions affect early root development and varietal yield in corn [37], and influence the response of hybrid corn to ammonium-based fertilizers under drought [38]. Table 10c showed that there is a significantly negative correlation between soil color chroma and exchangeable calcium and magnesium, and cation exchange capacity at  $\alpha < 0.05$  (5%) with  $r^2 = 0.9443$ ,  $0.8937$ , and  $0.7815$ , respectively (Table 10d), collected at 30-60 cm depth of the Silay soil series.

**Table 10a.** Correlated matrix for soil color chroma and soil fertility parameters of Silay Soil Series collected at 0 – 30 cm depth.

| Correlated Variables                          | Pearson Correlation Coefficient | Interpretation  | P-value |
|-----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Organic Carbon          | 0.6890                          | Medium positive | 0.1982  |
| Soil color chroma vs. pH (H <sub>2</sub> O)   | 0.8791                          | High positive   | 0.0495  |
| Soil color chroma vs. pH (CaCl <sub>2</sub> ) | 0.7889                          | Medium positive | 0.1127  |
| Soil color chroma vs. Total N                 | 0.7855                          | Medium positive | 0.1153  |
| Soil color chroma vs. Available P             | -0.6417                         | Medium negative | 0.2431  |
| Soil color chroma vs. Exchangeable K          | 0.2832                          | Low positive    | 0.6443  |
| Soil color chroma vs. Exchangeable Na         | -0.5864                         | Medium negative | 0.2987  |
| Soil color chroma vs. Exchangeable Ca         | -0.4386                         | Low negative    | 0.4601  |
| Soil color chroma vs. Exchangeable Mg         | -0.8975                         | High negative   | 0.0388  |
| Soil color chroma vs. CEC                     | -0.2368                         | Low negative    | 0.7014  |
| Soil color chroma vs. Exchangeable Al         | -0.2248                         | Low negative    | 0.7162  |
| Soil color chroma vs. Exchangeable Fe         | -0.2203                         | Low negative    | 0.7218  |
| Soil color chroma vs. % Clay                  | -0.8938                         | High negative   | 0.0409  |
| Soil color chroma vs. % Silt                  | -0.2786                         | Low negative    | 0.6499  |
| Soil color chroma vs. % Sand                  | 0.8349                          | High positive   | 0.0785  |

**Table 10b.** Regression matrix for soil color chroma and soil fertility parameters of Silay Soil Series collected at 0 – 30 cm depth.

| Soil Fertility Parameters | Regression Equation                                     | R <sup>2</sup> | Pr (>F) |
|---------------------------|---------------------------------------------------------|----------------|---------|
| pH (H <sub>2</sub> O)     | pH (H <sub>2</sub> O) = 4.47 + (0.65*soil color chroma) | 0.7729         | 0.0495  |
| Exchangeable Magnesium    | Exchangeable Mg = 679.07 + (-201.19 *soil color chroma) | 0.8055         | 0.0388  |
| Percent Clay              | Percent Clay = 50.19 + (-14.75 *soil color chroma)      | 0.7989         | 0.0409  |

**Table 10c.** Correlated matrix for soil color chroma and soil fertility parameters of Silay Soil Series collected at 30 – 60 cm depth.

| Correlated Variables                          | Pearson Correlation Coefficient | Interpretation  | P-value |
|-----------------------------------------------|---------------------------------|-----------------|---------|
| Soil color chroma vs. Organic Carbon          | 0.5602                          | Medium positive | 0.3260  |
| Soil color chroma vs. pH (H <sub>2</sub> O)   | -0.4544                         | Low negative    | 0.4420  |
| Soil color chroma vs. pH (CaCl <sub>2</sub> ) | -0.2246                         | Low negative    | 0.7164  |
| Soil color chroma vs. Total N                 | 0.6673                          | Medium positive | 0.2185  |
| Soil color chroma vs. Available P             | -0.1820                         | Low negative    | 0.7696  |
| Soil color chroma vs. Exchangeable K          | 0.2630                          | Low positive    | 0.6691  |
| Soil color chroma vs. Exchangeable Na         | -0.3962                         | Low negative    | 0.5091  |
| Soil color chroma vs. Exchangeable Ca         | -0.9718                         | High negative   | 0.0057  |
| Soil color chroma vs. Exchangeable Mg         | -0.9454                         | High negative   | 0.0152  |
| Soil color chroma vs. CEC                     | -0.8840                         | High negative   | 0.0466  |
| Soil color chroma vs. Exchangeable Al         | -0.1532                         | Low negative    | 0.8057  |
| Soil color chroma vs. Exchangeable Fe         | 0.7584                          | Medium positive | 0.1373  |
| Soil color chroma vs. % Clay                  | -0.8468                         | High negative   | 0.0703  |

**Table 10c.** Correlated matrix for soil color chroma and soil fertility parameters of Silay Soil Series collected at 30 – 60 cm depth. (continue)

| Correlated Variables         | Pearson Correlation Coefficient | Interpretation | P-value |
|------------------------------|---------------------------------|----------------|---------|
| Soil color chroma vs. % Silt | -0.4649                         | Low negative   | 0.4301  |
| Soil color chroma vs. % Sand | 0.8470                          | High positive  | 0.0701  |

**Table 10d.** Regression matrix for soil color chroma and soil fertility parameters of Silay Soil Series collected at 30 – 60 cm depth.

| Soil Fertility Parameters | Regression Equation                                      | R <sup>2</sup> | Pr (>F) |
|---------------------------|----------------------------------------------------------|----------------|---------|
| Exchangeable Calcium      | Exchangeable Ca = 3523.09 + (-710.38 *soil color chroma) | 0.9443         | 0.0057  |
| Exchangeable Magnesium    | Exchangeable Mg = 752.86 + (-140.16 *soil color chroma)  | 0.8937         | 0.0152  |
| Cation Exchange Capacity  | CEC = 11.36 + (-1.34 *soil color chroma)                 | 0.7815         | 0.0466  |

#### 4. Conclusions

This study demonstrates that soil color is a practical and informative indicator of soil physicochemical and fertility properties in sugarcane-cultivated soils. In the topsoil (0–30 cm depth), soil color value was significantly correlated with organic carbon, exchangeable magnesium, and exchangeable potassium, whereas in the subsoil (30–60 cm depth), it was significantly correlated with pH (CaCl<sub>2</sub>) and exchangeable magnesium, potassium, and iron. Chroma, on the other hand, showed broader associations correlating with organic carbon, exchangeable magnesium and sodium, particle-size fractions (clay and sand), and pH (H<sub>2</sub>O) in the topsoil, and with organic carbon, exchangeable magnesium, sodium, and calcium, total nitrogen, cation exchange capacity, and percentages of sand and clay in the subsoil layer. These findings indicate that Munsell color readings can complement conventional laboratory analyses by offering a rapid, low-cost field screening tool for fertility status, particularly where laboratory access is limited. Pending validation across larger, independent datasets, color-based assessment offers a scalable approach to support site-specific nutrient management in tropical sugarcane systems.

#### 5. Limitations

Several limitations of this study must be acknowledged. First, all correlation and regression analyses are based on  $n = 5$  data points (one composite sample per soil series and depth), severely limiting statistical power. At this sample size, achieving  $p < 0.05$  requires  $|r| > 0.878$ , so only the strongest associations are detected. Second, no correction for multiple comparisons was applied across the approximately 300 correlation tests performed; some reported significant correlations may represent Type I errors and should be treated as hypothesis-generating rather than confirmatory. Third, compositing 20 soil borings per farm into a single analytical sample eliminates within-series variability. Fourth, the regression equations presented have not been independently validated; leave-one-out cross-validation or testing on an independent dataset is required before these equations can be applied predictively. Fifth, hue was excluded from the analysis because all five series fell within the 7.5YR-10YR hue range, limiting its discriminatory power in this dataset. Future studies should increase sample size per series, include hue analysis, and validate regression equations before field-scale predictive use, ideally complemented by digital colorimetry approaches such as near-infrared spectroscopy [20].

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# Chitosan and Plant Extract-Based Edible Coatings in Postharvest Life of Bell Pepper (*Capsicum Annuum* L.) Under Tropical Ambient Conditions

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**Abstract:** Growing concerns over food sustainability and postharvest losses have made eco-friendly preservation strategies essential. Edible coatings offer an alternative to refrigeration, helping maintain fruit quality during storage. This study evaluated chitosan and plant extract-based coatings on the postharvest quality of bell pepper under ambient conditions. The research was conducted in two phases. Study 1 tested chitosan at 1%, 2%, 3%, and 4% concentrations against an uncoated control, and Study 2 combined the most effective concentration with Aloe vera and Moringa leaf extracts. In Study 1, 2% chitosan was most effective in reducing weight loss and shriveling while maintaining firmness and visual quality. Building on this result, Study 2 applied five treatments: T0 (2% chitosan), T1 (50 mL of 2% chitosan + 50 mL of 50% Aloe vera), T2 (33 mL of 2% chitosan + 67 mL of 50% Aloe vera), T3 (50 mL of 2% chitosan + 50 mL of 50% Moringa leaf extract), and T4 (33 mL of 2% chitosan + 67 mL of 50% Moringa leaf extract). Among these, the Aloe vera-enriched coating T2 best reduced weight loss, delayed shriveling, and extended marketable shelf life, whereas the Moringa-chitosan treatments better preserved firmness but showed earlier visual deterioration. Overall, 2% chitosan alone delayed postharvest deterioration, but combining it with a higher proportion of Aloe vera was most effective, underscoring the potential of natural coatings as sustainable alternatives to refrigeration.

**Keywords:** Aloe vera gel; biodegradable packaging; moringa leaf extracts; sustainable food storage

## 1. Introduction

Food security is one of the major challenges we face worldwide. Millions of people around the globe still battle hunger and inadequate nutrition. Governments and the private sector address this issue by boosting local food production, but this is not sufficient because, in general, those commodities, especially horticultural crops, easily deteriorate after harvesting. Bell pepper (*Capsicum annuum* L.), a horticultural crop also known as sweet pepper, is considered an economically important crop due to its high nutritive value, as it is an excellent source of antioxidants, including carotenoids, ascorbic acid, flavonoids, and phenolic acids. These compounds contribute to its potential health benefits, including immune-modulatory effects, valuable antioxidant properties, and the potential to counteract carcinogenic processes [1]. Like most of the horticultural crops, postharvest losses of this crop account for about 40%

annually, and because bell peppers are highly perishable, once harvested, they continue to respire and transpire, resulting in moisture loss, shriveling, discoloration, and microbial spoilage, thus requiring meticulous handling and proper attention to retain their postharvest quality [2].

Over the past decades, numerous conventional methods have been developed for food preservation, but only a few strategies have been employed to extend the shelf life of bell peppers in a more natural, cost-effective, and sustainable way. Nowadays, consumers are becoming more conscious of the food they eat, leading to a higher demand for healthy, chemical-free food. Because of this, researchers are seeking better, safer, and equally effective alternatives to prevent postharvest losses. Among these postharvest technology alternatives is the application of edible coatings. Edible coatings are environmentally sustainable strategies for food preservation [3], as they can replace synthetic chemicals and packaging materials. These are thin layers of natural biopolymers applied to the surfaces of fruits and vegetables that act as semipermeable barriers, reducing transpiration, moisture loss, and pathogen contamination while maintaining the produce's nutritional and visual quality. Edible coatings are generally 0.3 mm thick and are made from layer materials used for enrobing food to fortify or replace natural layers consumed with the food [4]. The application of edible coatings to vegetables appears to be a promising approach for minimizing postharvest losses due to deterioration and disease. It can be done by brushing, spraying, wrapping, and dipping.

Chitosan, a biodegradable polysaccharide biopolymer extracted from chitin found in crustacean shells, is widely recognized for its excellent film-forming ability, antimicrobial activity, and biodegradability, and has shown promising results in extending the shelf life and quality of produce [5, 6]. Aloe vera plant produces a mucilaginous gel obtained through extraction from the jelly-like substance of the parenchyma tissue of its leaves. It is used as a novel edible coating due to its moisturizing effect, exceptional barrier properties, and antifungal and antibacterial properties, which are biologically safe, helping extend the shelf life of fruits and vegetables. The commercial application of Aloe vera gel is considered an alternative to postharvest chemical treatments, as it is safer to consume at the recommended concentration. Moringa oleifera, as a medicinal tree, has numerous economic applications and uses in human consumption [7]. Its extracted leaves can be used in the food industry as a potential post-harvest treatment agent. The extract from leaves contains natural antioxidants, including ascorbic acid, phenolic compounds, and carotenoids, which contribute to reducing microbial activity in fruits and vegetables.

Chitosan alone as a coating material has remarkable properties; however, additional properties are essential when combined with bioactive plant components to develop multifunctional coatings with both barrier and antimicrobial properties, to achieve food security. Furthermore, less information is available on the combined application of a chitosan edible coating enriched with Aloe vera gel and moringa leaf extracts. Therefore, this study evaluated the synergistic effect of chitosan enriched with Aloe vera gel and moringa leaf extracts on the physico-chemical postharvest properties of bell pepper under ambient room conditions.

## 2. Materials and Methods

### 2.1 Location

The study was conducted at the Cebu Technological University-Argao Campus-Biodiversity, Environment and Natural Resources Research Center (CTU-BENRC), Argao, Cebu, under tropical ambient conditions.

### 2.2 Fruit Sample Preparation

Newly harvested, commercially mature, green "Emperor F1" bell peppers were purchased from the local farmer in Nug-as, Alcoy, Cebu, properly packed in suitable containers, and immediately transported to the CTU-Argao BENRC Laboratory. Only healthy, fresh, uniform-sized, and disease-free fruits were selected for application of various postharvest treatments. The collected fruit samples were washed with tap water, then decontaminated with 30% commercial bleach, and rinsed 3 times with sterile water.

### 2.3 Study 1: Effects of Different Chitosan Edible Coating Concentration on Physico-chemical Properties of Bell Pepper (*Capsicum annuum* L.) Fruits

The study was conducted in a series of experiments to determine optimal chitosan edible coating formulations for preserving quality and extending the shelf life of bell peppers. In these experiments, the

effects of different concentrations of edible coating formulations on bell pepper fruit were evaluated using general quality parameters and chemical properties. Then, the edible coating, which has the potential to preserve quality and extend the shelf life of these fruits, was selected and incorporated with Aloe vera gel and Moringa Leaf Extracts, and tested for general quality parameters and physico-chemical properties in Study 2.

#### 2.4. Preparation of Chitosan Edible Coating Solution

A study by [8] served as a guide for preparing the coating. To prepare 100 ml of chitosan solutions at concentrations 1, 2, 3, and 4 g/L, 1, 2, 3, and 4 g of chitosan powder were weighed using a sensitive balance. Each amount of chitosan was dissolved in 75 ml of distilled water containing 1 ml of glacial acetic acid, then placed on a magnetic stirrer for 60 minutes to ensure complete dissolution. The final pH of the solution was adjusted to 5.6 with 0.1 M NaOH, and the volume was made up to 100 mL with distilled water.

#### 2.5 Method of Coating Application

Food-grade chitosan was used to coat bell pepper fruits. A total of 150 freshly harvested, healthy, uncontaminated, and uniform-sized bell pepper fruits were selected for Study 1. The bell pepper fruits were dipped in the respective coating formulations for 7 minutes and left to dry at room temperature. After drying, the treated bell peppers were placed in a plastic mesh tray and stored under ambient room conditions.



Figure 1. Preparation of Chitosan Coating



Figure 2. Application of Chitosan Coatings

## 2.6 Experimental Design and Treatments

The study was laid out in a Completely Randomized Design (CRD) with five treatments and three replications, with ten samples per treatment per replicate.

The treatments were as follows:

C0- No coating

C1- 1g chitosan powder was dissolved in 100 ml of distilled water (1% w/v)

C2- 2g chitosan powder was dissolved in 100 ml of distilled water (2% w/v)

C3- 3g chitosan powder was dissolved in 100 ml of distilled water (3% w/v)

C4- 4g chitosan powder was dissolved in 100 ml of distilled water (4% w/v)

## 2.7 Study 2: Comparative Effects of Chitosan and Plant Extract- Based Edible Coating on Physicochemical Properties of Bell Pepper (*Capsicum annuum* L.) Fruits

For this study, the optimum chitosan concentration from study 1 was combined with different ratios of Aloe vera gel and moringa leaf extracts. These coatings were prepared to enrich the coating.

## 2.8 Aloe vera Gel Preparations

Preparations of Aloe vera extracts were based on the method of [9] with slight modifications, where Aloe vera leaves were obtained and prepared fresh from the field. All edges of the Aloe vera leaves were cut using a knife and soaked in water for 30 minutes before scraping the gel. The colorless, gel-like material obtained is called hydro parenchyma. Using a spoon, it was scraped into a blender and blended to a slimy consistency. This solution was then filtered through a strainer with 8 mm holes to remove water and the fibrous fraction. A pure, 100% aloe vera solution was stored at 5°C in a refrigerator until use to prevent oxidation of the phenolic compounds.

## 2.9 Moringa Leaf Extracts

The moringa leaf extraction process was carried out according to the method described by [10]. Young leaves below the apical portion of the moringa tree were collected. Moringa leaves were separated from stalks and sorted to remove the yellow and brown leaves. The leaves were washed with distilled water to remove dirt, and afterward spread out on clean tissue paper. The extract was obtained by grinding the young leaves with 1 ml of distilled water per 10g of fresh moringa leaves, as described by [10], using a conventional juicer blender. The solid materials were separated from the juice using a strainer with 8 mm holes. The extract was collected in a separate container and prepared following the recommended dilutions for moringa leaf extracts [10, 11].

## 2.10 Experimental Design and Treatment

The study used the 2% chitosan concentration, as it was the optimum concentration determined in Study 1. Additionally, the study was laid out in a single-factor experiment in a Completely Randomized Design (CRD) with five treatments and three replications with 10 samples per replicate. Each treatment used the optimal chitosan concentration solution, determined in Study 1, as a consistent base to assess the synergistic effects of Aloe vera and Moringa leaf extracts at varying percentages. T0 served as the control, with the optimum chitosan concentration (2%) used. T1 was prepared by homogenizing 50 mL of the optimal chitosan concentration with 50 mL of Aloe vera gel, as recommended by [12]. T2 was prepared by homogenizing 33 mL of the optimum chitosan concentration with 67 mL of Aloe vera gel, aiming to offer a low-cost technology that farmers can adopt by increasing the volume of Aloe vera extract. For T3, the solution was prepared by homogenizing 50 mL of the optimal chitosan concentration with 50 mL of 20% Moringa leaf extract, as recommended by [10, 11]. T4 was prepared by homogenizing 33 mL of the optimum chitosan concentration with 67 mL of Moringa leaf extract, also aiming to provide a low-cost technology by increasing the proportion of Moringa leaf extract. Homogenization time was standardized at 3 minutes for all preparations, as described by [12].

## 2.11 Data Collection

*Cumulative Weight Loss (%)*. The initial weight of the fruits was measured before the treatments were applied. The fruits' weights were evaluated every other day after treatments were applied to determine weight loss. The following formula calculates weight loss.



$$\text{Weight loss (\%)} = (W_i - W_t) / W_i \times 100$$

where:

$W_i$ - initial weight

$W_t$ - Weight after every other day observation

Fruit Shriveling - This was determined through the use of the shriveling index (SI) [13].

Firmness index- The degree of fruit firmness was evaluated every other day using the palpation-based firmness index method described by [13]. This method involves the manual palpation of sample fruits from each treatment per replicate to determine relative firmness levels and ensure consistency in assessment across the sampling period. To minimize experimental error and ensure reliable data, strict care was taken with the fruits during each evaluation.

Visual Quality Rating and Shelf Life - The physical appearance of bell pepper fruit was assessed every other day using the 9-point hedonic scale of [13].

*Decay Incidence/Spoilage.* Decay incidence was calculated as the number of decayed/diseased fruits divided by the total number of fruits. The fruits were visually inspected for rotting and microbial infection. Percent decay incidence was calculated using the formula.

$$\text{Decay Incidence (\%)} = \frac{\text{number of decayed fruits}}{\text{Total number of fruits}} \times 100$$

*pH level.* A gram of bell pepper fruit from each treatment was cut into small slices and pooled. Samples were homogenized in a blender, and portions of the homogenate were taken for quality assessment. The pH value of the fruit was measured with a pH meter.

*Total Soluble Solid (0Brix).* A composite sample of 5 g of fruit flesh per treatment was prepared, blended, homogenized with 50 mL of distilled water, and filtered. The filtrate was analyzed for TSS. TSS in Brix% was measured using a hand-held refractometer calibrated with distilled water by placing 1-3 drops of juice on the instrument's prism and taking the reading.

Titrateable Acidity (%) – A five (5) mL aliquot of fruit juice extract was taken from blended and homogenized samples and diluted with 45 mL of distilled water. The aliquot was measured, placed in an Erlenmeyer flask, and treated with 2 drops of 1% phenolphthalein indicator. This was titrated with 0.1% NaOH until the faint pink color, and the volume of NaOH was recorded, and TA was calculated as follows [13]:

$$\% \text{ TA of predominant acid}$$

Where:

V- Volume of NaOH added, ml

N- Concentration of NaOH, normality (N)

M- Milliequivalent weight of predominant acid, g/meq (citric acid- 0.064)

W- Weight equivalent of aliquot, g

## 2.12 Relative Humidity and Temperature

The ambient storage temperature and relative humidity (RH) were monitored twice daily, in the morning (AM; 07:00–08:00 h) and the afternoon (PM; 13:00–14:00 h) throughout the 16-day storage period, for both Study 1 and Study 2. Recordings were made using a calibrated digital thermo-hygrometer placed inside the storage room of the CTU-Argao Campus BENRC Laboratory, Argao, Cebu, Philippines, where all treatment groups were stored under identical, uncontrolled tropical ambient conditions with no refrigeration or modified atmosphere.

## 2.13 Statistical Analysis

Statistical analysis was performed using the Statistical Tool for Agricultural Research (STAR) software. One-way ANOVA test at a significance level of 5%, and Least Significant Difference (LSD) Test for comparisons among treatments were performed.

### 3. Results and Discussion

#### 3.1 Weight Loss

Weight loss is one of the most critical postharvest factors influencing the marketability of fresh produce. The evaluation of the percentage weight loss in bell peppers over a period of sixteen (16) days under five (5) treatments with different concentrations of chitosan (T0–T4) is shown in Table 1. The weight loss of bell pepper fruits increased progressively with storage time, and significant differences among treatments were observed, particularly during the early to mid-storage period. Table 1 shows that at 2 days of storage, the control (T0) already lost 7.45% of its weight, while the 2% chitosan coating (T2) showed a much lower weight loss of only 4.43%. The analysis confirmed that these differences were statistically significant, indicating that even at the start of storage, coating concentration had a measurable impact on fruit dehydration. Interestingly, the higher concentrations (T3 – 8.06% and T4 – 7.58%) recorded values comparable to or even greater than the control, suggesting that overly thick coatings in T3 and T4 may have failed to regulate water vapor loss effectively. This aligns with reports by [14], who emphasized that excessively thick coatings can hinder normal gas exchange, which may explain why T3 and T4 did not outperform the control in this study. By day 6, treatment differences became more pronounced. Fruits under T2 maintained the lowest weight loss (14.54%), significantly lower than the control (21.73%), while T1 (18.57%) provided moderate protection. This trend continued through days 8 and 10, where T2 fruits consistently had 6–8% less weight loss than the control and other treatments. By day 12, the advantage of T2 remained evident (31.39%) compared to T0 (40.56%), confirming that chitosan coatings remained highly effective through this stage. However, by day 14 and day 16, treatment differences were no longer significant, with all fruits exceeded 50% weight loss. It is important to acknowledge that the commercially acceptable threshold for bell pepper weight loss is typically 5–8%; fruit exceeding this threshold would be unmarketable under commercial standards. In this study, shelf life is therefore defined operationally based on Visual Quality Rating (VQR  $\geq 5.0$  on the hedonic scale) rather than weight loss alone, as VQR integrates multiple visible quality attributes. At this stage, natural senescence and tissue breakdown dominated storage behavior, overriding the protective effects of the coatings. These observations highlight that while chitosan delays water loss, it cannot indefinitely suppress the natural progression of postharvest deterioration.

**Table 1.** Percent (%) weight loss of the bell pepper fruit (*Capsicum annuum* L.) as influenced by the different concentrations of chitosan edible coating.

| Treatments         | Means (Per day)    |                     |                    |                    |                    |                     |        |        |
|--------------------|--------------------|---------------------|--------------------|--------------------|--------------------|---------------------|--------|--------|
|                    | 2                  | 4                   | 6                  | 8                  | 10                 | 12                  | 14     | 16     |
| T0- Uncoated       | 7.45 <sup>a</sup>  | 14.32 <sup>ab</sup> | 21.73 <sup>a</sup> | 28.33 <sup>a</sup> | 34.47 <sup>a</sup> | 40.56 <sup>a</sup>  | 52.98  | 60.05  |
| T1- 1% Chitosan    | 6.20 <sup>ab</sup> | 12.75 <sup>b</sup>  | 18.57 <sup>b</sup> | 25.68 <sup>a</sup> | 32.03 <sup>a</sup> | 37.94 <sup>b</sup>  | 53.82  | 59.88  |
| T2- 2% Chitosan    | 4.43 <sup>b</sup>  | 9.26 <sup>c</sup>   | 14.54 <sup>c</sup> | 20.70 <sup>b</sup> | 26.28 <sup>b</sup> | 31.39 <sup>c</sup>  | 53.57  | 59.12  |
| T3- 3% Chitosan    | 8.06 <sup>a</sup>  | 14.43 <sup>ab</sup> | 21.46 <sup>a</sup> | 27.99 <sup>a</sup> | 33.73 <sup>a</sup> | 40.33 <sup>ab</sup> | 52.74  | 59.25  |
| T4- 4% Chitosan    | 7.58 <sup>a</sup>  | 15.19 <sup>a</sup>  | 21.67 <sup>a</sup> | 28.38 <sup>a</sup> | 34.53 <sup>a</sup> | 42.10 <sup>a</sup>  | 54.76  | 62.16  |
| <b>F Value</b>     | 5.57               | 11.38               | 15.93              | 12.80              | 13.96              | 27.08               | 0.75   | 1.87   |
| <b>Pr (&gt; F)</b> | 0.0127             | 0.0010              | 0.0002             | 0.0006             | 0.0004             | 0.0000              | 0.5827 | 0.1922 |
| <b>CV (%)</b>      | 15.91              | 9.22                | 6.91               | 6.05               | 4.98               | 3.66                | 2.97   | 2.58   |

Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.



**Figure 3.** Weighing of Bell Pepper Fruit

### 3.2 Fruit Shriveling

Table 2 presents the shriveling scores of bell peppers treated with different concentrations of chitosan over a 16-day storage period. Shriveling, often associated with moisture loss and loss of firmness, directly impacts visual quality and consumer acceptance. The application of chitosan coatings significantly influenced the shriveling behavior of bell peppers during storage. Statistical analysis revealed significant differences in shriveling at 2, 10, and 16 days, indicating that treatment formulations influenced bell pepper moisture retention during storage and suggesting that chitosan concentration plays a crucial role in maintaining fruit quality over time. Data show that the control (T0) consistently had the highest shriveling scores throughout the storage period, reaching 4.93 by day 16, indicating rapid deterioration in the absence of a protective coating. In contrast, coated fruits exhibited delayed shriveling, with T2 (2% chitosan) consistently performing best, achieving the lowest shriveling score of 3.67. This demonstrates that chitosan effectively served as a semipermeable film, reducing water vapor loss and maintaining firmness. [15] Similarly, it was reported that chitosan coatings delay shriveling in bell peppers by preserving water content and reducing desiccation symptoms. By day 10, significant differences among treatments became more apparent. The control (T0) registered a shriveling score of 2.90, which was markedly higher than T1 (2.50), T2 (2.67), and T3 (2.53). Among all treatments, T2 maintained the numerically lowest mean shriveling score (2.21) across the entire storage period, outperforming T0 (2.48), T1 (2.33), T3 (2.38), and T4 (2.43) in overall moisture retention. Interestingly, while chitosan was effective overall, higher concentrations (T3 = 3% and T4 = 4%) did not always outperform 2% chitosan. In fact, excessively thick coatings may hinder gas exchange, reducing oxygen availability and triggering anaerobic respiration, thereby accelerating quality decline [16]. Also noted that moderate concentrations (~2%) yield optimal effects due to proper film formation and permeability, while very low or very high concentrations compromise effectiveness. This pattern aligns with previous findings by [17, 18] and [19], who emphasized the concentration-dependent response of chitosan, where excessively thick coatings may disrupt physiological balance, while thin coatings may fail to provide adequate protection. The average shriveling scores over the entire storage duration further support this trend. T0 had the highest mean shriveling (2.48), while T2 maintained the lowest average (2.21), followed closely by T1 (2.33). Treatments T3 (2.38) and T4 (2.43) did not show improvement beyond the effectiveness of 2% chitosan. This suggests that selecting an optimal coating level to balance moisture retention and gas exchange, and a moderate chitosan concentration (2%) provides the best protection against shriveling, whereas higher concentrations may not yield additional benefits.

**Table 2.** Shriveling of the bell pepper fruit (*Capsicum annuum* L.) as influenced by the different concentrations of chitosan edible coating.

| Treatments         | Means (Per day)    |        |        |        |                     |        |        |                    |
|--------------------|--------------------|--------|--------|--------|---------------------|--------|--------|--------------------|
|                    | 2                  | 4      | 6      | 8      | 10                  | 12     | 14     | 16                 |
| T0- Uncoated       | 1.00 <sup>b</sup>  | 1.17   | 1.47   | 2.00   | 2.90 <sup>a</sup>   | 3.03   | 3.33   | 4.93 <sup>a</sup>  |
| T1- 1% Chitosan    | 1.03 <sup>b</sup>  | 1.20   | 1.50   | 2.07   | 2.50 <sup>c</sup>   | 2.80   | 3.20   | 4.30 <sup>b</sup>  |
| T2- 2% Chitosan    | 1.00 <sup>b</sup>  | 1.10   | 1.43   | 2.03   | 2.67 <sup>abc</sup> | 2.80   | 3.00   | 3.67 <sup>c</sup>  |
| T3- 3% Chitosan    | 1.10 <sup>ab</sup> | 1.23   | 1.67   | 1.97   | 2.53 <sup>bc</sup>  | 2.90   | 3.17   | 4.47 <sup>ab</sup> |
| T4- 4% Chitosan    | 1.17 <sup>a</sup>  | 1.47   | 1.63   | 2.03   | 2.80 <sup>ab</sup>  | 2.87   | 3.00   | 4.47 <sup>ab</sup> |
| <b>F Value</b>     | 4.70               | 2.65   | 0.88   | 0.93   | 3.76                | 1.01   | 2.07   | 7.23               |
| <b>Pr (&gt; F)</b> | 0.0215             | 0.0962 | 0.5086 | 0.4853 | 0.0407              | 0.4460 | 0.1602 | 0.0053             |
| <b>CV (%)</b>      | 5.45               | 12.03  | 12.43  | 3.38   | 5.70                | 5.74   | 5.45   | 6.74               |

\*Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.

**Figure 4.** First day of Bell Pepper fruit storage (Study 1)**Figure 5.** Final day of Bell Pepper fruit storage (Study 1)

### 3.3 Firmness

Fruit firmness is one of the most important quality attributes influencing consumer preference and marketability. In this study, significant differences in firmness were observed at multiple storage intervals (days 2, 4, 6, 10, and 16). At day 6, fruits treated with 3% and 4% chitosan (T3 and T4, respectively) maintained significantly higher firmness (2.03 and 2.00, respectively) than the uncoated control (1.87). However, by day 10, fruits coated with 4% chitosan (T4) exhibited lower firmness (2.73) than those in other treatments, suggesting that excessively thick coatings may interfere with normal gas exchange, thereby accelerating softening. By day 16, the highest firmness was recorded in T0 (5.00), while coated fruits exhibited slightly lower values, with T2 (4.10) being the lowest. This may be attributed to natural textural changes during ripening, where chitosan coatings delayed but could not fully prevent the eventual breakdown of cell wall polysaccharides. It should be noted that in this palpation-based firmness index, lower scores reflect better-quality fruit (less dehydration), and higher scores indicate desiccation and tissue collapse. The overall average firmness index of coated fruits (2.54–2.66) was numerically lower than that of the uncoated control (2.70),



suggesting that chitosan coatings reduced desiccation-driven hardening during the early stages of storage. The marginal numerical difference, however, should be interpreted with caution, given the subjective nature of the measurement method.

The overall average firmness was highest in coated treatments, with T2 (2% chitosan) showing the most stable firmness across storage days. This confirms that moderate chitosan concentrations act as effective semipermeable barriers, delaying cell wall degradation and maintaining tissue integrity [20, 21]. These findings also agree with [17], who reported that chitosan coatings maintained firmness in tomatoes by reducing respiration and slowing the enzymatic degradation of cell wall structures, and align with [21], who noted that chitosan delays the activity of softening enzymes, such as polygalacturonase, thereby preserving texture. Similarly, [22] found that sweet peppers treated with chitosan retained firmness longer than untreated controls, due to the semipermeable nature of the coating, which modifies the fruit's internal microenvironment.

**Table 3.** Firmness of bell pepper fruit (*Capsicum annuum* L.) as influenced by different concentrations of an edible chitosan coating.

| Treatments         | Means (Per day) |                   |        |        |        |        |        |                    |
|--------------------|-----------------|-------------------|--------|--------|--------|--------|--------|--------------------|
|                    | 2               | 4                 | 6      | 8      | 10     | 12     | 14     | 16                 |
| T0- Uncoated       | 1.20            | 1.50 <sup>b</sup> | 1.87   | 2.00   | 3.00   | 3.00   | 4.00   | 5.00 <sup>a</sup>  |
| T1- 1% Chitosan    | 1.33            | 1.50 <sup>b</sup> | 2.10   | 2.10   | 3.00   | 3.07   | 4.03   | 4.17 <sup>cd</sup> |
| T2- 2% Chitosan    | 1.03            | 1.33 <sup>b</sup> | 1.87   | 2.00   | 2.97   | 3.00   | 4.00   | 4.10 <sup>d</sup>  |
| T3- 3% Chitosan    | 1.27            | 1.40 <sup>b</sup> | 2.03   | 2.00   | 3.00   | 3.00   | 4.00   | 4.60 <sup>b</sup>  |
| T4- 4% Chitosan    | 1.37            | 1.73 <sup>a</sup> | 2.00   | 2.00   | 2.73   | 3.00   | 4.00   | 4.40 <sup>bc</sup> |
| <b>F Value</b>     | 1.60            | 6.09              | 1.62   | 1.00   | 0.94   | 1.00   | 1.00   | 19.24              |
| <b>Pr (&gt; F)</b> | 0.2483          | 0.0095            | 0.2448 | 0.4516 | 0.4804 | 0.4516 | 0.4516 | 0.0001             |
| <b>CV (%)</b>      | 14.58           | 7.13              | 7.17   | 3.83   | 7.08   | 1.71   | 0.6444 | 3.23               |

Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.

### 3.4 Visual Quality Rating and Shelf Life

Table 4 presents the Visual Quality Rating (VQR) of bell peppers treated with various chitosan-based coatings over a 16-day storage period. VQR is a direct indicator of marketability and consumer acceptability, where higher scores reflect better visual appearance and delayed senescence. Statistical analysis revealed significant differences in VQR among treatments at several time points, particularly at 6, 8, 12, 14, and 16 days. These results suggest that the applied coatings had a marked influence on maintaining visual quality, especially during the mid- to late-storage period. The application of chitosan coatings significantly influenced the visual quality rating (VQR) and shelf life of bell peppers during storage. During the initial storage period (2–4 days), no significant differences were observed among treatments (T0–T4), and all fruits maintained high VQR values (>8.5). This indicates that during the early stage, both coated and uncoated bell peppers retained acceptable marketable quality, suggesting that chitosan's protective effect becomes more evident as storage duration increases. Similar findings were reported by [18], who emphasized that coatings have a greater impact during prolonged storage. From day 6 onward, significant differences emerged among treatments. T2 (2% chitosan) consistently exhibited the highest VQR among the treatments, including the control and other coating concentrations. At 6 and 8 days, bell peppers treated with 2% chitosan maintained superior visual appeal, showing less shriveling and discoloration. This result agrees with [23], who noted that moderate chitosan concentrations form a semipermeable film that reduces respiration rate and moisture loss, thereby delaying senescence. By the later storage period (12–16 days), the effectiveness of chitosan became more apparent. The uncoated fruits (T0) deteriorated rapidly, with VQR values dropping below the marketable quality threshold (<4.0), whereas all coated treatments (T1–T4) maintained significantly higher ratings. Among them, T2 (2% chitosan) stood out with the highest VQR, maintaining consumer-acceptable quality for up to 2 weeks of storage. Treatments T1 (1%) and T3 (3%) also maintained acceptable quality but were less effective than T2, whereas T4 (4%) offered no substantial improvement and, in some cases, performed slightly worse.



This trend supports the observation of [24], who reported that excessive coating thickness may impede gas exchange, potentially causing anaerobic conditions and hastening deterioration.

### 3.5 Decay Incidence

Decay incidence is a key indicator of microbial spoilage and overall postharvest disease resistance in fresh produce. As shown in Table 5, the decay incidence of bell peppers varied significantly among treatments ( $p < 0.001$ ), highlighting the influence of coating composition on microbial protection. The untreated control (T0) exhibited the highest decay incidence at 26.67%, with an average of 2.67 out of 10 fruits showing visible signs of decay by the end of the storage period. In contrast, all chitosan-based coatings were effective in reducing decay to varying degrees. The most notable result was observed in treatment T2 (2% Chitosan), which completely inhibited decay, recording a 0% incidence. This suggests a strong antimicrobial effect of 2% chitosan. Higher concentrations (T3 and T4) still reduced decay compared to the control but were less effective than T2, possibly because coating thickness affected gas balance. The antimicrobial properties of chitosan, attributed to its polycationic nature, which disrupts microbial cell membranes, explain this reduction [16, 21].

**Table 4.** VQR and shelf life of the bell pepper fruit (*Capsicum annuum* L.) as influenced by the different concentrations of chitosan edible coating.

| Treatments         | Total No. of Fruits | Decayed Fruits | Decay Incidence     |
|--------------------|---------------------|----------------|---------------------|
| T0- Uncoated       | 30                  | 8              | 26.67 <sup>a</sup>  |
| T1- 1% Chitosan    | 30                  | 4              | 13.33 <sup>ab</sup> |
| T2- 2% Chitosan    | 30                  | 0              | 0.00 <sup>b</sup>   |
| T3- 3% Chitosan    | 30                  | 7              | 23.33 <sup>a</sup>  |
| T4- 4% Chitosan    | 30                  | 6              | 20.00 <sup>a</sup>  |
| <b>F Value</b>     |                     |                | 5.56                |
| <b>Pr (&gt; F)</b> |                     |                | 0.0128              |
| <b>CV (%)</b>      |                     |                | 46.48               |

Means within the same column in a block followed by a common letter and/or without letter designations are not significantly different from each other at 5% level of significance.

**Table 5.** Decay incidence of bell pepper fruit (*Capsicum annuum* L.) as influenced by different concentrations of edible chitosan coating.

| Treatments         | Means (Per day) |        |                   |                   |        |                   |                   |                   |
|--------------------|-----------------|--------|-------------------|-------------------|--------|-------------------|-------------------|-------------------|
|                    | 2               | 4      | 6                 | 8                 | 10     | 12                | 14                | 16                |
| T0- Uncoated       | 8.73            | 8.67   | 7.40 <sup>b</sup> | 7.00 <sup>b</sup> | 6.60   | 5.90 <sup>c</sup> | 5.10 <sup>c</sup> | 3.47 <sup>b</sup> |
| T1- 1% Chitosan    | 8.97            | 8.70   | 7.80 <sup>b</sup> | 7.40 <sup>b</sup> | 7.10   | 6.67 <sup>b</sup> | 6.30 <sup>b</sup> | 5.33 <sup>a</sup> |
| T2- 2% Chitosan    | 9.00            | 9.00   | 8.60 <sup>a</sup> | 8.13 <sup>a</sup> | 7.53   | 7.37 <sup>a</sup> | 6.97 <sup>a</sup> | 6.33 <sup>a</sup> |
| T3- 3% Chitosan    | 8.93            | 8.80   | 7.77 <sup>b</sup> | 7.33 <sup>b</sup> | 7.13   | 6.63 <sup>b</sup> | 6.23 <sup>b</sup> | 5.37 <sup>a</sup> |
| T4- 4% Chitosan    | 8.90            | 8.63   | 7.53 <sup>b</sup> | 7.27 <sup>b</sup> | 7.13   | 6.63 <sup>b</sup> | 6.13 <sup>b</sup> | 5.27 <sup>a</sup> |
| <b>F Value</b>     | 0.62            | 0.81   | 5.47              | 4.71              | 3.00   | 5.58              | 42.17             | 6.63              |
| <b>Pr (&gt; F)</b> | 0.6574          | 0.5480 | 0.0135            | 0.0213            | 0.0723 | 0.0126            | 0.0000            | 0.0071            |
| <b>CV (%)</b>      | 2.56            | 3.26   | 4.42              | 4.55              | 4.67   | 5.73              | 2.91              | 13.57             |

Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.



**Figure 6.** Fungus-infected bell pepper (*Capsicum annuum* L.) fruits

### 3.6 pH

Table 6 shows that the pH values of bell peppers increased gradually throughout storage across all treatments, indicating the natural ripening process marked by the degradation of organic acids. Statistical analysis revealed significant differences among treatments at all storage intervals. On the final day, the control (T0) recorded the highest pH (5.20a), while the 2% chitosan treatment (T2) maintained the lowest (5.04c). This demonstrates that chitosan coatings, especially at 2%, effectively slowed down the rise in pH by reducing the consumption of organic acids. [23] and [19] explained that chitosan acts as a semipermeable barrier, lowering respiration and ethylene production, and significantly slowing pH increases by acting as a gas barrier, thus conserving acidity and delaying senescence. Similar findings were reported by [19] for coated strawberries and by [17, 18] for bell peppers, where coatings helped maintain lower pH levels than in untreated fruits. Additionally, treatments T3 (3% chitosan) and T4 (4% chitosan) showed intermediate values (5.17ab). These results suggest that chitosan coatings delayed the rise in pH by conserving organic acids, with T2 showing the most pronounced effect. The effect of chitosan on pH is attributed to its ability to form a semipermeable film that modifies the fruit's internal atmosphere by limiting oxygen diffusion and slowing respiration [21]. This reduced metabolic rate delays the consumption of organic acids such as citric, malic, and ascorbic acid, which are major contributors to the fruit's acidity.

**Table 6.** Initial, 5<sup>th</sup> day, 10<sup>th</sup> day, and final pH of bell pepper fruit (*Capsicum annuum* L.) as influenced by the different concentrations of chitosan edible coating.

| Treatments         | pH at              |                     |                      |                    |
|--------------------|--------------------|---------------------|----------------------|--------------------|
|                    | Initial            | 5 <sup>th</sup> day | 10 <sup>th</sup> day | Final              |
| T0- Uncoated       | 4.91 <sup>c</sup>  | 5.04 <sup>a</sup>   | 5.13 <sup>a</sup>    | 5.20 <sup>a</sup>  |
| T1- 1% Chitosan    | 4.95 <sup>ab</sup> | 5.01 <sup>a</sup>   | 5.06 <sup>b</sup>    | 5.11 <sup>b</sup>  |
| T2- 2% Chitosan    | 4.91 <sup>c</sup>  | 4.94 <sup>b</sup>   | 4.96 <sup>c</sup>    | 5.04 <sup>c</sup>  |
| T3- 3% Chitosan    | 4.98 <sup>a</sup>  | 5.03 <sup>a</sup>   | 5.12 <sup>a</sup>    | 5.17 <sup>ab</sup> |
| T4- 4% Chitosan    | 4.95 <sup>b</sup>  | 5.01 <sup>a</sup>   | 5.08 <sup>ab</sup>   | 5.17 <sup>ab</sup> |
| <b>F Value</b>     | 7.55               | 6.67                | 17.40                | 9.82               |
| <b>Pr (&gt; F)</b> | 0.0045             | 0.0070              | 0.0002               | 0.0017             |
| <b>CV (%)</b>      | 0.3696             | 0.5235              | 0.5578               | 0.6688             |

Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.

### 3.7 Total Soluble Solids

TSS, expressed in °Brix, reflects the sugar content of the fruit and is a key determinant of sweetness and overall taste quality. Table 7 showed variations across treatments from the first day until the last day of storage. During storage, the TSS of bell peppers generally increased until day 10, then declined at the final evaluation. This trend corresponds to the conversion of starches and organic acids into sugars during ripening, followed by sugar utilization in respiration at later stages [25]. The different chitosan coating concentrations influenced the total soluble solids (TSS) of bell pepper fruits during storage. Initial TSS values were similar across treatments (5.10–5.30 °B), with no significant differences. By the 5th day, TSS values started to vary slightly, ranging from 5.47 °B (T2) to 6.13 °B (T0), although differences remained statistically non-significant. Significant differences became apparent on the 10th day. The control (T0) registered the highest TSS (6.93), followed by T4 (6.40) and T3 (6.27). In contrast, T2 (5.87) maintained the lowest TSS value, while T1 (6.10) was intermediate. This trend indicates that uncoated fruits ripened more rapidly, leading to higher sugar accumulation, whereas chitosan coatings, especially at 2%, slowed the conversion of starch and organic acids into soluble sugars. By the final day of storage, highly significant differences were observed, with the control (T0) still exhibiting the highest TSS (5.93), while T2 decreased sharply to 4.07. Treatments T3 (5.50) and T4 (5.60) were statistically comparable but lower than the control. This pattern highlights that chitosan-coated fruits not only accumulated sugars more slowly but also retained them for a longer period by reducing respiration-driven sugar depletion, whereas the uncoated control experienced rapid ripening followed by sugar decline. The TSS trend is consistent with the pH and TA results. The control (T0), which showed the highest TSS values, also recorded the highest pH (5.20) and lowest TA (0.18) at the end of storage, reflecting advanced ripening and metabolic acid depletion. Conversely, T2 fruits exhibited the lowest TSS (4.07), lowest pH (5.04), and highest TA (0.26), indicating delayed ripening and preservation of the acid–sugar balance.

These findings confirm the interconnected role of sugar accumulation and acid degradation during fruit maturation. Similar results were reported by [26] for cold-stored fruit and by [27] for litchis, where chitosan coatings reduced sugar accumulation by slowing respiration. [28] Further explained that coatings limit oxygen availability, thereby decreasing enzymatic activities responsible for sugar metabolism. In bell peppers, [17]. Also observed that chitosan significantly reduced the rise in TSS, supporting the present findings.

**Table 7.** Initial, 5<sup>th</sup> day, 10<sup>th</sup> day, and final TSS of bell pepper fruit (*Capsicum annuum* L.) as influenced by different concentrations of edible chitosan coating.

| Treatments         | TSS (°Brix) at |                     |                      |                    |
|--------------------|----------------|---------------------|----------------------|--------------------|
|                    | Initial        | 5 <sup>th</sup> day | 10 <sup>th</sup> day | Final              |
| T0- Uncoated       | 5.30           | 6.13                | 6.93 <sup>a</sup>    | 5.93 <sup>a</sup>  |
| T1- 1% Chitosan    | 5.30           | 5.63                | 6.10 <sup>bc</sup>   | 5.30 <sup>b</sup>  |
| T2- 2% Chitosan    | 5.30           | 5.47                | 5.87 <sup>c</sup>    | 4.07 <sup>c</sup>  |
| T3- 3% Chitosan    | 5.10           | 5.70                | 6.27 <sup>bc</sup>   | 5.50 <sup>ab</sup> |
| T4- 4% Chitosan    | 5.20           | 5.80                | 6.40 <sup>b</sup>    | 5.60 <sup>ab</sup> |
| <b>F Value</b>     | 0.71           | 2.63                | 7.73                 | 24.28              |
| <b>Pr (&gt; F)</b> | 0.6059         | 0.0977              | 0.0042               | 0.0000             |
| <b>CV (%)</b>      | 3.52           | 4.60                | 3.94                 | 4.77               |

Means within the same column in a block followed by a common letter and/or without letter designations are not significantly different from each other at 5% level of significant.

### 3.8 Titratable Acidity

TA is another critical indicator of fruit quality, reflecting the concentration of organic acids, such as citric, malic, and ascorbic acids. Acidity provides peppers with their characteristic tangy flavor and contributes to their microbial stability. Table 8 shows that the titratable acidity (TA) of bell pepper fruits declined progressively during storage across all treatments, reflecting the natural utilization of organic acids as substrates in respiration and metabolic reactions. Statistical analysis revealed significant differences among treatments starting at the 5th day, with the differences becoming more pronounced at the 10th and final days. Initial TA values were comparable (0.38–0.41%) and did not differ significantly. By the 5th day, however,

statistical differences emerged; the highest TA was observed in T2 (0.36a), while the lowest was in T4 (0.30b) and T0 (0.31b). This early variation suggests that chitosan coatings, particularly at 2%, were effective in slowing down the metabolic depletion of organic acids. Moreover, differences became more pronounced at the 10th day, where the control (T0) showed a sharp decline to 0.23c, while T2 (0.31a) and T3 (0.30a) maintained significantly higher acidity. By the final day, TA values ranged from 0.18c (T0) to 0.26a (T2), with T3 (0.24ab) and T4 (0.23ab) maintaining intermediate levels. These results clearly indicate that 2% chitosan (T2) was most effective in preserving acidity, delaying senescence, and maintaining postharvest quality. Table 8 clearly shows that the TA trend is closely associated with the observed pH and TSS results. The control (T0), which exhibited the lowest TA (0.18c), also recorded the highest pH (5.20a) and TSS (5.93a) at the end of storage, reflecting advanced ripening characterized by acid depletion and sugar accumulation. Conversely, T2 maintained the highest TA (0.26a), the lowest pH (5.04c), and the lowest TSS (4.07c), suggesting delayed metabolic activity and preservation of the acid–sugar balance. This interdependence confirms that chitosan reduced the degradation of organic acids into sugars and lowered sugar consumption during respiration. Coatings such as chitosan act as semipermeable barriers that slow respiration and ethylene production, thereby conserving organic acids and delaying their conversion into sugars [19, 23]. This mechanism has also been reported in strawberries [28], cherries [29], and plums [30], where coated fruits exhibited higher TA and lower TSS compared to controls, and it was noted by [30] that coatings act as protective barriers that limit oxygen diffusion, slowing enzymatic activities involved in organic acid metabolism.

**Table 8.** Initial, 5<sup>th</sup> day, 10<sup>th</sup> day, and final Titratable Acidity (TA) of bell pepper fruit (*Capsicum annuum* L.) as influenced by the different concentrations of chitosan edible coating.

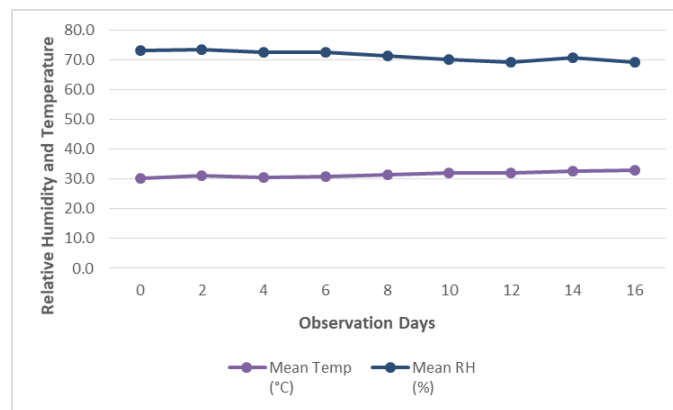
| Treatments         | TA (%) at |                     |                      |                    |
|--------------------|-----------|---------------------|----------------------|--------------------|
|                    | Initial   | 5 <sup>th</sup> day | 10 <sup>th</sup> day | Final              |
| T0- Uncoated       | 0.38      | 0.31 <sup>b</sup>   | 0.23 <sup>c</sup>    | 0.18 <sup>c</sup>  |
| T1- 1% Chitosan    | 0.39      | 0.32 <sup>b</sup>   | 0.28 <sup>ab</sup>   | 0.21 <sup>bc</sup> |
| T2- 2% Chitosan    | 0.41      | 0.36 <sup>a</sup>   | 0.31 <sup>a</sup>    | 0.26 <sup>a</sup>  |
| T3- 3% Chitosan    | 0.39      | 0.33 <sup>ab</sup>  | 0.30 <sup>a</sup>    | 0.24 <sup>ab</sup> |
| T4- 4% Chitosan    | 0.39      | 0.30 <sup>b</sup>   | 0.26 <sup>bc</sup>   | 0.23 <sup>ab</sup> |
| <b>F Value</b>     | 0.90      | 3.97                | 7.25                 | 6.97               |
| <b>Pr (&gt; F)</b> | 0.4994    | 0.0349              | 0.0042               | 0.0060             |
| <b>CV (%)</b>      | 5.10      | 6.17                | 7.25                 | 8.93               |

Means within the same column in a block followed by a common letter and/or without letter designations are not significantly different from each other at 5% level of significant.

### 3.9 Relative Humidity and Temperature

Ambient temperature and relative humidity (RH) were monitored twice daily throughout the 16-day storage period at the CTU-Argao BENRC Laboratory under uncontrolled tropical indoor conditions. Mean daily temperature ranged from 30.3°C (Day 0) to 33.0°C (Day 16), with an overall mean of 31.6°C, while mean daily RH ranged from 69.2% to 73.4% (overall mean: 71.5%). Morning readings were consistently cooler and more humid (AM temperature: 29.6–31.5°C; AM RH: 72.3–78.1%), while afternoon readings were warmer and drier (PM temperature: 31.0–34.4°C; PM RH: 65.8–69.9%). These conditions followed a clear progressive trend over the storage period mean daily temperature increased gradually from Day 0 through Day 16, while RH declined correspondingly, a pattern that closely mirrors the deterioration trajectories observed across all Study 1 quality parameters. From Day 8 onward, mean temperatures consistently exceeded 31.5°C, and by Day 16, afternoon readings reached 34.4°C, coinciding with the period of accelerated pH rise and TSS increase across all treatments, as organic acids were consumed more rapidly and starches were converted into soluble sugars. The concurrent decline in afternoon RH to as low as 65.8% reinforced transpirational water loss from fruit surfaces, directly consistent with the escalating weight loss and shriveling observed across all treatments, most notably from Day 10 onward, where quality deterioration accelerated sharply in the uncoated control.

The chitosan-coated treatments, particularly T2 (2% chitosan), demonstrated better buffering against worsening ambient conditions, maintaining lower pH and higher titratable acidity, and significantly reducing weight loss relative to the control throughout storage. These environmental data therefore provide the necessary quantitative context for interpreting treatment differences in Study 1, and results obtained at ~31.6°C and ~71.5% RH should be considered when making comparisons with studies conducted under refrigerated or temperate storage conditions.



**Figure 7.** Relative humidity (%) and temperature (°C) during storage of bell pepper (*Capsicum annuum* L.) fruits

**Table 9.** Percent (%) weight loss of the bell pepper fruit (*Capsicum annuum* L.) as influenced by the different chitosan and plant extract-based edible coating.

| Treatments                                                  | Means (Per day)    |        |        |        |        |        |        |        |
|-------------------------------------------------------------|--------------------|--------|--------|--------|--------|--------|--------|--------|
|                                                             | 2                  | 4      | 6      | 8      | 10     | 12     | 14     | 16     |
| T0- 2% Chitosan                                             | 6.06 <sup>bc</sup> | 14.08  | 23.10  | 28.60  | 38.30  | 44.69  | 52.84  | 62.02  |
| T1- 50ml of 2% Chitosan + 50ml of 50% Aloe Vera             | 7.03 <sup>a</sup>  | 14.07  | 23.77  | 31.98  | 39.88  | 46.19  | 54.97  | 61.55  |
| T2- 33ml of 2% Chitosan + 67ml of 50% Aloe Vera             | 5.90 <sup>c</sup>  | 13.69  | 21.56  | 30.84  | 38.12  | 44.59  | 52.67  | 60.88  |
| T3- 50ml of 2% Chitosan + 50ml of 50% Moringa Leaf Extracts | 7.32 <sup>a</sup>  | 15.61  | 23.41  | 31.64  | 39.69  | 46.81  | 55.42  | 63.38  |
| T4- 33ml of 2% Chitosan + 67ml of 50% Moringa Leaf Extracts | 6.88 <sup>ab</sup> | 15.58  | 23.10  | 31.99  | 39.45  | 45.60  | 53.47  | 61.63  |
| <b>F Value</b>                                              | 4.73               | 1.38   | 0.73   | 1.22   | 0.33   | 0.36   | 0.44   | 0.19   |
| <b>Pr (&gt; F)</b>                                          | 0.0211             | 0.3094 | 0.5918 | 0.3624 | 0.8499 | 0.8297 | 0.7807 | 0.9369 |
| <b>CV (%)</b>                                               | 7.51               | 9.25   | 7.46   | 7.22   | 6.30   | 6.03   | 6.12   | 5.91   |

Means within the same column in a block followed by a common letter and/or without letter designations are not significantly different from each other at 5% level of significant.

### 3.10 Weight Loss

The weight loss of bell pepper fruits was monitored over a 16-day storage period to evaluate the effects of different chitosan-based treatments combined with Aloe vera and Moringa leaf extracts. The results are presented in Table 9. At 2 days, significant differences were observed among the treatments. The lowest weight loss was recorded in T2 (5.90%), which combined 33 mL of 2% chitosan with 67 mL of 50% Aloe vera gel. This suggests that the increased proportion of Aloe vera contributed positively to reducing moisture loss, likely due to its gel-like, hygroscopic nature and film-forming ability. In contrast, T3 (7.32%), which used chitosan and Moringa leaf extract in equal volumes, showed the highest weight loss at this time point, although it was not statistically different from T0, T1, and T4. From day 4 onward, no statistically significant differences were



observed among the treatments, although slight trends were noted. This aligns with the findings of [31], who reported that while chitosan reduces respiration and transpiration, additional plant extracts may not always significantly improve weight-loss control. Table 9 shows that T2 consistently exhibited slightly lower weight loss than other treatments, particularly at 6 and 8 days. However, by day 10 and beyond, the weight loss values across all treatments began to converge, with final readings at 16 days ranging from 60.88% (T2) to 63.38% (T3). Despite this convergence, T2 generally maintained the lowest weight loss throughout the storage period, indicating that a higher Aloe vera ratio may enhance the barrier properties of the chitosan coating. The slight reduction in weight loss observed in T2 (33ml of 2% chitosan + 67ml of 50% of aloe vera) suggests that Aloe vera gel may enhance moisture barrier properties due to its high polysaccharide and mucilaginous content, as previously observed in mango and tomato fruits coated with Aloe vera-based films [18]. The lack of significant differences at later stages suggests that the effectiveness of the chitosan-based coatings in reducing weight loss diminishes over extended storage periods, potentially due to film degradation or increased respiration rates in the fruit.

### 3.11 Fruit Shriveling

Shriveling is a key indicator of moisture loss and overall postharvest deterioration in fresh produce. Table 10 presents the shriveling scores of bell peppers treated with various chitosan-based coatings over a 16-day storage period. Shriveling was assessed using a subjective scale, with higher values indicating greater visible wrinkling and surface collapse. The progression of shriveling in bell pepper fruits increased across storage duration in all treatments, as shown in Table 10. At the early stages of storage (2 and 4 days), shriveling scores were low across all treatments, with no significant differences observed. This suggests that during the initial storage period, moisture loss was minimal and not visibly distinguishable among the treatments. However, significant differences emerged on the 6th day, with T2 (33ml of 2% chitosan + 67ml of 50% aloe vera) consistently exhibiting the lowest shriveling scores (1.07 at day 6 and 1.23 at day 8), compared to T0, T1, T3, and T4. On average, T2 recorded a 1.85 shriveling score, significantly lower than the other treatments, which ranged from 2.25 to 2.31. This suggests that the higher proportion of aloe vera enhanced chitosan's protective effect. Aloe vera gel is known for its hydrophilic polysaccharides and moisture-retaining capacity, which minimize transpirational water loss [32]. Similarly, [14] highlighted that aloe vera coatings reduce water vapor permeability and effectively delay dehydration in fresh produce, effectively delaying shriveling and dehydration in peppers by reducing transpiration losses [33]. Thus, T2 effectively reduced fruit shriveling by combining the film-forming property of chitosan with the water-retentive capability of aloe vera.

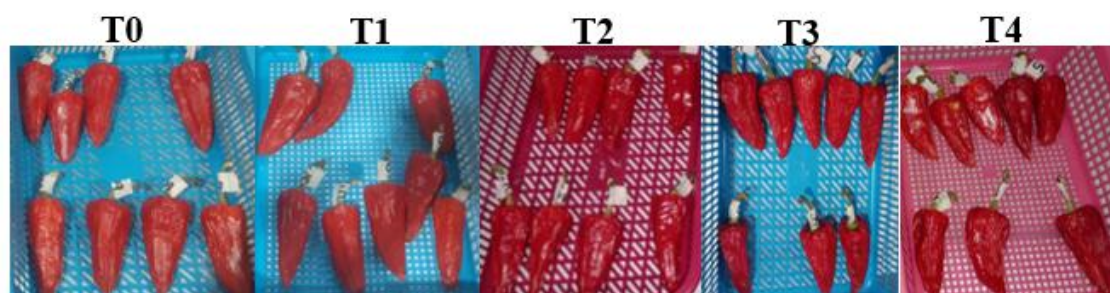
**Table 10.** Shriveling of the bell pepper fruit (*Capsicum annuum* L.) as influenced by the different chitosan and plant extract-based edible coating.

| Treatments                                                  | Means (Per day) |        |                   |                   |        |                   |                   |                    |
|-------------------------------------------------------------|-----------------|--------|-------------------|-------------------|--------|-------------------|-------------------|--------------------|
|                                                             | 2               | 4      | 6                 | 8                 | 10     | 12                | 14                | 16                 |
| T0- 2% Chitosan                                             | 1.13            | 1.17   | 1.40 <sup>a</sup> | 1.93 <sup>a</sup> | 2.27   | 2.77 <sup>a</sup> | 3.30 <sup>a</sup> | 4.30 <sup>a</sup>  |
| T1- 50ml of 2% Chitosan + 50ml of 50% Aloe Vera             | 1.10            | 1.23   | 1.40 <sup>a</sup> | 1.83 <sup>a</sup> | 2.37   | 2.77 <sup>a</sup> | 3.43 <sup>a</sup> | 4.37 <sup>a</sup>  |
| T2- 33ml of 2% Chitosan + 67ml of 50% Aloe Vera             | 1.00            | 1.03   | 1.07 <sup>b</sup> | 1.23 <sup>b</sup> | 1.87   | 2.17 <sup>b</sup> | 2.80 <sup>b</sup> | 3.67 <sup>b</sup>  |
| T3- 50ml of 2% Chitosan + 50ml of 50% Moringa Leaf Extracts | 1.10            | 1.20   | 1.57 <sup>a</sup> | 1.73 <sup>a</sup> | 2.40   | 2.77 <sup>a</sup> | 3.40 <sup>a</sup> | 4.03 <sup>ab</sup> |
| T4- 33ml of 2% Chitosan + 67ml of 50% Moringa Leaf Extracts | 1.07            | 1.17   | 1.40 <sup>a</sup> | 1.67 <sup>a</sup> | 2.30   | 2.67 <sup>a</sup> | 3.47 <sup>a</sup> | 4.27 <sup>a</sup>  |
| <b>F Value</b>                                              | 0.82            | 1.04   | 3.66              | 6.16              | 3.31   | 3.83              | 5.25              | 3.69               |
| <b>Pr (&gt; F)</b>                                          | 0.5403          | 0.4337 | 0.0438            | 0.0091            | 0.0568 | 0.0388            | 0.0153            | 0.0429             |
| <b>CV (%)</b>                                               | 8.95            | 11.13  | 12.10             | 11.19             | 9.15   | 8.79              | 6.35              | 6.26               |

Means within the same column in a block followed by a common letter and/or without letter designations are not significantly different from each other at 5% level of significant.



**Figure 8.** First day of Bell Pepper fruit storage (Study 2)



**Figure 9.** Final day of Bell Pepper fruit storage (Study 2)

### 3.12 Firmness

Firmness is a critical quality attribute in bell peppers. In this study, firmness was measured using a subjective palpation-based index, with higher scores indicating greater dehydration and tissue deterioration, not improved quality—consistent with the scale interpretation in Study 1. Loss of desirable texture during postharvest storage is primarily caused by cellular degradation, enzymatic activity, and moisture loss. The effects of various chitosan- and plant extract-based treatments on bell pepper firmness were assessed over a 16-day storage period, and the results are summarized in Table 11. Firmness index values increased progressively during storage for all treatments. Consistent with Study 1, higher index values in this palpation-based scale reflect greater desiccation and tissue deterioration, not improved quality. In bell pepper, higher firmness index readings are associated with moisture loss, tissue collapse, and increased shriveling, all of which negatively affect textural quality and consumer acceptability. Treatments yielding lower firmness index values thus indicate better preservation of quality. During the first 4 days of storage (2 and 4 days), there were no significant differences in firmness among treatments, indicating that all treatments were similarly effective at retaining initial fruit texture in the early postharvest stage. Statistical analysis confirmed significant differences among treatments on day 6. At this stage, T2 (1.10) recorded the lowest firmness, significantly lower than T3 (1.63) and T4 (1.50), which exhibited the greatest firmness loss. At day 16, the highest firmness values were observed in T4 (4.43) and T3 (4.40), followed by T1 (4.37) and T0 (4.20), while the lowest was recorded in T2 (3.87). On average, T2 (2.01) was significantly lower than T3 (2.40) and T4 (2.35), whereas T0 (2.23) and T1 (2.32) showed intermediate values.

Across the storage period, the average firmness ranged from 2.01 in T2 to 2.40 in T3, indicating that fruits in T2 consistently showed slower firmness increases, thus indicating better quality preservation. On the other hand, the relatively higher firmness values in T1, T3, T4, and even in the control T0 indicate that these fruits deteriorated faster, with more pronounced dehydration and tissue hardening. The effectiveness of T2 (33ml of 2% chitosan + 67ml of 50% aloe vera) in maintaining a lower firmness trend can be attributed to the hydrophilic polysaccharides and bioactive compounds in aloe vera, which delay water loss and maintain cell turgidity [32]. Conversely, treatments containing moringa leaf extracts (T3 and T4) recorded higher firmness values, suggesting that while moringa provides antioxidant protection [34, 35], it was less effective in controlling transpiration losses compared to aloe vera. Similarly, T1 (50 ml of 2% chitosan + 50ml of 50% aloe vera) maintained intermediate firmness values but still showed faster hardening than T2, likely due to the lower aloe vera concentration. The control (T0, 2% chitosan alone) exhibited firmness values similar to those

of T1, confirming the protective effect of chitosan, although aloe vera supplementation provided a greater advantage. These findings are consistent with [33], who reported that aloe vera-based coatings slowed down textural deterioration by preventing excessive water loss in coated fruits. Therefore, while all treatments delayed firmness increase, T2 formulation (33ml of 2% chitosan + 67ml of 50% aloe vera) emerged as the most effective in preserving desirable texture by maintaining the lowest firmness values throughout storage.

### 3.13 Visual Quality Rating and Shelf Life

The Visual Quality Rating (VQR), a subjective measure based on visual attributes such as color, freshness, shriveling, and decay, was used to assess the shelf life of bell pepper fruits over 16 days of storage (Table 12). Visual quality rating is a key parameter that reflects consumer acceptability of fresh produce during storage. Table 10 shows that the VQR remained relatively high across all treatments during the first 4 days, with no significant differences observed. However, by the 6th day, a trend emerged in which T2 (33ml of 2% chitosan + 67ml of 50% aloe vera) recorded a higher VQR (8.87) than T3 (7.93) and T4 (8.27), although the differences were not yet statistically significant. Significant variation emerged on the 8th day, with T2 maintaining the highest rating (8.67), while T3 (7.87) and T4 (8.07) had noticeably lower scores. This pattern persisted on days 10, 12, and 14, with T2 consistently recording significantly higher VQR scores than T3 and T4. By day 16, T2 still had the highest VQR (5.27), which was significantly greater than the other treatments, whose scores ranged from 4.07 to 4.53. Biologically, the superior VQR of T2 reflects its effectiveness in delaying external symptoms of deterioration such as shriveling, discoloration, and surface pitting. Aloe vera's natural polysaccharides form a thin, semipermeable film that reduces moisture loss and modifies internal gas

exchange, thereby slowing respiration and senescence [32, 33]. Chitosan also contributes by lowering ethylene production and providing antimicrobial protection [21], but its effect was clearly enhanced when combined with a higher proportion of aloe vera. By contrast, T3 and T4 (chitosan + moringa extracts) exhibited lower VQR values as storage progressed, indicating earlier deterioration. Although moringa leaf extracts contain antioxidants that may slow oxidative damage [34], they were less effective in preserving external appearance compared to aloe vera, possibly due to weaker film-forming properties. Similarly, T0 (2% chitosan alone) and T1 (50ml of 2% chitosan + 50ml of 50% of aloe vera) performed moderately well but did not maintain marketable quality as long as T2, suggesting that the higher aloe vera concentration was critical in extending shelf life.

**Table 11.** Firmness of the bell pepper fruit (*Capsicum annuum* L.) as influenced by the different chitosan and plant extract-based edible coatings.

| Treatments                                                  | Means (Per day) |        |                    |        |        |        |                    |                    |
|-------------------------------------------------------------|-----------------|--------|--------------------|--------|--------|--------|--------------------|--------------------|
|                                                             | 2               | 4      | 6                  | 8      | 10     | 12     | 14                 | 16                 |
| T0- 2% Chitosan                                             | 1.13            | 1.20   | 1.33 <sup>b</sup>  | 1.87   | 2.27   | 2.60   | 3.20 <sup>ab</sup> | 4.20 <sup>ab</sup> |
| T1- 50ml of 2% Chitosan + 50ml of 50% Aloe Vera             | 1.10            | 1.23   | 1.43 <sup>b</sup>  | 1.87   | 2.30   | 2.90   | 3.33 <sup>ab</sup> | 4.37 <sup>a</sup>  |
| T2- 33ml of 2% Chitosan + 67ml of 50% Aloe Vera             | 1.00            | 1.03   | 1.10 <sup>c</sup>  | 1.50   | 2.07   | 2.50   | 3.03 <sup>b</sup>  | 3.87 <sup>b</sup>  |
| T3- 50ml of 2% Chitosan + 50ml of 50% Moringa Leaf Extracts | 1.10            | 1.20   | 1.63 <sup>a</sup>  | 2.03   | 2.43   | 2.90   | 3.47 <sup>a</sup>  | 4.40 <sup>a</sup>  |
| T4- 33ml of 2% Chitosan + 67ml of 50% Moringa Leaf Extracts | 1.07            | 1.10   | 1.50 <sup>ab</sup> | 1.90   | 2.47   | 2.83   | 3.50 <sup>a</sup>  | 4.43 <sup>a</sup>  |
| <b>F Value</b>                                              | 0.82            | 1.58   | 10.00              | 2.69   | 2.64   | 1.41   | 4.01               | 3.57               |
| <b>Pr (&gt; F)</b>                                          | 0.5403          | 0.2549 | 0.0016             | 0.0932 | 0.0972 | 0.2987 | 0.0340             | 0.0466             |
| <b>CV (%)</b>                                               | 8.95            | 10.01  | 7.82               | 11.44  | 7.34   | 9.81   | 5.06               | 5.04               |

Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.

### 3.14 Decay Incidence

Decay incidence is one of the most critical indicators of postharvest quality, as it directly affects marketability, food safety, and consumer acceptance. Table 11 presents the total number of decayed fruits and the decay percentages among bell peppers treated with different combinations of chitosan and plant-based extracts after storage. All treatments began with 10 fruits per treatment, ensuring equal sample sizes for accurate comparison. Table 13 statistical analysis revealed no significant differences among treatments. Despite this, a clear pattern emerged, showing that coating composition influenced the degree of decay. The lowest decay incidence was observed in T2 (6.67%), while higher incidences were recorded in T1 (26.67%), T4 (23.33%), and both T0 and T3 (16.67%). Although not statistically different, these numerical variations suggest that treatment combinations exerted differential effects on disease suppression. As treatment differences were not statistically significant ( $p = 0.0567$ ), specific mechanistic conclusions cannot be drawn. However, the numerical trend toward lower T2 decay is biologically plausible, given that both chitosan and Aloe vera possess antimicrobial properties. Chitosan disrupts microbial cell membranes through its polycationic nature, potentially inhibiting spore germination [21, 36]. Aloe vera complements this by providing additional antimicrobial compounds, such as anthraquinones, acemannan, and saponins, which have been reported to inhibit fungal growth and bacterial proliferation [37]. The higher proportion of aloe vera in T2 likely enhanced this protective effect, contributing to its reduced decay incidence. By comparison, T1 (50 ml of 2% chitosan + 50 ml of 50% aloe vera) showed higher decay incidence, which may be attributed to the lower aloe vera concentration, offering less bioactive antimicrobial protection than T2. Similarly, T3 and T4 (chitosan + moringa) exhibited moderate to high decay levels, indicating that while moringa extracts are rich in antioxidants and have some antimicrobial potential [34], they were less effective than aloe vera in controlling postharvest decay. The control treatment (T0, 2% chitosan alone) provided intermediate protection, consistent with previous studies reporting chitosan's ability to delay microbial infection in fruits [36].

**Table 12.** VQR and shelf life of the bell pepper fruit (*Capsicum annuum* L.) as influenced by the different chitosan and plant extract-based edible coating.

| Treatments                                                  | Total No. of Fruits | Decayed Fruits | Decay Incidence |
|-------------------------------------------------------------|---------------------|----------------|-----------------|
| T0- 2% Chitosan                                             | 30                  | 5              | 16.67           |
| T1- 50ml of 2% Chitosan + 50ml of 50% Aloe Vera             | 30                  | 8              | 26.67           |
| T2- 33ml of 2% Chitosan + 67ml of 50% Aloe Vera             | 30                  | 2              | 6.67            |
| T3- 50ml of 2% Chitosan + 50ml of 50% Moringa Leaf Extracts | 30                  | 5              | 16.67           |
| T4- 33ml of 2% Chitosan + 67ml of 50% Moringa Leaf Extracts | 30                  | 7              | 23.33           |
| <b>F Value</b>                                              |                     |                | 3.31            |
| <b>Pr (&gt; F)</b>                                          |                     |                | 0.0567          |
| <b>CV (%)</b>                                               |                     |                | 40.57           |

Means within the same column in a block followed by a common letter and/or without letter designations are not significantly different from each other at 5% level of significance.



**Table 13.** Decay incidence of the bell pepper fruit (*Capsicum annuum* L.) as influenced by the different chitosan and plant extract-based edible coating.

| Treatments                                                  | Means (Per day) |        |        |                     |                     |                    |                    |                   |
|-------------------------------------------------------------|-----------------|--------|--------|---------------------|---------------------|--------------------|--------------------|-------------------|
|                                                             | 2               | 4      | 6      | 8                   | 10                  | 12                 | 14                 | 16                |
| T0- 2% Chitosan                                             | 8.67            | 8.67   | 8.47   | 8.33 <sup>ab</sup>  | 7.80 <sup>ab</sup>  | 7.20 <sup>ab</sup> | 6.33 <sup>a</sup>  | 4.40 <sup>b</sup> |
| T1- 50ml of 2% Chitosan + 50ml of 50% Aloe Vera             | 8.67            | 8.53   | 8.40   | 8.27 <sup>abc</sup> | 7.73 <sup>abc</sup> | 7.00 <sup>b</sup>  | 6.20 <sup>ab</sup> | 4.53 <sup>b</sup> |
| T2- 33ml of 2% Chitosan + 67ml of 50% Aloe Vera             | 8.67            | 8.93   | 8.87   | 8.67 <sup>a</sup>   | 7.93 <sup>a</sup>   | 7.40 <sup>a</sup>  | 6.67 <sup>a</sup>  | 5.27 <sup>a</sup> |
| T3- 50ml of 2% Chitosan + 50ml of 50% Moringa Leaf Extracts | 8.33            | 8.60   | 7.93   | 7.87 <sup>c</sup>   | 7.33 <sup>c</sup>   | 6.73 <sup>c</sup>  | 5.73 <sup>b</sup>  | 4.07 <sup>b</sup> |
| T4- 33ml of 2% Chitosan + 67ml of 50% Moringa Leaf Extracts | 8.67            | 8.67   | 8.27   | 8.07 <sup>bc</sup>  | 7.40 <sup>bc</sup>  | 6.67 <sup>c</sup>  | 5.73 <sup>b</sup>  | 4.33 <sup>b</sup> |
| <b>F Value</b>                                              | 0.20            | 1.04   | 2.79   | 4.41                | 3.64                | 21.50              | 7.30               | 4.15              |
| <b>Pr (&gt; F)</b>                                          | 0.9327          | 0.4337 | 0.0855 | 0.0259              | 0.0443              | 0.0001             | 0.0051             | 0.0308            |
| <b>CV (%)</b>                                               | 6.71            | 2.97   | 4.18   | 3.01                | 3.10                | 1.65               | 4.21               | 8.47              |

Means within the same column in a block followed by a common letter and/or without letter designations are not significantly different from each other at 5% level of significant.

### 3.15 pH

The pH values showed significant differences across treatments at all storage stages. Table 14 shows that T3 (50ml of 2% chitosan + 50ml of 20% moringa leaf extract) consistently recorded the highest pH values (4.73–4.90), followed by T4 (33ml of 2% chitosan + 67ml of 20% moringa extract), while T0 (2% chitosan) and aloe vera-based coatings (T1 and T2) exhibited relatively lower values. While all treatments exhibited an increase in pH over time, consistent with the natural metabolism of organic acids into sugars and other compounds during ripening [17], the degree of increase varied. Treatments with aloe vera (T1 and T2) and pure 2% chitosan (T0) maintained relatively lower pH values compared to the moringa-based treatments (T3 and T4). This is notable since lower pH values typically indicate better preservation of fruit acidity and greater metabolic stability, as fruits tend to become more alkaline during ripening and microbial spoilage. Additionally, the T1 and T2 treatments, both incorporating Aloe vera, were more effective at maintaining lower pH, likely due to Aloe vera's antimicrobial properties, which slow microbial activity and acid degradation. Although T3 (50ml of 2% chitosan + 50ml of 20% moringa extract) and T4 (33ml of 2% chitosan + 67ml of 20% moringa extract) recorded the highest pH values, suggesting faster reduction in acidity, the aloe vera-based coatings slowed the rise in pH more effectively. Aloe vera's gel matrix likely acted as a semipermeable barrier, reducing respiration rates and microbial activity, thereby maintaining higher acid content and lower pH levels for a longer duration [32, 33]. Similarly, chitosan itself has been widely reported to delay pH increases by reducing enzymatic and microbial activity in coated fruits [17, 18, 23, 38, 39].

### 3.16 Total Soluble Solids (TSS)

The TSS values in bell pepper fruits showed no statistically significant differences among treatments across storage time, as summarized in Table 15. Despite this, a clear trend was observed: TSS levels increased until the 10th day, then declined slightly by the final observation. This pattern is typical of ripening fruits, in which starch reserves and complex carbohydrates are hydrolyzed into simple sugars, leading to an increase in soluble solids. At the same time, the subsequent decline reflects their utilization as substrates for respiration and other metabolic processes [17, 23]. Among the treatments, T2 (33 ml of 2% chitosan + 67 ml of 50% aloe vera) recorded the highest final TSS value (5.93 °Brix), followed by T3 (5.67 °Brix). Although not statistically significant, this suggests that a higher proportion of aloe vera gel may have contributed to delayed sugar depletion. Aloe vera gel has been reported to act as a semipermeable barrier that reduces water loss, respiration rate, and oxidative stress, thereby slowing down sugar metabolism [32, 33]. Similarly, chitosan



coatings are known to reduce respiration and ethylene production, thereby helping preserve sugars during storage [36, 40]. The slight variations in TSS among treatments may also be linked to the differing physiological effects of aloe vera and moringa extracts. Aloe vera-based coatings (T1 and T2) tended to support higher sugar retention, while moringa-based coatings (T3 and T4) maintained stable but relatively lower TSS levels. This could be attributed to moringa's higher antioxidant activity, which focuses more on reducing oxidative degradation rather than directly influencing sugar preservation.

**Table 14.** Initial, 5<sup>th</sup> day, 10<sup>th</sup> day and final pH of bell pepper fruit (*Capsicum annuum* L.) as influenced by the different chitosan and plant extract-based edible coating.

| Treatments                                                  | pH at             |                     |                      |                    |
|-------------------------------------------------------------|-------------------|---------------------|----------------------|--------------------|
|                                                             | Initial           | 5 <sup>th</sup> day | 10 <sup>th</sup> day | Final              |
| T0- 2% Chitosan                                             | 4.57 <sup>c</sup> | 4.64 <sup>c</sup>   | 4.67 <sup>b</sup>    | 4.73 <sup>bc</sup> |
| T1- 50ml of 2% Chitosan + 50ml of 50% Aloe Vera             | 4.53 <sup>c</sup> | 4.62 <sup>c</sup>   | 4.68 <sup>b</sup>    | 4.74 <sup>bc</sup> |
| T2- 33ml of 2% Chitosan + 67ml of 50% Aloe Vera             | 4.52 <sup>c</sup> | 4.59 <sup>c</sup>   | 4.63 <sup>b</sup>    | 4.68 <sup>c</sup>  |
| T3- 50ml of 2% Chitosan + 50ml of 50% Moringa Leaf Extracts | 4.73 <sup>a</sup> | 4.82 <sup>a</sup>   | 4.85 <sup>a</sup>    | 4.90 <sup>a</sup>  |
| T4- 33ml of 2% Chitosan + 67ml of 50% Moringa Leaf Extracts | 4.66 <sup>b</sup> | 4.72 <sup>b</sup>   | 4.77 <sup>a</sup>    | 4.83 <sup>ab</sup> |
| <b>F Value</b>                                              | 23.26             | 15.39               | 12.31                | 5.75               |
| <b>Pr (&gt; F)</b>                                          | 0.0000            | 0.0003              | 0.0007               | 0.0115             |
| <b>CV (%)</b>                                               | 0.6964            | 0.8661              | 0.9476               | 1.32               |

Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.

**Table 15.** Initial, 5<sup>th</sup> day, 10<sup>th</sup> day, and final TSS of bell pepper fruit (*Capsicum annuum* L.) as influenced by the different chitosan and plant extract-based edible coating.

| Treatments                                                  | TSS (°Brix) at |                     |                      |        |
|-------------------------------------------------------------|----------------|---------------------|----------------------|--------|
|                                                             | Initial        | 5 <sup>th</sup> day | 10 <sup>th</sup> day | Final  |
| T0- 2% Chitosan                                             | 5.00           | 6.00                | 6.00                 | 5.43   |
| T1- 50ml of 2% Chitosan + 50ml of 50% Aloe Vera             | 4.50           | 5.30                | 6.10                 | 5.63   |
| T2- 33ml of 2% Chitosan + 67ml of 50% Aloe Vera             | 5.30           | 6.50                | 6.43                 | 5.93   |
| T3- 50ml of 2% Chitosan + 50ml of 50% Moringa Leaf Extracts | 5.20           | 6.10                | 6.40                 | 5.67   |
| T4- 33ml of 2% Chitosan + 67ml of 50% Moringa Leaf Extracts | 5.10           | 5.60                | 6.30                 | 5.63   |
| <b>F Value</b>                                              | 0.35           | 0.78                | 0.33                 | 0.32   |
| <b>Pr (&gt; F)</b>                                          | 0.8384         | 0.5658              | 0.8533               | 0.8571 |
| <b>CV (%)</b>                                               | 18.17          | 15.46               | 9.18                 | 9.63   |

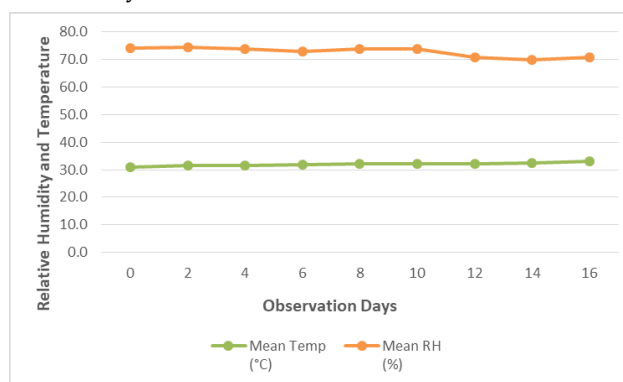
Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.

### 3.17 Titratable Acidity (TA)

Table 16 showed that titratable acidity (TA) decreased progressively during storage across all treatments, a trend commonly observed in ripening fruits as organic acids are metabolized into sugars and other compounds through respiration [30]. At the initial stage, TA values ranged between 0.34% and 0.41%, with T0 (2% chitosan) and T2 (33 ml of 2% chitosan + 67 ml of 50% Aloe Vera) recording the highest values (0.41%). By the final day, TA decreased to 0.26–0.30%, with T2 again retaining the highest acid content (0.30%). Statistical analysis revealed significant differences among treatments up to the 10th day, but none at the final measurement. This indicates that coating effects were more evident in the earlier stages of storage, while acid metabolism became uniform toward the end of storage. Aloe vera-based coatings showed contrasting results at different concentrations. T1 (50ml of 2% chitosan + 50ml of 50% aloe vera) consistently recorded the lowest TA values (0.34% initial to 0.26% final), suggesting faster acid utilization and ripening. In contrast, T2 (33ml of 2% chitosan + 67ml of 50% aloe vera) maintained higher TA values, performing comparably to pure chitosan (T0). This suggests that a greater concentration of aloe vera gel enhanced the barrier effect, reduced respiration, and slowed down acid degradation. This is consistent with findings of [33] and [32], who reported that aloe vera gel coatings delay acid loss in grapes and papaya by limiting gas exchange and reducing oxidative stress. Moringa-based coatings (T3 and T4) performed moderately, with final TA values of 0.27% and 0.29%, respectively. While not as high as T2, they maintained better acidity levels than T1. The antioxidant and antimicrobial compounds in moringa, such as flavonoids and phenolics, likely contributed to delaying acid breakdown. However, their effect was less pronounced than that of aloe vera at higher concentrations.

### 3.18 Relative Humidity and Temperature

Ambient temperature and relative humidity (RH) were monitored twice daily throughout the 16-day storage period at the CTU-Argao BENRC Laboratory under uncontrolled tropical indoor conditions. Mean daily temperature ranged from 30.8°C to 33.2°C (overall mean: 32.1°C), while RH ranged from 66.8% to 72.8% (mean: 69.7%), with afternoon readings consistently warmer and drier than morning readings. These conditions followed a clear, progressive trend over the storage period: temperature increased gradually from Day 0 to Day 16, while RH declined correspondingly, a pattern that closely aligns with the deterioration trends observed across all Study 2 quality parameters. As mean daily temperatures climbed beyond 30°C from Day 6 onward, the accelerated enzymatic activity and respiration rates are reflected in the progressive rise in pH and TSS recorded across treatments, as organic acids were consumed more rapidly and starches converted into soluble sugars. The concurrent decline in RH over the same period exacerbated water loss from fruit surfaces, consistent with the escalating weight loss observed across all treatments, most notably beyond Day 10, when weight loss accelerated sharply across all treatment groups. The Aloe vera-enriched coatings, particularly T2, demonstrated better buffering against worsening ambient conditions, maintaining lower pH, higher titratable acidity, and lower weight loss than Moringa-based treatments, whose weaker film-forming matrices offered less protection as temperature rose and RH fell progressively throughout the storage period. These environmental data, therefore provide critical context for understanding not only the magnitude of quality deterioration observed in Study 2, but also the differential effectiveness of coating treatments over time.



**Figure 10.** Relative humidity (%) and temperature (°C) during storage of bell pepper (*Capsicum annuum* L.) fruits as influenced by the different chitosan and plant extract-based edible coating.

**Table 16.** Initial, 5<sup>th</sup> day, 10<sup>th</sup> day, and final Titratable Acidity (TA) of bell pepper fruit (*Capsicum annuum* L.)

| Treatments                                                  | TA (%) at         |                     |                      |        |
|-------------------------------------------------------------|-------------------|---------------------|----------------------|--------|
|                                                             | Initial           | 5 <sup>th</sup> day | 10 <sup>th</sup> day | Final  |
| T0- 2% Chitosan                                             | 0.41 <sup>a</sup> | 0.38 <sup>a</sup>   | 0.33 <sup>ab</sup>   | 0.28   |
| T1- 50ml of 2% Chitosan + 50ml of 50% Aloe Vera             | 0.34 <sup>b</sup> | 0.30 <sup>c</sup>   | 0.28 <sup>c</sup>    | 0.26   |
| T2- 33ml of 2% Chitosan + 67ml of 50% Aloe Vera             | 0.41 <sup>a</sup> | 0.38 <sup>a</sup>   | 0.34 <sup>a</sup>    | 0.30   |
| T3- 50ml of 2% Chitosan + 50ml of 50% Moringa Leaf Extracts | 0.37 <sup>b</sup> | 0.35 <sup>ab</sup>  | 0.30 <sup>bc</sup>   | 0.27   |
| T4- 33ml of 2% Chitosan + 67ml of 50% Moringa Leaf Extracts | 0.37 <sup>b</sup> | 0.34 <sup>b</sup>   | 0.31 <sup>abc</sup>  | 0.29   |
| <b>F Value</b>                                              | 7.94              | 8.25                | 4.27                 | 1.87   |
| <b>Pr (&gt; F)</b>                                          | 0.0038            | 0.0033              | 0.0284               | 0.1915 |
| <b>CV (%)</b>                                               | 4.85              | 5.71                | 6.41                 | 7.14   |

Means within the same column in a block, followed by a common letter and/or without letter designations, are not significantly different from each other at 5% level of significance.

#### 4. Conclusions

This study demonstrates that, among the tested concentrations, 2% chitosan was the most effective for preserving bell pepper quality. It significantly reduced shriveling, maintained firmness, delayed the rise in pH, and completely inhibited decay incidence (0%) relative to the other concentrations, while also maintaining higher titratable acidity and lower total soluble solids—together reflecting delayed ripening and extended shelf life. In Study 2, where chitosan was combined with Aloe vera and Moringa leaf extracts, treatment T2 (33 mL of 2% chitosan + 67 mL of 50% Aloe vera) consistently outperformed the other formulations, producing the lowest weight loss, minimal shriveling, slower firmness loss, superior visual quality ratings, lower decay incidence (6.67%), and higher retention of acidity. Several limitations should be acknowledged to guide future work. First, firmness was assessed using a subjective palpation-based index; instrumental penetrometer measurements should be adopted in future studies. Second, ambient storage temperature and relative humidity were not recorded, and these parameters must be reported to allow meaningful comparison across studies. Third, the Moringa extract concentration requires clarification and consistent labeling throughout (20% vs. 50%). Fourth, the decay-incidence differences in Study 2 were not statistically significant, so antimicrobial claims require validation through microbiological enumeration. Fifth, future studies should include an uncoated negative control in Study 2 to enable direct comparison. Practically, the T2 coating (33 mL of 2% chitosan + 67 mL of 50% Aloe vera) represents a low-cost, farmer-accessible postharvest technology suited to smallholder systems in tropical environments. Further research should assess its economic feasibility, consumer acceptability, and potential for scale-up.

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# Utilization of A mixed Mode Solar Dryer Assisted with An Auxiliary Electric Heater for Drying Sweet Potato Slices

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**Abstract:** Reducing post-harvest losses of perishable fresh products is a key strategic approach to increasing income, national security, and nutrition in the world. A hybrid solar dryer equipped with an auxiliary electric heater was developed and used to dry sweet potato slices, maximizing the use of available solar energy for food drying and producing a high-quality dried product. During the experiments, two different sweet potato cultivars (Abis and Balady) were dried at three different drying air temperatures (50, 60, and 70°C) and a constant slice thickness of  $3\pm0.5$ mm after pre-treatment with sodium metabisulfite and citric acid. Eleven mathematical models were evaluated to determine the most accurate description of the drying behavior of potato slices. The effective moisture diffusivity, activation energy, and solar dryer efficiency were also calculated. Results indicated that the dryer reached the required temperature and maintained it throughout the experiment. The drying of sweet potato slices occurred during the falling rate period. The logistics model best describes the drying behavior of sweet potato slices from both varieties under the study conditions, followed by the Page model. The effective moisture diffusivity values were in the ranges of  $(1.8329\times10^{-10}$  to  $2.2888\times10^{-10})$  and  $(1.8055\times10^{-10}$  to  $2.2341\times10^{-10})$  m<sup>2</sup>/s for the Abis and Balady varieties, respectively. The activation energies were 10.274 and 9.772 kJ/mol for the Abis and Balady varieties, respectively. The overall thermal efficiency of the solar collector ranged from 65.1% to 70.4%. The overall thermal efficiency of the dryer ranged from 11.1245 to 12.5004%.

**Keywords:** Sweet potato; hybrid solar dryer; mathematical modeling.

## 1. Introduction

Sweet potato (*Ipomoea batatas*) has been cultivated in Egypt for many years. The area planted with sweet potatoes in Egypt in 2023 reached about 29,588 feddan, with a total production of approximately 423,423 tons [1]. In general, sweet potatoes are orange, though they can also be white, red, pink, violet, yellow, or purple. Typically, it serves as a foundational component for snacks, flour, and traditional confections. It is also processed into chips, which are deep-fried after being finely sliced. It is rich in minerals, ascorbic acid, and  $\beta$ -carotene, making it a healthy food [2]. Sweet potatoes are a great source of complex carbohydrates and simple starches. In addition to low levels of vitamins and minerals, the tuber contains useful substances such as polyphenols, anthocyanins, and beta-carotene, a provitamin A carotenoid [3]. Vitamins, minerals, dietary fiber, and antioxidants, including  $\beta$ -carotene, tocopherol, anthocyanins, and phenolic acids, are abundant in sweet potatoes. Sweet potato

phytochemicals have been shown to have radical-scavenging properties and several health-promoting effects on human conditions such as cancer, heart disease, and age-related neuronal degeneration [4]. Fresh sweet potato tubers contain high levels of moisture, up to about 70%, which makes them perishable and difficult to transport from one place to another. Therefore, it was necessary to use appropriate preservation technologies to extend their shelf life [5]. Drying is a food processing method that has been used safely for centuries to preserve fruits and vegetables for long-term storage. Drying methods aim to remove the most moisture from food at the lowest cost and highest efficiency [6]. The availability of dried sweet potato roots will increase their shelf life and facilitate reconstitution. The process of turning sweet potato tuber into powder is a remarkable opportunity to provide the product in a stable form [7].

The food drying process involves heat and mass transfer, which is accompanied by changes in the product's structure, shape, and biological quality. Therefore, it is necessary to understand and study the drying properties of various food materials to produce high-quality dried products [8]. Solar drying is one of the oldest methods used in food drying. This technology is very popular because it saves energy costs and is environmentally friendly [9]. However, there are limitations to the use of solar drying, such as the long drying time, low efficiency, difficulty in controlling drying conditions (e.g., temperature), and dependence on time of day, climate, and season [10,11]. Solar dryers can be divided into three categories: direct, indirect, and hybrid (mixed-mode) [3, 9]. Continuous research and development are being conducted on the use of hybrid solar dryers. These dryers use an additional heating system to accelerate the drying process, reduce the time required to complete drying, and control drying conditions such as temperature.

Several different heating technologies have been used to support solar dryers, such as heat from electric coils and other auxiliary heaters [12-15], waste heat [16], storage heat [17-19], and heat from biomass combustion [20-22]. Thin-layer drying models are mathematical models that are used to create and enhance current drying systems as well as to gain a better understanding of the thermal process [23,24]. Models of thin-layer drying offer a basis for comprehending the drying properties of any food item [25]. This work aims to study the drying kinetics of two different sweet potato varieties using a hybrid solar dryer equipped with an auxiliary electric heating unit at three different air temperature levels. In addition, effective moisture diffusion, activation energy, and mathematical modeling were studied by testing and evaluating 11 models to determine the most accurate for describing the drying behavior of sweet potato slices in the hybrid dryer. The study also aims to evaluate the thermal performance and calculate the efficiency of both the solar collector and the hybrid solar dryer.

## 2. Materials and Methods

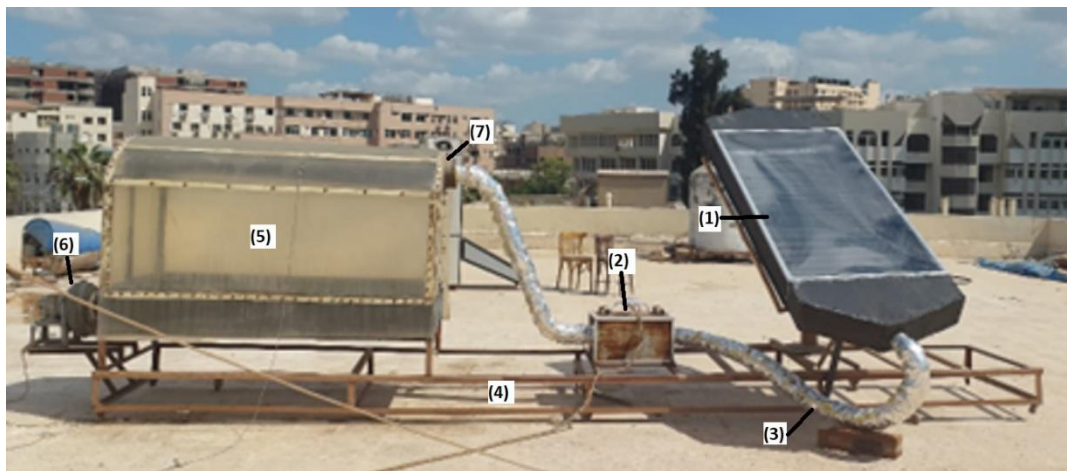
To achieve the objective of this study, an experimental-scale mixed-mode hybrid solar dryer was developed and tested at the Agricultural Engineering Department, Faculty of Agriculture, Mansoura University. The latitude and longitude angles and the altitude above sea level of the experimental location are 31.045 oN, 31.37 oE, and 19.05 m, respectively. The dryer was used for conducting experimental work during July and August 2025.

### 2.1 Sample preparation:

Orange-colored sweet potatoes (Abis variety) and yellow (Balady variety) were obtained from a local farmer in Damietta and Kafr El-Sheik governorates during the 2025 harvest season and stored at room temperature until use. Before laboratory experiments, sweet potato roots were washed with water to remove dirt and foreign particles stuck to the root surface. Peeling was then performed to remove the outer skin of the potato roots, as well as any parts damaged by weevils and other pests, using hand knives. After peeling, the sweet potato roots were cut into  $3 \pm 0.5$  mm-thick slices using a manual home slicer to increase their surface area during drying. After slicing, the sweet potato slices were immediately soaked in a 0.5% sodium metabisulfite and 1% citric acid solution for 30 minutes to prevent browning and preserve their color during drying [26, 27].

## 2.2 Solar dryer:

A mixed-mode hybrid solar dryer assisted by an auxiliary electrical heater was used for the experimental work. The forced-convection solar dryer consists of the parts shown in Figure 1.



(1) Solar collector; (2) auxiliary heating unit; (3) insulated air duct; (4) metallic frame; (5) solar dryer - drying chamber; (6) AC suction fan; (7) perforated pipe.

**Figure 1.** Overall features of the mixed-mode hybrid solar dryer.

The developed dryer consists of the following parts:

1. Solar collector: The solar collector consists of a frame made of steel angle (L) measuring 30 x 30 mm 2000 mm long, 1000 mm wide, and 250 mm high. Its transparent surface is made of acrylic with a transmittance coefficient of 95%, and the absorber plate is a 1 mm-thick corrugated aluminum plate painted matte black and insulated from below with a layer of glass wool. The solar collector is connected to both bases via a pyramid-shaped air inlet and outlet with a length of about 460 mm, covered from above with black matte-coated sheet metal, and equipped with air inlet and outlet pipes with a diameter of 76.2 mm. The collector's slope was set at 31° relative to the horizontal, calculated based on the dryer's location (the average optimum tilt angle for all months for the Department of Agricultural Engineering, Faculty of Agriculture - Mansoura University).

2. Auxiliary heating unit: a metal box measuring 550 mm long, 360 mm wide, and 310 mm high. It is internally insulated to reduce heat loss and equipped with 4 kW electric heaters to raise the drying air temperature to the required levels. It has an air inlet and outlet.

3. Insulated air duct :an externally insulated pipe 76.2 mm in diameter that transports air from the solar collector outlet to the auxiliary heating unit inlet and from the heating unit outlet to the air distribution pipe at the top of the drying chamber.

4. Solar dryer – drying chamber: The solar dryer is a rectangular chamber with a metal frame measuring 2000 mm long, 1000 mm wide, and 700 mm high, and a semicircular roof measuring 300 mm high, covered on the outside with a polycarbonate sheet with an effective transmittance of 90%. On each side of the drying chamber is a door measuring 960 x 700 mm for loading and unloading potato slices. The drying chamber consists of a perforated stainless-steel sheet fixed over an iron frame with dimensions of 1000 x 2000 mm to keep the sweet potato slices from falling down and to permit air to move through it. The frame rested on a plenum chamber made of iron angles covered with 0.5 mm-thick iron sheets.

5. AC Suction Fan: A 0.367 kW AC suction fan at 3000 rpm is employed to facilitate air circulation within the dryer, aiding the drying process.

6. Frame: all parts of the dryer are mounted on a metal frame measuring 6060 mm long by 1060 mm wide for easy movement and orientation.

7. Control unit: it contains the operating switches for the exhaust fan and electric heaters, a thermostat to adjust the air temperature inside the dryer, and an electric meter to measure the electric energy consumed by the electric heaters during the trial period.

### 2.3 Measurements

**Air temperature and relative humidity:** A temperature and relative humidity meter model HT-315, USA, with measuring range 0 to 80 °C± 0.1 °C for temperature and 0 to 95± 1.5% for air relative humidity, was used for measuring both parameters of the drying air and exhaust air during the experimental work. Also, a 12-channel datalogger model (No. 92000-05 Benchtop 230V) connected to 12 thermocouple sensors was used to read and record air temperature at different locations. **Air velocity:** The velocity of air passing through the dryer was measured using a digital air velocity instrument model 9515 (USA), with a measuring range of 0 to 20 ± 0.025 m/s, connected to a velocity probe. **Moisture content:** The initial and final moisture content of sweet potato slices were determined using a 24-hour oven drying method at 105°C [28]. **Sample weight:** A digital balance with an accuracy of 0.01g and a maximum weight of 200g was used to weigh the samples. **Intensity of solar radiation:** the solar radiation meter model SPM-1116SD, with an accuracy of ±0.1 W/m<sup>2</sup>, was used to measure the incident solar radiation flux during the experimental work.

### 2.4 Experimental Procedure

All tests associated with the drying process were carried out at the Agricultural Engineering Department, Faculty of Agriculture, Mansoura University. The dryer was used for conducting experimental work during July and August 2025. After the sweet potato slices are prepared and soaked, they are arranged on a drying tray and placed in three sub-trays to measure the samples' weight at time intervals. The tray is placed inside the dryer, the doors are tightly closed, the fan and heaters are turned on, and the thermostat is set to the air temperature required for the experiment. The experiment starts at 8:00 a.m., and the sub-trays are weighed every half hour. The experiment ends when the sub-samples reach a constant weight, indicating an equilibrium moisture content with the drying air. To determine the moisture content of potato slices during drying, three small trays, 300 mm long and 150 mm wide, made of galvanized wire mesh, were placed inside the dryer. Each tray contained a sample of sweet potato slices weighing approximately 150 g. The trays were weighed every 30 minutes, and the weights were converted to moisture content.

### 2.5 Calculations

**Moisture ratio:** (MR) represents the dimensionless moisture ratio, which was calculated using the following formula [29]

$$MR = \frac{M_t - M_f}{M_o - M_f} \quad (1)$$

Where: MR is the moisture ratio, dimensionless;  $M_t$  is the moisture content at a specific time, (d. b.);  $M_f$  is the final moisture content, (d. b.);  $M_o$  is the initial moisture content, (d. b.).

**Drying rate:** the drying rate (DR) of sweet potato slices was calculated using the following equation, [29]

$$DR = \frac{M_{t+\Delta t} - M_t}{\Delta t} \quad (2)$$

Where:  $M_t$  is moisture content at  $t$ ;  $M_{t+\Delta t}$  is moisture content at  $t + \Delta t$  (g water/g dry matter), and  $t$  is time (min).

### 2.6 Mathematical Modeling of Sweet Potato Drying

The present study aims to evaluate the effectiveness of eleven selected thin-layer drying models in the context of drying data analysis. The models are assessed based on their ability to accurately and efficiently predict the drying behavior of various products. Table 1 shows numerous thin-layer drying models used to analyze and describe the experimental data gathered during the drying process. These models were fitted to the experimental data for each drying process. The coefficients of the models were obtained using nonlinear regression analysis in Microsoft Excel and SigmaPlot 14. The most effective model was chosen based on parameters such as the lowest standard error (SE), chi-square ( $\chi^2$ ), root-mean-square error (RMSE), and the highest  $R^2$  (coefficient of determination). The model that best matches the experimental data was identified using the previously indicated criteria [30, 31].



The following equations can be used to compute these parameters:

$$R^2 = 1 - \frac{\sum_{i=1}^N (MR_{exp,i} - MR_{calc,i})^2}{[\sum_{i=1}^N (MR_{calc,i} - MR)^2]} \quad (3)$$

$$\chi^2 = \frac{\sum_{i=1}^N (MR_{exp,i} - MR_{calc,i})^2}{N-n} \quad (4)$$

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^N (MR_{calc,i} - MR_{exp,i})^2} \quad (5)$$

Where  $MR_{exp,i}$  stands for the observed moisture ratio found in any measurement,  $MR_{calc,i}$  is the calculated moisture ratio for this measurement, and  $MR$  represents the average predicted value.  $N$  and  $n$  are the number of observations and constants, respectively [32, 33].

**Table 1.** Selected mathematical models to demonstrate the drying process of sweet potato.

| Model name                                 | Model equation                                          | Ref. | Eq   |
|--------------------------------------------|---------------------------------------------------------|------|------|
| Lewis                                      | $MR = \exp(-k \cdot t)$                                 | [34] | (6)  |
| Henderson and Pabis                        | $MR = a \exp(-k \cdot t)$                               | [35] | (7)  |
| Logarithmic                                | $MR = a \exp(-k t) + c$                                 | [36] | (8)  |
| Page                                       | $MR = \exp(-k t^n)$                                     | [37] | (9)  |
| Elfar et al model                          | $MR = a + b \exp(-k t) + c t$                           | [38] | (10) |
| Logistics                                  | $MR = \frac{b}{b \exp(-k t) + 1}$                       | [39] | (11) |
| Modified Page III                          | $MR = k \exp(-\frac{t}{d^2})^n$                         | [40] | (12) |
| The two terms                              | $MR = a \exp(-k_0 t) + b \exp(-k_1 t)$                  | [41] | (13) |
| Modified Henderson and Pabis (three terms) | $MR = a \exp(-k_0 t) + b \exp(-k_1 t) + c \exp(-k_2 t)$ | [42] | (14) |
| Modified two terms III                     | $MR = a \exp(-k t) + (1 - a) \exp(-k a t)$              | [43] | (15) |
| Aghbashlo                                  | $MR = \exp\left(-\frac{k_1 t}{1 + k_2 t}\right)$        | [44] | (16) |

Where:  $MR$  is the moisture ratio (dimensionless);  $k$ ,  $k_0$ ,  $k_1$ , and  $k_2$  are the drying constants ( $\text{min}^{-1}$ );  $a$ ,  $b$ ,  $c$ , and  $n$  are the model constants (dimensionless);  $t$  is the drying time (min).

## 2.7 Effective moisture diffusivity and activation energy

In most studies on food drying, diffusion is considered the primary mechanism for moisture transfer from the interior of the material to its surface, where it evaporates [45]. To calculate effective moisture diffusivity, a solution to the Fick equation was developed, assuming moisture transfer by diffusion, negligible shrinkage, constant diffusion coefficients, and constant temperature for the laminates [46]. The equation was modified to the following form [47]:

$$MR = \left(\frac{8}{\pi^2}\right) \exp\left[\frac{-\pi^2 D_{eff} t}{4L^2}\right] \quad (17)$$

where  $L$  is half the thickness of the sweet potato slice ( $3 \pm 0.5 \text{ mm}$ );  $t$  is the drying time (sec), and  $D_{eff}$  is the effective moisture diffusivity ( $\text{m}^2/\text{s}$ ).

The effect of temperature on effective moisture diffusion can be described by an Arrhenius-type relationship as follows [48]:

$$D_{eff} = D_o \exp\left[\frac{-E_a}{RT}\right] \quad (18)$$

Where:  $D_o$ : pre-exponential factor of the Arrhenius equation ( $\text{m}^2\text{s}^{-1}$ );  $R$ : universal gas constant ( $8.314 \text{ Jmol}^{-1} \text{ K}^{-1}$ );  $E_a$ : activation energy ( $\text{kJ.mol}^{-1}$ );  $T$ : the absolute temperature (K).

## 2.8 Thermal efficiency of the solar collector:

To evaluate the thermal performance of a solar collector, a set of parameters must be calculated. These parameters can be calculated as follows [49]:

$$\text{Solar energy available} \quad Q = R \times A_c \quad (19)$$

Where:  $Q$  is Solar energy available, W;  $R$ : Solar energy flux incident on the surface of the solar collector, W/m<sup>2</sup>;  $A_c$ : Surface area of the solar collector, m<sup>2</sup>

$$\text{Absorbed solar energy:} \quad Q_a = \tau \cdot \alpha \cdot R \cdot A_c \quad (20)$$

Where:  $Q_a$  is Absorbed solar energy, W;  $\tau$ : Effective transmittance of solar collector cover system, decimal;  $\alpha$  = Effective absorptance of the absorber plate of the collector, decimal

$$\tau = \tau_{\max} - 0.00437 \exp [0.0936 (\theta - 30)] \quad (21)$$

$$\alpha = \alpha_{\max} - 0.00476 \exp [0.0940 (\theta - 35)] \quad (22)$$

Where:  $\theta$  is Solar incident angle

$$\text{Absorption efficiency } (\eta_a): \quad \eta_a = \frac{Q_a}{Q} \times 100, \% \quad (23)$$

$$\text{Useful heat gain to storage } (Q_c): \quad Q_c = m_a \cdot c_p \cdot (T_{a_{oc}} - T_{a_{ic}}) \quad (24)$$

Where:  $Q_c$  is the useful heat gain to storage, W;  $m_a$  is the mass flow rate of air, kg/s;  $C_p$  is the specific heat of air, J/kg/ °C;  $T_{a_{oc}}$  is the temperature of air just leaving the solar collector, °C;  $T_{a_{ic}}$  is the air temperature before entering the solar collector, °C.

$$\text{Heat transfer efficiency:} \quad \eta_h = \frac{Q_c}{Q_a} \times 100, \% \quad (25)$$

$$\text{Solar collector heat losses:} \quad Q_L = Q_a - Q_c, \text{ Watt} \quad (26)$$

$$\text{Overall thermal efficiency:} \quad \eta_o = \frac{Q_c}{Q} \times 100, \% \quad (27)$$

## 2.9 Thermal efficiency of the solar dryer:

The thermal efficiency of a solar dryer is defined as the ratio of the energy required to evaporate moisture from the product ( $Q_{ev}$ ) to the total energy available inside the dryer( $Q_I$ ), which is represented by the energy acquired by the air from the solar collector ( $Q_U$ ), the energy acquired by the air from the auxiliary electric heating unit ( $Q_E$ ), and the solar energy falling directly on the drying chamber ( $Q_d$ ). It could be calculated as follows [50]:

$$\eta_{thd} = \frac{Q_{ev}}{Q_I} \times 100, \% \quad (28)$$

$$Q_{ev} = m_p C_{pp}(\Delta T_p) + m_{ev} \cdot h_{fg} \quad (29)$$

Where:  $Q_{ev}$  is the energy required to evaporate moisture from the product, kJ;  $m_p$  is the mass of the product, kg;  $C_{pp}$  is the specific heat of the product, kJ/kg °C;  $\Delta T_p$  is a change in bulk temperature over a period of time, °C;  $m_{ev}$  is the mass of moisture evaporated, kg;  $h_{fg}$  is the latent heat of vaporization of water, kJ/kg.

$$C_{pp} = 1.672 + 0.025 MC_{wb} \quad (30)$$

$$h_{fg} = 2393.673(1 + 23 \exp(-0.4 MC_{db})) \quad (31)$$

Where:  $MC_{wb}$  and  $MC_{db}$  are the moisture content of the product [51].

$$Q_I = Q_U + Q_E + Q_d \quad (32)$$

**Where:**  $Q_I$  is the total energy available inside the dryer, kJ;  $Q_U$  is the Useful heat gain by the solar collector, kJ;  $Q_E$  is the electric energy of heaters, kJ;  $Q_d$  is the solar energy falling directly on the drying chamber, kJ

### 3. Results and Discussion

This experiment was conducted to evaluate the thermal performance and drying kinetics of two different varieties of sweet potato slices using a hybrid solar dryer equipped with an additional electric air heater at three different drying air temperature levels (50, 60, and 70°C), a constant air flow rate inside the dryer of (0.0721 m<sup>3</sup>/s), and a constant potato slice thickness (3 ± 0.5 mm). The moisture content of the potato slices was monitored over time until a constant value was reached.

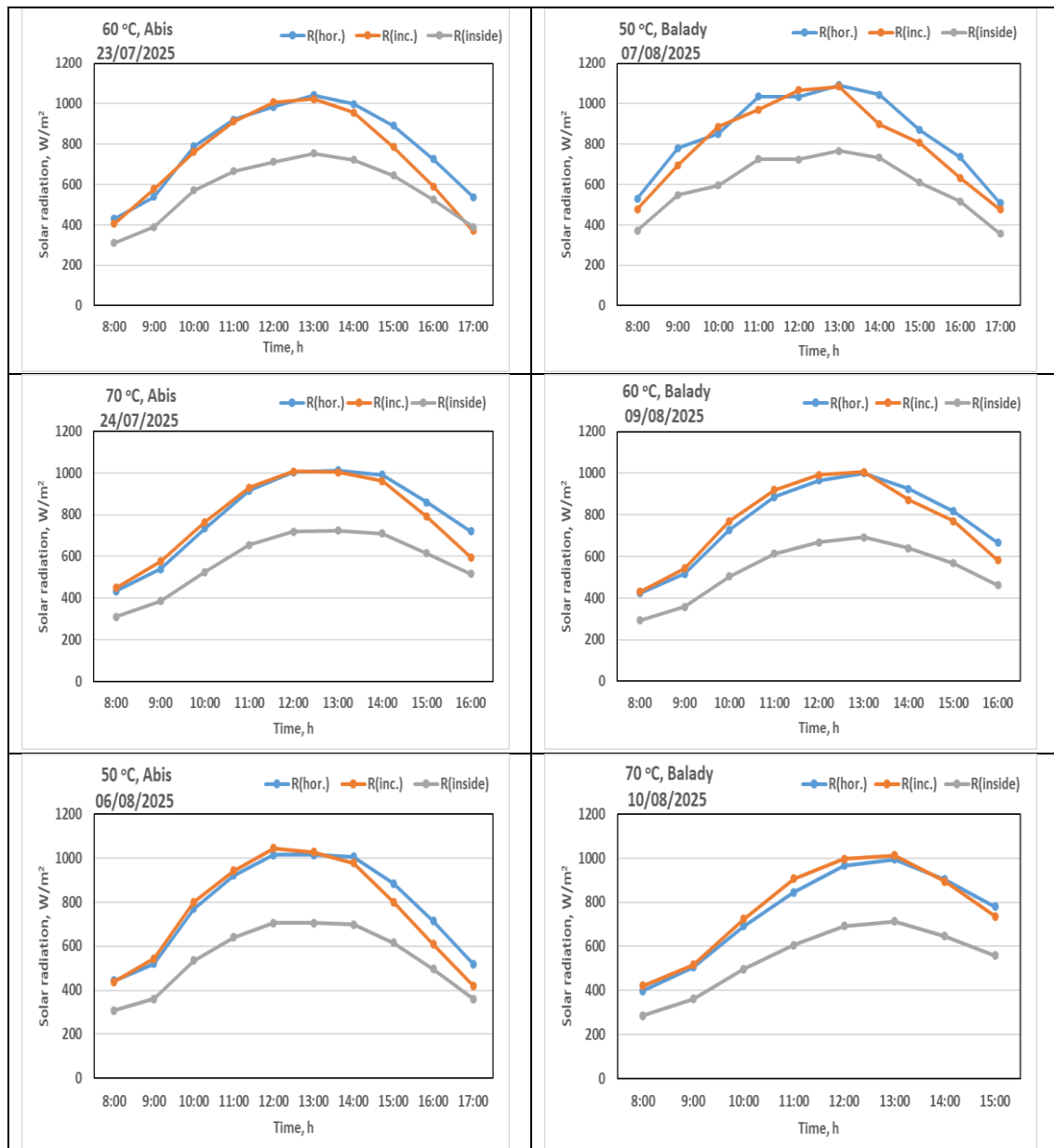
#### 3.1 Solar radiation flux incident:

To study the thermal performance of the solar dryer, it was necessary to determine the amount of incident solar radiation during the drying process. The average available solar hourly radiation was measured and recorded during the experimental work on July 23 and 24 and August 6, 7, 9, and 10, 2025, in Mansoura, Egypt. Table 2 shows the average solar radiation incident on a horizontal surface outside and inside the solar dryer and the solar radiation incident on the solar collector surface during laboratory experiments. The average incident solar radiation on a horizontal surface outside the solar dryer during the experimental period ranged between 398 and 1092 W/m<sup>2</sup>. At the same time, the average incident solar radiation on the collector surface ranged between 370 and 1085 W/m<sup>2</sup>. Meanwhile, the average incident solar radiation on a horizontal surface inside the solar dryer ranged from 285 to 765 W/m<sup>2</sup>. Also, the average incident solar radiation on the horizontal surface outside the solar dryer, on the solar collector surface, and on the horizontal surface inside the solar dryer was 791, 770, and 559 W/m<sup>2</sup>, respectively. Figure 2 shows the measured incident solar energy flux during the experimental work.

**Table 2.** Solar radiation flux incident during the experimental period.

| Experiment day |      | R(hor.) | R(inc.) | R(inside) |
|----------------|------|---------|---------|-----------|
| 23/07/2025     | Max  | 1043.0  | 1023.0  | 754.2     |
|                | Min  | 430.0   | 370.0   | 310.9     |
|                | Mean | 786.0   | 738.8   | 568.3     |
|                | SD   | ±219.4  | ±242.2  | ±158.6    |
| 24/07/2025     | Max  | 1012.0  | 1008.0  | 724.4     |
|                | Min  | 433.0   | 450.0   | 309.9     |
|                | Mean | 800.9   | 786.4   | 573.3     |
|                | SD   | ±210.1  | ±206.7  | ±150.4    |
| 06/08/2025     | Max  | 1016.0  | 1045.0  | 705.8     |
|                | Min  | 443.1   | 420.0   | 307.8     |
|                | Mean | 781.1   | 760.3   | 542.6     |
|                | SD   | ±223.0  | ±241.9  | ±154.9    |
| 07/08/2025     | Max  | 1092.0  | 1085.0  | 765.9     |
|                | Min  | 507.0   | 475.0   | 355.6     |
|                | Mean | 847.8   | 799.1   | 594.6     |
|                | SD   | ±211.5  | ±222.9  | ±148.3    |
| 09/08/2025     | Max  | 1000.0  | 1006.0  | 692.5     |
|                | Min  | 423.0   | 430.0   | 292.9     |
|                | Mean | 769.4   | 764.6   | 532.8     |
|                | SD   | ±202.3  | ±206.3  | ±140.1    |
| 10/08/2025     | Max  | 995.0   | 1013.0  | 712.7     |
|                | Min  | 398.0   | 422.0   | 285.1     |
|                | Mean | 760.7   | 776.4   | 544.9     |
|                | SD   | ±216.1  | ±218.1  | ±154.8    |

In general, solar radiation gradually increased from sunrise to noon, then gradually decreased to its minimum at sunset. The marked variation in available solar energy during the drying period affected the dryer's air-heating efficiency and the differences in air temperature and relative humidity between inside and outside the solar dryer. This demonstrated the need for auxiliary heaters to maintain a constant air temperature inside the drying chamber. In this case, drying behavior can be accurately described using well-studied mathematical models.



**Figure 2.** Solar radiation flux incident on the horizontal surface inside and outside the solar dryer, and on the solar collector surface, as a function of time during the experimental period.

### 3.2 Air temperature outside and inside the hybrid solar dryer:

As the air flows through the dryer, it passes through the solar collector where it touches the absorber plate. This causes its temperature to rise above the ambient temperature outside the dryer but below the level required for drying. It then passes through an additional heating unit to raise its temperature to the required level. It then passes into the drying chamber, where it evaporates moisture from the material and carries the vapor to the outside of the dryer as the suction fan draws it. Figure (3) presents the air temperature profile at different positions outside and inside the examined solar dryer (ambient  $T_{a,c}$ , leave the solar collector  $T_{a,oc}$ , and

inside the drying chamber  $T_{ad}$ ). It can be seen from the Figure that the system maintained a nearly constant temperature inside the drying chamber from 10 am until the end of the experimental period. Table 3 shows the average air temperatures outside and inside the solar dryer, as well as the air leaving the solar collector during laboratory experiments. The table shows that the average air temperatures outside the dryer during laboratory experiments for drying the Abis variety were 36.6, 36.4, and 37°C. The solar collector raised the air temperature to 47.20, 48.8, and 47.1°C, with an average increase of 30.11%. During laboratory experiments on drying the Balady variety, the average air temperatures outside the dryer were 36.1, 39.3, and 41.5°C. The solar collector raised the air temperature to 47.4, 50.1, and 52.3°C, with an average increase of 28.27%. It is also shown that the system reached a temperature between  $51.8 \pm 1.48$  and  $51.8 \pm 1.78$  when the thermostat was set to 50°C. While the average temperature inside the dryer was  $58.8 \pm 2.13$  to  $59.5 \pm 1.46$  °C when the thermostat was set to 60°C. Meanwhile, the average temperature inside the dryer was in the range  $68.9 \pm 2.0$  and  $69.2 \pm 1.57$  when the thermostat was set at 70°C

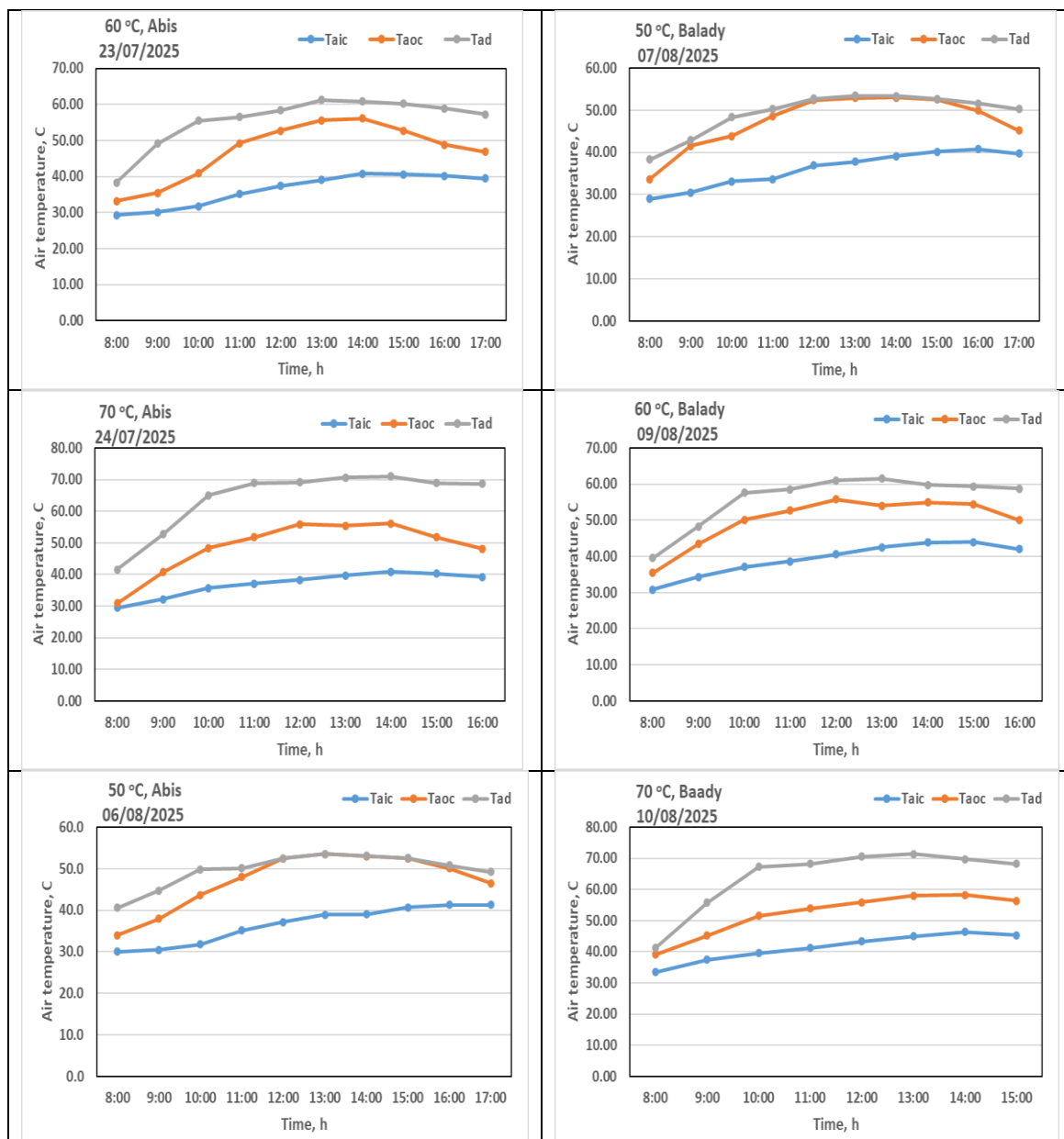


Figure 3. Air temperatures at different positions over time during the experimental period.

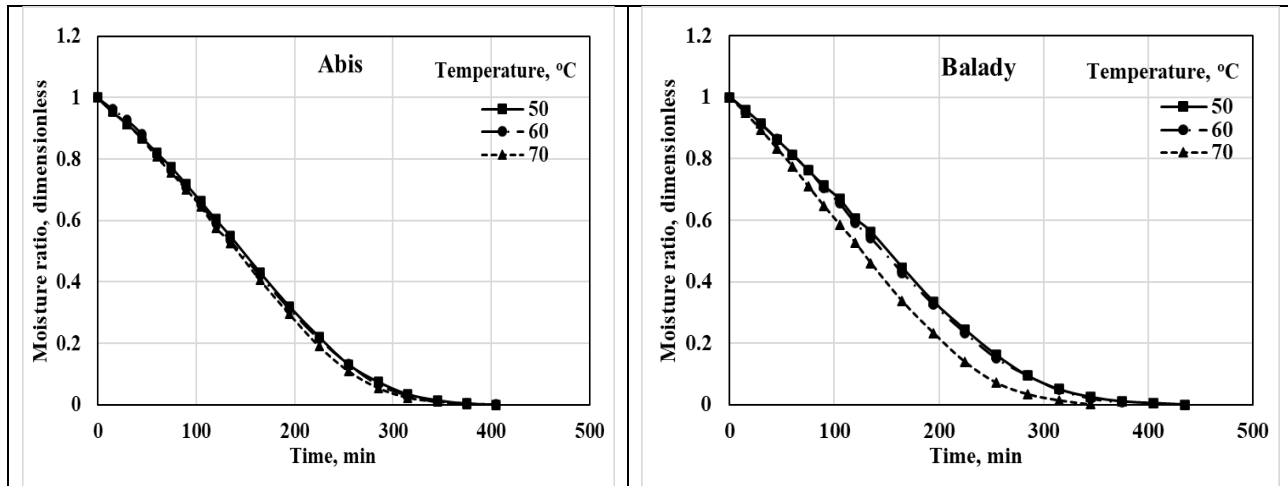


**Table 3.** Air temperature during the experimental period.

| Experiment day |      | Ta <sub>ic</sub> | Ta <sub>oc</sub> | Ta <sub>d</sub> |
|----------------|------|------------------|------------------|-----------------|
| 23/07/2025     | Max  | 40.8             | 56.1             | 61.8            |
|                | Min  | 29.3             | 33.1             | 54.3            |
|                | Mean | 36.4             | 47.1             | 58.8            |
|                | SD   | ±4.49            | ±8.10            | ±2.13           |
| 24/07/2025     | Max  | 40.8             | 56.2             | 71.8            |
|                | Min  | 29.5             | 30.9             | 64.1            |
|                | Mean | 37.0             | 48.8             | 68.9            |
|                | SD   | ±3.9             | ±8.31            | ±2.0            |
| 06/08/2025     | Max  | 41.3             | 53.5             | 53.8            |
|                | Min  | 30.0             | 34.0             | 48.5            |
|                | Mean | 36.6             | 47.2             | 51.8            |
|                | SD   | ±4.45            | ±6.76            | ±1.48           |
| 07/08/2025     | Max  | 40.8             | 53.0             | 53.9            |
|                | Min  | 29.0             | 33.6             | 48.0            |
|                | Mean | 36.1             | 47.4             | 51.8            |
|                | SD   | ±4.23            | ±6.36            | ±1.78           |
| 09/08/2025     | Max  | 44.0             | 55.8             | 61.9            |
|                | Min  | 30.8             | 35.4             | 56.3            |
|                | Mean | 39.3             | 50.1             | 59.5            |
|                | SD   | ±4.52            | ±6.68            | ±1.46           |
| 10/08/2025     | Max  | 46.4             | 58.3             | 71.6            |
|                | Min  | 33.4             | 39.1             | 66.1            |
|                | Mean | 41.5             | 52.3             | 69.2            |
|                | SD   | ±4.47            | ±6.81            | ±1.57           |

### 3.3 Drying curves

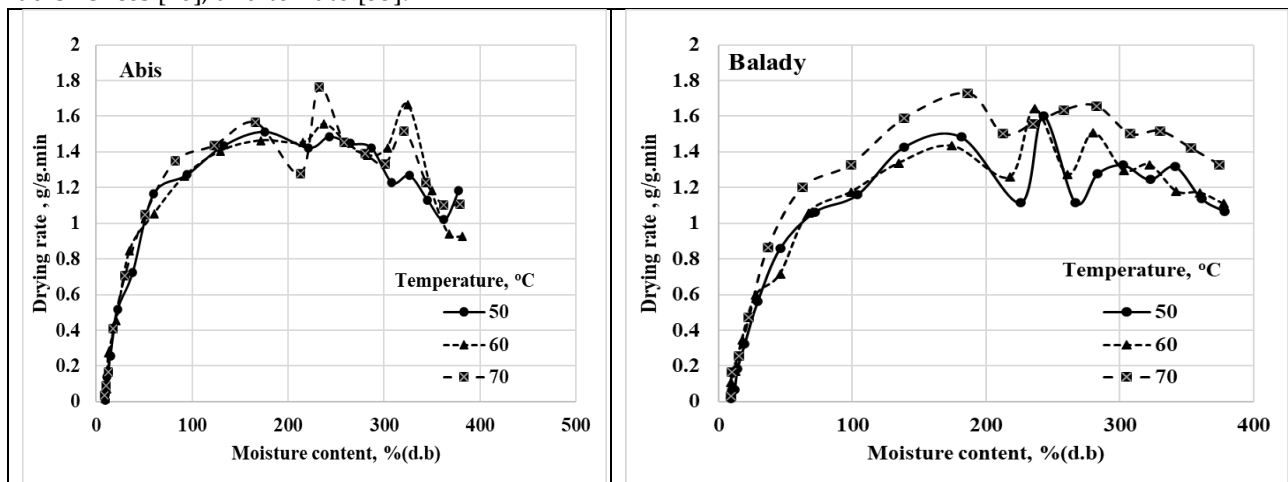
Sweet potato slices of Abis and Balady cultivars were dried in a hybrid solar dryer at three air temperatures in the drying chamber: 50, 60, and 70°C. Figure 4 shows the relationship between moisture ratio (MR) and time (t) at different air temperatures inside the drying chamber for both varieties. It could be observed that air temperature has a clear effect on moisture ratio MR, with MR decreasing as air temperature increases. The drying time required to reduce the moisture content of sweet potato slices of the Abis variety from an initial moisture content of 395.45 % (d.b.) to final moisture contents of 9.25, 9.06, and 8.85 % (d.b.) was 435, 435, and 405 min at 50, 60, and 70°C, respectively. While the drying time required to reduce the moisture content of sweet potato slices of Baladi variety from an initial moisture content of 394.11 % (d.b.) to a final moisture content of 9.60, 9.35, and 9.07 % (d.b.) was 465, 405, and 375 min at 50, 60, and 70°C, respectively. It can be observed that the drying time decreased with increasing drying air temperature.



**Figure 4.** Variation of moisture ratio MR for sweet potato slices at different air temperatures.

### 3.4 Drying rate

The changes in the drying rates versus moisture content for the drying air temperatures of 50, 60, and 70 °C for both sweet potato varieties are shown in Figure 5. The drying rate increased with increasing drying air temperature. It can also be observed that there was no constant-rate drying period in the drying of sweet potato slices for both studied varieties, and all drying processes occurred in the falling-rate period. This showed that diffusion was the dominant physical mechanism governing moisture movement in the samples [45]. These results are consistent with those obtained for some vegetables, such as pumpkin slices [52], white radish slices [48], and tomato [53].



**Figure 5.** Drying rate of sweet potato versus moisture content at different air temperature levels.

### 3.5 Evaluation of drying models

Using mathematical models to determine the drying kinetics of agricultural products is crucial. Understanding the underlying transport mechanisms involved in thin-layer drying of agricultural materials is essential for simulating the entire process and scaling it up to improve or control operating conditions. Experimental drying practices that do not account for the mathematical aspects of drying kinetics significantly reduce dryer efficiency, increase production costs, and degrade the quality of the dried product. Therefore, to ensure effective process design, optimization, energy integration, and control, an effective mathematical model is essential [43]. Moisture content readings at different times were converted to moisture ratio (MR), and eleven mathematical models were used to evaluate them and determine the most accurate one for describing the drying behavior of sweet potato slices under experimental conditions. The mathematical models were compared using the coefficient of determination ( $R^2$ ), standard error (SE), chi-square ( $\chi^2$ ), and root mean square error (RMSE). The best model to describe the drying behavior in a thin layer is the one that

gives a higher value for ( $R^2$ ) and lower values for (SE), ( $\chi^2$ ), and (RMSE) [30, 31, 54]. The results of the statistical analysis show that all the drying models had an overall high coefficient of determination ( $R^2$ ) within the range (0.9587 to 0.9994) for the Abis variety and (0.9302 to 0.9995) for the Balady variety, as shown in Tables 4 and 5. From the results shown in Tables 3 and 4, it can be said that the logistics model is the best in describing the drying behavior of sweet potato slices of both Abis and Balady varieties under the study conditions, followed by the Page model.

Figures (6 and 7) represent the comparison between the observed moisture ratio (MR) and the calculated MR obtained from the first and second appropriate drying models for both varieties of sweet potato slices at different drying temperatures.

**Table 4.** Statistical analysis of the examined mathematical thin-layer models for the Abis variety.

|                        | T, C    | k       |         |         |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|------------------------|---------|---------|---------|---------|--------|----------------|--------|----------------|--------|
| Lewis                  | 50      | 0.0057  |         |         |        | 0.9791         | 0.0533 | 0.0095         | 0.0950 |
|                        | 60      | 0.0058  |         |         |        | 0.9799         | 0.0505 | 0.0093         | 0.0939 |
|                        | 70      | 0.0059  |         |         |        | 0.9802         | 0.0514 | 0.0094         | 0.0942 |
|                        | Average |         |         |         |        | 0.9797         | 0.0517 | 0.0094         | 0.0944 |
| Henderson<br>and Pabis |         | a       | k       |         |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | 1.1291  | 0.0065  |         |        | 0.9818         | 0.0726 | 0.0071         | 0.0798 |
|                        | 60      | 1.1350  | 0.0066  |         |        | 0.9829         | 0.0703 | 0.0066         | 0.0771 |
|                        | 70      | 1.1275  | 0.0067  |         |        | 0.9828         | 0.0702 | 0.0070         | 0.0789 |
|                        | Average |         |         |         | 0.9825 | 0.0710         | 0.0069 | 0.0786         |        |
| Logarithmic            |         | a       | k       | c       |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | 1.6186  | 0.003   | -0.5608 |        | 0.9954         | 0.0427 | 0.0027         | 0.0480 |
|                        | 60      | 1.5614  | 0.0032  | -0.4953 |        | 0.9956         | 0.0409 | 0.0024         | 0.0453 |
|                        | 70      | 1.7042  | 0.0029  | -0.6501 |        | 0.9962         | 0.0470 | 0.0025         | 0.0460 |
|                        | Average |         |         |         | 0.9957 | 0.0435         | 0.0025 | 0.0465         |        |
| Page                   |         | k       | n       |         |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | 0.00039 | 1.5539  |         |        | 0.9947         | 0.0430 | 0.0026         | 0.0481 |
|                        | 60      | 0.00029 | 1.6097  |         |        | 0.9963         | 0.0359 | 0.0017         | 0.0396 |
|                        | 70      | 0.00067 | 1.4299  |         |        | 0.9964         | 0.0308 | 0.0016         | 0.0376 |
|                        | Average |         |         |         | 0.9958 | 0.0366         | 0.0020 | 0.0418         |        |
| Elfar                  |         | a       | b       | k       | c      | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | -40.630 | 41.6909 | 0.0005  | 0.0160 | 0.9945         | 0.0384 | 0.0034         | 0.0523 |
|                        | 60      | -41.002 | 42.0698 | 0.0005  | 0.0169 | 0.9587         | 0.0290 | 0.0251         | 0.1418 |
|                        | 70      | -38.474 | 39.5310 | 0.0005  | 0.0156 | 0.9728         | 0.0304 | 0.0163         | 0.1135 |
|                        | Average |         |         |         | 0.9753 | 0.0326         | 0.0150 | 0.1026         |        |
| logistics              |         | a       | b       | k       |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | 0.1202  | 1.0945  | 0.0159  |        | 0.9994         | 0.0137 | 0.0002         | 0.0144 |
|                        | 60      | 0.1376  | 1.1244  | 0.0156  |        | 0.9993         | 0.0145 | 0.0003         | 0.0151 |
|                        | 70      | 0.1273  | 1.1047  | 0.0163  |        | 0.9993         | 0.0146 | 0.0003         | 0.0155 |
|                        | Average |         |         |         | 0.9993 | 0.0143         | 0.0003 | 0.0150         |        |
| Mod page 3             |         | k       | d       | n       |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | 1.1291  | 2.8367  | 0.0522  |        | 0.9818         | 0.0726 | 0.0075         | 0.0798 |
|                        | 60      | 1.1350  | 2.8387  | 0.0534  |        | 0.9829         | 0.0703 | 0.0070         | 0.0771 |
|                        | 70      | 1.1275  | 2.8512  | 0.0547  |        | 0.9828         | 0.0702 | 0.0074         | 0.0789 |
|                        | Average |         |         |         | 0.9825 | 0.0710         | 0.0073 | 0.0786         |        |

**Table 4.** Statistical analysis of the examined mathematical thin-layer models for the Abis variety. (continue)

|                |         | a              | k <sub>o</sub> | b      | k <sub>1</sub> |        |                | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|----------------|---------|----------------|----------------|--------|----------------|--------|----------------|----------------|--------|----------------|--------|
| Two terms      | 50      | 0.5927         | 0.0064         | 0.5335 | 0.0064         |        |                | 0.9819         | 0.0711 | 0.0080         | 0.0799 |
|                | 60      | 0.5969         | 0.0066         | 0.5354 | 0.0066         |        |                | 0.9829         | 0.0698 | 0.0074         | 0.0771 |
|                | 70      | 0.5907         | 0.0067         | 0.5333 | 0.0067         |        |                | 0.9828         | 0.0695 | 0.0079         | 0.0790 |
|                | Average |                |                |        |                |        |                | 0.9825         | 0.0701 | 0.0078         | 0.0787 |
|                |         | a              | k <sub>o</sub> | b      | k <sub>1</sub> | c      | k <sub>2</sub> | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
| Three terms    | 50      | 0.3922         | 0.0064         | 0.3778 | 0.0064         | 0.3562 | 0.0064         | 0.9819         | 0.0711 | 0.0091         | 0.0799 |
|                | 60      | 0.3951         | 0.0066         | 0.3798 | 0.0066         | 0.3575 | 0.0066         | 0.9829         | 0.0698 | 0.0085         | 0.0771 |
|                | 70      | 0.3915         | 0.0067         | 0.3769 | 0.0067         | 0.3557 | 0.0067         | 0.9828         | 0.0695 | 0.0091         | 0.0790 |
|                | Average |                |                |        |                |        |                | 0.9825         | 0.0701 | 0.0089         | 0.0787 |
| Mod two term 3 |         | a              | k              |        |                |        |                | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                | 50      | 1.00           | 0.00568        |        |                |        |                | 0.9791         | 0.0533 | 0.0100         | 0.0950 |
|                | 60      | 1.00           | 0.00577        |        |                |        |                | 0.9799         | 0.0505 | 0.0098         | 0.0939 |
|                | 70      | 1.00           | 0.00589        |        |                |        |                | 0.9787         | 0.0514 | 0.0099         | 0.0942 |
| Average        |         |                |                |        |                |        | 0.9792         | 0.0517         | 0.0099 | 0.0944         |        |
| Aghbashlo      |         | k <sub>1</sub> | k <sub>2</sub> |        |                |        |                | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                | 50      | 0.0057         | 0              |        |                |        |                | 0.9791         | 0.0533 | 0.0100         | 0.0950 |
|                | 60      | 0.0058         | 0              |        |                |        |                | 0.9799         | 0.0505 | 0.0098         | 0.0939 |
|                | 70      | 0.0059         | 0              |        |                |        |                | 0.9802         | 0.0514 | 0.0099         | 0.0942 |
| Average        |         |                |                |        |                |        | 0.9797         | 0.0517         | 0.0099 | 0.0944         |        |

**Table 5.** Statistical analysis of the examined mathematical thin-layer models for the Balady variety.

|                        | T, C    | k      |        |         | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|------------------------|---------|--------|--------|---------|----------------|--------|----------------|--------|
| Lewis                  | 50      | 0.0056 |        |         | 0.9813         | 0.0506 | 0.0081         | 0.0877 |
|                        | 60      | 0.0056 |        |         | 0.9842         | 0.0474 | 0.0079         | 0.0866 |
|                        | 70      | 0.0065 |        |         | 0.9838         | 0.0469 | 0.0074         | 0.0834 |
|                        | Average |        |        | 0.9831  | 0.0483         | 0.0078 | 0.0859         |        |
| Henderson<br>and pabis |         | a      | k      |         | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | 1.1221 | 0.0063 |         | 0.9839         | 0.0687 | 0.0059         | 0.0734 |
|                        | 60      | 1.1172 | 0.0064 |         | 0.9863         | 0.0648 | 0.0058         | 0.0722 |
|                        | 70      | 1.1121 | 0.0074 |         | 0.9858         | 0.0631 | 0.0056         | 0.0705 |
|                        | Average |        |        | 0.9853  | 0.0655         | 0.0058 | 0.0720         |        |
| Logarithmic            |         | a      | k      | c       | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | 1.3883 | 0.0037 | -0.3242 | 0.9954         | 0.0384 | 0.0021         | 0.0420 |
|                        | 60      | 1.7693 | 0.0026 | -0.7264 | 0.9972         | 0.0366 | 0.0017         | 0.0377 |
|                        | 70      | 1.5798 | 0.0035 | -0.5352 | 0.9962         | 0.0403 | 0.0019         | 0.0399 |
|                        | Average |        |        | 0.9963  | 0.0385         | 0.0019 | 0.0399         |        |
| Page                   |         | k      | n      |         | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|                        | 50      | 0.0004 | 1.5141 |         | 0.9954         | 0.0400 | 0.0020         | 0.0429 |
|                        | 60      | 0.0006 | 1.4612 |         | 0.9972         | 0.0326 | 0.0014         | 0.0355 |
|                        | 70      | 0.0006 | 1.4870 |         | 0.9973         | 0.0357 | 0.0017         | 0.0389 |
|                        | Average |        |        | 0.9966  | 0.0361         | 0.0017 | 0.0391         |        |

**Table 5.** Statistical analysis of the examined mathematical thin-layer models for the Balady variety. (continue)

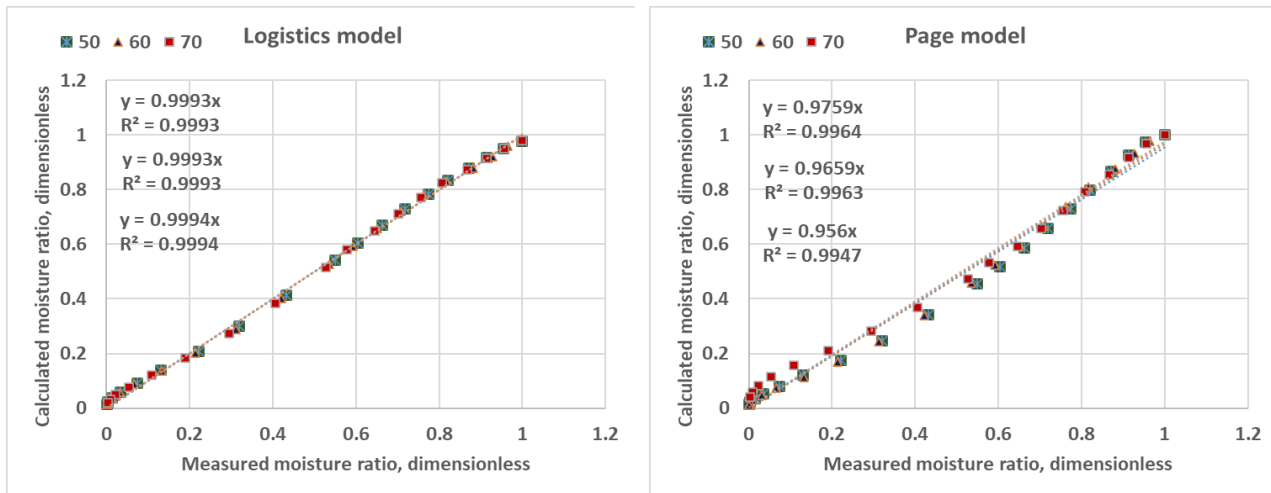
|                |         | a       | b      | k      | c      |        |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
|----------------|---------|---------|--------|--------|--------|--------|--------|----------------|--------|----------------|--------|
| Elfar          | 50      | -42.839 | 43.900 | 0.0005 | 0.0173 |        |        | 0.9962         | 0.0319 | 0.0015         | 0.0353 |
|                | 60      | -29.669 | 30.713 | 0.0005 | 0.0121 |        |        | 0.9302         | 0.0198 | 0.0550         | 0.2084 |
|                | 70      | -33.665 | 34.710 | 0.0006 | 0.0163 |        |        | 0.9691         | 0.0236 | 0.0194         | 0.1228 |
|                | Average |         |        |        |        |        |        | 0.9652         | 0.0251 | 0.0253         | 0.1222 |
|                |         | a       | b      | k      |        |        |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
| logistics      | 50      | 0.1463  | 1.1208 | 0.0146 |        |        |        | 0.9993         | 0.0148 | 0.0003         | 0.0148 |
|                | 60      | 0.1610  | 1.1409 | 0.0145 |        |        |        | 0.9995         | 0.0129 | 0.0002         | 0.0137 |
|                | 70      | 0.1661  | 1.1431 | 0.0166 |        |        |        | 0.9994         | 0.0137 | 0.0003         | 0.0145 |
|                | Average |         |        |        |        |        |        | 0.9994         | 0.0138 | 0.0002         | 0.0143 |
|                |         | k       | d      | n      |        |        |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
| Mod page 3     | 50      | 1.1221  | 2.8376 | 0.0509 |        |        |        | 0.9839         | 0.0687 | 0.0063         | 0.0734 |
|                | 60      | 1.1173  | 2.8390 | 0.0513 |        |        |        | 0.9863         | 0.0648 | 0.0062         | 0.0722 |
|                | 70      | 1.1121  | 2.9144 | 0.0625 |        |        |        | 0.9858         | 0.0631 | 0.0060         | 0.0705 |
|                | Average |         |        |        |        |        |        | 0.9853         | 0.0655 | 0.0061         | 0.0720 |
|                |         | a       | ko     | b      | k1     |        |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
| Two terms      | 50      | 0.5925  | 0.0063 | 0.5296 | 0.0063 |        |        | 0.9839         | 0.0683 | 0.0066         | 0.0734 |
|                | 60      | 0.5814  | 0.0063 | 0.5314 | 0.0063 |        |        | 0.9863         | 0.0635 | 0.0066         | 0.0722 |
|                | 70      | 0.5799  | 0.0073 | 0.5286 | 0.0073 |        |        | 0.9858         | 0.0622 | 0.0064         | 0.0705 |
|                | Average |         |        |        |        |        |        | 0.9853         | 0.0646 | 0.0066         | 0.0720 |
|                |         | a       | ko     | b      | k1     | c      | k2     | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
| Three terms    | 50      | 0.3926  | 0.0063 | 0.3765 | 0.0063 | 0.353  | 0.0063 | 0.9839         | 0.0683 | 0.0075         | 0.0734 |
|                | 60      | 0.3858  | 0.0063 | 0.3733 | 0.0063 | 0.3538 | 0.0063 | 0.9863         | 0.0635 | 0.0076         | 0.0722 |
|                | 70      | 0.3848  | 0.0073 | 0.3721 | 0.0073 | 0.3516 | 0.0073 | 0.9858         | 0.0622 | 0.0075         | 0.0705 |
|                | Average |         |        |        |        |        |        | 0.9853         | 0.0646 | 0.0075         | 0.0720 |
|                |         | a       | k      |        |        |        |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
| Mod two term 3 | 50      | 1.0     | 0.0056 |        |        |        |        | 0.9813         | 0.0506 | 0.0085         | 0.0877 |
|                | 60      | 1.0     | 0.0056 |        |        |        |        | 0.9842         | 0.0474 | 0.0084         | 0.0866 |
|                | 70      | 1.0     | 0.0065 |        |        |        |        | 0.9838         | 0.0469 | 0.0078         | 0.0834 |
|                | Average |         |        |        |        |        |        | 0.9831         | 0.0483 | 0.0082         | 0.0859 |
|                |         | k1      | k2     |        |        |        |        | R <sup>2</sup> | SE     | χ <sup>2</sup> | RMSE   |
| Aghbashlo      | 50      | 0.0056  | 0      |        |        |        |        | 0.9813         | 0.0506 | 0.0085         | 0.0877 |
|                | 60      | 0.0056  | 0      |        |        |        |        | 0.9824         | 0.0474 | 0.0084         | 0.0866 |
|                | 70      | 0.0065  | 0      |        |        |        |        | 0.9838         | 0.0469 | 0.0078         | 0.0834 |
|                | Average |         |        |        |        |        |        | 0.9825         | 0.0483 | 0.0082         | 0.0859 |

### 3.6 Effective Moisture Diffusivity

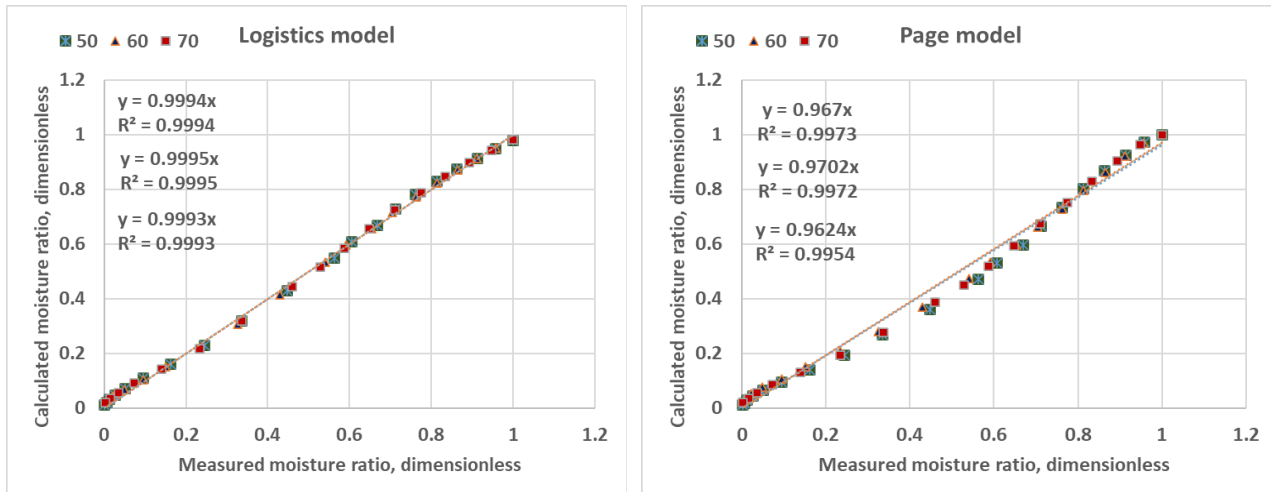
The drying process of food materials generally occurs during the falling rate period. To calculate effective moisture diffusivity values at different levels of drying air temperature, the relationship between the logarithm of the moisture ratio (lnMR) and time (t) was established as shown in Figure 8. The results presented in Table 6 showed that the effective moisture diffusivity values were in the range of  $1.8329 \times 10^{-10}$  to  $2.2888 \times 10^{-10}$  m<sup>2</sup>/s for Abis variety and  $1.8055 \times 10^{-10}$  to  $2.2341 \times 10^{-10}$  m<sup>2</sup>/s for Balady variety, which are generally within the range of  $10^{-11}$  to  $10^{-6}$  m<sup>2</sup>/s given for food materials' moisture diffusion coefficient [55-57]. It can also be seen that the effective moisture diffusivity increased with increasing drying air temperature. This can be explained by the fact that increasing the heat to raise the drying temperature increases the mobility of water molecules, thereby increasing the rate of moisture diffusion. It can also be noted that the moisture diffusivity values of the Balady



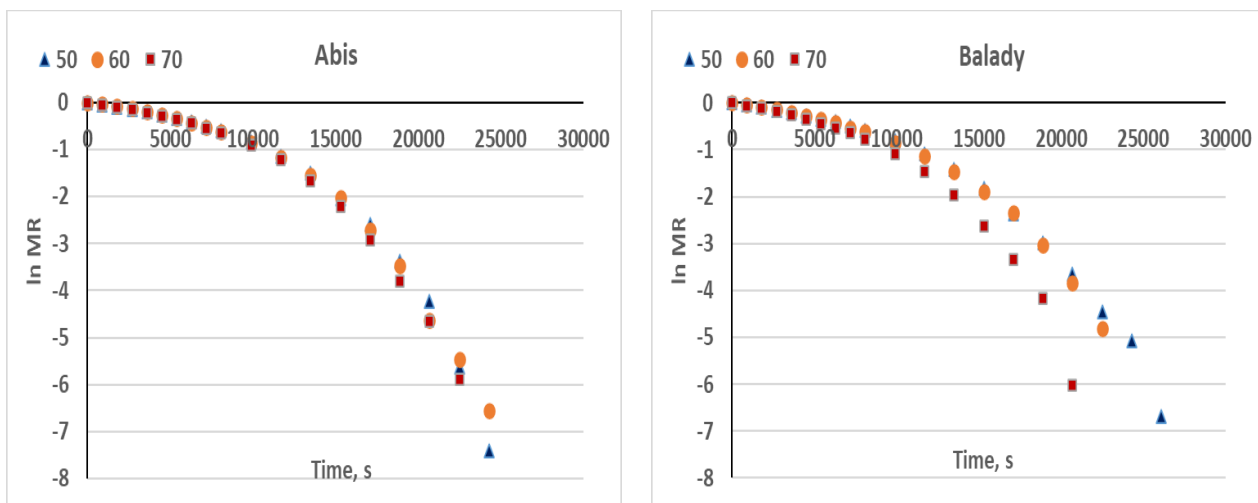
variety were lower than those of the Abis variety at all levels of drying air temperature. This can be attributed to the internal composition of both varieties.



**Figure 6.** The observed and calculated moisture ratios (MRs) for the Abis variety at different drying temperatures.



**Figure 7.** The observed and calculated moisture ratios (MRs) for the Balady variety at different drying temperatures.



**Figure 8.** The relation between  $\ln(MR)$  and drying time at different drying air temperatures.

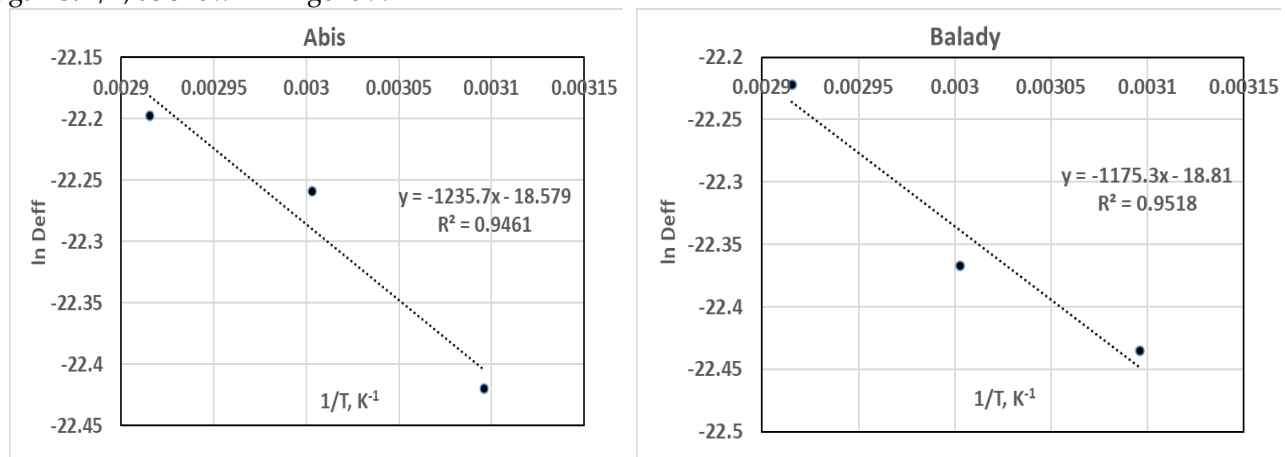
**Table 6.** Values of ( $D_{eff}$ ) for Abis and Balady sweet potato at different levels of drying air temperature.

| Variety | Drying temperature, °C | Linear equation                  | R <sup>2</sup> | Deff, m <sup>2</sup> /s  |
|---------|------------------------|----------------------------------|----------------|--------------------------|
| Abis    | 50                     | $\ln(MR) = 0.9396 - 0.000201(t)$ | 0.8509         | $1.8329 \times 10^{-10}$ |
|         | 60                     | $\ln(MR) = 0.8755 - 0.000236(t)$ | 0.8863         | $2.1521 \times 10^{-10}$ |
|         | 70                     | $\ln(MR) = 0.7616 - 0.000251(t)$ | 0.8872         | $2.2888 \times 10^{-10}$ |
| Balady  | 50                     | $\ln(MR) = 0.8017 - 0.000198(t)$ | 0.8946         | $1.8055 \times 10^{-10}$ |
|         | 60                     | $\ln(MR) = 0.5688 - 0.000212(t)$ | 0.8995         | $1.9332 \times 10^{-10}$ |
|         | 70                     | $\ln(MR) = 0.7180 - 0.000245(t)$ | 0.8750         | $2.2341 \times 10^{-10}$ |

These results are also consistent with the results obtained by [58] when drying sweet potato slices using a hot air dryer with a temperature ranging between 50 and 70°C, where the results they obtained showed that the average values of effective moisture diffusivities of sweet potato slices ranged from  $3.66 \times 10^{-10}$  to  $2.11 \times 10^{-9}$  m<sup>2</sup>/s within the given drying temperature range.

### 3.7 Activation Energy (Ea)

The activation energies for the examined varieties of sweet potato were obtained by plotting  $\ln D_{eff}$  against  $1/T$ , as shown in Figure 9.

**Figure 9.** Plot of  $\ln D_{eff}$  against  $1/T$  for both sweet potato varieties.

As shown in Equations (33) and (34), the drying air temperature has a noticeable effect on the effective moisture diffusivity ( $D_{eff}$ ) of sweet potatoes.

$$D_{eff} = 8.54 \times 10^{-9} \exp \left( -\frac{1235.7}{T} \right) \quad (R^2 = 0.9461) \quad \text{for Abis variety} \quad (33)$$

$$D_{eff} = 6.76 \times 10^{-9} \exp \left( -\frac{1175.3}{T} \right) \quad (R^2 = 0.9518) \quad \text{for Balady variety} \quad (34)$$

The coefficients of determination for the fitted lines based on the experimental data were mostly close to unity, indicating a strong correlation. The activation energy was found to be 10.274 kJ/mol for the Abis variety and 9.772 kJ/mol for the Balady variety. The values obtained in this study were in a close range to 12.7–110 kJ/mol for various foods reported by [59]. The values obtained from this study are also close to those obtained by [45] for sweet potato cubes (11.38 kJ/mol) and close to the values obtained by [60] for sweet potato slices (8.74 – 34.76 kJ/mol).

### 3.8 Thermal efficiency of the solar collector:

Table 7 shows the calculated thermal efficiency of the solar collector. As shown in the table, the solar collector's absorption efficiency ranged from 77.25 to 81.88%, and its heat transfer efficiency ranged from 81.6 to 84.0%. In contrast, the overall thermal efficiency of the solar collector ranged from 65.1% to 70.4%.

**Table 7.** Solar collector thermal performance and efficiency.

| Date       | Variety | Air temperature, C | solar radiation W/m <sup>2</sup> | Ambient air temperature, C | Air temperature at the outlet of solar | Solar energy available Q, W | Absorbed solar energy Q <sub>a</sub> , W | absorption efficiency $\zeta_a$ | Useful heat gain Q <sub>c</sub> , W | Heat transfer efficiency, $\zeta_h$ | Solar collector heat losses, Q <sub>l</sub> , W | Overall thermal efficiency $\zeta_o$ |
|------------|---------|--------------------|----------------------------------|----------------------------|----------------------------------------|-----------------------------|------------------------------------------|---------------------------------|-------------------------------------|-------------------------------------|-------------------------------------------------|--------------------------------------|
| 06/08/2025 | Abis    | 50                 | 760.3                            | 36.6                       | 47.2                                   | 1520.7                      | 1202.1                                   | 0.7729                          | 989.7                               | 0.825                               | 212.4                                           | 0.651                                |
| 23/07/2025 |         | 60                 | 738.8                            | 36.4                       | 47.1                                   | 1477.5                      | 1204.3                                   | 0.8188                          | 1007.3                              | 0.816                               | 197.0                                           | 0.682                                |
| 24/07/2025 |         | 70                 | 786.4                            | 37.0                       | 48.8                                   | 1572.8                      | 1263.9                                   | 0.7962                          | 1106.7                              | 0.836                               | 157.2                                           | 0.704                                |
| 07/08/2025 | Balady  | 50                 | 799.1                            | 36.1                       | 47.4                                   | 1598.2                      | 1261.0                                   | 0.7725                          | 1057.9                              | 0.840                               | 203.1                                           | 0.662                                |
| 09/08/2025 |         | 60                 | 764.6                            | 39.3                       | 50.1                                   | 1529.2                      | 1226.1                                   | 0.7923                          | 1011.7                              | 0.832                               | 214.3                                           | 0.662                                |
| 10/08/2025 |         | 70                 | 776.4                            | 41.5                       | 52.3                                   | 1552.8                      | 1258.4                                   | 0.8026                          | 1013.4                              | 0.823                               | 245.0                                           | 0.653                                |

### 3.9 Energy consumption and thermal efficiency of the solar dryer:

Results in Table 8 showed that the total heat energy provided varied across treatments due to differences in the solar energy available and the energy required for drying. Table 8 also showed that the overall thermal efficiency of the developed solar dryer with the auxiliary heaters ranged from 11.1245 to 12.5004%, and the solar collector provided 95.60 – 96.18%, 91.88 – 93.28%, and 89.04 – 91.61% of the total heat energy consumption for air heating to the level of 50, 60, and 70 OC respectively. It can be said that the main parameters affecting the proportions of heat energy consumption in the form of electrical and solar energy were solar collector surface area, ambient air temperature, available solar energy, and the drying air temperature required for the drying process. Previous studies have shown that the overall efficiency of hybrid dryers is low because the total available energy is much greater than the energy required to dry and evaporate moisture from the product. The overall efficiency of the hybrid solar dryer equipped with a biomass thermal heating unit was 15.59% when drying ginger [61]. The overall drying efficiency of the hybrid solar dryer with a biomass combustion unit for drying fresh pineapples was 13% [62]. The overall drying efficiency of chili was 11.06% using a hybrid dryer system that combines an unglazed transpired solar collector, a rock bed, and a biomass gasifier stove with a heat exchanger [63]. In addition, climate change has led to an increase in solar radiation, with intensity exceeding 1000 W/m<sup>2</sup> during laboratory experiments, thereby increasing the total available energy inside the dryer.

**Table 8.** Thermal efficiency and energy consumption of the solar dryer.

| Date       | Variety | Useful heat gain by the collector, kJ | Sensible heat, kJ | mass of moisture removed, kg | Average latent heat of evaporation, kJ/kg | Latent heat of evaporation, kJ | Total heat energy required for drying, kJ | Average heat energy gain by auxiliary heaters, kJ | Useful heat gain by the solar dryer, kJ | Total heat energy available, kJ | average thermal efficiency of the dryer | average energy saving by solar collector and solar dryer | Average energy saving by solar collector and dryer |
|------------|---------|---------------------------------------|-------------------|------------------------------|-------------------------------------------|--------------------------------|-------------------------------------------|---------------------------------------------------|-----------------------------------------|---------------------------------|-----------------------------------------|----------------------------------------------------------|----------------------------------------------------|
| 06/08/2025 | Abis    | 35628.4                               | 167.669           | 3.7805                       | 2569.884                                  | 9110.351                       | 9278.020                                  | 2880                                              | 36853.56                                | 75361.96                        | 12.3113                                 | 96.1784                                                  |                                                    |
| 23/07/2025 |         | 36261.4                               | 190.744           | 3.7824                       | 2581.225                                  | 9118.311                       | 9309.055                                  | 5400                                              | 38681.92                                | 80343.32                        | 11.5866                                 | 93.2788                                                  | 93.69                                              |
| 24/07/2025 |         | 35857.1                               | 173.374           | 3.8625                       | 2554.214                                  | 9314.356                       | 9487.730                                  | 6480                                              | 34918.27                                | 77255.37                        | 12.2810                                 | 91.6122                                                  |                                                    |
| 07/08/2025 | Balady  | 38084.9                               | 171.868           | 3.7120                       | 2528.261                                  | 8930.485                       | 9102.353                                  | 3600                                              | 40138.04                                | 81822.94                        | 11.1245                                 | 95.6003                                                  |                                                    |
| 09/08/2025 |         | 32780.2                               | 140.970           | 3.6209                       | 2484.492                                  | 8728.984                       | 8869.954                                  | 5760                                              | 32416.98                                | 70957.18                        | 12.5004                                 | 91.8824                                                  | 92.18                                              |
| 10/08/2025 |         | 29184.7                               | 146.328           | 3.2728                       | 2538.02                                   | 7898.114                       | 8044.442                                  | 7200                                              | 29331.97                                | 65716.67                        | 12.2411                                 | 89.0439                                                  |                                                    |

#### 4. Conclusions

This research was conducted to examine the drying behavior of two varieties of sweet potato slices in a mixed-mode hybrid solar dryer assisted by an electric heating unit. The regression analysis showed that the Logistics models, among eleven thin-layer drying models, provide the best prediction of the moisture ratio for sweet potato slices, followed by the Page model. The effective moisture diffusivity values were in the ranges of  $(1.8329 \times 10^{-10}$  to  $2.2888 \times 10^{-10})$  and  $(1.8055 \times 10^{-10}$  to  $2.2341 \times 10^{-10})$  m<sup>2</sup>/s for the Abis and Balady varieties, respectively. The activation energies were 10.274 and 9.772 kJ/mol for the Abis and Balady varieties, respectively. The overall thermal efficiency of the solar collector ranged from 65.1% to 70.4%. The overall thermal efficiency of the dryer ranged from 11.1245 to 12.5004%. This indicates that the total available thermal energy inside the dryer was much greater than the energy required for drying, and therefore, the amount of product to be dried could be increased to maximize the use of this energy.

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# Institutional Barriers and Stakeholder Perceptions in Agricultural Education: A Descriptive-Correlational Study of the BSA Program at Cebu Technological University–Barili Campus

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**Abstract:** The declining enrollment in agricultural degree programs among Filipino youth presents a critical challenge to long-term food security and agricultural sustainability. This study assessed the level of awareness, perceptions, and perceived barriers toward the Bachelor of Science in Agriculture program among Senior High School students, parents, and administrators in Barili, Cebu. Utilizing a descriptive-correlational research design, the study analyzed quantitative data through weighted means and p-value significance tests. The findings revealed a "Low" overall level of awareness among all respondent groups (mean = 2.02), with students demonstrating the lowest familiarity regarding diverse career opportunities in the sector (mean = 1.88). While general perceptions toward the program were "Moderate" (mean = 2.75), the analysis identified significant relationships ( $p < 0.05$ ) between respondent profiles—specifically student age, parental occupation, and family size—and their perceptions of the field. The most critical "Very Serious Barrier" to program enrollment was the lack of information and promotional campaigns (mean = 3.65), followed by negative perceptions of agriculture (mean = 3.42), and the belief that agriculture is not a financially viable career (mean = 3.31). These results underscore a pervasive informational gap that hinders the recruitment of the next generation of agricultural professionals. The study concludes that institutional interventions must prioritize strategic rebranding and modernized outreach to correct societal biases and showcase the technology-driven professional viability of the 21st-century agricultural industry.

**Keywords:** Agriculture education; BSA program; student perceptions; enrollment barriers; career choice

## 1. Introduction

Agriculture remains the backbone of the Philippine economy, yet it faces a critical demographic crisis as the average age of Filipino farmers continues to rise, currently estimated at 57-59 years [1]. Despite the sector's vital role in national food security, there is a marked decline in youth interest in farming, with many students viewing agricultural careers as labor-intensive, low-paying, and lacking social prestige [2-5]. This "status gap" is particularly evident in rural areas like Barili, Cebu, where the youth often perceive non-

agricultural degrees as the only viable pathway for upward social mobility [6, 7]. While the Bachelor of Science in Agriculture program is designed to professionalize the sector by integrating science and technology into traditional farming, enrollment remains stagnant due to persistent misconceptions held by students and their primary influencers—parents and school administrators [8]. Research suggests that parental discouragement, often rooted in the desire for children to escape manual labor, significantly steers students toward urban-centric professions [9, 10]. Therefore, this study aims to evaluate the levels of awareness and perceptions toward the BSA program among key stakeholders in Barili, Cebu, providing a foundation for localized interventions and promotional strategies to revitalize interest in agricultural education [11, 12].

## 2. Materials and Methods

### 2.1 Research Design and Location of the Study

This study employed a descriptive-correlational research design to systematically describe current levels of awareness and perceptions of the BSA program, and to investigate relationships between respondent profiles and their attitudes toward the field. This design is appropriate for identifying patterns in stakeholder opinions and determining if demographic variables, such as age or parental occupation, significantly influence career-related perceptions [9] [3]. The study was conducted in the municipality of Barili, Cebu, a locality with a significant agricultural footprint. The respondents included Senior High School students, who represent the target demographic for BSA enrollment; parents, identified as the primary decision-influencers in the Filipino family structure; and school administrators, who are responsible for career guidance and the implementation of agricultural curricula at the secondary level [10-11, 13]. A total of 200 respondents participated in the study, comprising 120 Senior High School students, 60 parents, and 20 administrators, selected through purposive sampling. The overall response rate was 95%. The primary data collection tool was a researcher-developed, structured survey questionnaire divided into three parts: a respondent profile section, an awareness and perceptions section measured using a 4-point Likert Scale, and a section dedicated to identifying barriers to program implementation and enrollment [11].

### 2.2 Data Gathering Procedure

The data gathering process followed a systematic four-stage approach to ensure ethical compliance and data integrity. It began with preliminary coordination, during which formal letters of request were sent to the Department of Education district office and school principals in Barili, Cebu, to secure permission for student and staff involvement. Following approval, an orientation was conducted for all participants to explain the study's objectives, and informed consent was obtained, emphasizing that participation was voluntary and that all personal information would remain confidential under the Data Privacy Act. The survey administration was then carried out, with questionnaires distributed to students and administrators during scheduled school visits, while parents received their surveys through their children or during parent-teacher association meetings. Finally, the researchers conducted a data retrieval and quality check, reviewing all completed forms for accuracy and completeness to ensure that only fully accomplished surveys were included in the final data set for statistical analysis.

### 2.3 Data Analysis

The gathered data were encoded and analyzed using a combination of descriptive and inferential statistical tools to answer the research questions. The weighted mean was calculated to determine the average levels of awareness and perceptions, as well as the severity of perceived barriers, using a scale ranging from "Very Low/Not a Barrier" to "Very High/Very Serious Barrier." To investigate the significant relationship between respondents' demographic profiles—such as age, parental occupation, and family size, and their perceptions toward the BSA program, the researchers employed Pearson's Square ( $\chi^2$ ) tests for categorical variables (gender, parental occupation, family size) and Pearson Correlation ( $r$ ) for continuous or ordinal variables (age) where data distribution was confirmed as approximately normal. A  $p$ -value threshold of less than 0.05 ( $p < 0.05$ ) was used to determine statistical significance, and effect sizes were calculated using Cramér's  $V$  for Chi-Square tests and  $r$  for Pearson Correlation, aligning with established methodologies in agricultural education research. The researcher-developed questionnaire was subjected to content validation



by three subject matter experts and pilot-tested with 30 respondents not included in the main study. Cronbach's alpha coefficients were calculated for each Likert-scale section to confirm internal consistency reliability (awareness:  $\alpha = 0.82$ ; perceptions:  $\alpha = 0.79$ ; barriers:  $\alpha = 0.85$ ) [3]. Furthermore, a ranking system was used to identify the most critical challenges and barriers reported by stakeholders, enabling clear prioritization of the factors hindering enrollment in the BSA program [1].

### 3. Results and Discussion

The overall mean score of 2.02 indicates a "Low Awareness" among students, particularly regarding career opportunities (1.88). This critical informational gap aligns with findings that the Filipino youth's intention to enroll in agricultural programs is heavily influenced by limited exposure to modern practices [3], [14]. Research suggests that agriculture remains undervalued in the national curriculum, where it is often categorized as a vocational "laborer" track rather than a scientific profession [2]. Consequently, students fail to recognize the transition toward a technology-driven agribusiness sector [15]. This lack of knowledge about the "Nature and Scope" (2.15) of the program perpetuates the cycle of low enrollment as students dismiss the field based on incomplete information [8].

**Table 1.** Level of Awareness of Senior High School Students Toward the BSA Program in Terms of Its Nature and Scope

| Indicator                           | Mean Score | Verbal Interpretation |
|-------------------------------------|------------|-----------------------|
| Nature and Scope of the BSA Program | 2.15       | Low Awareness         |
| Career Opportunities in Agriculture | 1.88       | Low Awareness         |
| Overall Mean                        | 2.02       | Low Awareness         |

Legend: Awareness was measured on a 4-point scale where 1.00-1.75 = Very Low, 1.76-2.50 = Low, 2.51-3.25 = Moderate, 3.26-4.00 = High.

#### 3.1 Level of Awareness of Parents and Administrators towards the BSA Program

The low awareness among parents (2.12) and administrators (2.45) is significant because these groups are the primary influencers in a student's career decision-making process [15]. Studies in the Philippines highlight a concerning trend in which farmers themselves notice that their children lack interest and often encourage them to pursue non-agricultural degrees for perceived greater stability [16]. Furthermore, in many households, fathers act as the dominant decision-makers, and their perception of agriculture as a "low-status" vocation can actively steer students away from the BSA program [9]. Improving awareness among these adult influencers is essential to correct misconceptions about the modern viability of the agricultural profession [2].

**Table 2.** Level of Awareness of Parents and Administrators towards the BSA Program

| Respondent Group | Mean Score | Verbal Interpretation |
|------------------|------------|-----------------------|
| Parents          | 2.12       | Low Awareness         |
| Administrators   | 2.45       | Low Awareness         |
| Overall Mean     | 2.28       | Low Awareness         |

Legend: Awareness was measured on a 4-point scale where 1.00-1.75 = Very Low, 1.76-2.50 = Low, 2.51-3.25 = Moderate, 3.26-4.00 = High

#### 3.2 Level of Perceptions of Senior High School Students, Parents, and Administrators towards the BSA Program

The moderate perception (2.75) across all groups indicates that while the field is not viewed entirely negatively, it still lacks the prestige associated with other degrees. This "status gap" is a common challenge in the ASEAN region, where agriculture is perceived as riskier and more difficult to achieve than other sectors [11, 17, 18]. Even when students are initially interested, the absence of a modern curriculum that emphasizes "Agricultural Entrepreneurship," combining cultivation mastery with managerial skills, makes the degree less appealing to those seeking innovation-driven careers [9, 12, 19]. Rebranding is necessary to shift the view of traditional labor from a traditional labor to a professionalized industry [9].

**Table 3.** Level of Perceptions of Senior High School Students, Parents, and Administrators towards the BSA Program



| Respondent Group | Mean Score | Verbal Interpretation |
|------------------|------------|-----------------------|
| Students         | 2.55       | Moderate Perception   |
| Parents          | 2.68       | Moderate Perception   |
| Administrators   | 3.01       | Moderate Perception   |
| Overall Mean     | 2.75       | Moderate Perception   |

### 3.3 Relationship between the Profile of the Respondents and their Perceptions toward the BSA Program

The significant relationship between parental occupation/family size and perception ( $p < 0.05$ ) reflects economic pragmatism; parents often discourage agriculture if they perceive it cannot provide financial security for a large household [10]. For students, the significance of age ( $p < 0.05$ ) suggests that as they approach graduation, they become more pragmatic about employability [3]. These findings are supported by longitudinal studies in the Philippines, where personal and economic perceptions were found to be statistically significant ( $p < 0.05$ ) in determining a youth's intention to enroll in agricultural programs [3, 8].

**Table 4.** Relationship between the Profile of the Respondents and their Perceptions toward the BSA Program

| Respondent Group | Demographic Profile Variable         | p-value  | Decision        | Verbal Interpretation              |
|------------------|--------------------------------------|----------|-----------------|------------------------------------|
| Administrators   | Age, Gender, Length of Service       | $> 0.05$ | Not Significant | No significant relationship.       |
| Parents          | Age, Gender, Educational Attainment  | $> 0.05$ | Not Significant | No significant relationship.       |
|                  | Occupation, Number of Family Members | $< 0.05$ | Significant     | A significant relationship exists. |
| Students         | Gender, Socio-economic Status        | $> 0.05$ | Not Significant | No significant relationship.       |
|                  | Age                                  | $< 0.05$ | Significant     | A significant relationship exists. |

Legend: A p-value less than 0.05 indicates a statistically significant relationship.

### 3.4 Challenges and Barriers to the Implementation of the BSA Program

The ranking of "Lack of information and promotional campaigns" (3.65) as a very serious barrier confirms that disinterest in agriculture stems from insufficient knowledge rather than personal preference [1, 20]. Addressing this requires a shift from traditional lectures to social-media-driven marketing that showcases success stories to inspire the younger generation [12, 21]. Furthermore, the serious barrier of "lack of facilities" (3.19) mirrors regional findings that many agricultural schools lack classrooms for specific purposes and the necessary smart-farming infrastructure, such as IoT tools, to attract tech-savvy students [15], [22, 23].

**Table 5.** Challenges and Barriers to the Implementation of the BSA Program

| Rank | Challenges and Barriers                                | Mean Score | Verbal Interpretation |
|------|--------------------------------------------------------|------------|-----------------------|
| 1    | Lack of information and promotional campaigns          | 3.65       | Very Serious Barrier  |
| 2    | Negative perceptions of agriculture                    | 3.42       | Very Serious Barrier  |
| 3    | Belief that agriculture is not a lucrative career      | 3.31       | Very Serious Barrier  |
| 4    | Absence of clear career opportunities after graduation | 3.19       | Serious Barrier       |
| 5    | Lack of government support for agriculture education   | 2.98       | Serious Barrier       |

Legend: The barrier level was measured on a 4-point scale where 1.00-1.75 = Not a Barrier, 1.76-2.50 = Less Serious, 2.51-3.25 = Serious, 3.26-4.00 = Very Serious.

## 4. Conclusion

The study confirms that the primary barrier to the successful implementation and promotion of the Bachelor of Science in Agriculture (BSA) program at Cebu Technological University-Barili Campus is a

widespread lack of awareness and information among public Senior High School students, parents, and administrators. The findings revealed that all three groups have low levels of awareness, a direct consequence of inadequate promotional campaigns and the institution's lack of outreach. This informational gap, coupled with persistent negative perceptions of agriculture as a low-paying and labor-intensive profession, actively discourages potential students from pursuing the program. The study's results are critical for informing strategic interventions. The solution lies not just in providing more information but in actively rebranding agriculture as a modern, technology-driven, and financially viable career path. Future efforts should focus on designing and implementing targeted campaigns that address parents' specific concerns about economic stability and showcase the diverse career opportunities available to BSA graduates. By bridging the informational divide and challenging negative social biases, educational institutions can help secure the future of agriculture by attracting the next generation of professionals to the field.

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# Molecular Detection of Emerging Begomoviruses Associated with Pepper (*Capsicum* spp.) in the Philippines

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**Abstract:** Several *Begomoviruses* have become significant plant viruses affecting pepper crops worldwide. In the Philippines, begomoviruses infecting pepper were first documented in 2011, but since then, no further studies have been conducted. As new and emerging begomoviral diseases continue to threaten and hamper crop production, it is important to deal with them proactively to mitigate losses. Here, begomoviruses associated with pepper in the country were successfully detected and identified using a combination of conventional molecular methods and next-generation sequencing (NGS). Pepper leaf samples exhibiting disease symptoms were gathered from various locations across the country. Presence of begomovirus infection was initially detected through visual observation of its characteristic symptoms (leaf curling/cupping/crinkling, interveinal yellowing of leaves, and apical leaf size reduction) and subsequently verified through polymerase chain reaction (PCR) using begomovirus-specific degenerate primers, together with rolling circle amplification (RCA). Emerging begomoviruses in pepper were not detected by restriction enzyme digestion but were identified through NGS-derived sequences. Two known and five unknown begomoviruses were identified from NGS sequences and provided evidence of the presence of emerging known begomoviruses in new areas, as well as new begomoviruses of pepper in the Philippines. This is the first study to employ Illumina-NGS technology for the molecular surveillance of begomoviruses infecting pepper in the Philippines.

**Keywords:** Bioinformatics; next-generation sequencing; phylogenetic analysis; polymerase chain reaction; rolling circle amplification

## 1. Introduction

Peppers (*Capsicum* spp.) rank among the key spice and vegetable crops grown in tropical and subtropical regions, valued for their characteristic spicy taste and for being excellent sources of vitamins A and C, along with various phenolic acids [1, 2]. They are produced in numerous varieties, exhibiting considerable variation in fruit form, size, color, and pungency [3]. Although considered a minor crop in the Philippines, pepper is cultivated year-round and continues to face increasing market demand [4]. Virus infections remain among the most significant limitations to pepper production globally [5, 6]. To date, 49 virus species have been identified as infecting pepper [6, 7], and approximately 20 of them, representing 15 distinct taxonomic groups, have been documented

to cause substantial losses in pepper-growing areas [6]. The appearance of new viral problems in pepper production is not unexpected, as outbreaks of newly identified viruses or the increasing incidence and severity of established ones are often associated with intensified farming systems and expanding global trade [8]. Because peppers are cultivated across broad geographic regions, they are exposed to diverse pathogens [9]. The movement of contaminated propagative materials through trade promotes the introduction of viruses and their insect vectors into previously unaffected areas [10]. Moreover, large-scale cultivation of single pepper varieties promotes the adaptation, transmission, and survival of begomoviruses across successive cropping seasons [11]. In addition, shifts in climate patterns can alter vector distributions and increase plant vulnerability, thereby heightening the risk of emerging viral diseases in pepper-growing areas.

Several begomoviruses (genus *Begomovirus*, family *Geminiviridae*) have become prominent viral pathogens affecting pepper crops worldwide. The whitefly *Bemisia tabaci* transmits the genus *Begomovirus*, the largest genus of plant viruses, which now comprises over 400 identified species [12, 13]. Members of this genus possess circular, single-stranded DNA (ssDNA) genomes arranged either as a single component (monopartite) or as two separate components (bipartite), each approximately 2.6-3.0 kb in size. These genomes are enclosed within characteristic twinned (geminata) icosahedral particles measuring about 22 x 38 nm [13]. More recent studies have further described the structural organization, genome architecture, and evolutionary dynamics of begomoviruses, emphasizing their genomic plasticity and recombination-driven diversification [14, 15]. In the Western Hemisphere, begomoviruses infecting pepper (*Capsicum* spp.) were initially documented in the United States [16] and subsequently in Mexico [17]. Recent reports confirmed the continued occurrence and expanding diversity of begomoviruses infecting pepper across the Americas, highlighting their ongoing evolution and geographic spread [14, 15]. In Oman, *Tomato yellow leaf curl virus* (TYLCV; recently reclassified as *Begomovirus coheni* by the ICTV [12]) was reported infecting sweet pepper, producing typical begomovirus symptoms such as upward leaf curling, chlorosis, and stunting [18]. In India, TYLCV-associated leaf curl disease in pepper has been widely documented and remains prevalent [19]. In the Philippines, begomoviruses were first detected in pepper in 1994 using a TYLCV-based DNA probe [20]. Subsequent reports confirmed the presence of *Tomato leaf curl Cebu virus* infecting pepper [21].

Accurate detection and identification of viruses are critical for effective disease management, especially in breeding and developing resistant cultivars. To date, only one study [21] has been done on the molecular identification of begomoviruses associated with pepper in the Philippines. As new and emerging begomoviral diseases continue to threaten and hamper pepper crop production, it is imperative to be proactive in dealing with them rather than reactive. Early detection and precise identification of emerging begomoviruses infecting pepper are crucial for implementing appropriate management strategies and minimizing potential yield losses. In several countries, next-generation sequencing (NGS) has been used effectively to detect and identify emerging viral pathogens, including begomoviruses rapidly. In this study, emerging begomoviruses infecting pepper were detected and identified by conventional and next-generation sequencing to assess their occurrence and geographic distribution across the country's crop-producing provinces.

## 2. Materials and Methods

### 2.1 Survey and Sample Collection

Field surveys and sampling were conducted to determine the occurrence of *Begomovirus* in the major crop-growing provinces of Luzon (Laguna, Quezon, Bulacan, Batangas, Nueva Ecija, Pangasinan, Ilocos Sur, and Ilocos Norte), Visayas (Cebu, Iloilo, and Leyte), and Mindanao (Butuan and Davao). In total, 160 leaf samples were obtained from 32 pepper farms across the surveyed areas. Leaf samples were randomly collected from five to ten plants exhibiting virus-like symptoms per site. Relevant field data, including GPS coordinates of field location, crop growth stage, observed whitefly populations, cultivar planted, and other agronomic details, were documented. All samples were brought to the Plant Virology Laboratory, Institute of Weed Science, Entomology and Plant Pathology (IWEP), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), and stored at 40 °C before molecular analysis.



## 2.2 Field Assessment of Begomovirus Symptoms

Begomovirus infection in pepper fields was evaluated by visual inspection for characteristic leaf curl symptoms, including leaf curling, cupping, or crinkling, accompanied by interveinal yellowing. Other virus-associated symptoms such as mosaic or mottle patterns, chlorosis, leaf distortion, and plant stunting were also noted. All symptomatic plants were photographed, and symptoms were categorized based on symptom type, crop growth stage, and sampling location.

## 2.3 Molecular Detection of Begomovirus Using Polymerase Chain Reaction (PCR)

Begomovirus infection in symptomatic pepper leaves was confirmed through polymerase chain reaction (PCR) using two sets of degenerate primers, TY1F/TY2R and PAL1v1978B/PAR1c715H. The TY1F/TY2R primer pair amplified an approximately 585 bp fragment corresponding to the coat protein gene [22] while PAL1v1978B/PAR1c715H generated approximately 1.5 kb of the DNA-A component [21, 23]. Total genomic DNA was isolated from symptomatic leaf tissues following the Dellaporta et al. protocol [24]. DNA quality and concentration were assessed spectrophotometrically using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) by measuring the A260/A280 and A260/A230 absorbance ratios; only samples with A260/A280 ratios between 1.8 and 2.0 were used for downstream molecular analyses. PCR reactions were prepared in a 25.0  $\mu$ L mixture containing PCR buffer (2.5  $\mu$ L 1X),  $MgCl_2$  (1.0  $\mu$ L, 2 mM), dNTPs (0.5  $\mu$ L), forward and reverse primers (1.0  $\mu$ L, 10.0  $\mu$ M), *Taq* DNA Pol (0.2  $\mu$ L, 1U, Invitrogen, Carlsbad, CA, USA), sterile nuclease-free water (16.8  $\mu$ L), and DNA template (2.0  $\mu$ L, 50.0 ng/ $\mu$ L). The composition of the reaction mixture was modified as needed based on the primer pair used. Each PCR assay included DNA from a healthy plant as the negative control, sterile distilled water as the blank control, and DNA from a confirmed ToLCPV-infected plant as the positive control. PCR amplification was carried out in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). For the TY1R/TY2R primer pair, the cycling profile included an initial denaturation at 94 °C for 1 minute, followed by 35 cycles of denaturation at 94 °C for 15 seconds, annealing at 55 °C for 15 seconds, and extension at 72 °C for 1 minute, with final extension at 72 °C for 10 minutes. For the PAL1v1978B/PAR1c715H primers, the protocol began with an extended initial denaturation at 94 °C for 5 minutes, followed by 35 cycles incorporating a longer annealing step at 55 °C for 2 minutes and extension at 72 °C for 2 minutes, and concluded with a final extension at 72 °C for 10 minutes.

## 2.4 Rolling Circle Amplification (RCA)

Circular single-stranded DNA was amplified from total nucleic acids extracted from symptomatic pepper leaves using the TempliPhi Amplification Kit (GE Healthcare Life Sciences, Chicago, IL, USA) in accordance with the manufacturer's instructions. Briefly, 1.0  $\mu$ L of undiluted total DNA was combined with 5.0  $\mu$ L of sample buffer, incubated at 95 °C for 3 minutes in a thermoshaker, and immediately chilled on ice. Thereafter, 5  $\mu$ L of reaction buffer and 0.2  $\mu$ L of enzyme mix were added to each sample, followed by incubation at 30 °C for 18 hours in a Biometra Thermoshaker (Analytik Jena, Jena, Germany). The amplification reaction was terminated by heating at 65 °C for 10 minutes to inactivate the enzyme. The resulting rolling circle amplification (RCA) products were analyzed by agarose gel electrophoresis. Specifically, 1.0  $\mu$ L of the RCA product was mixed with 5.0  $\mu$ L of 1x loading dye and loaded onto a 0.8% agarose gel pre-stained with GelRed (Biotium, Fremont, CA, USA) (1.0%), then electrophoresed at 95 V for 40 minutes.

## 2.5 Restriction Enzymes (RE) Digestion

RCA-restriction enzyme digestion analysis [25] was performed on RCA products from representative PCR-positive and PCR-negative pepper samples to detect Begomovirus(es). These four enzymes were selected based on the restriction fragment profiles previously described for begomoviruses [25, 21], where *Sal*I (recognition site: G<sup>^</sup>TCGAC), *Hind*III (A<sup>^</sup>AGCTT), *Eco*RI (G<sup>^</sup>AATTC), and *Bam*HI (G<sup>^</sup>GATCC) are known to generate diagnostic fragments of approximately 2.7 kb (*Sal*I), 0.8 and 1.9 kb (*Hind*III), 1.5 and 1.2 kb (*Eco*RI), and 2.7 kb (*Bam*HI) for a typical ~2.7-kb begomovirus genome, thereby enabling species-level discrimination based on their distinct restriction site distributions. Rolling circle amplification (RCA) products were digested with *Sal*I, *Hind*III, *Eco*RI, and *Bam*HI. Each digestion reaction was performed according to the manufacturer's protocol, using 0.5  $\mu$ L of RCA-amplified viral DNA and incubating the reaction at 37 °C for 1 hour in a Biometra ThermoShaker (Analytik Jena, Jena, Germany). The restriction enzyme-digested products were

subsequently analyzed by agarose gel electrophoresis. A total of 10 µL of the digested RCA product was mixed with 2 µL of 6x loading dye and loaded onto a 0.8% agarose gel prepared in 1x TBE (Tris-borate-EDTA) buffer and pre-stained with 1.0% GelRed. Electrophoresis was performed at 95 V for 30 minutes. Detection of potential emerging Begomovirus was based on comparison of the resulting restriction fragment patterns with those of previously characterized begomovirus species.

## 2.6 Illumina MiSeq Sequencing

A total of 10 RCA-amplified DNA samples (5.0 µL) from pepper (5 from bell pepper and 5 from chili pepper) were submitted to the DNA Sequencing Laboratory at the Philippine Genome Center (PGC) (UP Diliman, Quezon City, Philippines) for next-generation sequencing. The RCA-amplified DNA samples were purified using the Ampure DNA purification kit (Beckman Colter, Brea, CA, USA), followed by library preparation using Nextera XT and deep sequencing on the Illumina MiSeq (2x300 V3) (Illumina, San Diego, CA, USA).

## 2.7 Viral Genome Assembly and Analysis

NGS sequences were submitted to the Core Facility for Bioinformatics (PGC, UP Diliman, Quezon City) for quality control assessment, de novo assembly, annotation, and phylogenetic analysis. Figure 1 shows the PGC bioinformatics pipeline (PGC) employed for the assembly and analysis of begomoviral genome sequences. Briefly, each sample was trimmed, clipped, and filtered using fastp [26]. Quality-filtered sequencing reads were assembled into contigs using SPAdes [27], and the resulting contigs were subsequently annotated through BLASTn. For contigs that did not map to begomovirus sequences, they were filtered out. From the remaining contigs, “genes” were predicted using GeneMark [28]. These “genes” were re-mapped and re-annotated using BLASTn. The sequences of annotations that were present in the greatest number of samples were then used for alignment and downstream analysis.

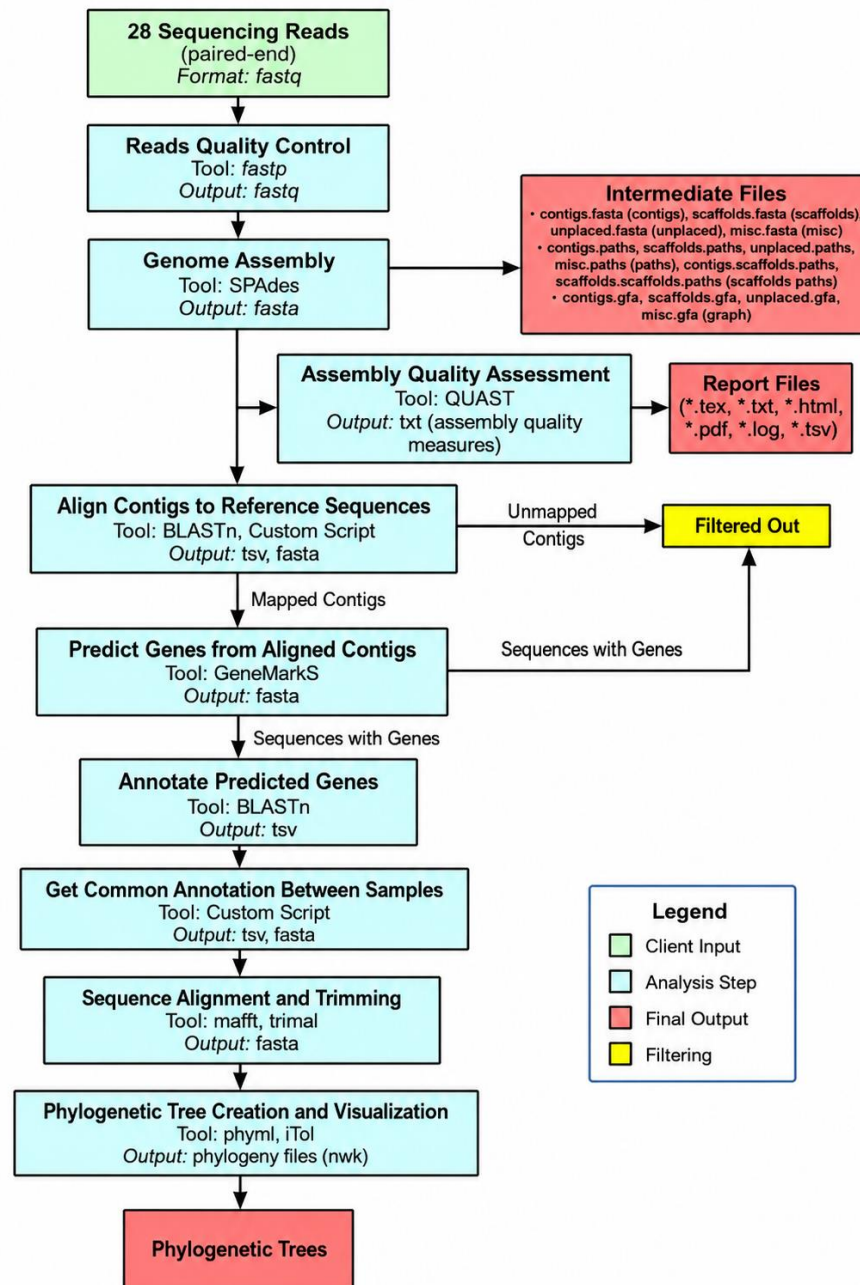
## 2.8 Sequence and Phylogenetic Analyses

Genes mapped to or annotated as a part of the begomovirus genome were then used as the basis for the sequence alignment. The annotations for all samples were then compared, and the annotation with the most samples was used to construct the phylogenetic tree. Representative sequences obtained from the samples were combined into a single multi-FASTA file. The sequences were aligned using MAFFT [29], subsequently trimmed using trimAl [30], and converted into PHYLIP format using an online generic file converter before phylogenetic tree construction. The phylogenetic trees were created using jmodelTest2 and PhyML [31], and iTOL [32] was used for visualization.

# 3. Results and Discussion

## 3.1 Symptoms of Begomovirus and other diseases

Field inspections during farm visits revealed begomovirus infection in pepper crops, as indicated by characteristic foliar symptoms. Infected plants typically exhibited upward leaf curling, interveinal chlorosis, generalized yellowing, and crinkling of the young apical leaves. These symptoms were noted in both bell and chili pepper crops across different growth stages. In bell peppers, infection was associated with pronounced leaf deformations, including curling, cupping, puckering, and crinkling, as well as interveinal yellowing or mosaic patterns, particularly during the vegetative to early fruiting stages (Figure 2). Similarly, infected chili pepper plants exhibited leaf curling and crinkling, interveinal chlorosis, reduced apical leaf size (little leaf), shortened internodes, leaf crowding, and overall stunting during the vegetative to early fruiting stages (Figure 3). The symptomatology documented in this study is consistent with previous descriptions of begomovirus infection in pepper [18] and aligns with more recent reports highlighting typical leaf curl, chlorosis, vein thickening, and growth suppression in begomovirus-affected *Capsicum* spp. [15]. Sweet pepper plants infected with *Tomato yellow leaf curl virus* (TYLCV) have been reported to exhibit curling of leaf margins, interveinal and marginal chlorosis, vein thickening, and noticeable leaf thickening, with severe infections leading to pronounced yellowing [18]. In Indonesia, yellow leaf curl disease has been documented in nearly all major pepper-producing regions, where infected plants commonly display little leaf symptoms, yellow mosaic, shortened internodes, and stunted growth [33]. In Costa Rica, begomovirus-infected sweet pepper plants exhibited symptoms ranging from mild to severe, including mosaic patterns, chlorosis, upward or downward



**Figure 1.** Bioinformatics pipeline (Core Facility of Bioinformatics, PGC) employed for assembly and analysis of begomoviral genome sequences.

leaf curling, vein-associated yellowing, and overall plant stunting. In the Philippines, [21] likewise reported an association between begomoviruses and pepper, describing symptomatic plants with leaf curling, mosaic, yellowing, blistering, and growth reduction. More recent studies further confirm that these foliar distortions, chlorosis, and growth abnormalities remain hallmark symptoms of begomovirus infections in *Capsicum* spp. across diverse agroecological regions [15]. Other symptoms of virus infection were also observed on chili peppers, including mosaic/mottle, curling or severe leaf distortion, particularly on apical or emerging leaves, and shoestring. These virus-like symptoms were primarily observed during the flowering and fruiting stages of chili crops. The manifestations resembled those commonly associated with pepper-infecting viruses such as *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV), and *Tomato/Tobacco mosaic virus* (ToMV), which have previously been reported in the Philippines [21].





**Figure 2.** Begomovirus-like symptoms observed in bell peppers. (A-D) Upward leaf curling and crinkling, interveinal chlorosis, and puckering of leaves.



**Figure 3.** Begomovirus-like symptoms observed in chili peppers. (A-D) Leaf curling and crinkling, interveinal chlorosis, and reduced size of apical leaves.

### 3.2 Incidence of Begomovirus and Other Diseases

The occurrence of Begomovirus and other virus-like diseases in pepper crops, as determined by visual symptom assessment, varied among sampling sites (Table 1). Begomovirus-like symptoms were observed in 78.1% (125/160) of the total samples across Luzon, Visayas, and Mindanao. In Luzon, begomovirus-like symptoms were present in all samples from Laguna, Quezon, Batangas, and Nueva Ecija, totaling 40 out of 75 samples (53.3%). Begomovirus-like symptoms occurred in 100% of the samples from all provinces in the

Visayas (Cebu, Leyte, and Iloilo) and Mindanao (Butuan and Davao del Sur). Other virus-like diseases were observed exclusively in Luzon, with a recorded incidence of 46.6%. Most of the samples from Bulacan, Batangas, Pangasinan, Ilocos Sur, and Ilocos Norte showed symptoms of other viruses. In Bulacan (San Idelfonso) and Ilocos Sur (Sinait), these symptoms were observed alongside begomovirus-like symptoms. Begomovirus infection in symptomatic pepper plants was verified through PCR amplification using the primer pairs TY1F/TY2R and PAL1v1978/PAR1c715H. Using genomic DNA as the template, amplification of the begomoviral coat protein gene (~585 bp) was obtained in 43.1 % of the samples (69 out of 160) with the TY1F/TY2R primer pair (Table 1). However, the PCR products detected in positive samples were generally weak in intensity. No amplification was observed in the healthy pepper control or in the no-template control containing distilled water, confirming the primers' specificity for begomoviral DNA. The lack of PCR products in the remaining samples indicated the absence of detectable begomovirus infection in those tissues. Conversely, amplification with the PAL1v1978/PAR1c715H primer pair did not produce any detectable DNA bands in the pepper samples upon gel electrophoresis. The expected ~1.5 kb DNA-A fragment was observed only in the positive control derived from a ToLCPV-infected plant.

The faint amplification obtained with the coat protein primers, along with the lack of amplification using the DNA-A primers, may be due to a low concentration of viral DNA in the total plant DNA extracts, potentially falling below the detection threshold of conventional PCR. Similar observations have been reported, where begomovirus amplification was faint or undetectable in samples with low viral titers [34]. Under such conditions, more sensitive approaches, such as rolling circle amplification coupled with PCR (RCA-PCR), are recommended for the reliable detection of viruses at low concentrations [34]. Indeed, RCA-PCR is significantly more sensitive than the standard PCR, enabling detection of Tomato leaf curl New Delhi virus (ToLCNDV) in samples that tested negative by conventional PCR, thereby reducing false-negative results during virus indexing [34]. Similarly, [35] reported a higher detection rate of begomoviruses with RCA-PCR than with PCR alone. In this study, selected representative samples were first processed using rolling circle amplification (RCA) to increase the amount of circular viral DNA before conducting PCR analysis. Specifically, the 20 samples selected for RCA analysis were those that had tested negative by conventional PCR using genomic DNA as template, thus representing cases in which viral titers were potentially below the detection threshold of standard PCR. Using RCA-amplified DNA as the template, 55 % (11 out of 20) of the samples tested positive for Begomovirus with the same primer pairs (Table 1). All positive samples yielded the expected amplicons of approximately 585 bp and 1.5 kb following amplification with the TY1F/TY2R and PAL1v1978/ PAR1c715H primer pairs, respectively. These results confirmed the presence of Begomovirus in several pepper samples and demonstrated that RCA effectively enriched viral DNA, thereby enhancing the sensitivity and reliability of subsequent PCR detection. The findings further indicate that viral DNA levels in the original extracts were below the detection threshold of conventional PCR but became detectable following RCA enrichment. The remaining 45% (9 out of 20) of the samples (9/20) remained negative after RCA-PCR, suggesting the absence of begomovirus infection in those plants. Overall, the results highlight the usefulness of RCA-PCR in detecting Begomovirus present at very low titers in pepper samples.

Published information on the occurrence of begomoviruses infecting pepper, especially in the Philippines, is still scarce. In Spain, *Capsicum annuum* was identified as a host of Tomato yellow leaf curl virus (TYLCV) [36]. Similarly, in the Caribbean region, molecular detection techniques such as PCR, restriction enzyme analysis, and nucleic acid hybridization successfully confirmed TYLCV infection in field-grown *C. annuum* and *C. chinense* in the Dominican Republic, Cuba, and Jamaica [37]. These findings reinforced earlier reports recognizing *Capsicum* species as natural hosts of TYLCV. Evidence of susceptibility in *C. frutescens*, *C. baccatum*, and *C. chinense* was later documented by [38]. More recent studies have further demonstrated the expanding distribution and diversity of begomoviruses infecting pepper in various regions, including Costa Rica [39] and Indonesia [33], as well as broader global assessments highlighting the increasing host range and recombination-driven evolution of begomoviruses in solanaceous crops [15]. In the Philippines, begomoviruses infecting pepper—specifically *Tomato leaf curl Philippines virus* (ToLCPV) and *Tomato leaf curl Cebu virus* (ToLCCeV)—were previously detected in 3 out of 18 samples collected from Laguna (Los Baños), Cebu, and Bukidnon [21].

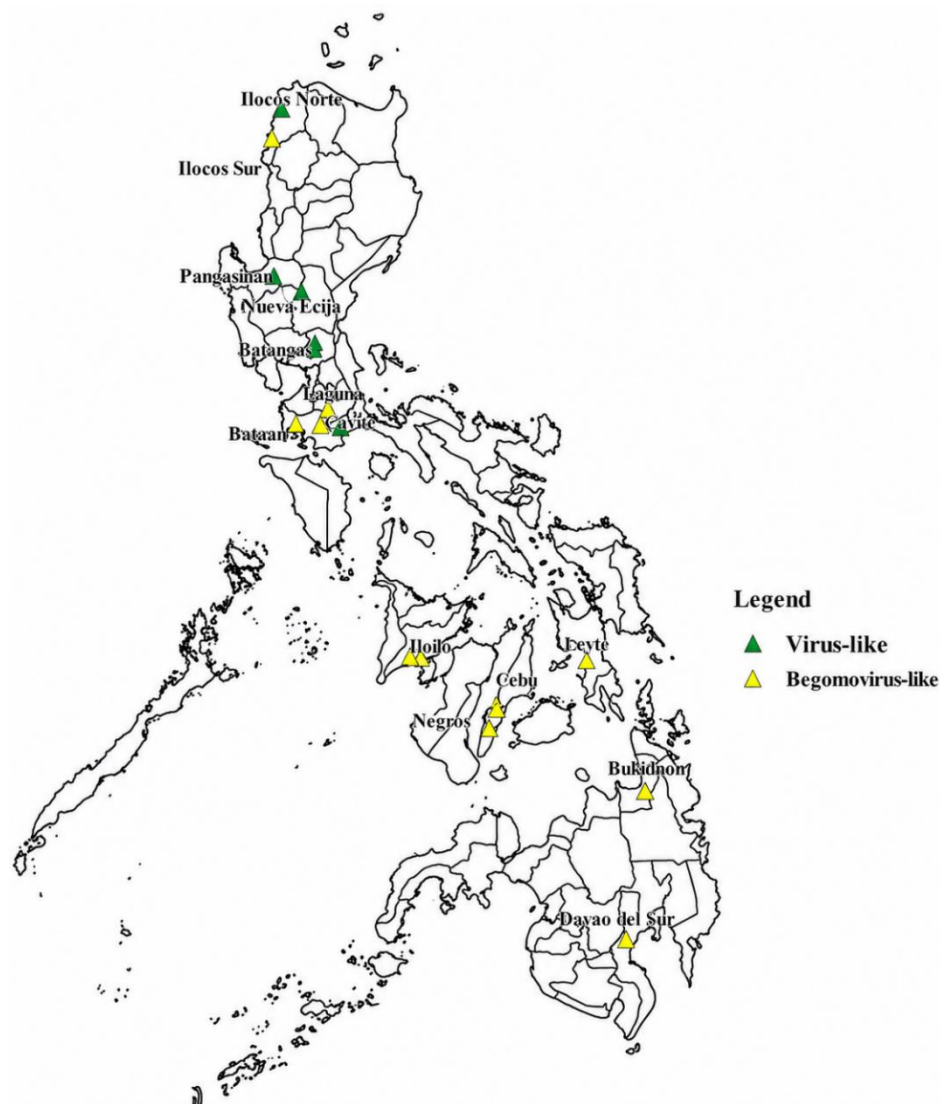


**Table 1.** Incidence of Begomovirus and other virus-like diseases in pepper (bell pepper and chili pepper) by sampling location across Luzon, Visayas, and Mindanao, based on visual symptom assessment, and corresponding detection results from conventional PCR and rolling circle amplification (RCA)-PCR using the TY1F/TY2R and PAL1v1978/PAR1c715H primer pairs.

| Province        | Location<br>(No. of Farm) | No. of<br>samples | Disease/<br>symptom observed |                             | No. of Positive Samples     |                                |                                      |
|-----------------|---------------------------|-------------------|------------------------------|-----------------------------|-----------------------------|--------------------------------|--------------------------------------|
|                 |                           |                   | Begomo-<br>virus-like        | Other<br>virus-<br>like     | TY1F/<br>TY2R <sup>a</sup>  | TY1F/<br>TY2R <sup>b</sup>     | PAL1v1978/<br>PAR1c715H <sup>b</sup> |
| <b>Luzon</b>    | <b>9 (15)</b>             | <b>75</b>         | <b>40</b>                    | <b>35</b>                   | <b>10</b>                   | <b>4/11</b>                    | <b>5/11</b>                          |
| Laguna          | Los Baños (2)             | 10                | 10                           | 0                           | 5                           | 1/3                            | 1/3                                  |
| Quezon          | Candelaria (1)            | 5                 | 5                            | 0                           | 0                           | -                              | -                                    |
| Bulacan         | San Idelfonso (4)         | 20                | 5                            | 15                          | 0                           | 0/2                            | 0/2                                  |
| Batangas        | Lipa (1)                  | 5                 | 5                            | 0                           | 4                           | 1/1                            | 1/1                                  |
|                 | Calaca (1)                | 5                 | 5                            | 0                           | 1                           | 1/1                            | 1/1                                  |
| Nueva Ecija     | Muños (1)                 | 5                 | 5                            | 0                           | 0                           | 0/1                            | 0/1                                  |
| Pangasinan      | Urdaneta (2)              | 10                | 0                            | 10                          | 0                           | 0/1                            | 0/1                                  |
| Ilocos Sur      | Sinait (2)                | 10                | 5                            | 5                           | 0                           | 1/2                            | 2/2                                  |
| Ilocos Norte    | Bacarra (1)               | 5                 | 0                            | 5                           | 0                           | -                              | -                                    |
| <b>Visayas</b>  | <b>6 (13)</b>             | <b>65</b>         | <b>65</b>                    | <b>0</b>                    | <b>42</b>                   | <b>5/6</b>                     | <b>4/6</b>                           |
| Cebu            | Cebu City (1)             | 5                 | 5                            | 0                           | 2                           | 2/2                            | 2/2                                  |
|                 | Barili (5)                | 25                | 25                           | 0                           | 9                           | -                              | -                                    |
|                 | Dalaguete (2)             | 10                | 10                           | 0                           | 6                           | 1/2                            | 0/2                                  |
| Leyte           | Baybay (1)                | 5                 | 5                            | 0                           | 5                           | 1/1                            | 1/1                                  |
| Iloilo          | Leon (2)                  | 10                | 10                           | 0                           | 10                          | 1/1                            | 1/1                                  |
|                 | Pavia (2)                 | 10                | 10                           | 0                           | 10                          | -                              | -                                    |
| <b>Mindanao</b> | <b>2 (4)</b>              | <b>20</b>         | <b>20</b>                    | <b>0</b>                    | <b>17</b>                   | <b>2/3</b>                     | <b>2/3</b>                           |
| Butuan          | Ampayon (2)               | 10                | 10                           | 0                           | 7                           | 2/2                            | 1/2                                  |
| Davao del Sur   | Digos (2)                 | 10                | 10                           | 0                           | 10                          | 0/1                            | 1/0                                  |
| <b>Total</b>    | <b>17 (32)</b>            | <b>160</b>        | <b>125</b><br><b>(78.1%)</b> | <b>35</b><br><b>(21.9%)</b> | <b>69</b><br><b>(43.1%)</b> | <b>11/20</b><br><b>(55.0%)</b> | <b>11/20</b><br><b>(55.0%)</b>       |

### 3.3 Geographical Distribution of Begomovirus

In this study, begomovirus infection was observed in pepper crops, including both bell peppers and chili peppers, across multiple regions of the country (Figure 4). Begomovirus-like infections were found in more than 20 locations of the pepper farms surveyed. In Luzon, begomovirus infections were present in Laguna, Batangas, Bulacan, and Ilocos Sur, while in other provinces surveyed, such as Quezon, Nueva Ecija, Pangasinan, and Ilocos Norte, other virus-like symptoms were also present. In Visayas, begomovirus-like infections were detected in the majority of the farms in Cebu (Cebu City, Barili, and Dalaguete), Iloilo (Pavia and Leon), and Leyte (Baybay). In Mindanao, Begomovirus was present in Butuan and Davao del Sur.



**Figure 4.** Geographical distribution of *Begomovirus* and other viruses in the provinces surveyed across the Philippines, as detected by visual observation and PCR.

### 3.4 Emerging Begomoviruses Detected in Pepper Crops by RCA and RE Digestion

RCA was performed on DNA extracts from representative bell pepper and chili pepper samples that were PCR-positive and PCR-negative. All samples showed successful amplification, evidenced by a distinct high-molecular-weight band on the agarose gel corresponding to the begomovirus genome. All RCA-amplified products were digested with *Sall*, *HindIII*, *BamHI*, and *EcoRI* to detect the presence of begomoviruses. However, no digestion product of any specific length was generated after digestion with the four restriction enzymes; hence, the presence of begomoviruses could not be confirmed. These results may suggest the absence of Begomovirus in the sample. However, PCR analysis using RCA-amplified DNA as a template revealed Begomovirus in several pepper samples. Therefore, the complete absence of Begomovirus in the tested samples cannot be conclusively established. The findings may suggest the occurrence of different begomovirus species or viral variants lacking the specific restriction sites targeted in previously reported profiles. These observations can be further validated through next-generation sequencing analysis.

### 3.5 Identification of Emerging Begomoviruses Associated with Pepper Crops by Next-Generation Sequencing (NGS) and Sequence Analysis

#### 3.5.1 Begomoviruses Identified from mapped/annotated genes

The NGS-Illumina MiSeq sequencing of pepper DNA libraries generated raw reads ranging from 226,929 (P1La) to 310,725 (C1Bul) and were reduced to 216,706 (P1La) and 295,422 (C1Bul) reads after quality control. Quality-filtered reads were assembled de novo with SPAdes, yielding a large number of contigs ranging from 24,056 to 39,548. After the contigs were annotated using BLASTn, the remaining contig numbers only ranged from 0 to 6. From these contigs, “genes” were predicted and then mapped and annotated again using BLASTn. BLAST analysis identified several known and putative begomoviruses, along with an associated betasatellite infecting pepper (Tables 2 and 3). A total of seven partial sequences obtained from Cebu (P2Ce), Leyte (P3Le), Iloilo (P4Ilo), and Butuan (C5But) isolates had sequence identity ranging from 87% to 98% with *Tomato leaf curl Cebu virus* (ToLCCeV) (Tables 2 and 3). Isolates from Cebu, Leyte, and Butuan showed high sequence similarity (96-98%) with the known Tomato leaf curl Cebu virus, whereas the Iloilo isolates were below 95% and 89%, suggesting strain or species variation in the samples. Only two sequences (from Iloilo, P4Ilo, and Butuan, C5But) corresponded to the known *Tomato leaf curl Philippines virus* (ToLCPV) with 92% and 81%, indicating a different strain or species present in the sample.

In addition to known begomoviruses identified from pepper NGS sequences, five new or unknown begomoviruses of pepper in the country were noted (Tables 2 and 3). The identified viral species and associated elements included *Ageratum yellow vein coronavirus*, *Tobacco leaf curl Yunnan virus*, *Papaya leaf curl coronavirus*, *Ageratum yellow vein virus* (AYVV), *Tomato yellow leaf curl Thailand virus*, and *Malvastrum leaf curl Philippines betasatellites* (Table 3). Several NGS-derived sequences from the Butuan isolate (C5But) showed high nucleotide similarity (96%) to *Ageratum yellow vein coronavirus*, but shared less than 95% identity with *Papaya leaf curl coronavirus*. Partial sequences from the Cebu (P2Ce) and Butuan (C5But) isolates exhibited 96% sequence similarity to *Tobacco leaf curl Yunnan virus*, a species not previously reported in the country. Additionally, partial sequences from the Cebu isolate demonstrated 90% identity with *Ageratum yellow vein virus* (AYVV), a virus earlier documented in tomato but not in pepper [21]. Meanwhile, sequences from Leyte (P3Le) exhibited relatively low similarity to *Tomato yellow leaf curl Thailand virus*, whereas the Butuan isolate (C5But) showed 98% sequence identity to the same virus. A betasatellite was also detected from partial sequences obtained from pepper in Cebu (P2Ce), but with low sequence similarity (86%) with *Malvastrum leaf curl Philippines betasatellites*.

Although several of the detected viruses have been previously reported in pepper, comprehensive information regarding the host range of many of these viruses remains limited. Nonetheless, the present findings suggest the possible occurrence of additional begomoviruses in the country that may also infect pepper. The partial sequences generated in this study point to the presence of potentially novel viral variants; however, these data are only indicative and not yet conclusive. Such sequence variation may be attributed to the high mutation rates and frequent recombination events characteristic of begomoviruses [38], among other evolutionary factors. To verify the existence of these putative new viruses, partial sequences can be used to design primers specific to the remaining genomic regions. Complete genome sequencing will ultimately confirm the identity and presence of these begomoviruses in the country. Furthermore, all annotated sequences generated in this study, including those corresponding to the putative novel strains and the known begomoviruses identified, should be submitted to the NCBI GenBank database to establish citable molecular records and facilitate future research on begomovirus diversity in the Philippines.

#### 3.5.2 Phylogenetic Analysis of Mapped/Annotated Genes from Pepper

Phylogenetic analysis was conducted using a representative annotation of *Tomato leaf curl Cebu virus* (ToLCCeV; isolate P2-1, complete genome), which had the highest number of corresponding annotated sequences. Based on phylogenetic tree reconstruction using ToLCCeV as the reference alignment, isolates from Butuan (C5But), Cebu (P2Ce), Iloilo (P4Ilo), and Leyte (P3Le) grouped into a single clade, indicating close genetic relatedness to the reported ToLCCeV strain (Figure 5). *Tomato leaf curl Cebu virus* has previously been documented infecting tomato in the Philippines [21] and was also reported in pepper from Cebu, where infected plants exhibited yellowing and mild leaf curling [21]. These findings document, for the first time, the

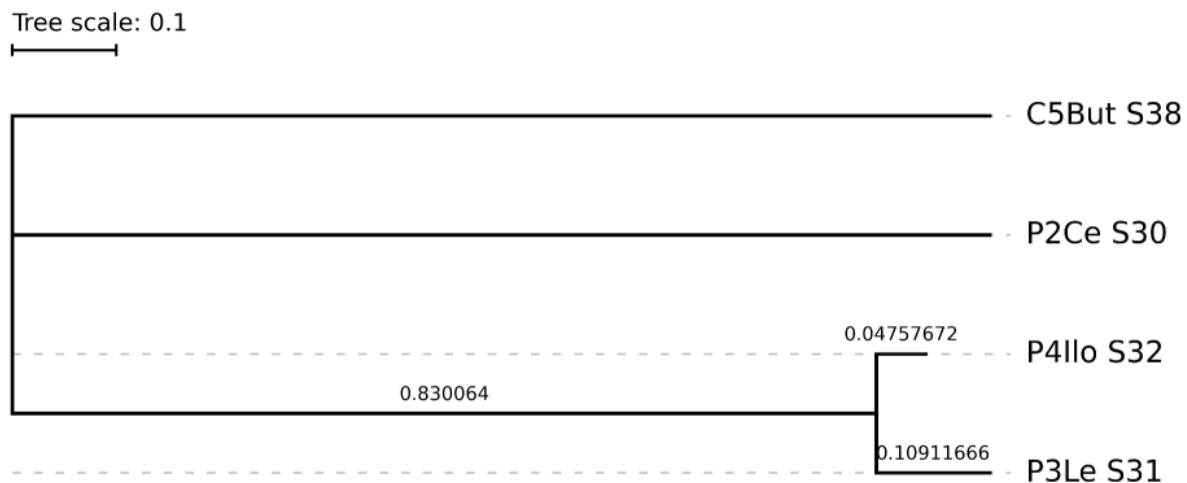
occurrence of ToLCCeV infecting chili pepper in Butuan and bell pepper in Iloilo and Leyte. These results indicate the wider distribution of this known Begomovirus in additional pepper-growing regions of the country.

**Table 2.** Known and unknown begomoviruses and betasatellites identified from mapped/ annotated genes from pepper samples.

| Sample | Location   | No. of Genes<br>Mapped to<br>BGV | Begomovirus                                           | %<br>Identity |
|--------|------------|----------------------------------|-------------------------------------------------------|---------------|
| P1La   | Laguna     | 0                                | None                                                  | -             |
| P2Ce   | Cebu       | 4                                | <i>Tobacco leaf curl Yunnan virus</i>                 | 96            |
|        |            |                                  | <i>Tomato leaf curl Cebu virus</i>                    | 98            |
|        |            |                                  | <i>Ageratum yellow vein virus</i>                     | 90            |
|        |            |                                  | <i>Malvastrum leaf curl Philippines betasatellite</i> | 86            |
| P3Le   | Leyte      | 3                                | <i>Tomato yellow leaf curl Thailand virus</i>         | 82            |
|        |            |                                  | <i>Tomato leaf curl Cebu virus</i>                    | 98            |
|        |            |                                  | <i>Tomato leaf curl Cebu virus</i>                    | 97            |
| P4Ilo  | Iloilo     | 3                                | <i>Tomato leaf curl Cebu virus</i>                    | 93            |
|        |            |                                  | <i>Tomato leaf curl Cebu virus</i>                    | 87            |
|        |            |                                  | <i>Tomato leaf curl Philippines virus</i>             | 92            |
| P5Da   | Davao      | 0                                | None                                                  | -             |
| C1Bul  | Bulacan    | 0                                | None                                                  | -             |
| C2Ba   | Batangas   | 0                                | None                                                  | -             |
| C3Pa   | Pangasinan | 1                                | <i>Tobacco leaf curl Yunnan virus</i>                 | 92            |
| C4IS   | Ilocos Sur | 0                                | None                                                  | -             |
| C5But  | Butuan     | 9                                | <i>Ageratum yellow vein China virus</i>               | 96            |
|        |            |                                  | <i>Ageratum yellow vein China virus</i>               | 96            |
|        |            |                                  | <i>Tobacco leaf curl Yunnan virus</i>                 | 96            |
|        |            |                                  | <i>Tomato leaf curl Cebu virus</i>                    | 98            |
|        |            |                                  | <i>Tomato leaf curl Cebu virus</i>                    | 96            |
|        |            |                                  | <i>Tobacco leaf curl Yunnan virus</i>                 | 96            |
|        |            |                                  | <i>Tomato yellow leaf curl Thailand virus</i>         | 98            |
|        |            |                                  | <i>Papaya leaf curl China virus</i>                   | 91            |
|        |            |                                  | <i>Tomato leaf curl Philippines virus</i>             | 81            |

**Table 3.** Summary of known and unknown begomoviruses as well as betasatellites from infected pepper samples obtained through NGS.

| Begomovirus                                           | No. of Sample<br>Present | % Identity |
|-------------------------------------------------------|--------------------------|------------|
| <b>Known</b>                                          |                          |            |
| <i>Tomato leaf curl Cebu virus</i>                    | 7                        | 87-98      |
| <i>Tomato leaf curl Philippines virus</i>             | 2                        | 81-92      |
| <b>New/unknown</b>                                    |                          |            |
| <i>Ageratum yellow vein China virus</i>               | 2                        | 96         |
| <i>Tobacco leaf curl Yunnan virus</i>                 | 4                        | 92-96      |
| <i>Papaya leaf curl China virus</i>                   | 1                        | 91         |
| <i>Ageratum yellow vein virus</i>                     | 1                        | 90         |
| <i>Tomato yellow leaf curl Thailand virus</i>         | 2                        | 82-98      |
| <i>Malvastrum leaf curl Philippines betasatellite</i> | 1                        | 86         |



**Figure 5.** Unrooted maximum likelihood phylogenetic tree of pepper-derived begomovirus isolates based on sequences annotated as “*Tomato leaf curl Cebu virus* isolate P2-1, complete genome”. The tree was constructed using PhyML following model selection in jModelTest2 and visualized using iTOL. Bootstrap support values (1,000 replicates) are indicated at each node. P2Ce = bell pepper from Cebu, P3Le = bell pepper from Leyte, P4Ilo = bell pepper from Iloilo, C5But = chili pepper from Butuan. No outgroup sequence was included; therefore, the tree is presented as an unrooted phylogeny. [Authors: please confirm whether an outgroup sequence was included and identify it in the caption; if no outgroup was used, note this limitation. Also provide a key to the sample codes used in the tree: P2Ce = bell pepper from Cebu, P3Le = bell pepper from Leyte, P4Ilo = bell pepper from Iloilo, C5But = chili pepper from Butuan.]

#### 4. Conclusions

Begomovirus infections and emerging begomoviruses are evident in pepper crops grown in the country. Known and unknown begomoviruses in pepper crops are detected visually and by traditional molecular techniques (PCR, RCA, and RE digestions), and confirmed with NGS data. Present NGS data provide evidence of the presence of emerging begomoviruses, including known Begomoviruses in new areas and new/unknown Begomoviruses of pepper in the country. Although the findings are not definitive due to the availability of only partial sequences, they offer important insights into the begomovirus population present in the plant samples. Further analysis, including complete genome sequencing and a comprehensive phylogenetic analysis, is necessary to confirm species identity accurately. This study is the first to utilize next-generation sequencing (NGS) for the molecular surveillance of begomoviruses infecting pepper in the Philippines. The findings highlight the effectiveness of NGS combined with bioinformatics tools in detecting both established and potentially novel begomoviruses associated with pepper. This approach provides a proactive strategy for the early detection of emerging viral pathogens, thereby reducing potential yield losses and facilitating the development of effective management measures, including the breeding of disease-resistant pepper cultivars.

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**Author Contributions:** MLDP designed and conducted the research, analyzed datasets, wrote the manuscript; and FCSC aided in designing the research, supervised all processes, and proofread and edited the manuscript.



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# Predicting User Acceptance of Solar-Powered Vending Machines Based on Quality Dimensions

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**Abstract:** With the increasing demand for sustainable technologies in educational institutions, solar-powered vending machines have emerged as a promising solution to meet this need. This study assesses the perceived quality and user acceptance of solar-powered vending machines at North Eastern Mindanao State University (NEMSU) in Bislig City, Philippines. The research focuses on two key dimensions of perceived quality: attribute quality (performance, features, aesthetics) and reliability quality (reliability, serviceability, perceived quality), and evaluates how these factors influence user acceptance, based on the Technology Acceptance Model (TAM). A structured questionnaire was used to gather data from 200 participants, including students, faculty, and staff. The findings indicate that users generally have a positive perception of the vending machines, with high ratings for features and aesthetics, and satisfactory ratings for performance and reliability. Performance and aesthetics were identified as significant predictors of user acceptance, while serviceability emerged as the most important factor in reliability quality. The study concludes that solar-powered vending machines have strong potential for acceptance in educational settings. However, improvements in Wi-Fi stability and maintenance features could further enhance user satisfaction and long-term adoption. The results offer valuable insights for universities and policymakers seeking to implement sustainable technologies on their campuses.

**Keywords:** Perceived quality; user acceptance; solar-powered vending machine; renewable energy; educational technology

## 1. Introduction

The push for smart infrastructure and sustainable solutions within higher education has become increasingly urgent in the context of environmental responsibility and institutional modernization. Renewable energy systems are playing a central role in this transformation, enabling campuses to reduce carbon footprints while providing enhanced services to users [1, 2]. Technological innovations, such as solar-powered systems, smart vending machines, and integrated service outlets, reflect this dual focus on service convenience and environmental stewardship [3, 4]. The use of solar-powered vending machines offering amenities such as Wi-Fi access, mobile device charging, and clean drinking water aligns with this trend by enabling tertiary institutions to integrate green technology into everyday campus life.

Despite the growing adoption of sustainable technologies, several issues hinder their widespread and effective use in campus settings. User acceptance

remains a critical barrier: technologies deployed without adequate user engagement, perceived usefulness, or ease of use are less likely to deliver the intended benefits [5]. Research on renewable energy technology adoption reveals that, although many systems are technically feasible, user perceptions of quality, reliability, and design significantly influence actual uptake [6]. Some investigations into technology acceptance in green or energy-augmented contexts suggest that traditional models, such as the Technology Acceptance Model (TAM), may lack constructs specifically tailored to capture system quality or reliability in renewable technologies [7, 8].

In the Philippine context, particularly on island or regional campuses like North Eastern Mindanao State University (NEMSU) in Bislig City, innovations such as solar-powered vending machines are still emerging, and research on how users perceive these technologies remains limited. While some studies address renewable energy acceptance more broadly [8], few focus on user acceptance of solar-powered vending machines in higher education settings, especially in developing regions. Moreover, even fewer studies incorporate perceived quality dimensions, such as performance, aesthetics, and serviceability, which are crucial in understanding the factors that drive user adoption. "More importantly, existing studies predominantly examine single-function technologies (e.g., standalone charging stations or basic vending units) [9, 10], thereby overlooking the growing emergence of multi-functional systems that integrate services such as Wi-Fi access, device charging, and water dispensing into a single solar-powered unit." This lack of attention to how quality attributes and reliability influence acceptance in this specific and integrated context creates a significant gap. "Thus, the novelty of this study lies in its focus on a multi-functional solar-powered vending machine and in examining how distinct quality dimensions, specifically attribute-based and reliability-based factors, interact within the Technology Acceptance Model (TAM) to explain user adoption." As a result, institutions may implement solar-powered vending machines, but without a full understanding of how users evaluate quality and reliability, their long-term viability and user uptake may be compromised.

To address this gap, it is crucial to investigate the role of perceived quality dimensions in the adoption of solar-powered vending machines. [11] underscore the importance of integrating perceived quality with technology acceptance models to better understand user adoption of renewable energy technologies. Specifically, focusing on attributes such as performance, aesthetics, and serviceability provides deeper insight into how users evaluate and ultimately accept such technologies. "In contrast to prior studies, this research explicitly distinguishes between attribute quality (e.g., functionality and design) and reliability quality (e.g., consistency and dependability), and integrates these dimensions into the TAM framework." Incorporating both attribute and reliability quality dimensions, as suggested in [12], addresses the existing gap in the literature and provides valuable insights into the factors driving user acceptance in developing educational settings, such as NEMSU. "This approach advances existing models that typically treat quality as a single construct and instead proposes a more nuanced, multi-dimensional perspective tailored to complex, multi-functional systems." This approach ensures that solar-powered vending machines are not only technically feasible but also well-received, fostering long-term adoption and success.

This study, therefore, aims to investigate how perceived quality, encompassing both attribute quality (performance, features, aesthetics) and reliability quality (reliability, serviceability, perceived quality), predicts user acceptance of solar-powered vending machines at NEMSU. Integrating traditional Technology Acceptance Model (TAM) constructs, such as perceived usefulness and perceived ease of use, with quality dimensions tailored to renewable energy systems, this research contributes to both theory and practice. It addresses the gap in the literature on technology acceptance within sustainable campus infrastructure, offering actionable insights for university administrators, designers, and policymakers overseeing the deployment of green technologies in educational settings. Ultimately, this study enhances our understanding of how engineering and design factors, combined with user perception, drive the acceptance of renewable energy technologies in higher education.



## 2. Literature Review and Theoretical Framework

### 2.1 The Technology Acceptance Model (TAM) as a Framework for User Adoption

To understand the factors that drive the adoption of new technologies, researchers frequently turn to the Technology Acceptance Model (TAM). Initially developed by Davis, TAM provides a parsimonious yet powerful framework for explaining how users come to accept and utilize new systems, “and is widely recognized for its theoretical simplicity and robustness, as well as its consistent ability to explain user adoption behavior across various domains [13, 14]”. The model posits that a user's decision to adopt a technology is primarily determined by two core perceptual constructs: Perceived Usefulness (PU) and Perceived Ease of Use (PEOU). [15], define Perceived Usefulness as the degree to which an individual believes that using a particular system will enhance their job performance or task effectiveness. Concurrently, they define Perceived Ease of Use as the degree to which an individual believes that using the system will be free of physical and mental effort [16].

Within the TAM framework, these two constructs have distinct but interrelated roles. Perceived Usefulness is consistently identified as the strongest and most direct predictor of a user's behavioral intention to adopt a technology [17, 18]. In contrast, Perceived Ease of Use exerts its influence both directly on a user's attitude and indirectly by positively affecting their perception of the technology's usefulness; a system that is easy to operate is more likely to be considered useful [16, 19]. Together, these beliefs shape a user's attitude and intention, which ultimately lead to the actual adoption and use of the technology.

The efficacy of TAM as a predictive tool is firmly established in the literature. Numerous systematic reviews and meta-analyses confirm that TAM is a robust, highly validated framework for predicting user adoption across a wide range of contexts, including education, healthcare, and e-commerce [3, 12, 20]. For instance, [21] found, in a systematic review of telemedicine, that TAM consistently outperformed many alternative models in explaining user acceptance. While the core model is powerful on its own, researchers also note that its predictive accuracy can be further enhanced by integrating relevant external variables such as social influence, trust, or system quality, “which [13] and [22] have shown to significantly influence behavioral intention and actual usage, and improve the model's explanatory power across different technological contexts,” making it a versatile and adaptable foundation for technology adoption research [20, 23, 24].

While the DeLone & McLean Information Systems Success Model is widely used in technology adoption studies, its reliance on constructs such as 'System Quality' and 'Information Quality' is primarily tailored to software-based or digital information environments. In contrast, the solar-powered vending machine evaluated in this study is predominantly a physical, multi-functional hardware unit. Consequently, software-centric dimensions do not fully capture the physical user experience. Therefore, employing 'Attribute Quality'—which evaluates physical aesthetics and hardware functionality—and 'Reliability Quality'—which measures mechanical serviceability and consistent power output—provides a more robust, context-specific framework for this technology than the traditional DeLone & McLean dimensions.

#### 2.1.1 The Role of Perceived Quality in Influencing Consumer Behavior

While technology acceptance models provide a strong foundation for understanding user adoption, they are often enhanced by considering external variables that shape a user's core perceptions. Among the most critical of these variables is perceived quality. In marketing and consumer behavior literature, researchers define perceived quality not as an objective or technical attribute of a product, but as the consumer's subjective judgment of its overall excellence or superiority [25, 26]. [27] and [28] characterize this as a holistic evaluation based on an individual's unique expectations and experiences. This subjectivity means that a consumer's perception of quality can be influenced by a range of tangible and intangible cues, such as brand reputation, price, and product design, particularly when product knowledge is limited [29, 30]. The relationship between this subjective perception and a consumer's ultimate decision-making is direct and powerful. A substantial body of research has firmly established that a higher level of perceived product quality is a significant driver of consumer purchase intention [31, 32]. [30] explain that this effect is particularly strong in e-commerce, where consumers rely heavily on quality cues to reduce perceived risk and build trust [33]. This principle extends directly into the domain of technology adoption. Within the context of the Technology Acceptance Model (TAM), perceived quality is considered a key antecedent to its core constructs. [34] and [35] demonstrate that

when users perceive a technology as high quality, they are significantly more likely to also perceive it as useful and easy to use. [36] found that the perceived quality of service robots in the food industry had a strong positive influence on their acceptance, mediated by perceived usefulness and ease of use. Therefore, perceived quality does not operate in isolation; it is a foundational belief that directly shapes user perceptions, which ultimately determine whether a new technology will be accepted or rejected.

## 2.2 Key Dimensions of Perceived Product Quality

The concept of perceived quality is best understood through its core dimensions, commonly categorized into attribute-based and reliability-based factors. These dimensions serve as key cues that shape users' quality judgments and adoption intentions, as supported in consumer behavior literature [37, 38]. This study adopts these dimensions because they align closely with user-centered evaluation of tangible, multifunctional technologies such as solar-powered vending machines. Research grounded in the Technology Acceptance Model shows that users rely on direct interaction with system features and performance outcomes in forming acceptance decisions [22, 39]. Attribute-based factors capture immediate functional and experiential evaluation, whereas reliability-based factors reflect sustained performance and trust. In contrast, System Quality and Information Quality from the DeLone and McLean Information Systems Success Model are more suited to information systems focused on data and processing, as cited by [40] and [41]. Solar-powered vending machines, as physical service systems, are better evaluated through observable features and operational dependability, making attribute and reliability dimensions more appropriate for this study.

### 2.2.1 Attribute-Based Quality: Performance, Features, and Aesthetics

The most immediate drivers of quality perception are a product's tangible attributes. [42] and [43] assert that high product performance is a fundamental determinant of perceived quality and purchase intention, as consumers expect a product to function effectively and reliably. Beyond basic performance, the product's features also play a critical role. However, [44] cautions that the sheer quantity of features can be detrimental if it reduces usability. Their research reveals that the interrelatedness of features—how well they work together to create a seamless experience—is a more significant driver of positive quality perception than the total number of features alone [45]. Complementing function and features is the powerful influence of aesthetics. Product design has a significant impact on first impressions and can create a "beauty premium" that directly enhances perceived quality and willingness to adopt [46, 47]. [48] confirm in a meta-analysis that visual aesthetics are strongly linked to consumer preference. This effect is significant for innovative products, where [49] argue that strong aesthetics can help buffer consumer uncertainty about performance. While an attractive design is a powerful initial draw, long-term satisfaction ultimately depends on the product's functional performance living up to its aesthetic promise. In this context, attribute-based dimensions were selected because they capture users' immediate functional and sensory evaluation of the system, as highlighted by [50], which is critical for public-facing technologies that rely on direct interaction rather than purely digital outputs.

### 2.2.2 Reliability-Based Quality: Reliability, Durability, and Serviceability

Beyond immediate attributes, a user's perception of quality is profoundly shaped by factors related to a product's long-term dependability. Reliability, which is the ability to function consistently without failure, is a cornerstone of user trust [51]. [25] and [52] demonstrate that when users experience reliable performance, their dependence on and confidence in the technology grow, directly influencing long-term acceptance. This is closely linked to durability, which refers to a product's ability to maintain its performance over time. A durable product signals quality, reduces the perceived risk of a poor investment, and can foster stronger trust and even emotional attachment [53, 54].

Finally, serviceability, which is the perceived ease of maintenance and repair, plays a crucial role in building and maintaining trust. The assurance that potential issues can be resolved efficiently and effectively protects users against the risk of failure, thereby supporting long-term retention and positive word of mouth [55, 56]. [29] argue that for emerging technologies, strong reliability and clear serviceability are essential for overcoming brand barriers and establishing a foothold in the market. Together, these reliability-based

reliability-based dimensions were included because they reflect sustained operational performance and maintainability as noted by [57], which are essential for ensuring continuous service delivery and long-term usability of solar-powered vending machines in real-world institutional environments.

### ***2.2.3 Consumer Adoption of Sustainable and Solar-Powered Technologies***

The successful introduction of a solar-powered vending machine depends not only on its inherent quality and usability but also on the broader context of consumer attitudes toward sustainable technologies. The literature identifies a complex interplay of factors that drive or hinder public adoption of "green" innovations. A primary driver identified by [58] and [28] is the consumer's perceived benefits, which include not only potential cost savings but also a sense of contributing to environmental well-being [59, 60]. Social influence also plays a critical role [61, 62], and [62] notes that social norms and peer recommendations significantly increase adoption intentions. Furthermore, government support in the form of financial incentives and clear policies is consistently found to be a powerful catalyst for adoption, reducing the primary barrier: high initial costs [63, 64, 65]. Conversely, significant barriers can impede the adoption of solar-powered products. The most frequently cited obstacle is the high upfront financial investment, which can be prohibitive for many consumers [4, 62]. [58]. Also highlight that perceived risks related to the technology's long-term performance, reliability, and value can create significant consumer hesitation. This uncertainty is often compounded by a lack of awareness or technical knowledge, underscoring the importance of clear information and marketing campaigns in building consumer confidence [66, 67, 68]. These dynamics are directly relevant to the acceptance of solar-powered vending machines, which exist at the intersection of sustainable technology and automated retail. Public perception of automated retail is generally positive, driven largely by its convenience, 24/7 accessibility, and speed [69, 70]. [71] and [72] found that modern technological features, such as contactless payment and a user-friendly interface, further enhance acceptance. However, just as with sustainable technologies, barriers related to trust and risk are prevalent. [73] notes that consumer concerns about payment security, data privacy, and machine reliability can hinder adoption, particularly among those unfamiliar with such systems [74]. Therefore, the acceptance of a solar-powered vending machine will likely depend on its ability to deliver the convenience expected of automated retail while simultaneously overcoming the perceived risks associated with a novel, sustainable technology.

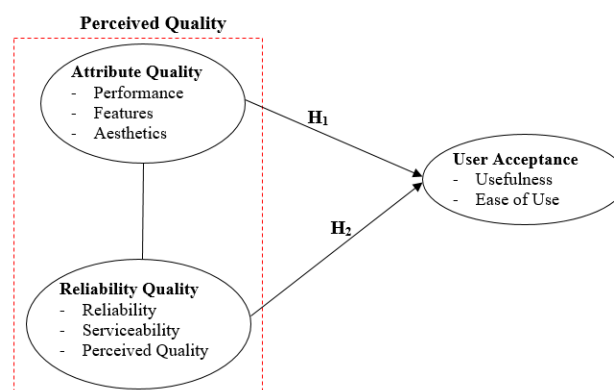
### ***2.2.4 Technological Advancements Supporting Solar-Powered Vending Adoption***

Recent advancements in renewable energy and smart retail technologies strongly suggest that the adoption of solar-powered vending machines is no longer constrained by the limitations identified in earlier studies but is instead supported by a rapidly evolving technological landscape. Improvements in photovoltaic efficiency, battery storage, and IoT-enabled system monitoring have significantly enhanced the reliability and operational stability of solar-powered devices, directly addressing prior concerns related to performance and maintenance. As argued by [75], the continuous decline in solar technology costs, combined with increasing environmental awareness, has shifted consumer perception from skepticism to acceptance, making sustainable technologies more accessible and desirable. At the same time, the transformation of traditional vending machines into smart retail systems further strengthens this adoption argument. The integration of features such as cashless payment, real-time inventory management, and data-driven personalization has redefined vending machines as efficient, user-centered platforms rather than mere automated dispensers. According to [76], these technological enhancements not only improve operational efficiency but also increase user trust and perceived value, which are critical determinants of technology acceptance. Taken together, these developments indicate that solar-powered vending machines are uniquely positioned at the convergence of sustainability and intelligent retail innovation. This convergence supports the argument that their adoption is not only feasible but increasingly inevitable, as they align with both environmental priorities and modern consumer expectations for convenience, efficiency, and technological sophistication.

## **2.3 Conceptual Framework**

The conceptual framework in Figure 1 highlights the relationship between Perceived Quality and User Acceptance of solar-powered vending machines. It posits that users' perceptions of quality, specifically Attribute Quality and Reliability Quality, are crucial factors in determining whether they will accept and

engage with the technology. Attribute Quality, which includes performance, features, and aesthetics, plays a significant role in shaping user satisfaction. A well-performing vending machine, with useful features such as mobile charging and Wi-Fi access, alongside an aesthetically pleasing design, is more likely to attract users. These elements contribute to the perceived functionality and visual appeal of the machine, which can strongly influence user acceptance [8]. On the other hand, Reliability Quality, encompassing factors like reliability, serviceability, and perceived quality, ensures that users feel confident in the machine's ability to function consistently and be easily maintained, which also affects their willingness to adopt it [51]. The framework further integrates these quality dimensions with User Acceptance, which is primarily influenced by Usefulness and Ease of Use—key components of the Technology Acceptance Model (TAM). According to TAM, a system's usefulness and ease of use directly determine whether users will embrace it. In the case of solar-powered vending machines, their usefulness in providing convenient services and ease of operation are critical for fostering widespread use. The framework suggests that both Attribute Quality (H1) and Reliability Quality (H2) significantly predict User Acceptance, with each dimension influencing users' overall willingness to engage with the technology. Recognizing the importance of both functional attributes and reliability, the model provides valuable insights into the design and deployment of renewable energy technologies in educational settings, ensuring they meet users' expectations and support sustainable, long-term adoption.



**Figure 1.** Conceptual Framework of Perceived Quality and User Acceptance of Solar-Powered Vending Machines

### 3. Methodology

#### 3.1 Research Design

This study employed a quantitative research design to evaluate the perceived quality and user acceptance of solar-powered vending machines, with a focus on the relationships among quality dimensions and technology adoption. A non-experimental, descriptive-correlational design was employed to collect data on users' perceptions of attribute and reliability quality, as well as their acceptance of the technology. A structured survey gathered data on key quality attributes (performance, aesthetics, features, reliability, serviceability) and perceptions of the vending machines' usefulness and ease of use. This design was ideal as it allowed for the collection of objective, measurable data, offering insights into user perceptions. [77] and [78] demonstrated the effectiveness of survey-based quantitative designs in capturing attitudes and behaviors related to technology acceptance, particularly in the context of renewable energy systems. Data were collected through online and on-site surveys to ensure wide representation.

#### 3.2 Participants and Sampling

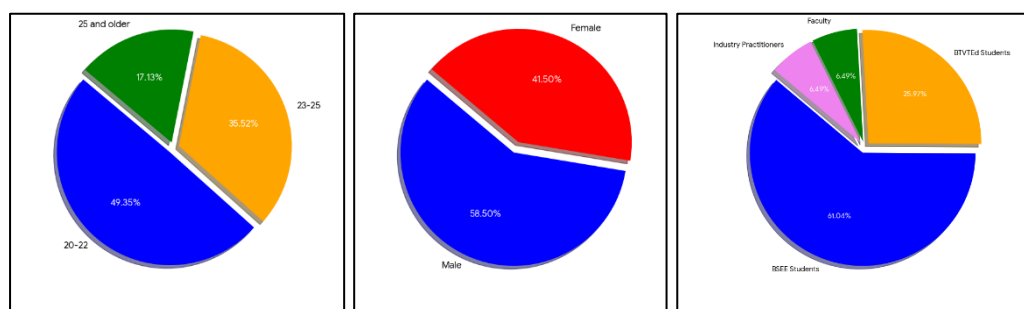
The participants in this study were users of solar-powered vending machines at North Eastern Mindanao State University (NEMSU) in Bislig City, Philippines, including students, faculty, and staff who regularly interacted with the machines. As shown in Table 1, 200 participants were selected using convenience sampling, which was ideal for accessing users on the NEMSU campus. The survey was administered both on-site and through online platforms to ensure accessibility for all potential users. Participants were required to be at least 18 years old, have used the solar-powered vending machines at least once, and agree to participate voluntarily. The sample aimed to include a diverse range of users, balancing students, faculty, and staff to

provide a comprehensive understanding of user acceptance across different demographic groups. This approach ensured that the study gathered relevant insights into how various groups perceive and engage with solar-powered technology on campus.

**Table 1.** Profile of the Respondents (n=200)

| Profile              | Category               | Frequency | Percentage |
|----------------------|------------------------|-----------|------------|
| Age                  | 20-22                  | 99        | 49.35%     |
|                      | 23-25                  | 67        | 33.52%     |
|                      | 25 and older           | 34        | 17.13%     |
| Gender               | Male                   | 117       | 58.44%     |
|                      | Female                 | 83        | 41.56%     |
| Academic Affiliation | BSEE Students          | 122       | 61.04%     |
|                      | BTVTEd Students        | 52        | 25.97%     |
|                      | Faculty                | 13        | 6.49%      |
|                      | Industry Practitioners | 13        | 6.49%      |

Regarding demographics as shown in Table 1 and Figure 1, most respondents were young, with 80.52% aged 25 or younger, and the largest group was those aged 20–22 (49.35%). Males comprised the majority of respondents (58.44%), while females accounted for 41.56%. In terms of academic affiliation, the sample was predominantly composed of Bachelor of Science in Electrical Engineering (BSEE) students (61.04%), with a significant percentage from Bachelor of Technical Vocational Teacher Education (BTVTEd) students (25.97%), and smaller proportions from faculty (6.49%) and industry practitioners (6.49%). This demographic distribution aligns with the perspectives of a younger, more technically-minded audience, making them ideal evaluators of renewable energy technologies in educational settings.



**Figure 1.** Profile of the Respondents

However, while this technically minded demographic provided valuable insights into the system's performance, the heavy reliance on engineering students through convenience sampling introduces a potential technical bias. Specifically, their inherent familiarity with complex mechanical and electrical systems may have positively skewed the ratings for 'Perceived Ease of Use.' Therefore, this demographic concentration is acknowledged as a limitation of the current study. Future research should employ a more randomized sampling approach to include a larger proportion of non-technical staff, students from other disciplines, and general campus visitors to further validate these findings.

### 3.3 Instrument

In this study, a structured questionnaire consisting of a three-part instrument was used. The first part included Informed Consent, where participants were provided with information about the study's purpose, the voluntary nature of participation, and their rights, ensuring ethical transparency and compliance. Following the informed consent, participants completed the survey questionnaires. The second part of the instrument was the Perceived Quality Scale, which consisted of two dimensions: (1) Attribute Quality, assessing performance, functionality, and aesthetics, and (2) Reliability Quality, which included reliability,



serviceability, and perceived quality. A pilot test was conducted to assess the instrument's internal consistency, yielding excellent results. Cronbach's alpha for this scale was 0.92, and the Composite Reliability (CR) was 0.91, both exceeding the recommended threshold, indicating strong internal consistency and reliability. The third part of the instrument was the User Acceptance Scale, based on the Technology Acceptance Model (TAM) dimensions of Perceived Usefulness and Perceived Ease of Use. Similar to the Perceived Quality Scale, a pilot test was conducted for this section, yielding a Cronbach's alpha of 0.94 and a Composite Reliability (CR) of 0.92, both exceeding the recommended threshold and confirming the scale's excellent reliability. All respondents were asked to rate their agreement with statements on a 5-point Likert scale, ranging from 1 (Strongly Disagree) to 5 (Strongly Agree). Pilot testing confirmed the instrument's robustness, ensuring it could reliably measure the constructs of perceived quality and user acceptance.

### 3.4 Data Collection and Analysis

The data-gathering procedure followed a systematic, ethical approach. Formal permission to conduct the study was first secured from North Eastern Mindanao State University (NEMSU), including approval from the Research Ethics Committee. The study's purpose, methods, and requirements were presented, and all necessary documents, such as the ethical review, were submitted. Once approved, participants were informed through an Informed Consent Form outlining the study's objectives, the voluntary nature of participation, anonymity of responses, and their right to withdraw at any time. Only those who provided consent were included in the study. Participants were recruited both online and on-site to ensure accessibility. The survey link was distributed through NEMSU's official communication channels, such as social media groups and email lists, while on-site recruitment targeted users of the solar-powered vending machines. After consenting, respondents completed a structured Likert-type questionnaire comprising three parts: Perceived Quality (performance, functionality, aesthetics, reliability, serviceability, and perceived quality), User Acceptance based on the Technology Acceptance Model (TAM), and demographic information. Items were rated on a 5-point scale from 1 (Strongly Disagree) to 5 (Strongly Agree). All responses were anonymized and stored in password-protected files accessible only to authorized researchers. Data were analyzed using IBM SPSS 26. Descriptive statistics (mean and standard deviation) were used to summarize responses. Pearson correlation tested the relationships between perceived quality dimensions and user acceptance, while multiple regression identified significant predictors of acceptance. All analyses used a 0.05 significance level. Reliability tests, including Cronbach's alpha and Composite Reliability (CR), confirmed strong internal consistency of the scales. Final results were presented using tables and graphs for clarity.

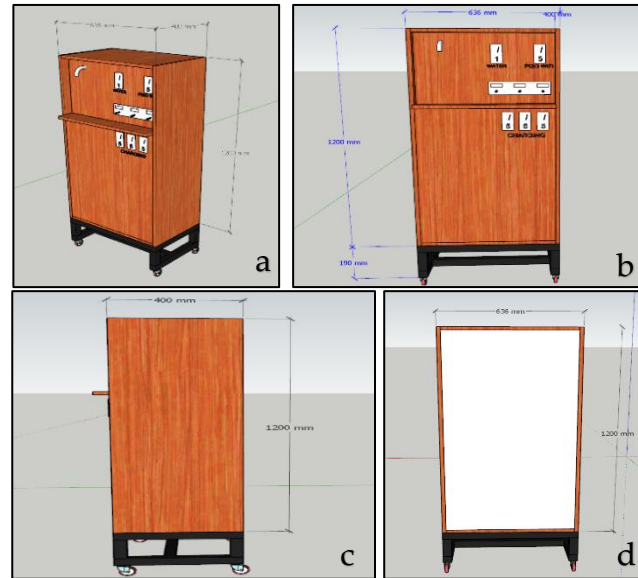
### 3.5 Ethical Considerations

Ethical standards were strictly followed throughout the data collection process. Participants were given full disclosure about the study's objectives, the voluntary nature of their participation, and their right to withdraw at any point. The Research Ethics Committee at NEMSU reviewed and approved the research design, ensuring compliance with ethical guidelines for research involving human subjects. All participants provided informed consent before their participation. Moreover, the study adhered to the principles of respect, beneficence, and justice, ensuring that participants were not harmed and promoting the fair distribution of research benefits. After completing the survey, participants were debriefed on the study's aims and were allowed to ask questions. Participants were informed of how the results would be used, and their contact information was kept confidential. A follow-up was conducted to ensure all data were collected and any concerns regarding the process were addressed.

## 4. Results and Discussion

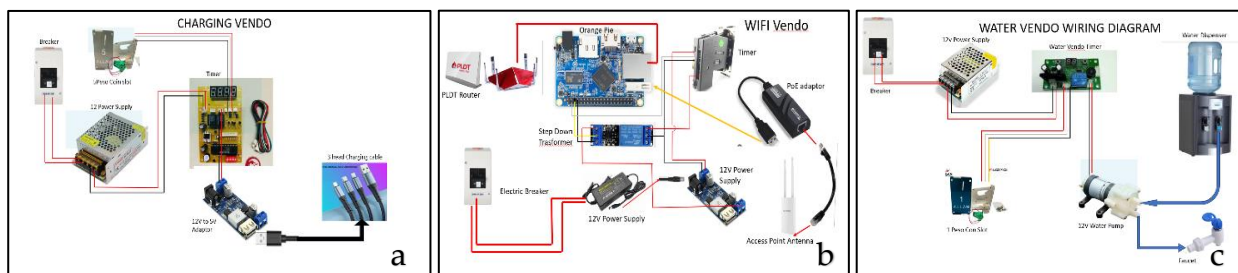
### 4.1 Aesthetics

The design of the solar-powered vending machine is not only functional but also thoughtfully aesthetic, as demonstrated in the provided figures.



**Figure 2.** Dimensions of Solar-Powered Vendo Machine

As shown in Figure 2, the solar-powered vending machine features a compact, ergonomic design measuring 1,700 mm in height, 600 mm in width, and 400 mm in depth. This size ensures space efficiency while maintaining user accessibility. Its design combines functionality and aesthetics through a wood finish and a black base, creating a balance between eco-friendly and modern industrial appeal. The natural texture enhances visual attractiveness and reflects sustainability, making it suitable for public spaces. The solar panel positioned on top highlights its environmental purpose while contributing to its overall design. Rounded edges soften the machine's appearance, making it more approachable and less bulky compared to traditional vending machines. The front interface features a large touchscreen with a clean, user-friendly layout. Clear labels and interactive icons improve usability, while the modern design enhances both functionality and visual appeal.



**Figure 3.** Wiring Diagram of Solar-Powered Vendo Machine

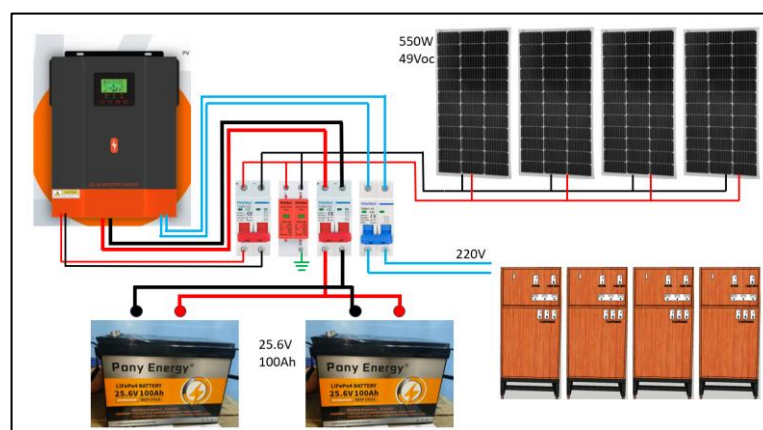
The three diagrams in Figure 2 show the internal wiring and system architecture for three models of the solar-powered vending machine, each serving a unique function and utilizing solar energy. The Charging Vendo Diagram illustrates (see figure 2.a) how the machine functions as a charging station, using a solar power supply, transformers, and timer control to manage the charging of devices. This setup ensures that the machine can operate in locations without a direct power supply, making it eco-friendly and energy-efficient. The Wi-Fi Vendo Wiring Diagram (see figure 2.b), demonstrates how the machine connects to a Wi-Fi network using an access point antenna and Wi-Fi module. The diagram also highlights the inclusion of step-down transformers for voltage regulation, enabling the machine to function in areas requiring internet connectivity for remote control or data monitoring. Lastly, the Water Vendo Wiring Diagram (see figure 2.c) outlines the integration of a water pump and UV filter system, ensuring the machine dispenses clean water efficiently and is powered entirely by solar energy. These wiring configurations underscore the versatility and sustainability

of the solar-powered vending machine, demonstrating its ability to support a range of needs, including device charging, internet connectivity, and clean water dispensing.



**Figure 4.** Actual Campus Set-up of Solar-Powered Vendo Machine

Figure 4 shows four units strategically deployed across different areas of the campus, including hallways and waiting areas. These placements ensure high visibility and easy access for students and staff. Positioning the units in high-traffic locations, such as common spaces, ensures they are readily available for use, enhancing convenience and accessibility. The setup optimizes functionality, enabling users to quickly engage with units where they are most needed, thereby contributing to efficient campus operations.



**Figure 5.** Wiring Set-up of Solar-Powered Vendo Machine

Figure 5 illustrates the wiring setup for a solar-powered vending machine, showing the connections between the various components. The system consists of four solar panels (550W each) connected to a charge controller that regulates power flow to the battery bank. The batteries (25.6V, 100Ah) store the energy generated by the panels for use. The charge controller is connected to an inverter that converts the DC power

into AC (220V) to operate the vending machine. The wiring includes safety features, such as fuses and breakers, to protect the system from overcurrent and ensure safe, efficient operation. This setup highlights a sustainable, off-grid power solution for the vending machine that uses solar energy and energy storage for continuous operation.

#### 4.2 Perceived Quality of Solar-Powered Vending Machines in terms of Attribute Quality

The data in Table 2 shows a strongly positive perception of the solar-powered vending machines, reflected in the high overall mean of 4.39 ("Agree") and a standard deviation of 0.81. This favorable baseline is important because perceived quality heavily shapes user attitudes and their willingness to adopt new technologies [79, 80]. A closer look at the dimensions reveals what users value most. The features dimension earned the highest rating (4.56, "Strongly Agree"), showing that multi-functionality, portability, and convenient payment options are strongly appreciated. The integrated functions—Wi-Fi, cold drinking water, and smartphone charging—received the highest individual score, suggesting strong user preference for systems that offer multiple services in one unit. However, the regression analysis indicates that features alone do not drive acceptance as strongly as performance and aesthetics. This aligns with findings that multi-functionality may initially attract users, but long-term adoption depends more on how consistently and effectively core functions operate [44]. The performance dimension supports this pattern, with a mean of 4.17 ("Agree") and strong approval of the cold-water feature, yet a notable weakness in Wi-Fi stability. Since performance reliability is central to perceived usefulness, disruptions—particularly in Wi-Fi—pose a real threat to continued use and acceptance [52, 71]. This lower rating may be attributed to technical constraints in solar-powered systems, where energy intermittency affects continuous power delivery to network components such as routers and signal amplifiers [81]. During periods of low solar irradiance or nighttime operation, limited battery storage capacity can result in unstable voltage supply, leading to intermittent connectivity or reduced router performance. To address this, engineering solutions such as optimizing battery storage capacity, integrating power management systems with load prioritization for communication modules, and implementing energy-efficient routers with low-power standby modes may enhance Wi-Fi stability and overall system reliability. Hybrid charging support or auxiliary backup power systems may also be considered to ensure uninterrupted connectivity [82]. Meanwhile, the aesthetics dimension (4.42, "Agree") demonstrates that users view the machines as visually appealing and well-suited to the campus environment, a factor known to enhance perceived value and promote willingness to engage with technology in shared public spaces [47, 72, 83].

The consistently high ratings across all attributes signal strong acceptance potential within educational settings, yet targeted improvements are needed to sustain long-term user loyalty. Strengthening performance, especially Wi-Fi stability, should be the top priority because performance was identified as the strongest predictor of acceptance. Enhancing this single area would significantly improve perceived quality and usefulness. The strong aesthetic appeal of the machines should continue to be leveraged, as the design contributes to positive first impressions and helps attract new users. At the same time, expectations around features must be carefully managed. While the multi-functionality is well-received, the priority should be to refine the reliability of existing services—water dispensing, phone charging, and Wi-Fi connectivity—rather than adding new functions. Focusing on consistent performance for these core features will build trust and repeat use more effectively than expanding the feature set. Overall, the machines have established a solid perception of quality, and addressing the identified performance gap will allow the institution to convert this positive perception into long-term adoption, user satisfaction, and sustainability of the service.

**Table 2.** Mean Distribution of the Respondents' Ratings on the Perceived Quality of Solar-Powered Vending Machines in terms of Attribute Quality

| Attribute Quality                                                                                                                                                          | Mean        | SD          | Description           |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|-------------|-----------------------|
| <b>Performance</b>                                                                                                                                                         |             |             |                       |
| 1. The Wi-Fi vending feature provides a stable and fast internet connection.                                                                                               | 3.92        | 1.02        | Agree                 |
| 2. The cold-water feature consistently dispenses water at the desired temperature.                                                                                         | 4.39        | 0.67        | Agree                 |
| 3. The smartphone charging feature charges devices quickly and reliably.                                                                                                   | 4.00        | 1.06        | Agree                 |
| 4. The solar-powered vending machines exhibit strong performance and cost-effectiveness.                                                                                   | 4.36        | 0.67        | Agree                 |
| <b>Mean</b>                                                                                                                                                                | <b>4.17</b> | <b>0.71</b> | <b>Agree</b>          |
| <b>Features</b>                                                                                                                                                            |             |             |                       |
| 5. The 3-in-1 vending machine's multi-functionality addresses diverse user needs by offering Wi-Fi vending, cold drinking water, and smartphone charging in a single unit. | 4.63        | 0.63        | Strongly Agree        |
| 6. The machine's wheels make it portable and easy to move to different locations.                                                                                          | 4.46        | 0.74        | Agree                 |
| 7. The machine's compact size makes it suitable for small spaces without being obtrusive.                                                                                  | 4.53        | 0.64        | Strongly Agree        |
| 8. The machine's coin slot payment option is convenient and easy to use.                                                                                                   | 4.62        | 0.61        | Strongly Agree        |
| 9. The smartphone charging feature supports multiple device types, ensuring compatibility and convenience for a wide range of users.                                       | 4.57        | 0.52        | Strongly Agree        |
| <b>Mean</b>                                                                                                                                                                | <b>4.56</b> | <b>0.50</b> | <b>Strongly Agree</b> |
| <b>Aesthetics</b>                                                                                                                                                          |             |             |                       |
| 10. The overall design of the 3-in-1 vending machine is visually appealing.                                                                                                | 4.36        | 0.81        | Agree                 |
| 11. The machine's appearance complements its surroundings well (e.g., a campus environment).                                                                               | 4.47        | 0.77        | Agree                 |
| 12. I find the machine's design to be modern, elegant, attractive, and aesthetically pleasing.                                                                             | 4.42        | 0.80        | Agree                 |
| <b>Mean</b>                                                                                                                                                                | <b>4.42</b> | <b>0.72</b> | <b>Agree</b>          |
| <b>Weighted Mean</b>                                                                                                                                                       | <b>4.39</b> | <b>0.81</b> | <b>Agree</b>          |

Legend: 4.50 – 5.00 – Strongly Agree; 3.50 – 4.49 – Agree; 2.50 – 3.49 – Neutral; 1.50 – 2.49 – Disagree; 1.00 – 1.49 – Strongly Disagree

### 4.3 Perceived Quality of Solar-Powered Vending Machines in terms of Reliability Quality

The results in Table 3 show that respondents generally perceive the solar-powered vending machines as reliable, reflected in the high overall mean rating of 4.24. This positive perception is important because reliability consistently emerges in research as a major driver of technology adoption, shaping user trust, purchase intentions, and long-term satisfaction [29, 52]. When users believe a system performs dependably, they are more likely to embrace it and continue using it. The results reveal that serviceability received the highest rating at 4.29. This means users believe the machines are easy to maintain, repair, and install, which is a strong indicator of perceived durability and long-term value. Studies emphasize that products designed for easy maintenance tend to be perceived as higher in quality and more dependable, thereby increasing acceptance of technological innovations [53, 54]. Strong serviceability also enhances user confidence because it signals that any potential issues can be resolved quickly, supporting uninterrupted use and reducing concerns about risk or inconvenience. The reliability dimension had a mean of 4.13, supported by a high overall dependability rating of 4.34. This aligns with technology acceptance research, which recognizes



dependability as a major factor in the Technology Acceptance Model, strengthening perceived usefulness and reducing hesitation to adopt new tools [52, 77]. However, the feature-level results reveal an important concern. Although the cold drinking water feature scored well at 4.25, the Wi-Fi vending feature received the lowest rating at 3.79, suggesting occasional disruptions. Research on self-service technologies shows that even minor interruptions in heavily used features can lower user satisfaction and weaken behavioral intentions [51, 71]. This highlights that the reliability of each core feature is essential to shaping the overall user experience. The lower Wi-Fi rating may be associated with unstable energy supply during periods of low solar input, which can affect the continuous operation of network components supported by the system's storage capacity [84]. Enhancing battery efficiency, prioritizing energy allocation for communication modules, and using low-consumption networking devices may help stabilize connectivity and improve overall system reliability.

The perceived quality dimension also scored high at 4.30, with strong ratings for the cold-drinking-water feature at 4.39 and for overall machine quality at 4.35. These findings suggest that users believe the machines offer stable performance and solid construction. Consumer behavior studies consistently show that high perceived quality strengthens trust, encourages positive attitudes, and predicts stronger loyalty and likelihood of continued use [33, 85, 86]. Positive judgments of quality serve as a strong precursor to adoption in technology acceptance research [3].

In summary, the results indicate that the vending machines are viewed favorably for reliability, serviceability, and overall quality. The high ratings in both serviceability and water dispensing demonstrate a strong foundation for user acceptance. The lower Wi-Fi stability rating presents a clear area for improvement. Enhancing the reliability of this feature could significantly strengthen overall acceptance, as consistent performance across all functions reinforces perceptions of quality and usefulness [44], ultimately supporting long-term adoption.

#### 4.4 User Acceptance of Solar-Powered Vending Machines

The results in Table 4 show very high acceptability of the solar-powered vending machines, with an overall mean of 4.52 and a standard deviation of 0.69. This strong response aligns with the core principles of the Technology Acceptance Model, as both Perceived Usefulness (mean = 4.53, standard deviation = 0.55) and Perceived Ease of Use (mean = 4.52, standard deviation = 0.70) fell within the "Strongly Agree" range. These findings demonstrate that users recognize the machines as both highly beneficial and convenient to operate, a combination supported in technology adoption research [3, 77].

Usefulness indicators highlight a clear driver of acceptance. The highest rating was given to the machine's environmental contribution, with a mean of 4.65 and a standard deviation of 0.56, showing strong user appreciation for its eco-friendly design. This quantitative finding is supported by open-ended survey responses, which indicate strong appreciation for the machine's green integration. For instance, one student noted, "*Seeing the solar panel on top makes me feel good about using it. It shows our campus is actually doing something real for the environment.*" Another participant highlighted, "*It's great that we can charge our phones using clean energy right in the hallway.*" These sentiments confirm that environmental advantages significantly boost the adoption. Studies confirm that environmental advantages significantly boost the adoption of sustainable technologies by increasing perceived usefulness and adding moral or social value to the user experience [87, 88]. High ratings for the cold drinking water feature (mean 4.58, standard deviation 0.57) and overall comfort and convenience (mean 4.57, standard deviation 0.68) also suggest that users see the technology as both practical and aligned with sustainable values. Research notes that green innovations succeed most when ecological benefits are paired with personal convenience, reinforcing this pattern [28].

Ease-of-use indicators reveal equally positive results. Strong agreement was recorded across all items, with high ratings for the intuitiveness of the cold drinking water feature (mean = 4.62, standard deviation = 0.57) and the accessibility of the Wi-Fi function (mean = 4.51, standard deviation = 0.69). These findings show that the machines achieve a user-friendly interface that reduces effort and supports repeated use, consistent with studies on usability and self-service technologies [71, 80, 89]. However, it is important to note that these exceptionally high ease-of-use ratings may be partially influenced by the sample's technical bias, as the majority of respondents were engineering students who are naturally adept at navigating new interfaces. The slightly lower score for "requires little assistance," with a mean of 4.40 and a standard deviation of 0.96, signals

that some first-time users may still need brief guidance. Research in educational technology shows that concise instructions and simple onboarding strategies can meaningfully improve ease-of-use perceptions [17].

Thus, the strong results across both TAM dimensions indicate exceptional user acceptance and high potential for long-term use. The environmental appeal provides an added advantage in sustainability-focused environments. Enhancing support for new users through visual guides or simple onboarding approaches, as validated in previous technology adoption studies [74, 90], can further strengthen long-term user engagement and sustained adoption.

**Table 3.** Mean Distribution of the Respondents' Ratings on the Perceived Quality of Solar-Powered Vending Machines in terms of Reliability Quality

| Reliability Quality                                                                            | Mean        | SD          | Description           |
|------------------------------------------------------------------------------------------------|-------------|-------------|-----------------------|
| <b>Reliability</b>                                                                             |             |             |                       |
| 1. The Wi-Fi vending feature works without frequent disconnections or interruptions.           | 3.79        | 0.91        | Agree                 |
| 2. The cold drinking water feature operates without breakdowns or malfunctions.                | 4.25        | 0.75        | Agree                 |
| 3. The smartphone charging feature consistently charges devices without issues.                | 4.16        | 0.93        | Agree                 |
| 4. The solar-powered vending machines are dependable.                                          | 4.34        | 0.68        | Agree                 |
| <b>Mean</b>                                                                                    | <b>4.13</b> | <b>0.67</b> | <b>Agree</b>          |
| <b>Serviceability</b>                                                                          |             |             |                       |
| 5. The Wi-Fi vending feature is easy to troubleshoot and repair if issues arise.               | 4.23        | 0.79        | Strongly Agree        |
| 6. The cold drinking water system can be serviced or maintained without significant downtime.  | 4.36        | 0.65        | Agree                 |
| 7. The smartphone charging feature is easy to repair or replace if damaged.                    | 4.29        | 0.79        | Strongly Agree        |
| 8. The solar-powered vending machines are easy to maintain, install, and repair.               | 4.29        | 0.79        | Strongly Agree        |
| <b>Mean</b>                                                                                    | <b>4.29</b> | <b>0.64</b> | <b>Strongly Agree</b> |
| <b>Perceived Quality</b>                                                                       |             |             |                       |
| 9. Overall, I believe the Wi-Fi vending feature is of high quality.                            | 4.18        | 0.93        | Agree                 |
| 10. Overall, I believe the cold-water feature is of high quality.                              | 4.39        | 0.73        | Agree                 |
| 11. Overall, I believe the smartphone charging feature is of high quality.                     | 4.26        | 0.93        |                       |
| 12. The overall quality of the solar-powered vending machine meets or exceeds my expectations. | 4.35        | 0.79        | Agree                 |
| <b>Mean</b>                                                                                    | <b>4.30</b> | <b>0.79</b> | <b>Agree</b>          |
| <b>Weighted Mean</b>                                                                           | <b>4.24</b> | <b>0.88</b> | <b>Agree</b>          |

Legend: 4.50 – 5.00 – Strongly Agree; 3.50 – 4.49 – Agree; 2.50 – 3.49 – Neutral; 1.50 – 2.49 – Disagree; 1.00 – 1.49 – Strongly Disagree

**Table 4.** Mean Distribution of the User Acceptance of Solar-Powered Vending Machines

| Indicators                                                                                                                                     | Mean        | SD          | Description           |
|------------------------------------------------------------------------------------------------------------------------------------------------|-------------|-------------|-----------------------|
| <i>Perceived Usefulness</i>                                                                                                                    |             |             |                       |
| 1. The Wi-Fi vending feature is useful for my internet needs                                                                                   | 4.39        | 0.83        | Agree                 |
| 2. The cold-water feature is useful for staying hydrated.                                                                                      | 4.58        | 0.57        | Strongly Agree        |
| 3. The smartphone charging feature is useful for keeping my devices powered.                                                                   | 4.44        | 0.80        | Agree                 |
| 4. Overall, solar-powered vending machines help make the environment cleaner and safer.                                                        | 4.65        | 0.56        | Strongly Agree        |
| 5. Overall, I find solar-powered vending machines useful, as they offer comfort and convenience.                                               | 4.57        | 0.68        | Strongly Agree        |
| <i>Mean</i>                                                                                                                                    | 4.53        | 0.55        | Strongly Agree        |
| <i>Perceived Ease of Use</i>                                                                                                                   |             |             |                       |
| 6. The Wi-Fi vending feature is easy to access and use.                                                                                        | 4.51        | 0.69        | Strongly Agree        |
| 7. The cold-water feature is easy to operate.                                                                                                  | 4.62        | 0.57        | Strongly Agree        |
| 8. The smartphone charging feature is easy to use.                                                                                             | 4.53        | 0.72        | Strongly Agree        |
| 9. Overall, I require little assistance to use the 3-in-1 solar-powered vending machines.                                                      | 4.40        | 0.96        | Agree                 |
| 10. Overall, I feel positive and comfortable using the 3-in-1 solar-powered vending machines because of their user-friendly, intuitive design. | 4.55        | 0.70        | Strongly Agree        |
| <i>Mean</i>                                                                                                                                    | 4.52        | 0.70        | Strongly Agree        |
| <b>Weighted Mean</b>                                                                                                                           | <b>4.52</b> | <b>0.69</b> | <b>Strongly Agree</b> |

Legend: 4.50 – 5.00 – Strongly Agree; 3.50 – 4.49 – Agree; 2.50 – 3.49 – Neutral; 1.50 – 2.49 – Disagree; 1.00 – 1.49 – Strongly Disagree

#### 4.5 Attribute-Quality as a Predictor of User Acceptance

The regression analysis in Table 5 provides key insights into the factors driving user acceptance of solar-powered vending machines. Performance ( $\beta = 0.201$ ,  $p = 0.0005$ ) and aesthetics ( $\beta = 0.265$ ,  $p < 0.001$ ) significantly influence acceptance, while features ( $\beta = 0.067$ ,  $p = 0.4563$ ) do not. With 59.6 percent of the variance explained ( $R^2 = 0.596$ ), users rely more on how well the machine functions and how visually appealing it is when forming acceptance decisions, showing that certain attributes carry more weight than others in shaping adoption intent.

Performance emerges as a strong driver, emphasizing that reliable operation, including stable Wi-Fi, consistent cold-water dispensing, and dependable charging, is central to acceptance. This aligns with the Technology Acceptance Model, where functional performance is a key antecedent of Perceived Usefulness [77]. Dependability strongly influences technology trust [52], and research on smart retail and self-service technologies confirms that performance reliability is essential for user satisfaction and sustained use [51, 73]. Users are more likely to adopt technologies that consistently deliver without interruptions, making reliability a foundational determinant of acceptance.

Aesthetics is the strongest predictor, highlighting the importance of visual appeal, modern design, and environmental fit in shaping positive user attitudes. Attractive design enhances perceived value and quality [47, 48], creates positive first impressions, reduces perceived complexity, and signals modernity and professionalism [72, 83]. Design aesthetics also function as a post-adoption buffer, helping sustain continued use even when minor issues arise [49], reinforcing the influence of visual appeal on overall acceptance.

The non-significance of features provides an important insight. While users appreciate multi-functionality and portability, these aspects do not substantially shape acceptance once performance and aesthetics are accounted for. This reflects the “Feature Fatigue” concept [44], which warns that too many features can overwhelm users or fail to add meaningful value. Users expect a baseline level of functionality,

but acceptance depends more on the reliability of core features and visual satisfaction than on the number of features offered.

These findings suggest clear practical implications. Prioritizing performance reliability is essential, ensuring stable Wi-Fi, consistent water dispensing, and dependable charging. Investing in superior aesthetics is equally critical, as visual appeal strongly influences perceptions of quality and value. Introducing new features should be secondary to perfecting existing ones, following a “features-second” approach. Maximizing user acceptance requires a disciplined focus on operational excellence and thoughtful design, ensuring interactions with the vending machine are both reliably functional and visually rewarding.

**Table 5.** Regression Analysis Attribute-Quality Dimensions Predicting User Acceptance

| Predictor                 | Beta Coefficient | p-value | Interpretation  |
|---------------------------|------------------|---------|-----------------|
| Performance               | 0.201            | 0.0005  | Significant     |
| Features                  | 0.067            | 0.4563  | Not Significant |
| Aesthetics                | 0.265            | 0.0000  | Significant     |
| $R^2 = 0.596; F = 35.475$ |                  |         |                 |

Dependent variable: User Acceptance of Solar-Powered Vending Machines

#### 4.6 Reliability-Quality as Predictor of User Acceptance

The regression analysis in Table 6 provides a clear understanding of how reliability-related dimensions shape user acceptance. Serviceability emerges as the only significant predictor ( $\beta = 0.316$ ,  $p = 0.0029$ ), while reliability ( $\beta = 0.208$ ,  $p = 0.102$ ) and perceived quality ( $\beta = 0.091$ ,  $p = 0.4903$ ) do not significantly influence acceptance when considered alongside it. The model explains 37.0 percent of the variance in user acceptance ( $R^2 = 0.370$ ), highlighting that serviceability has unique explanatory power and serves as a distinct driver of adoption intent relative to other reliability-related attributes.

The significance of serviceability underscores that users place high value on how easily the machines can be maintained, repaired, and restored to full operation. This aligns with research showing that ease of maintenance is a core element of perceived durability and contributes to long-term consumer satisfaction [53, 54]. In public-facing technologies, rapid and effective recovery from service failures builds trust and strengthens user loyalty [51, 91]. Even if a machine is generally reliable, users are more willing to adopt it when they know issues can be resolved quickly and with minimal inconvenience, thereby lowering the perceived risk of adopting the technology.

The non-significance of reliability and perceived quality provides an important insight into user cognition. When serviceability is high, users may perceive a strong maintenance and support system as sufficient protection, reducing concerns about inherent reliability or product quality. This reflects a dynamic understanding of reliability, where the surrounding support system becomes as crucial as the technology’s initial performance. Research in other fields supports this, showing that robust operational support acts as a safety net that outweighs baseline reliability concerns in adoption decisions [55].

These findings have clear practical implications. Institutions should prioritize developing a responsive support ecosystem, ensuring spare parts availability, and establishing clear maintenance protocols. Communicating serviceability to users strengthens confidence and encourages adoption. Future iterations should incorporate design-for-serviceability principles, such as modular components, accessible parts, and diagnostic features to streamline repair processes [55].

In conclusion, while inherent reliability and perceived quality remain important, accessible and effective serviceability is the key factor that strengthens user confidence and drives acceptance of solar-powered vending machines. A technology that is not only well-built but also supported by a dependable maintenance system minimizes downtime, enhances user experience, and fosters long-term adoption.

**Table 6.** Regression Analysis of the Reliability-Quality Dimensions Predicting User Acceptance

| Predictor                 | Beta Coefficient | p-value | Interpretation  |
|---------------------------|------------------|---------|-----------------|
| Reliability               | 0.208            | 0.102   | Not Significant |
| Serviceability            | 0.316            | 0.0029  | Significant     |
| Perceived Quality         | 0.091            | 0.4903  | Not Significant |
| $R^2 = 0.370; F = 13.911$ |                  |         |                 |

Dependent variable: User Acceptance of Solar-Powered Vending Machines

## 5. Conclusions

This study evaluated the perceived quality and user acceptance of solar-powered vending machines at North Eastern Mindanao State University, focusing on attribute quality (performance, features, aesthetics) and reliability quality (reliability, serviceability, perceived quality). Results showed generally positive perceptions, with users valuing multi-functionality, portability, ease of use, and environmental sustainability. In particular, performance and aesthetics emerged as significant predictors of acceptance, underscoring the importance of both functional efficiency and appealing design in shaping user approval. Serviceability also proved to be a key determinant, underscoring the need for easy maintenance and repair to sustain system use over time. However, areas such as Wi-Fi stability were identified as needing improvement, as connectivity issues can negatively affect user satisfaction and overall adoption experience. Overall, high ratings for perceived usefulness and ease of use suggest strong potential for widespread adoption of the technology in educational settings. These findings provide practical guidance for administrators and policymakers in deploying and maintaining such systems to ensure alignment with user needs and sustainability goals. Consequently, addressing technical challenges while strengthening serviceability remains essential in maximizing system impact and sustaining user trust and engagement.

Future development of this technology should extend beyond system enhancement toward more rigorous and comprehensive evaluation frameworks. Future studies may adopt longitudinal research designs to track actual usage behavior over time, shifting focus from behavioral intention to sustained, real-world utilization. This approach would provide stronger evidence on long-term adoption patterns and system effectiveness in operational settings. In addition, future research may include financial viability indicators such as return on investment (ROI), cost recovery period, and long-term operational sustainability to assess the economic feasibility of scaling solar-powered vending machines. Environmental performance measures, such as carbon offset reductions, energy savings, and greenhouse gas mitigation, may also be incorporated to quantify contributions to institutional sustainability and climate goals. Collectively, these directions support a more holistic evaluation of solar-powered vending machines as both technological innovations and sustainability solutions in educational environments. The university may also integrate these systems into a broader “Green Campus” initiative, using them as instructional tools in renewable energy-related courses to strengthen practical learning in solar technology and applications.

This study also acknowledges several limitations that should be considered in interpreting the findings. The sample was drawn from a single institution, North Eastern Mindanao State University, which may restrict the generalizability of the results to other contexts. The study used convenience sampling with 200 participants, mostly engineering students (61.04%), which may have introduced technical bias and inflated ease-of-use ratings due to greater technology familiarity. Future research should use randomized or stratified sampling that includes non-technical students and staff to achieve a more balanced perspective. Participation was also limited to individuals with prior experience with the solar-powered vending machines, thereby excluding potential users who had not yet interacted with the system. The reliance on self-reported survey data may introduce response biases such as social desirability and recall bias, despite efforts to ensure anonymity and objectivity. Moreover, the study did not capture long-term usage behavior or post-adoption patterns, limiting insights into sustained engagement. External influences, such as socio-cultural factors and environmental conditions, were also not fully examined. While technical concerns such as Wi-Fi stability were identified, they were not deeply analyzed in terms of system engineering solutions. Furthermore, it is important to clarify that this research serves as an initial, cross-sectional baseline evaluation of the newly



deployed solar-powered vending machines in their current state. Because no prior versions of the machine existed on campus, pre- and post-upgrade satisfaction surveys and comparative technical matrices were not applicable. Future iterations of the system should utilize this baseline data to measure the effectiveness of subsequent technical upgrades. Similarly, while evaluating the economic impact is valuable, conducting a break-even point analysis or detailed financial assessment fell outside the scope of this user-centric behavioral study. Therefore, as recommended, future researchers should pair this acceptance data with economic viability metrics to fully assess the project's institutional value. Accordingly, future research may expand across multiple institutions, integrate qualitative approaches, incorporate longitudinal tracking of actual usage, and explore technical refinements to provide a more comprehensive understanding of the adoption and sustained use of solar-powered vending machines.

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# Development and Evaluation of a Laboratory-scale Pyrolysis Reactor for Bio-oil Production

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**Abstract:** Biomass pyrolysis is gaining interest as a renewable energy conversion pathway that valorizes agricultural residues while mitigating environmental hazards from open burning. This study aimed to design and fabricate a batch-fed laboratory-scale biomass pyrolysis reactor and evaluate its technical performance for bio-oil production using rice straw as feedstock. A split-plot experiment in a completely randomized design was employed, varying reactor insulation thickness (25, 50, and 75 mm), pyrolysis temperature (400°C, 450°C, and 500°C), and biomass loading density (60, 90, and 120 kg/m<sup>3</sup>). Analysis of variance and Scheffe's test were used to determine significant differences among treatments. Biomass loading density, pyrolysis temperature, and insulation thickness each had a significant effect on bio-oil production, whereas all two-way and three-way interactions were not significant. The combination of 25 mm insulation, 450°C pyrolysis temperature, and 90 kg/m<sup>3</sup> loading density yielded the highest mean bio-oil (78.07 g, 76.74 g, and 73.26 g, respectively). The developed reactor demonstrated temperature stability and operational integrity at 400°C–500°C. The reactor is a viable tool for biomass pyrolysis, and the established standard operating procedure provides a useful guide for optimizing bio-oil and bio-char production from various biomass types.

**Keywords:** Biomass; pyrolysis; batch-fed reactor; bio-oil; bio-char

## 1. Introduction

Interest in renewable energy sources is growing significantly due to concerns about global climate change, environmental pollution, and the depletion of fossil energy resources [1,2]. Among renewable resources, biomass is the only resource capable of producing liquid fuel. Biomass has favorable attributes: it is renewable and carbon-based, and it is low in sulfur and nitrogen contents [2,3]. Abundantly available worldwide [4], biomass utilization in mainstream energy applications has received significant attention due to environmental considerations and increasing energy demands [3]. Combustion of biofuel produces fewer harmful gas emissions, such as nitrogen oxides (NO<sub>x</sub>), sulfur dioxide (SO<sub>2</sub>), and soot, than conventional fossil fuels. Zero or negative carbon dioxide (CO<sub>2</sub>) emissions are possible from biomass fuel combustion because the released CO<sub>2</sub> can be recycled by plants through photosynthesis [5].

Biomass is a promising, eco-friendly alternative source of renewable energy. The current global energy supply is largely based on fossil fuels with

finite reserves. Given the growing world population, rising per capita energy consumption, and evidence of global warming, the need for long-term alternative energy sources is clear [1]. Biofuel, as an alternative fuel, has received considerable attention in the Philippines. Republic Act 9367 (Biofuels Act of 2006) mandates the use of biofuels as fuel blends and establishes a national biofuel program to reduce the Philippines' dependence on imported crude oil, increase rural employment, and reduce greenhouse gas emissions.

Among biomass-to-energy conversion processes, pyrolysis has attracted increased interest for producing liquid fuel due to advantages in storage, transport, and versatility in applications such as combustion engines, boilers, turbines, and power generation [6]. Pyrolysis is the thermal decomposition of biomass in the absence of oxygen, derived from Greek words “pyro” (fire) and “lysis” (decomposition) [7]. More than 5,500 years ago in Southern Europe and the Middle East, pyrolysis technology was used for charcoal production [8]. Pyrolysis technology can produce biofuel with high fuel-to-feed ratios, and its development continues worldwide [9,10]. Pyrolysis research in the Philippines has been focused primarily on industrial wastes such as rubber tires and plastics, with limited work on biomass resources. Rapsing [11] designed and fabricated a waste plastic oil converter, demonstrating conversion of waste plastics into fuel through pyrolysis. Pyrolysis of various agricultural and industrial biomass residues in the ASEAN region has also been investigated, including latex sediment from concentrated latex factories, demonstrating the potential of pyrolysis for regional waste valorization [12].

Maintaining critical design characteristics during scale-up enables prediction of process parameters from pilot-scale data [13]. A laboratory-scale pyrolysis system must be designed, constructed, and evaluated as the basis for commercial-scale development. This study, therefore, aimed to: (1) design and fabricate a batch-fed laboratory-scale biomass pyrolysis reactor; (2) evaluate its technical performance in terms of bio-oil and bio-char production using rice straw as feedstock under predetermined conditions; and (3) standardize the operating procedure for optimum bio-oil production.

## 2. Materials and Methods

### 2.1 Design and Fabrication of the Laboratory-Scale Pyrolysis Reactor

#### 2.1.1 Design considerations

The laboratory-scale reactor system was designed based on the principle of heating biomass in the absence of oxygen. A batch-fed configuration was adopted to maintain an oxygen-free environment during pyrolysis. The design targeted the pyrolysis of cellulosic biomass wastes, such as rice straw and other locally abundant agricultural residues. Flexibility in reactor insulation, biomass loading density, and heating temperature was incorporated to enable optimization of bio-oil production. Electric power was selected as the heat source, and a condenser was included to recover condensable pyrolytic gases as bio-oil. Fabrication was carried out using local materials and facilities [13].

#### 2.1.2 Designing the reactor and its components

The essential components of the pyrolysis system were identified based on the design reference from Henkel [14], with modifications to simplify construction: vertical reactor orientation for easy loading, a larger reactor volume to accommodate varied biomass types, and an Arduino-based temperature controller, while retaining induction heating. Pyrolysis reactor. The reactor was a cylindrical, fixed-bed type made from a 5-mm-thick block of iron pipe with a 12 cm diameter and 44 cm length. The top was provided with a cover for loading biomass feedstock and maintaining an oxygen-free environment. A solid residue (bio-char) collector was hinged at the bottom. An exit pipe at the upper side channeled evolved gases through a condenser to extract bio-oil. The reactor volume and mass capacity were calculated using a bulk density of 60 kg/m<sup>3</sup> for dried, chopped rice straw [15]:

$$V_r = \pi r^2 h = \pi (6 \text{ cm})^2 (44 \text{ cm}) = 4,976.28 \text{ cm}^3 \quad (1)$$

$$m_r = V_r \times D_{rs} = 4,976.28 \text{ cm}^3 \times 60 \text{ kg/m}^3 \approx 300 \text{ g} \quad (2)$$

The heater consisted of a 10-mm-diameter Canthel wire wound helically on the outer surface of the reactor. Heating was primarily through conduction, with a cylindrical metal casing and fiberglass wool insulation to minimize heat loss. The heater was rated based on total heat energy requirements, calculated as follows:

$$Q_T = Q_M + Q_L + SF \text{ (20\% safety factor)} \quad (3)$$

Where  $Q_T$  = total energy required (W·hr);  $Q_M$  = energy absorbed by reactor material and biomass;  $Q_L$  = energy lost by conduction from reactor surfaces. Calculations yielded a total power requirement of 3.08 kW for the reactor system. Reactor-heater insulation. Sheet pads of fiberglass wool were wrapped around the cylindrical metal casing to minimize heat loss during pyrolysis. Variable insulation thicknesses (25, 50, and 75 mm) were incorporated as experimental parameters for the volatile gas condenser. A vertical tube-type condenser was designed using a 12-mm pipe centrally inserted along a 150 × 300 mm cylindrical condenser body. Cold water at 10–20°C was circulated continuously to liquefy condensable pyrolytic gases into bio-oil. Pyrolysis system frame. An angular steel frame (45 cm × 60 cm × 70 cm) provided structural stability and safety for the assembled system. Temperature controller. The temperature control device consisted of an Arduino Uno circuit board, a 20-Amp electromechanical relay, K-type thermocouple wires, and a Max6675 digital integrated circuit. The relay automatically switched the power supply ON and OFF to maintain the set pyrolysis temperature.

### 2.1.3 Fabrication of the pyrolysis reactor system

Fabrication of the pyrolysis reactor and volatile gas condenser was job-ordered to a specialized machine shop in Cebu City. The reactor heater was fabricated at a specialized electrical shop in Lapu-Lapu City with a rated power of 3.0 kW. The Arduino temperature control device was constructed by the researcher with the assistance of an electronics professional. The system frame was fabricated locally by a skilled artisan. Total fabrication cost was PHP 60,370.00.

## 2.2 Operating Principles

The system operated on the principle of pyrolysis—heating biomass in the absence of oxygen. Each batch of biomass was loaded, then thermally degraded at a set controlled temperature. Pyrolytic gases were channeled through the condenser to collect bio-oil. The operation was stopped when no further significant bio-oil production was observed, after which end-products (bio-oil and bio-char) were collected and weighed separately. The sealed batch configuration maintained the oxygen-free condition during pyrolysis; the reactor was fully closed before heating and remained sealed throughout each run.

## 2.3 Technical Performance Evaluation

### 2.3.1 Experimental set-up

The experimental setup consisted of a cylindrical batch reactor externally heated by a 3-kW electrical coil, with temperature controlled using an external Arduino circuit controller. The reactor had two openings at the upper section: one covered opening for loading feedstock and one for vapor exit connected to the condenser. A third opening at the bottom, with a push-pull metal valve, discharged biochar. Ice-cooled water circulated in the condenser as the cooling medium. Condensed liquid and solid products were collected and weighed immediately after each pyrolysis run. Gaseous product yield was calculated by material balance.

### 2.3.2 Experimental procedure

Pyrolysis experiments were conducted by varying pyrolysis temperature, feedstock loading density, and insulation thickness. The operating conditions — batch loading, temperatures of 400–500°C, and residence times of 20–30 min — are consistent with slow pyrolysis mode, which is characterized by low heating rates and extended solid and vapor residence times that favor char and gas production over liquid bio-oil [16]. The measured heating rate of the 3-kW external coil should be recorded and reported for each temperature set point to ensure reproducibility and allow comparison with other systems. Based on preliminary tests, each set temperature was maintained for 20–30 min to ensure complete conversion of biomass. Three experimental

groups were conducted at insulation thicknesses of 25, 50, and 75 mm. Within each group, three pyrolysis temperatures (400°C, 450°C, and 500°C) and three loading densities (60, 90, and 120 kg/m<sup>3</sup>) were tested, with three replications per condition.

### 2.3.3 Experimental design and analyses

The experiment was carried out in a split-plot completely randomized design (CRD) with insulation thickness (H) as the main plot and biomass loading density (D) and pyrolysis temperature (T) as sub-plot factors. Insulation thickness was treated as a hard-to-change (HTC) factor. Insulation thickness was designated as the HTC factor because physically modifying the fiberglass wool wrapping (removing, adjusting thickness, and reinstalling around the reactor assembly) between experimental runs required significantly more time and effort than adjusting temperature set-points or biomass loading, consistent with the split-plot design rationale. The three insulation thickness levels were randomized at the whole-plot level; a single reactor unit was used, and insulation was changed between experimental groups (one group per insulation level) rather than between individual runs. Factor levels were:

$H_1 = 25$  mm,  $H_2 = 50$  mm,  $H_3 = 75$  mm thick fiberglass wool insulation

$D_1 = 60$  kg/m<sup>3</sup>,  $D_2 = 90$  kg/m<sup>3</sup>,  $D_3 = 120$  kg/m<sup>3</sup> biomass loading density

$T_1 = 400^\circ\text{C}$ ,  $T_2 = 450^\circ\text{C}$ ,  $T_3 = 500^\circ\text{C}$  pyrolysis temperature

Analysis of variance (ANOVA) was used to determine significant effects of variables and their interactions on bio-oil and bio-char production. Scheffe's test was employed to determine significant differences between factor levels. All statistical analyses were performed using SPSS software.

### 2.3.4 Preparation of feedstock

Rice straw samples were collected, cleaned, and sun-dried to a moisture content of approximately 10%. The straws were manually chopped to a maximum particle length of 10 mm. Samples were compacted to achieve target experimental densities using a compactor with the same inside diameter as the reactor. Proximate analysis (moisture, ash, volatile matter, fixed carbon) and ultimate analysis (C, H, N, O, S) of the rice straw feedstock should be determined and reported to allow yield contextualization and inter-study comparison; these data are standard inputs for pyrolysis studies and are needed to interpret the mass balance fully.

### 2.3.5 Data gathered

Temperature was measured inside the reactor using K-type thermocouples connected to the Arduino via the Arduino program. Bio-oil (condensable liquid yield) was collected in a container placed below the gas condenser throughout the pyrolysis process and weighed after each run using an analytical balance. Bio-char (solid residue) was removed from the reactor after each run, transferred to a metal container, covered to extinguish embers, cooled for approximately 5 min, and weighed.

### 2.3.6 Material mass balance calculation

The yield of each pyrolysis fraction was calculated using material balance equations [17]:

$$Y_{bo} = [(W_2 - W_1) / W_f] \times 100\% \quad (4)$$

$$Y_{bc} = [(W_4 - W_3) / W_f] \times 100\% \quad (5)$$

$$Y_g = 100\% - (Y_{bo} + Y_{bc}) \quad (6)$$

Where  $Y_{bo}$  = bio-oil yield (%);  $Y_{bc}$  = bio-char yield (%);  $Y_g$  = gas yield (%);  $W_1$  = weight of empty bio-oil container (g);  $W_2$  = weight of container with bio-oil after reaction (g);  $W_3$  = weight of empty bio-char container (g);  $W_4$  = weight of container with bio-char after reaction (g);  $W_f$  = weight of chopped rice straw fed to the reactor (g).

## 2.4 Standardization of the Operating Procedure

Results of preliminary tests and evaluations were analyzed to develop a standardized operating procedure (SOP) for optimum bio-oil production. The SOP covers: preparation of equipment and materials;

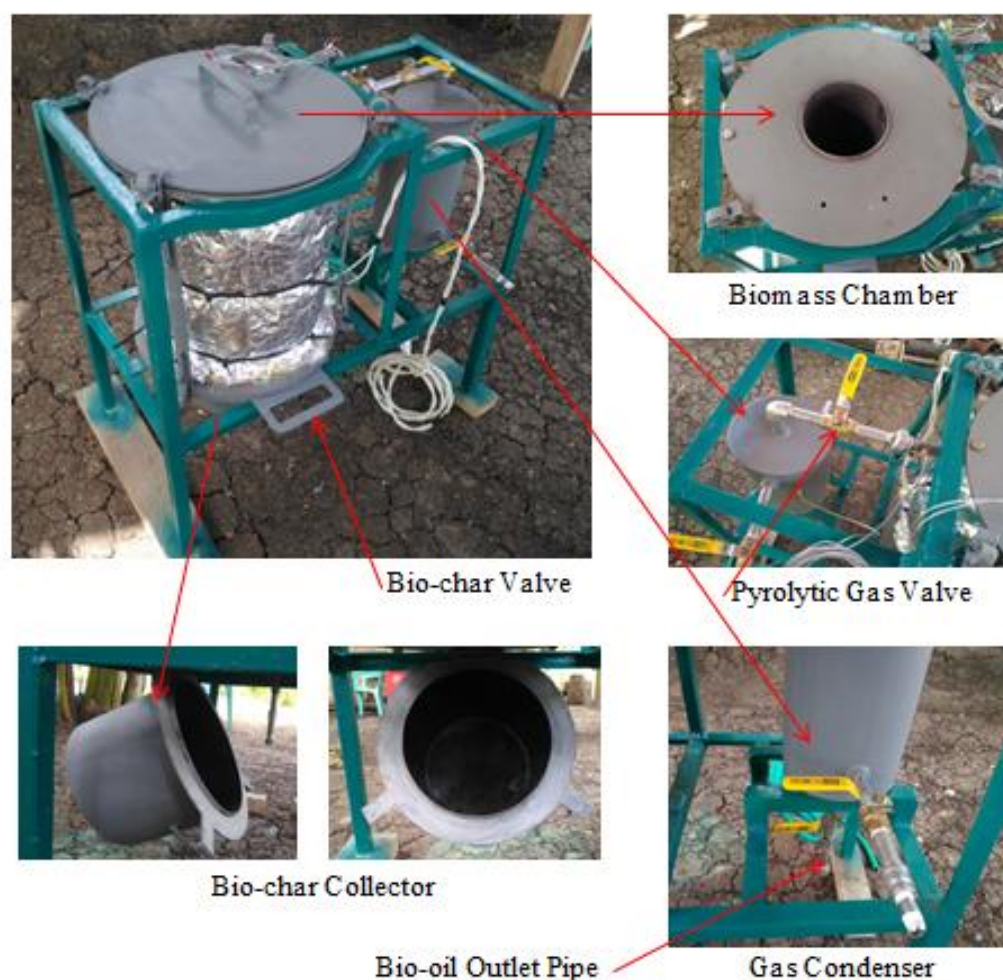


component connections; activation of the temperature controller; pre-heating; temperature monitoring; biomass loading; bio-oil collection; condenser cooling water maintenance; end-of-operation protocol; bio-char collection; and system maintenance for subsequent runs.

### 3. Results and Discussion

#### 3.1 The Laboratory-Scale Pyrolysis Reactor System

The designed pyrolysis reactor system consists of five main components: (1) pyrolysis reactor chamber, (2) electrical heater, (3) pyrolytic gas condenser, (4) Arduino temperature control device, and (5) system metal frame (Figure 1). The reactor vessel was fabricated from a 5-mm-thick block of iron pipe (12 cm diameter, 44 cm length) and mounted on an angle-bar frame for stability and safety. A helical 3-kW electric heating coil was mounted on the outer surface of the reactor chamber, enclosed by a 1-mm sheet-metal casing and insulated with fiberglass wool of variable thickness (25–75 mm). The Arduino-based temperature controller maintained stable pyrolysis temperatures by automatically switching an ON/OFF relay in response to signals from K-type thermocouple sensors.

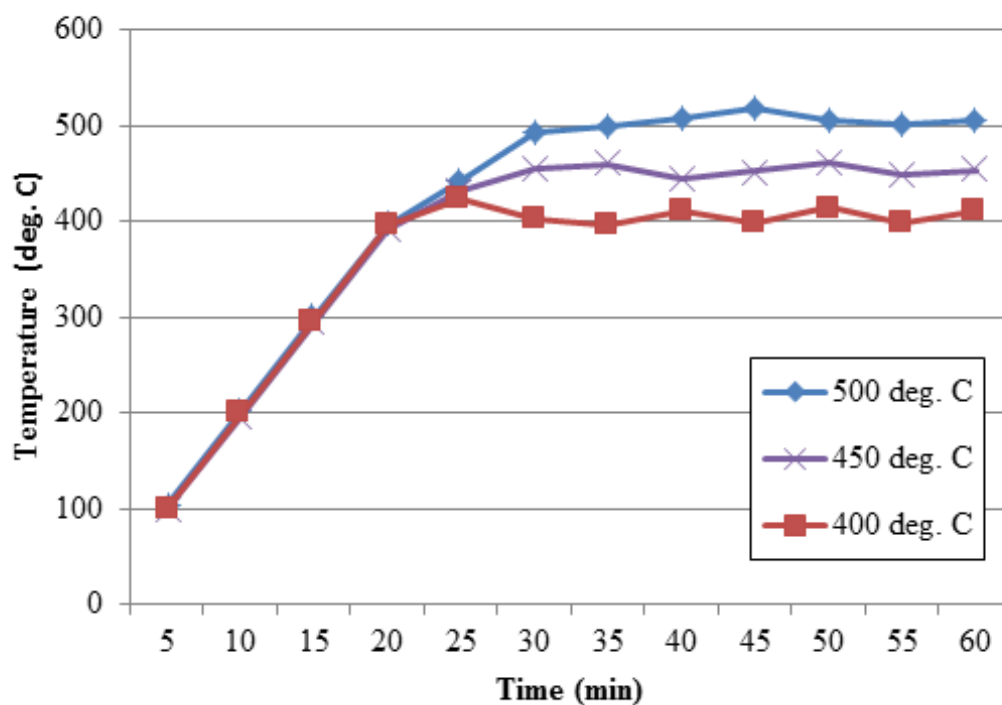


**Figure 1.** Perspective drawing of the pyrolysis reactor system components.

#### 3.2 Reactor Integrity and Operational Stability

Temperature stability was demonstrated by steady temperature profiles at all set points (Figure 2). Pyrolysis temperature steady-state conditions were reached on average in 20, 25, and 30 min after setting the temperature to 400°C, 450°C, and 500°C, respectively, upon activating the Arduino controller. The steady-state condition was maintained for at least 30 min, sufficient for complete rice straw pyrolysis. Loading the reactor with biomass did not disrupt the steady-state temperature, demonstrating the effective operation of the

temperature control device. The maintained functional condition through more than 5 hours of continuous operation confirmed the integrity of the pyrolysis system.



**Figure 2.** Temperature profiles of the pyrolysis reactor at 400°C, 450°C, and 500°C set points.

### 3.3 Bio-oil Production

Analysis of variance on bio-oil production showed that biomass loading density (D) had a highly significant effect ( $p < 0.010$ ), pyrolysis temperature (T) had a significant effect ( $p = 0.026$ ), and insulation thickness (H) had a highly significant effect ( $p < 0.010$ ) on bio-oil production (Table 1). In contrast, none of the two-way or three-way interaction terms was significant, indicating that each factor acted independently in influencing bio-oil production.

**Table 1.** Split-plot ANOVA in a completely randomized design on bio-oil production from pyrolysis experiments.

| Source of Variation | Factor          | df | Sum of Squares | Mean Square | F comp. | p-value |
|---------------------|-----------------|----|----------------|-------------|---------|---------|
| Main Plot           | Insulation (H)  | 2  | 4152.765       | 2076.383    | 15.652  | 0.000   |
|                     | Main plot error | 6  | 1294.444       | 215.741     | 1.626   | 0.160   |
| Sub-plot            | Density (D)     | 2  | 18723.877      | 9361.938    | 70.572  | 0.000   |
|                     | Temperature (T) | 2  | 1050.395       | 525.198     | 3.959   | 0.026   |
|                     | H × D           | 4  | 1049.901       | 262.475     | 1.979   | 0.113   |
|                     | H × T           | 4  | 198.272        | 49.568      | 0.374   | 0.826   |
|                     | D × T           | 4  | 1155.605       | 288.901     | 2.178   | 0.086   |
|                     | H × D × T       | 8  | 1498.617       | 187.327     | 1.412   | 0.216   |
|                     | Sub-plot error  | 48 | 6367.556       | 132.657     | —       | —       |

Further analysis using Scheffe's test (Table 2) showed that 25 mm insulation produced significantly higher bio-oil (78.07 g) than 50 mm (65.26 g) or 75 mm (61.30 g) insulation, though no significant difference existed between the latter two. This counterintuitive result — where thinner insulation yields more bio-oil despite higher expected heat loss — may be explained by the interaction between insulation thickness and the

Arduino-based ON/OFF relay controller. With thinner insulation, the heating element cycles more frequently to maintain the set temperature, resulting in a faster effective heating rate and shorter residence time in the intermediate temperature zone (250–350°C) where secondary char-forming reactions predominate. Conversely, thicker insulation may cause transient temperature overshoots during heater-ON cycles that briefly exceed the set point, promoting secondary cracking of condensable vapors into non-condensable gases and thereby reducing liquid yield. Additionally, moisture retained within thicker fiberglass wool at elevated temperatures may affect local vapor condensation dynamics. The significant interaction between insulation thickness and pyrolysis temperature observed in bio-char production (Table 3) further supports the conclusion that insulation effects are thermally mediated and not reducible to simple heat-loss reduction. For loading density, 90 kg/m<sup>3</sup> (76.74 g) produced significantly more bio-oil than 60 kg/m<sup>3</sup> (46.85 g), but was not significantly different from 120 kg/m<sup>3</sup> (81.04 g). This finding supports the observation of Weerachanchai et al. [18] that denser packing of smaller biomass particles impedes sweeping gas flow, ultimately reducing liquid fuel production. For the pyrolysis temperature, a significant difference was found only between 400°C (65.11 g) and 450°C (73.26 g). Production at 500°C (66.26 g) was intermediate and not significantly different from either extreme, likely because higher temperatures favor greater gas formation at the expense of bio-oil. The non-condensable gas produced was highly flammable, with characteristics similar to syngas [19].

**Table 2.** Scheffe's test of significance on mean bio-oil production (g) by factor level.

| Factor                      | Level                                                     | N  | Mean (g) |
|-----------------------------|-----------------------------------------------------------|----|----------|
| Biomass Loading Density (D) | D <sub>1</sub> = 60 kg/m <sup>3</sup> (300 g rice straw)  | 27 | 46.85 a  |
|                             | D <sub>2</sub> = 90 kg/m <sup>3</sup> (450 g rice straw)  | 27 | 76.74 b  |
|                             | D <sub>3</sub> = 120 kg/m <sup>3</sup> (600 g rice straw) | 27 | 81.04 b  |
| Pyrolysis Temperature (T)   | T <sub>1</sub> = 400°C                                    | 27 | 65.11 a  |
|                             | T <sub>2</sub> = 450°C                                    | 27 | 73.26 b  |
|                             | T <sub>3</sub> = 500°C                                    | 27 | 66.26 ab |
| Insulation Thickness (H)    | H <sub>1</sub> = 25 mm                                    | 27 | 78.07 a  |
|                             | H <sub>2</sub> = 50 mm                                    | 27 | 65.26 b  |
|                             | H <sub>3</sub> = 75 mm                                    | 27 | 61.30 b  |

Means within a factor followed by the same letter are not significantly different at  $\alpha = 0.05$ .

### 3.4 Biochar Production

ANOVA on bio-char production showed highly significant effects ( $p < 0.010$ ) for all three factors: loading density, pyrolysis temperature, and insulation thickness (Table 3). Significant two-way interactions were found between insulation thickness and pyrolysis temperature ( $p = 0.050$ ) and between loading density and pyrolysis temperature ( $p = 0.019$ ). The three-way interaction was also significant ( $p = 0.013$ ), indicating that the combined influence of factors affected biochar production.

**Table 3.** Split-plot ANOVA in a completely randomized design on bio-char production from pyrolysis experiments.

| Source of Variation | Factor          | df | Sum of Squares | Mean Square | F comp. | p-value |
|---------------------|-----------------|----|----------------|-------------|---------|---------|
| Main Plot           | Insulation (H)  | 2  | 4729.580       | 2364.790    | 8.312   | 0.001   |
|                     | Main plot error | 6  | 1330.000       | 221.667     | 0.779   | 0.590   |
| Sub-plot            | Density (D)     | 2  | 203755.728     | 101877.864  | 358.077 | 0.000   |
|                     | Temperature (T) | 2  | 3391.580       | 1695.790    | 5.960   | 0.005   |
|                     | H × D           | 4  | 2540.272       | 635.068     | 2.282   | 0.079   |
|                     | H × T           | 4  | 2927.309       | 731.827     | 2.572   | 0.050   |
|                     | D × T           | 4  | 3720.272       | 930.068     | 3.269   | 0.019   |
|                     | H × D × T       | 8  | 6311.728       | 788.966     | 2.773   | 0.013   |
|                     | Sub-plot error  | 48 | 13656.667      | 284.514     | —       | —       |

Scheffe's test results (Table 4) showed a continuous, significant increase in bio-char production across all loading density levels, unlike bio-oil, which plateaued at D<sub>2</sub>. This is consistent with the finding of Weerachanchai et al. [18] that denser biomass packing impedes sweeping gas flow, yielding higher bio-char. For pyrolysis temperature, significantly more bio-char was produced at 400°C (180.19 g) than at 500°C (164.81 g), indicating that higher temperatures direct more biomass toward gas formation rather than solid char. For insulation thickness, H<sub>2</sub> (50 mm, 161.04 g) produced significantly less bio-char than H<sub>1</sub> (179.26 g) or H<sub>3</sub> (173.85 g); this unexpected result may be attributed to the significant interaction between insulation thickness and pyrolysis temperature. The biochar produced in this study represents a potentially valuable co-product: biochar derived from thermochemical conversion of biomass has demonstrated benefits as a soil amendment, improving soil nutrient availability and supporting plant growth when applied at optimized rates [20].

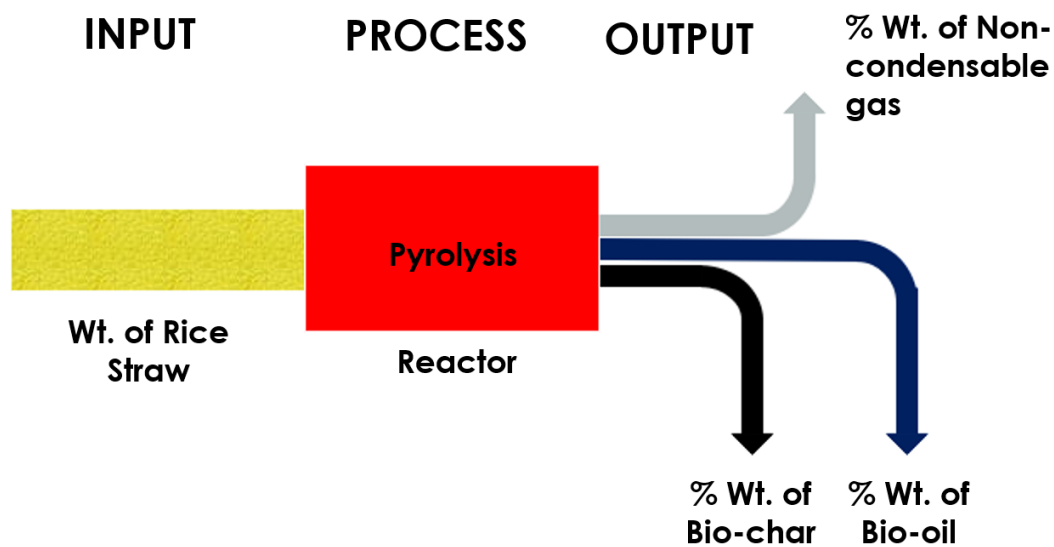
**Table 4.** Scheffe's test of significance on mean bio-char production (g) by factor level.

| Factor                      | Level                                                     | N  | Mean (g)  |
|-----------------------------|-----------------------------------------------------------|----|-----------|
| Biomass Loading Density (D) | D <sub>1</sub> = 60 kg/m <sup>3</sup> (300 g rice straw)  | 27 | 110.15 a  |
|                             | D <sub>2</sub> = 90 kg/m <sup>3</sup> (450 g rice straw)  | 27 | 171.00 b  |
|                             | D <sub>3</sub> = 120 kg/m <sup>3</sup> (600 g rice straw) | 27 | 233.00 c  |
| Pyrolysis Temperature (T)   | T <sub>1</sub> = 400°C                                    | 27 | 180.19 a  |
|                             | T <sub>2</sub> = 450°C                                    | 27 | 169.15 ab |
|                             | T <sub>3</sub> = 500°C                                    | 27 | 164.81 b  |
| Insulation Thickness (H)    | H <sub>1</sub> = 25 mm                                    | 27 | 179.26 a  |
|                             | H <sub>2</sub> = 50 mm                                    | 27 | 161.04 b  |
|                             | H <sub>3</sub> = 75 mm                                    | 27 | 173.85 a  |

Means within a factor followed by the same letter are not significantly different at  $\alpha = 0.05$ .

### 3.5 Material Mass Balance

The material mass balance showed that bio-oil yield was consistently lower than that of bio-char and syngas across all factor combinations (Figure 3). As the reaction temperature increased from 400°C to 500°C, gas yield increased, consistent with progressive thermal cracking of both primary volatile products and bio-char. The optimum temperature of 450°C produced the highest bio-oil yield (16.6%), and the optimum loading density of 90 kg/m<sup>3</sup> produced the highest bio-oil yield (17.1%). These values are substantially lower than the 28.4% reported by Kumar et al. [21] for similar agricultural biomass under comparable conditions. Two factors likely contribute to this difference. First, the present reactor operated under slow pyrolysis conditions (temperature range 400–500°C, batch-fed, 20–30 min residence time), which characteristically favors char and gas formation over liquid yield; fast pyrolysis systems optimized for bio-oil production typically achieve liquid yields of 40–60% through very high heating rates and short vapor residence times [16]. Second, condenser efficiency is a limiting factor: the tube-in-shell condenser used in this study (150 × 300 mm body, 12-mm central pipe, cooling water at 10–20°C) likely allowed a fraction of condensable vapors to escape uncollected, as no quantitative assessment of condensation efficiency was conducted. It should also be acknowledged that calculating gas yield by difference (Equation 6) accumulates measurement errors from bio-oil and bio-char weighing; direct gas measurement by gas chromatography in future studies would improve mass balance accuracy. The present reactor design is therefore more appropriate for slow pyrolysis applications and bio-char production studies than for optimizing bio-oil yield, and future design iterations should incorporate a higher-capacity condenser with quantified heat transfer area and cooling water flow rate.



**Figure 3.** Material mass balance input-process-output diagram for the pyrolysis reactor system.

### 3.6 Standardization of the Pyrolysis Operation

Based on a series of pyrolysis tests, evaluations, and experimental trials, a step-by-step SOP was drafted for optimum use of the developed laboratory-scale pyrolysis system. The SOP covers all operational phases: preparation of equipment and materials; component connections; activation of the temperature controller; pre-heating; temperature monitoring; biomass loading; bio-oil collection; maintenance of condenser cooling water; end-of-operation protocol; bio-char unloading and collection; and system maintenance for subsequent runs. Adherence to the SOP ensures consistent and reproducible pyrolysis results.

## 4. Conclusions

The developed laboratory-scale batch-fed pyrolysis reactor system is a viable tool for pyrolysis of rice straw and biomass with similar physical characteristics. The reactor demonstrated temperature stability and operational integrity at pyrolysis temperatures of 400°C–500°C. Among the experimental conditions evaluated, 25 mm reactor insulation thickness, 450°C pyrolysis temperature, and 90 kg/m<sup>3</sup> loading density produced the optimum bio-oil yield. All three factors independently influenced bio-oil and bio-char production, while their interactions were generally not significant for bio-oil. The established standard operating procedure provides an effective guide for using the system to pyrolyze various biomass types and optimize pyrolysis end products. The bio-oil extracted, together with the flammable non-condensable syngas co-product, represents a potential renewable energy resource. Further experiments using other biomass feedstocks and improved condenser designs are recommended to increase bio-oil recovery efficiency.

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3.1. Subsection  
3.1.1. Subsubsection

3.2. Figures, Tables, and Schemes

All figures and tables should be cited in the main text as Figure 1, Table 1, etc.



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| entry 2 | data    | data <sup>1</sup> |

<sup>1</sup> Table may have a footer.

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$$a = 1, \tag{1}$$

The text following an equation need not be a new paragraph. Please punctuate equations as regular text. This is example 2 of an equation:

$$a = b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u \tag{2}$$

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Concisely restate the hypothesis and most important findings. Summarize the significant findings, contributions to existing knowledge, and limitations. What are the future directions? Conclusions MUST be well stated, linked to original research question & limited to supporting results.

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