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Research article

Growth and Yield Response of Lettuce (*Lactuca sativa*) Using MykoPlus and Bio-N Biofertilizers

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Abstract: This study was conducted to determine the growth and yield response of lettuce (*Lactuca sativa)*, variety 'Black Simpson', using different amounts of MykoPlus and Bio-N biofertilizer. The experiment consisted of seven treatments: T1 (control), T2 (MykoPlus), T3 (Bio-N), T4 (50% MykoPlus and Bio-N), T5 (MykoPlus + ½ Recommended Rate or RR of synthetic fertilizer), T6 (Bio-N + ½ RR of synthetic fertilizer), and T7 (50% MykoPlus and Bio-N + ½ RR of synthetic fertilizer), arranged in a Randomized Complete Block Design (RCBD) from July to September 2022. Results revealed that T3 (Bio-N), T5 (MykoPlus + ½ RR of synthetic fertilizer), and T6 (Bio-N + ½ RR of synthetic fertilizer) had the best initial plant height response; however, in the final plant height gathered, T3, T4, T6, and T7 gave the best response. In terms of the initial leaf length and leaf width, T5 and T6 had the best responses and were comparable to T3, T4, and T7. In the final leaf length and width of lettuce, T6 and T7 showed the best results and were comparable with T2, T3, T4, and T5. No significant difference was observed in the number of leaves. On the yield parameter, T7 (50% MykoPlus and Bio-N + ½ RR of synthetic fertilizer) provided the highest weight of lettuce at harvest, comparable with T2, T3, T4, T5, and T6. The most feasible biofertilizer combination suitable for lettuce production in terms of growth parameters was T6 and T7, whereas in the yield parameter, application of T7 was recommended.

Keywords: Biofertilizer; Bio-N; MykoPlus; lettuce

1. Introduction

Lettuce (*Lactuca sativa*) is one of the high-value commercial crops in the country and a popular salad vegetable. Moreover, it is an ingredient for sandwiches, soups, and is used as a wrapper for food [1]. The leaves are a very low-calorie vegetable. Ausveg [2] stated that it is the seventh most popular fresh vegetable. It is also included as one of the most popular vegetables consumed globally. Nevertheless, its nutritional worth has been undervalued. It is low in fat, calories, and sodium, and a good source of iron, folate, fiber, and vitamin C [3]. Moreover, it is considered one of the healthy vegetables that has many benefits for the body. Nowadays, increasing agricultural production is the primary goal for the growing demand and necessity. Fertilizers play an important role in the crop's quality and quantity. Inorganic fertilizers are accessible to plants, providing essential nutrients in the form of nitrogen, phosphorus, and potassium. Crop production needs to be enhanced manifold due to the increase in population. Therefore, without taking into account the health of the soil or people, farmers are heavily using chemicals (pesticides and fertilizers) to maximize agricultural productivity. The traditional use of chemical fertilizers and pesticides harms the resident microflora, soil texture, and productivity, as well as human health, when used to boost agricultural output by nutrient enrichment or pathogen growth prevention [4]. Furthermore, the usage of synthetic fertilizer might kill beneficial microorganisms in the soil and increase the nitrate levels of the soil.

With the government's support of bioorganic farming and the active participation of research institutes, private persons, and businesses, the Philippines' biofertilizer sector has risen significantly. In this context, biofertilizers are used as an alternative to chemical fertilizers. They serve as a promising resource in agricultural ecosystems, offering a renewable, supplementary, and eco-friendly source of plant nutrients. They can convert essential elements from non-usable to highly absorbable forms without harming the natural environment [5]. Biofertilizers are beneficial to the soil since they help to restore its fertility. They can benefit both the environment and plants. The use of biofertilizers is widely adopted these days around the world due to the growing problems in the environment and concerns for food safety. In addition, biofertilizers are microbial inoculants, which are defined as preparations that contain live or dormant cells of efficient strains of microorganisms [4]. Microbiological inoculant, several types of microorganisms found in MykoPlus, a biological fertilizer product, aid plant roots in obtaining water and other nutrients from the soil, improving plant development, yield, and farmer income. It replaces up to 30% of the crop's chemical N, P, and K requirements while resulting in a 25-45% increase in crop yield [6]. Furthermore, MykoPlus consists of endomycorrhizal fungi, also known as arbuscular mycorrhizal fungi, or AMF, which are extremely beneficial to the environment, as they dramatically assist in improving plant health and maximizing the absorption of nutrients [7]. Arbuscular mycorrhizal fungi are natural biofertilizers, as they provide the host with nutrients, pathogen protection, and water, in exchange for photosynthetic products [8]. MykoPlus provides natural helpers to the plant's roots in the form of beneficial microorganisms. These helpers provide more phosphorus (P) from locked sources, greater access to free nitrogen (N) from the air, and microbially secreted substances. Through better uptake of water and nutrients, farmers save on chemical fertilizers, and soil becomes less acidic and more friable [6].

One of the biofertilizer products is Bio-N, which contains bacteria isolated from the roots of talahib (*Saccharum spontaneum* L.) grass. This bacterium is able to convert atmospheric nitrogen into a form usable by plants. The bacteria enhance growth, root development, and yield of the crops [9]. The beneficial effects of Bio-N include enhancing root development and shoot growth, and making plants resistant to pest attack and drought. It was observed to provide approximately 30%-50% of the nitrogen required by crops [10]. It is an inoculant for vegetables such as lettuce and pechay. Moreover, Bio-N, which contains *Azospirillum*, is considered the safest bacterium that can be used as a biofertilizer at a commercial level for several crops that are of economic importance for the whole world [11]. It can have a good impact on plant growth, agricultural yields, and N-content. Lettuce plants thrive in nutrient-rich soil. They are considered heavy feeder plants, along with kale, parsley, and collard. Their rapid growth leads to a high demand for nitrogen (N) fertilization to maintain adequate plant-available N concentrations in the soil over a short growing period. Nitrogen is considered the most limiting factor in crop production, and it receives the highest mark of all the major nutrients from inorganic sources applied by farmers [10]. Lettuce also requires a heavy feeding plant and additional nitrogen. It also favors slightly acidic soil.

One benefit of inorganic fertilizers is that they act quickly; however, they are not entirely composed of the nutrients needed by the plants. They also contain salts and other compounds that are not absorbed by the plants and are left behind in the soil, building up over time. According to Malone [12], inorganic fertilizers tend to lower the soil pH, making it more acidic. The plants tolerate different levels of soil pH, and some plants will do well in acidic soils. Furthermore, MykoPlus and Bio-N have favorable impacts on soil and plants, and are used to provide sustenance to crops directly. Hence, biofertilizers are recommended as an alternative or supplement for mineral nutrients. The purpose of this study is to investigate the growth response of lettuce utilizing biofertilizers such as MykoPlus and Bio-N, to evaluate the appropriate combination that supplies the correct ratio of plant nutrients.

2. Materials and Methods

2.1 Treatment and Experimental Design

The study was laid out in a Randomized Complete Block Design (RCBD). The treatments consisted of applying various levels of biofertilizers on lettuce. MykoPlus and Bio-N were employed as biofertilizers, and the specific treatments, which utilized these as growth enhancers, were:

Treatment 1 - Control (No fertilizers applied)
Treatment 2 – MykoPlus
Treatment 3 - Bio-N
Treatment 4 – 50% MykoPlus and Bio-N
Treatment 5 – MykoPlus plus 1/2 RR (side-dressing application throughout the growing season) farmer's practice
Treatment 6 - Bio-N plus 1/2 RR (side-dressing application throughout the growing season) farmer's practice
Treatment 7 - 50% MykoPlus and Bio-N plus 1/2 RR (side-dressing application throughout the growing season) farmer's practice

2.2 Application of Treatments

MykoPlus treatments were applied as a powder in a soil hole immediately before transplanting the lettuce. Bio-N treatments were applied as a solution by watering the plants, beginning on the day of transplanting and repeated every two weeks thereafter, according to the specific treatment ratio. Side-dressing applications began at 3 days after transplanting (DAT). Subsequent applications were performed every two weeks, starting 21 days after the first application. Treatment 1 (control): received no fertilizer. Treatment 2 received 5 grams of 100% Mykoplus before planting. Treatment 3 received 167 ml/plant of 100% of Bio-N every two weeks. Treatment 4 received 50% of both MykoPlus and Bio-N. Treatment 5 received MykoPlus plus side dressing (fertigation) with 70 ml per plant. Treatment 6 received Bio-N plus side dressing (fertigation) with 70 ml per plant. Treatment 7 received 50% of both MykoPlus and Bio-N plus side dressing (fertigation) with 70 ml per plant (Table 1).

Table 1. Total amount applied on treated plants

Treatments	MykoPlus	Bio-N	Urea Side Dressing as Fertigation
1	None	None	None
2	5 g/plant	None	None
3	None	167 ml/plant	None
4	2.5 g/plant	83 ml/plant	None
5	5 g/plant	None	70 ml/plant
6	None	167 ml/plant	70 ml/plant
7	2.5 g/plant	83 ml/plant	70 ml/plant

2.3 Installation of Protective Component

This study was conducted in a small greenhouse measuring 250 cm in length, 120 cm in width, and 180 cm in height. The front wall featured a 100 cm wide door. The roofing material consisted of a black shading net, and the framing material was bamboo.

2.4 Seedling Production

Lettuce seeds were sown in the seed boxes filled with a sterilized mixture of garden soil and vermicast at a 1:1 ratio. As part of the treatment, the seeds were pre-treated with their respective biofertilizers. The seed boxes were placed under the protective component with a shading net to protect the seedlings from direct sunlight and the impact of the rain. Seedlings were pricked out to modular seedling trays once they reached the first two true leaves. To harden the seedlings, they were subjected to gradual sunlight exposure one week before transplanting.

2.5 Pot Preparation

The soil used was first sterilized with hot water for five minutes in buckets. This was done to eradicate weed seeds, harmful organisms, and pathogens from the mineral soil. After sterilization, the soil was filled into pots measuring 8 inches in breadth, 4 inches in height, and 4 inches in width.

2.6 Transplanting

Seedlings were transplanted 13 days after sowing (DAS), in the late afternoon. A spacing of 15 cm between columns and 5 cm between rows was maintained during planting. Only healthy seedlings were used as the planting material.

2.7 Care Management and Harvesting

Watering was done as needed, based on the soil moisture content, to avoid disease infestation caused by microorganisms in extremely wet soil. Weeds were removed as soon as they appeared. Insects were controlled by hand-picking daily to ensure the lettuce foliage was not damaged. The lettuce was harvested pot by pot 35 days after sowing. Harvesting was accomplished by slowly and carefully uprooting the plants by hand.

2.8 Data gathered

The following parameters were used for the evaluation of lettuce:

- Plant height (cm) - This was obtained by using a ruler to measure the initial height (one week after transplanting) and the final height (during harvest), measuring from the ground level up to the tip of the main stem.
- Leaf length (cm) - This was determined by getting the initial and final length of the leaves from the base of the leaf blade to the topmost section of the leaf. Measurements were taken one week after transplanting and during harvest.
- Leaf width (cm) - This was determined by measuring the width of leaves, initial and final, across the widest section of the leaf blade. Measurements were determined one week after transplanting and during harvest.
- Number of leaves per plant - This was determined by counting the number of leaves during harvest.
- Yield of lettuce (g) - This was determined by measuring the fresh weight of lettuce after harvest.

2.9 Statistical Analysis

Analysis of variance (ANOVA) for the Randomized Complete Block Design was used for data analysis. Comparison of means was done using Least Significant Difference (LSD) at **the** 5% level of significance. All statistical analysis was performed using Statistical Tool for Agricultural Research (STAR) (version 2.0.1).

3. Results and Discussion

3.1 Initial Plant Height

The initial plant height (cm) was influenced by different application rates of the MykoPlus and Bio-N biofertilizers. The initial plant height of T3 was comparable to the means recorded for T5, T6, T4, T7, and T2. As shown in Figure 1, T3 gained the highest initial plant height (5.20 cm), followed by T5 (5.10 cm), T6 (4.97 cm), and T4 and T7, which recorded the same height (4.83 cm). T2 recorded 3.20 cm. The shortest plant height (2.67 cm) was observed in the T1 (control).

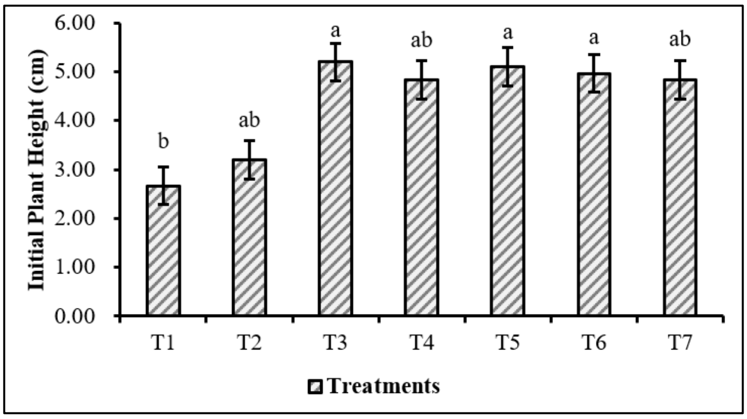

Figure 1. Initial plant height (cm) response from using MykoPlus and Bio-N biofertilizers.

The Analysis of Variance (ANOVA) revealed significant differences among treatments, resulting in the formation of three distinct groups. Group 1 consisted of T3, T5, and T6, which were found to be statistically comparable. Group 2 comprised T2, T4, and T7, which were also comparable to each other. The final group was T1 (control), which had the shortest plant height and was comparable only to the treatments in group 2 (T2, T4, and T7).

T3 (Bio-N) recorded the highest initial plant height mean of 5.20 cm. This superiority aligns with findings by Agapito and Mercado [13], who note that Bio-N, when applied as a root dip or seed inoculation, can supply 50% of the nitrogen requirements for high-value crops. In this study, Bio-N was applied both as a seed pre-treatment and as a solution for watering the plants. Bio-N's effectiveness is largely due to its component, *Azospirillum,* an associative nitrogen-fixing, aerobic bacterium. *Azospirillum* colonizes the root system of many vegetable plants, assisting the crops in better vegetative growth [14]. Furthermore, Bio-N produces plant growth promoters, including gibberellins and auxins [15]. Auxins, in particular, positively influence gibberellins, which stimulate cell elongation and consequently increase plant length [16]. Treatment 6 (T6) was statistically comparable to T3 and T5. T6, which combined Bio-N with a half rate of synthetic fertilizer, had a positive impact on initial plant height. This positive effect is likely due to the presence of *Azospirillum* in Bio-N, which produces growth promoters like auxins and gibberellins. Furthermore, the inclusion of nitrogen in T5 and T6 (via ½ Recommended Rate of synthetic fertilizer) is significant. Nitrogen fertilizer is known to ensure favorable conditions for the elongation and optimum vegetative growth of lettuce plants [17]. The findings suggest that the combination (T5 and T6) influenced the initial plant height just as effectively as the sole application of Bio-N (T3).

3.2 Final Plant Height

The final plant height (cm) was influenced by different rates of MykoPlus and Bio-N biofertilizers. The final plant height result for T3 was comparable to the plants in T4, T6, T7, T5, and T2. The highest plant height recorded during harvest was T7 at 22.83 cm, followed by T4 at 22.77 cm, T6 at 22.27 cm, T3 at 22.00 cm, T5 at 21.00 cm, and T2 at 20.40 cm. Among all the treatments, T1 had the lowest mean at 17.47 cm (Figure 2).

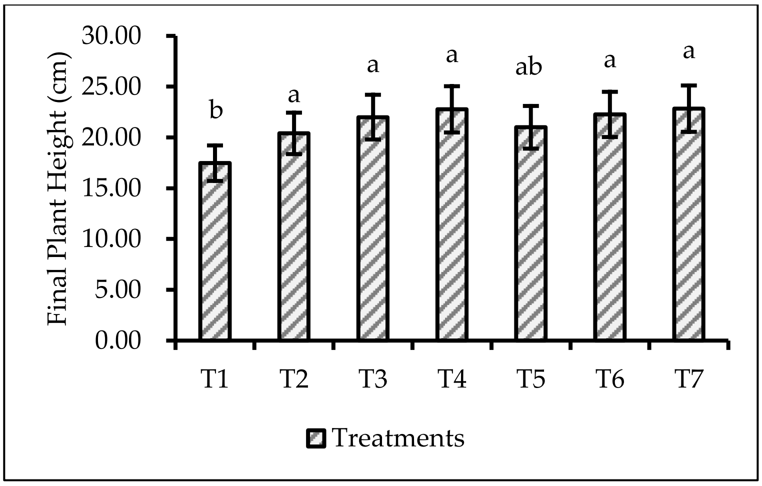

Figure 2. Final plant height (cm) response from using MykoPlus and Bio-N biofertilizers.

Plant height was significantly influenced by the different treatments applied. The analysis of variance showed significant differences among treatments. T3, T4, T6, and T7 resulted in the tallest plants, while T1 resulted in the shortest plants. The differences among T1, T2, and T5 were not statistically significant.

T2 and T5 were comparable to each other and influenced the height of the plant samples. As shown in Figure 2, T7 had the highest result for final plant height at 22.83 cm. The T7 plants were treated with 50% MykoPlus, Bio-N, plus ½ the Recommended Rate (RR) of synthetic fertilizer. In the study by Opeña and Sotto [18], MykoPlus increased root and shoot growth, which may be due to the beneficial effects of growth hormone secretors, mycorrhizal fungi, and nitrogen fixers contained in the biofertilizer. It also contains phosphorus solubilizers, which increase plant phosphorus (P) uptake, an important factor in stem elongation. Additionally, Bio-N produces plant growth promoters that result in increases in plant height. Synthetic fertilizer was also applied, which promotes better vegetative growth. T4 (50% of MykoPlus and Bio-N) and T6 (Bio-N plus 1/2 RR of synthetic fertilizer) gained 22.77 cm and 22.27 cm, respectively, indicating a positive impact on plant height. The combination of both biofertilizers, or solely Bio-N with synthetic fertilizer added, was evident in improving the plant height of lettuce. As part of the comparable treatments, T3 (Bio-N) gained 22.00 cm, and this study revealed that Bio-N can solely influence the height of lettuce up to harvest. The combination of both biofertilizers, with or without synthetic fertilizer, in this study proved to significantly increase the height of lettuce up to harvest.

3.3 Initial Leaf Length

The initial leaf length (cm) was influenced by different rates of MykoPlus and Bio-N biofertilizers. The analysis of variance result revealed that plants in T5 and T6 significantly performed better than T1 and T2 in terms of initial leaf length. The effectiveness of T3, T4, and T7 was not statistically distinguishable from either T5 and T6 or T1 and T2. The highest leaf length (3.00 cm) was gained from T5 and T6, followed by T3 (2.53 cm), T7 (2.43 cm), and T4 (2.33 cm). Furthermore, T2 (1.63 cm) and T1 (1.43 cm) were comparable and recorded the least leaf length (Figure 3).

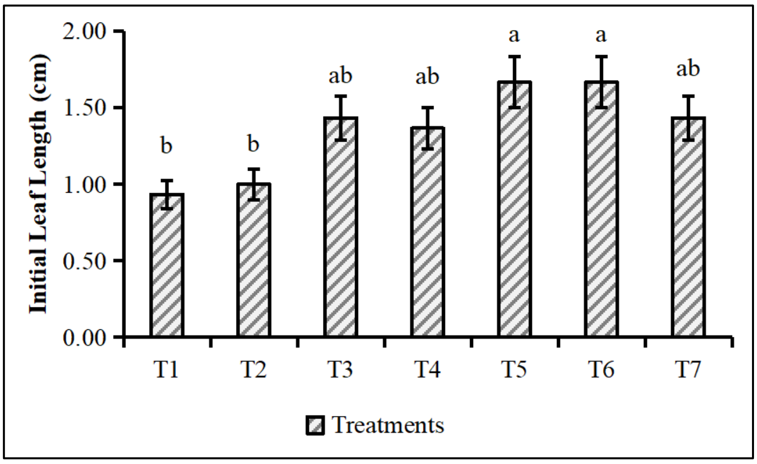

Figure 3. Initial leaf length (cm) response from using MykoPlus and Bio-N biofertilizers.

T5 and T6 were comparable and had the longest leaf length. The plants treated with T5 and T6 gained 3.00 cm in leaf length. This is because T5 has MykoPlus, which contains endomycorrhizal fungi that establish intercellular attachment with plants between the cell wall and cell membrane. The attachment helps the transfer of water and nutrients from the fungi to the plant. The fungi develop branched structures within the plant's root system. The branched structures increase the surface area of the roots, enabling the plant to gather essential nutrients and much more water [19]. The result of this study revealed that MykoPlus helps to absorb more water from the soil, which influenced the leaf length/size. Moreover, T5 has the application of nitrogen, which helps to improve the chlorophyll in the leaves that influences the leaf size. T6 was applied with Bio-N, which contains *Azospirillum*. Fukami et al. [20] state that the genus *Azospirillum* contains plant growth-promoting bacteria (PGPB). The benefits to plants by inoculating *Azospirillum* have been mainly attributed to its capacity to fix atmospheric nitrogen and its capacity to synthesize phytohormones. Additionally, T6 included the application of synthetic nitrogen fertilizer, which helps the leaf grow and influences the leaf size.

3.4 Final Leaf Length

The final leaf length (cm) was influenced by the different rates of MykoPlus and Bio-N biofertilizers. Figure 4 shows the result, indicating that the leaf length of the plant in T6 was comparable to that of the plants treated with T7. The highest final leaf length recorded was 12.73 cm from T6, followed by T7 with 12.47 cm, T4 with 12.27 cm, T3 with 11.67 cm, T5 with 11.63 cm, and T2 with 11.47 cm. Furthermore, T1 had the least final leaf length at 10.00 cm.

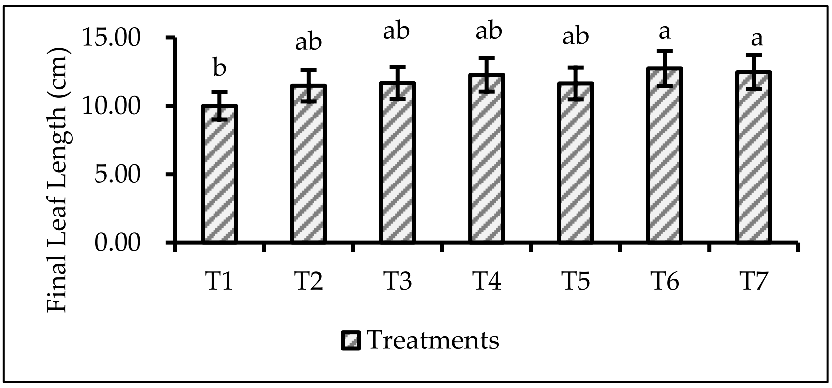

Figure 4. Final leaf length (cm) response from using MykoPlus and Bio-N biofertilizers.

Analysis of variance indicated a significant difference among the treatments in terms of final leaf length. T6 and T7 were statistically similar and demonstrated the greatest positive influence on leaf length. These two treatments were also comparable with T2, T3, T4, and T5. T1 had the shortest final leaf length and was statistically different from other treatments. T6 (Bio-N plus 1/2 RR of synthetic fertilizer) resulted in the longest leaf length (12.73 cm), supporting the initial data gathered. Bio-N was developed by isolating *Azospirillum* from the roots of the talahib grass. This bacterium can transform atmospheric nitrogen (N_2) into a form that plants can utilize [13]. A higher amount of chlorophyll in the leaves allows the plant to photosynthesize more effectively, which, in turn, results in bigger leaves. Additionally, increasing the nitrogen content will improve the chlorophyll concentration in the leaves. Consistent with this, the findings of Chamangasht et al. [21] showed that inoculating seeds with biofertilizers significantly increased the leaf area index compared with the control (no biofertilizer). This study revealed that T6 influences the leaf length up to harvest. T6 was comparable with T7 (50% of MykoPlus and Bio-N plus 1/2 RR of synthetic fertilizer). In T7, the application of 50% of MykoPlus provided an additional biofertilizer component, which contains beneficial bacteria and different species and strains of mycorrhizal fungi. This includes growth hormone secretors, nitrogen fixers, and phosphorus solubilizers [22]. The mycorrhizal fungi, along with the phosphorus-solubilizing microbes, promote plant growth not only through enhanced nutrient uptake but also by generating phytohormones, such as gibberellins, cytokinins, auxins, or polyamides. Wu et al. [23] reportedly found that leaf area is generally determined by the size and number of the cells in the leaf. Cytokinins stimulate cell division and enhance cell growth during the proliferation and expansion stages of leaf cell development. This study proved that the fertilizer combination used in T6 and T7 significantly improved the leaf length of lettuce.

3.5 Initial Leaf Width

The initial leaf width (cm) was influenced by the different rates of MykoPlus and Bio-N biofertilizers. The result for the plants in T5 was comparable to T6. The highest initial leaf width recorded was 1.67 cm, achieved by both T5 and T6. This was followed by T3 and T7 (both at 1.43 cm), T4 (1.37 cm), and T2 (1.00 cm). The least initial leaf width was observed from T1 at 0.93 cm (Figure 5).

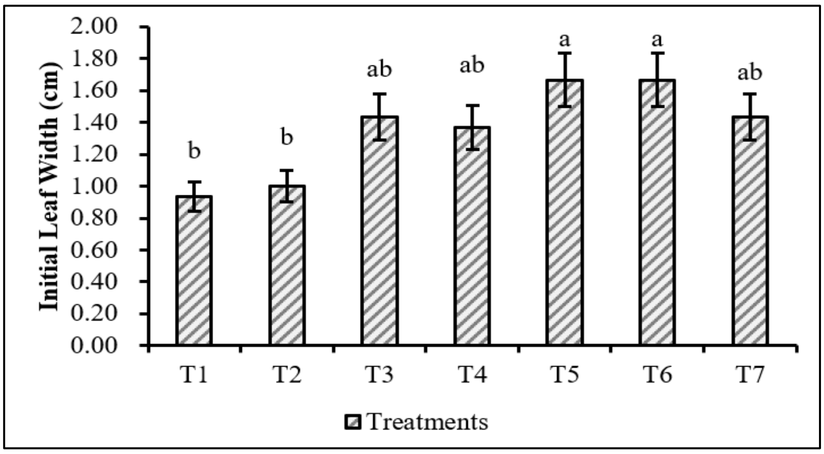

Figure 5. Initial leaf width (cm) response from using MykoPlus and Bio-N biofertilizers.

The analysis of variance showed significant differences among treatments. The result revealed during the first week, T5 and T6 achieved the highest leaf width. Meanwhile, T3, T4, and T7 performed better than T1 and T2. The low leaf width observed in T1 (control) confirms the positive impact of the biofertilizer treatments on early plant vigor. T5 (MykoPlus plus 1/2 RR of synthetic fertilizer) and T6 (Bio-N plus 1/2 RR of synthetic fertilizer) were comparable with each other, with a leaf width of 1.67 cm. This supports the initial leaf length data, where the same treatments also yielded the highest parameters. As stated by Mcrae [24], water availability is a major factor that limits the leaf size. T5's is partly due to the endomycorrhizal fungi found in MykoPlus. These fungi form an intercellular attachment with the plants, which facilitates the transfer of water and nutrients from the fungi to the plant. The branched structures formed within the plant's root system significantly increase the surface area of the roots, allowing the plant to collect essential nutrients and more water [19]. Furthermore, the combination of T5 with synthetic nitrogen fertilizer application also improves chlorophyll concentration in the leaves, which positively influences the leaf size. T6 was comparable to T5 and had a significant influence on leaf width. T6 involved the application of Bio-N combined with synthetic fertilizer. Bio-N contains *Azospirillum,* a plant growth-promoting bacterium that fixes atmospheric nitrogen and makes it available for plant use [20]. This study revealed that the combination of either MykoPlus or Bio-N with synthetic fertilizer has a great impact on the growth, specifically in terms of leaf width.

3.6 Final Leaf Width

The final leaf width (cm) was influenced by different rates of Mykoplus and Bio-N biofertilizers. The results showed that the plant with T6 was comparable to the plants treated with T7. The highest final leaf width was 6.60 cm from T6, followed by T7 with 6.37 cm, T4 with 6.23 cm, T3 with 6.17 cm, T5 with 5.97 cm, and T2 with 5.90 cm. The lowest final leaf width was obtained from plants treated with T1, with 5.17 cm (Figure 6).

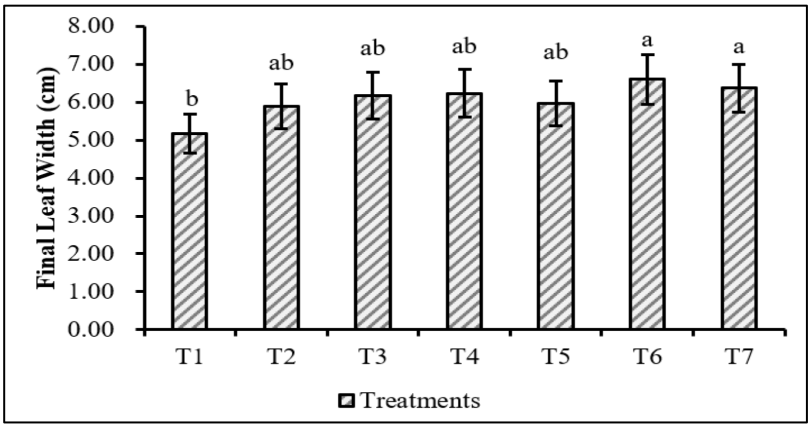

Figure 6. Final leaf width (cm) response from using MykoPlus and Bio-N biofertilizers.

The analysis of variance showed significant differences among treatments. The results indicated that T6 and T7 were comparable with each other, and these treatments influenced the actual leaf width by yielding the highest final leaf width. While T2, T3, T4, and T5 showed a leaf width higher than T1, but statistically failed to demonstrate a significant advantage over T1. The least effective treatment was T1. T6 (Bio-N plus 1/2 RR of synthetic fertilizer) obtained the highest result with 6.60 cm. As stated by Impello [25], fertilizers directly enhance plant growth and development by providing extra nutrients to the plant or the soil. Biofertilizers indirectly enhance plant growth and development by utilizing microorganisms to improve natural processes in the soil that affect plant growth. Bio-N contains native bacteria isolated from the roots of the talahib (*Saccharum spontaneum* L.) grass that can convert atmospheric nitrogen into a form usable by plants [9]. The T7 (50% MykoPlus and Bio-N plus 1/2 RR of synthetic fertilizer) yielded 6.37 cm and was observed in this study to have a positive impact on leaf width. Considering the combination of two biofertilizers used in this study improved the leaf size, the application of synthetic nitrogen fertilizer also plays an important role in leaf growth. Furthermore, increasing nitrogen fertilizer supply promotes leaf elongation and expansion growth by regulating the rate of cell size or cell division [26]. The combination of treatments applied in T7 evidently influences the actual leaf width compared to the non-treated plants.

3.7 Number of Leaves

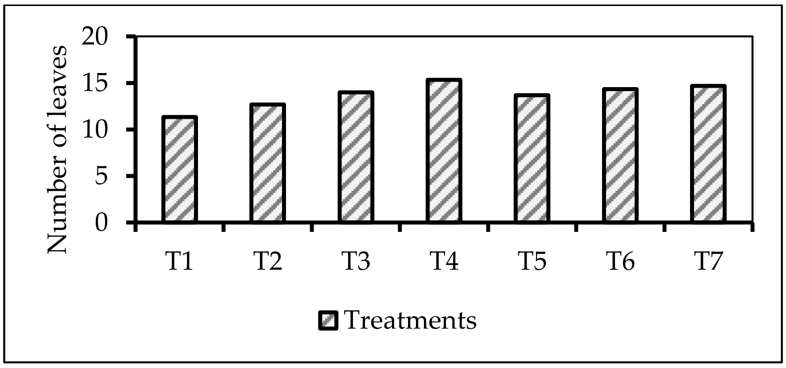

Figure 7. Number of leaves (cm) response from using MykoPlus and Bio-N biofertilizers.

Analysis of variance showed no significant difference among treatments. Application of different rates and combinations of biofertilizer together with synthetic fertilizer did not affect the number of leaves over the control.

3.8 Yield in grams

The yield (g) was influenced by different rates of MykoPlus and Bio-N biofertilizers [Figures 8 and 9]. Additionally, as shown in Figure 9, the highest yield was from T7, which yielded 78.00 g, followed by T4 with 66.67 g, T3 with 50.00 g, T6 with 48.67 g, T5 with 46.33 g, and T2 with 40.00 g. The lowest yield was obtained from plants treated with T1 (33.67 g).

Figure 8. Plant samples per block at harvest.

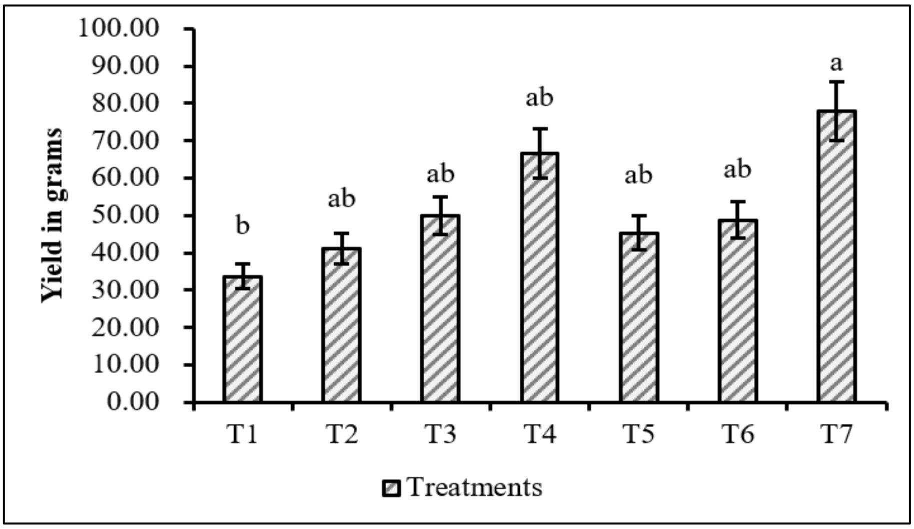

Figure 9. Yield response from using MykoPlus and Bio-N biofertilizers.

The analysis of variance revealed that T7 is the most effective treatment for increasing yield. Although T2, T3, T4, T5, and T6 show numerically higher yields than T1 (control), the statistical analysis indicated that these benefits are not significant enough to be separated from either T1 or T7. T7 (50% MykoPlus and Bio-N plus 1/2 RR of synthetic fertilizer) obtained the highest yield with 78.00 g. The study by Opeña and Sotto [18] revealed that the MykoPlus application significantly increased plant weights. Moreover, Pagcaliwagan [27] stated that farmers using MykoPlus observed that their crops had greater yields. Bio-N contains organisms that are capable of converting nitrogen from the air into ammonium nitrogen in the soil. It enhances root development and shoot growth, can replace 30 to 50% of the total chemical nitrogen needed for crops, and could increase crop yields by 11% [28]. According to Araguas [29], a microbial inoculant, Bio-N boosts nutrient absorption, provides resistance to pests and diseases, improves the plant's root system, and enhances soil conditions. Bio-N users reported improvement in grain quality and an increase in yield. The results of this study show that the use of MykoPlus together with the Bio-N and synthetic fertilizer can increase yield. T7, which had the highest result in the study, was combined with the synthetic fertilizer, specifically urea. The study by Kumar et al. [30] found that the different levels of nitrogen application influenced the total yield of lettuce. The highest yield was achieved with higher doses of nitrogen. As previously stated, in addition to the benefits of MykoPlus and Bio-N, synthetic nitrogen also helped the biofertilizers achieve the obtained yield in T7 by assisting plants with higher vegetative growth.

4. Conclusions

The most effective treatment for lettuce production was T7 (50% MykoPlus and Bio-N plus 1/2 RR of synthetic fertilizer), which is strongly recommended because it achieved the highest yield. Supporting this result, T7 was also identified as one of the top treatments for growth parameters in terms of final plant height, leaf length, and width. The T7 growth parameters contributed to the yield benefits observed. Therefore, the significant increase in yield driven by the T7 application confirms its superiority for application in lettuce production.

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