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RESEARCH ARTICLE

Impact of *Pseudomonas aeruginosa*, white fungus waste, and nano fertilizer on pyrophosphatase activity, growth characteristics, and yield of stevia plant

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Abstract

Aimed to know the effect of *Pseudomonas aeruginosa* bacteria, white fungus waste, and nano fertilizer on the pyrophosphatase enzyme, growth characteristics, and yield of stevia plants. The bacterial vaccine represented the first factor. It was added at two levels: B0 (without inoculum) and B1 (injecting 2 ml of liquid bio-inoculum represented by *P. aeruginosa*). The second factor was adding white fungus waste at three levels defined by Ab0 (without adding white mushroom waste), Ab1 (adding white mushroom waste at level 5 tons/h), and Ab2 (adding white mushroom waste at level 10 tons/h). Nano fertilizer was added as a third factor at four levels: N0 (without adding nano fertilizer), N1 (adding 4 kg/h of nano zinc), N2 (adding 2 kg/h of nano boron), and N3 (adding a mixture of 1 kg/h of nano boron + 2 kg/h of nano zinc). The triple combination B1Ab1N3 achieved a significant superiority in the activity of the pyrophosphatase enzyme in the first and second harvests and recorded (260.67 and 166.00) μg PO_4^{-3}-P/g soil 5/h, respectively, compared to the treatment without addition, which recorded (55.00 and 44.67) μg PO_4^{-3}-P/g soil 5/h, respectively. In contrast, the triple combination B1Ab2N3 achieved the highest growth and yield characteristics of the stevia plant and recorded the highest rate of plant height, dry weight of leaves and total yield, and recorded 83.90 and 76.00 cm/plant, 61.7 and 53.0 g/plant, 4933 and 4240 kg/h, respectively, compared to the treatment without addition, which recorded 68.98 and 60.63 cm/plant, 44.6 and 38.1 g/plant, 3563 and 3050 kg/h respectively.

Keywords: Pyrophosphatase; nano zinc; *P.aeruginosa*; Stevia.

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1. Introduction

Stevia plant has attracted economic and scientific interest due to the sweetness of its leaves and its many nutritional and health benefits. It is a delightful herb and is calories-free, so doctors recommend eating it instead of chemical sweeteners to avoid the damage caused by chemical sugars. Therefore, attention must be paid to growing this plant (Khiraoui et al., 2017). Stevia plant prefers soil that requires a sunny location and a suitable natural semi-tropical and semi-humid climate with temperatures between 21 to 43 °C. Stevia plant cultivation requires constant moist soil that is not flooded with water. High soil moisture can cause plant rot. The plant does not tolerate thirst or salinity of more than 1200 ppm (Ghaheri et al., 2018). Stevia plant cultivation is good in soil with a pH of 6 to 7. This depends on the characteristics of the cultivated type. It is preferable to use drip irrigation with an additional supply of water because Stevia has higher water needs after planting and cutting.

On the other hand, it requires adding organic fertilizers to reduce the pH to become suitable for the plant, so it is better to use the bacterial vaccine along with organic waste so that the bacteria can decompose the organic waste and thus improve the soil properties (Jain et al., 2014; Wagner & et al., 2025), as *Pseudomonas* bacteria can exploit many carbon sources as a source of energy. They favor the oxidase and catalase tests and do not ferment carbohydrates. Still, they consume them through oxidation processes (Virella, 1997; Karpiński et al., 2025), indirectly affecting plant growth by preparing nutrients (Mubarik et al., 2010). Hoseini et al. (2015) found that adding bio-inoculant to sugarcane plants resulted in an increase in vegetative growth, including plant height, stem diameter, leaf area, root length, fresh weight of both vegetative parts and roots, and dry weight of roots, which reached 91.26 cm, 7.14 mm, 15 mm², 18.19 cm, 90.2 g, 43.10 g, and 11.13 g, respectively. The control treatment recorded a significant decrease in the same traits, reaching 90.38 cm, 6.87 mm, 12.83 mm², 15.60 cm, 75.1 g, 33.92 g, and 8.05 g, respectively. Earanna (2007) found that adding bio-inoculant to sugarcane plants increased plant height, number of leaves per plant, dry weight of shoot, and dry weight of root system, which reached 44.53 cm, 33 leaves/plant, 2.78 g/plant, 2.03 g/plant compared to the control treatment, which recorded the following values for the same characteristics, reaching 29.33 cm 21.66 leaves/ plant, 1.3 g/plant, 1.14 g/plant, respectively.

The bio-inoculant improves plant growth, biomass, and yield by supplying the plant with nutrients and producing growth regulators. It also improves soil fertility by adding and accumulating nutrients in the soil in which the host plant grows. Organic waste is one of the primary forms of organic carbon and nutrients used by soil biomass. Retaining organic waste instead of burning it provides a practical way to increase soil biomass by increasing the amount of organic matter available. Biomass carbon was 45% to 64% higher in fields treated with organic waste than in fields treated with chemical fertilizers (Wichern et al., 2020; Shin et al., 2025).

On the other hand, adding nano elements in appropriate and limited quantities plays an important role, as zinc is the only essential element found in all six enzyme classes, in addition to its direct effect in improving plant resistance to soil conditions through its positive impact on soil bioindicators (Kekeli et al., 2025). A study found that adding low doses of nano-zinc and boron fertilizers can increase plant productivity, as this addition of nano-zinc and boron fertilizer increases the concentration of trace elements in plant leaves (Davarpanah et al., 2016; Anindita et al., 2025). All this would increase the effectiveness of the inorganic pyrophosphatase enzyme, which is one of the enzymes that catalyze the hydrolysis of inorganic pyrophosphate into orthophosphate. The phosphatase enzyme is divided into two parts: soluble pyrophosphatase enzyme and membrane pyrophosphatase enzyme, which differ in structure. Each has a different catalytic mechanism from the other. Thus, the pyrophosphatase enzyme works to regulate the level of pyrophosphate in the soil permanently by hydrolyzing orthophosphate according to the following equation (Kajander et al., 2013; Fernanda et al., 2025): It is a vital biological energy generator that gives other factors such as moisture, soil texture, organic content, and other structural properties which ultimately play a positive role in achieving the best yield of both quality and quantity for crops (Yang et al., 2009; Fan et al., 2025).

Therefore, the present investigation worked towards understanding the role of bacteria, *P. aeruginosa*, white fungus waste, and two nano fertilizers on pyrophosphatase enzyme in soli, growth parameters, and stevia yield.

2. Methodology

Soil samples were taken at a 0 to 30 cm depth from an agricultural site affiliated with the Agricultural Research Department / Diwaniyah Research Station in January 2024. The soil samples were then dried, and biological, chemical, and physical analyses of the soil were conducted (Table 1).

The field was divided into three sectors after the plowing, smoothing, and leveling operations were carried out, each sector contained 24 panels (Figure S2, Supplementary Material). Nitrogen fertilizer was added in the form of granular urea at a quantity of 48 kg N/h in two batches for each of the first and second mowings, and phosphate fertilizer was added in the form of single superphosphate at a quantity of 72 kg P/h in one batch for the first mowing. At the same time, potassium fertilizer was added as potassium sulfate at 60 kg K/h simultaneously with phosphate fertilizer. The following fertilizer treatments were used in the experiment:

1. Factor one: Injecting seedlings with two levels of bacterial inoculum: B0 = without inoculum and B1 = injecting 2 ml of liquid bio-inoculum represented by *P. aeruginosa* bacteria.
2. The second factor: Adding white mushroom waste (Spent Compost) at once during cultivation at three levels: Ab0 = without adding white mushroom waste, Ab1 = adding white mushroom waste at level 5 tons/h, and Ab2 = adding white mushroom waste at level 10 tons/h.

Table 1
Some soil chemical, physical, and biological analyses were carried out before planting the experiment

Parameter	*Value*	*Unit*
pH 1:1	7.23	-
EC 1:1	4.78	*ds/m*
CEC	15.18	*Centi mole + kg^{-1}*
Soil separators		
Sand	420.94	*g/kg soil*
Silty	310.74	*g/kg soil*
Clay	268.32	*g/kg soil*
Pyrophosphatase activity	18.70	*µg PO_4^{-3}-P/g soil 5/h*

3. The third factor: Adding nano fertilizer at once during cultivation at four levels: N0 = without adding nano fertilizer, N1 = adding 4 kg/h of nano zinc and N2 = adding 2 kg/h of nano boron and N3 = adding a mixture of 1 kg/h of nano boron + 2 kg/h of nano zinc.

Table 2 shows both nano fertilizers' physical and chemical properties. The treatments were repeated at a rate of three replications for each treatment so that the number of experimental units became (72) experimental units.

Table 2
Some physical and chemical properties of nano boron and zinc

Characteristic	*Boron nano*	*zinc nano*	*unit*
Form	powder	powder	-
Color	brown	light pink	-
Concentration	9	12	%
Particle Diameter	30	30	Nanometer
(APS) Molecular	10.81	65.41	g Mol^{-1}
Weight Density	2.46	7.14	g cm^{-3}

Agriculture and service operations of sugarcane leaf plant sugarcane leaf plant

Seedlings were planted on 2/15/2024 in experimental units, each containing 3 lines with 50 cm between them and 25 cm between each plant to reach a plant density of 80,000 plants/h, as the number of plants in each line was 5 plants, i.e., 15 plants in each experimental unit. Irrigation was carried out by calculating the values of ready water by the difference between the field capacity and the permanent wilting point, and a volumetric moisture level was maintained when 50% to 55% of the ready water was exhausted throughout the growing season. Irrigation was carried out through a network of drip pipes placed according to the field measurements between one dripper and another 25 cm and between one line and another 50 cm (Figure S1, Supplementary Material).

The good old practice of manual weeding was carried out once a week. The first harvest of the crop could be possible after four months, i.e., on 6/15/2024 and the second on 10/15/2024.

After planting, post planting samples were collected from the rhizosphere of the sugar leaf plant and two harvests. They were randomly sampled from the roots of three plants per experimental unit with a small brush meant to approximate the soil next to the root. The soil samples were kept in plastic jars and refrigerated until biological assessments were done (Figure S3, Supplementary Material).

Assessing the activity of the pyrophosphatase enzyme after the first and second harvests

The enzyme activity was assessed using a method developed by Dick & Tabatabai (1978), which involved incubating 1 g of soil with a sodium pyrophosphate solution at 37 °C for 5 hours. Following incubation, a buffer solution was added, and the mixture was shaken before centrifugation to separate the filtrate. To determine the amount of orthophosphate released, a series of reagents were added to the filtrate, and the resulting blue color was measured using a spectrophotometer at a wavelength of 700 nanometers.

Characteristics of sugar leaf plants

Three plants were randomly selected from the midline to study the characteristics mentioned below in the first and second plots:

1. Plant height (cm/plant): The height of each plant in the selected experimental plot was measured using a tape measure, and the average height was calculated.
2. The dry weight of leaves (g/plant): For the dry weight of leaves per plant, the leaves were sun-dried on cardboard sheets until desiccated, and the average weight was determined for each plant.
3. The total yield (kg/h): was also calculated using a specific equation, expressed in megagrams per hectare.

Total yield (kg/h) = yield per plant × plant density

Statistical analysis: The data for the measured indicators of the study were taken and statistically analyzed following the above-described design methods by the Genstat program. Means were compared using the least significant difference (LSD) test at a probability level 0.05 (Al-Rawi & Khalaf Allah, 1980).

3. Results and discussion

Effect of *P. aeruginosa*, white fungus waste, and nano fertilizer on the activity of pyrophosphatase enzyme (μg PO_4^{-3}-P/g soil 5/h) after the first and second harvests

Appendices 1 and 5, and Figure 1 show the study of the combined influence of *P. aeruginosa* bacteria, white fungus waste, and nano-fertilizer on the performance of pyrophosphatase in Stevia rhizosphere soil after the first harvest. Among all treatments, B1, as bio-inoculum, was much more effective in making pyrophosphatase active and recorded maximum activity during both the harvests at 177.39 and 129.44 μg PO_4^{-3}-P/g soil 5/h, respectively, which was lowest in the comparison treatment B0 at 98.92 and 77.94 μg PO_4^{-3}-P/g soil 5/h, respectively. This will help increase the efficiency of the pyrophosphatase enzyme because well-performing and viable isolation of added bacteria can produce chelates that increase the element binding capacity in the form of soluble complexes. The circumstances above generally strike via differing pathways in increasing the availability of these nutrients, besides producing organic acids and enzymes to a great extent. One of the enzymes presents might be the pyrophosphatase enzyme (Domenech et al., 1992; Aqeel et al., 2023). The results of statistical analysis divulge the marked superiority of the study factor, which embraces the incorporation of white mushroom waste at three levels (Ab0, Ab1, and Ab2); the level Ab2 performed the best as it unleashed the maximum pyrophosphatase enzyme activities in both of the genes to be 177.04 and 128.21 µg PO_4^{-3}-P g^{-1} soil $5h^{-1}$, respectively, as against the treatment Ab0 of the check 86.33 and 72.42 µg PO_4^{-3}-P g^{-1} soil $5h^{-1}$, respectively. The increased waste from white mushrooms explains the increased pyrophosphatase activity added to the soil. The waste has indirect effects on soil phosphorus availability. This is through increasing enzyme-secreting bacteria that work to dissolve some of the primary minerals found in mushroom waste and the insoluble phosphate compounds—the primary bacteria that secrete the pyrophosphatase enzyme and release phosphorus as they dissolve some of the primary minerals found in mushroom waste and the insoluble phosphate compounds, hence increasing the activity of the pyrophosphatase enzyme (Becher et al., 2021; Al-Taweel & Al-Budairy, 2021).

The analysis data showed that the study factor is included in adding nano fertilizer at four levels (N0, N1, N2, N3). The results show that the N3 level was significantly more superior over the N0 comparison treatment for both harvests with means 159.00 and 124.28 µg PO_4^{-3}-P/g soil 5/h, and 114.22 and 93.72 µg PO_4^{-3}-P/g soil 5/h for the first and second harvest, respectively. Stimulation elicited by such nanocomposites to promote root growth would increase up to a further quantity of mineral elements ready for uptake to meet the need of the plant and the continuance of its life activities due to increased pressure on them, thus also into enzyme actions that are responsible for the mobilization of these nutrients and mineral substances, with such a pyrophosphatase enzyme having a function of increasing the availability of the phosphorus element and its movement in response to providing an active transport system available in the plant and concomitant with the continuous stimulation of the root system positively reflected in the effectiveness of pyrophosphatase activity (Chaudhary et al., 2022).

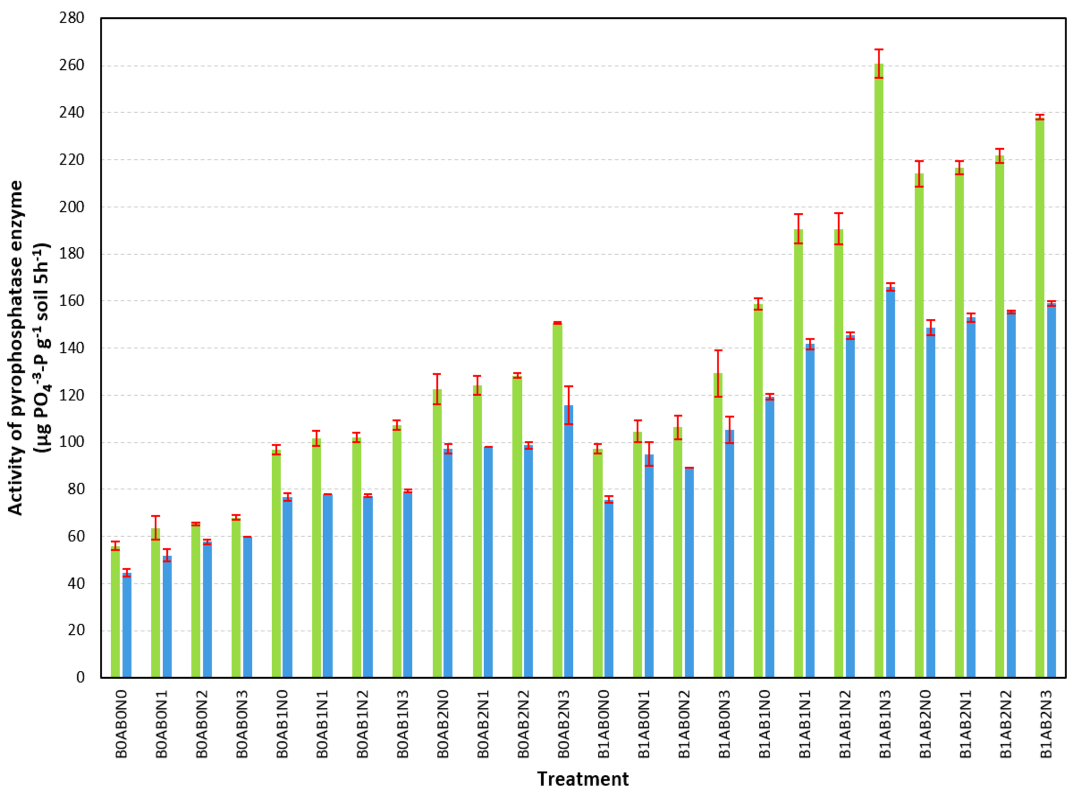

Figure 1. The combined effect of the three study factors on the activity of pyrophosphatase enzyme in the first and second genotypes. ■ Pyrophosphatase activity in soil during first harvest. ■ Pyrophosphatase activity in soil during the second harvest of the crop.

The interaction of bacteria with white fungus waste resulted in a marked superiority in increasing pyrophosphatase enzyme activity. Dual treatment B1Ab2 recorded the highest rate of enzyme activity, which was at 222.58 and 154.00 μg PO_4^{-3}-P/g soil 5/h, respectively, compared to the comparison treatment B0Ab0, which gave 63.25 and 53.58 μg PO_4^{-3}-P/g soil 5/h respectively. The performance of those added bacteria in nitrogen fixation and white fungus residues stimulates plant growth. It helps root growth enhance nutrient absorption by the plant, thus ensuring the balance of the nutrients the plant needs. This results in better plant growth and a denser root system. And for nutrients building up adequate enzyme secretion by the organism, this positively affects those organisms to produce pyrophosphatase enzyme: "The interaction of *P. aeruginosa* with the nano-fertilizer resulted in a significant effect, dual treatment B1N3 being the highest in two genotypes." It recorded rates of pyrophosphatase enzyme activity reaching 209.33 and 143.44 μg PO_4^{-3}-P/g soil 5/h, respectively, within both genotypes compared with the comparison treatment B0N0, which was 91.89 and 72.89 μg PO_4^{-3}-P/g soil 5/h for both genotypes, respectively. An increase could be traced to zinc and nano-boron through their addition and the dissolution of their compounds in the soil by bacteria added to the soil that elevated their absorption by the plant, which in turn magnified the density of the root system. Increasing cation exchange was also mentioned. Other additions of bacteria increased gibberellins, which in turn increased the production of enzymes, especially the pyrophosphatase enzyme (Kishore et al., 2005; Seyfouzadeh & Mohammadi., 2012; Daneshvar et al., 2007).

The results reveal that the binary combination of white mushroom supplements with nano-fertilizer gave a remarkable superiority in the pyrophosphatase enzyme activity. It was all due to Ab2N3 treatment that beat the two plants and recorded 194.33 and 137.33 μg of PO_4^{-3}-P/g soil 5/h in that order, 1 compared to the control therapy that registered the least activity of 76.67 and 60.17 μg of PO_4^{-3}-P/g soil 5/h. In photosynthesis, microelements greatly enhance efficiency and accelerate the formation of carbohydrates, which, through the descending sap, are conveyed to the absorption sites in the roots. Since carbohydrates are considered an essential energy source to ensure the bio-absorption of phosphorus through the origins, this text falls true. Zinc and nano-boron built the auxin hormone responsible for root growth and their nutrient uptake, enabling enzyme activity (Panhwar et al., 2011; Ralia & Tarafdar, 2013) in the initial stage of white fungus waste, improving structure and porosity that affects soil aeration, microbial respiration, and the growth of other beneficial microorganisms that require oxygen and CO_2 evolution in the soil solution, and there is nutrient availability. Therefore, the readiness of nutrients then increases the enzymes that those organisms secrete, including the pyrophosphatase enzyme (Joniec et al., 2022). Data discussed statistically (Appendices 1 and 5) revealed that the interaction of the three study factors was significantly superior to the rate of pyrophosphatase enzyme activity, as the triple combination B1Ab1N3 was superior in the two genes and recorded 260.67 and 166.00 μg PO_4^{-3}-P/g soil 5/h, respectively compared to the comparison treatment which recorded the lowest activity of 56.00 and 44.67 μg PO_4^{-3}-P/g soil 5/h, respectively. This may be due to the synergistic role between bacteria, nano fertilizer and white fungus waste that was used as organic fertilizer in improving plant growth and increasing its productivity, and that bacterial fertilizer encouraged the absorption of water and nutrients that play an essential role in changing pH. Microenvironments contribute to the dissolution of some nutrients and then increase their availability by secreting hormones, gibberellins, some organic acids and enzymes, such as pyrophosphatase (Durairaj et al., 2017, Abdel-Dayem et al., 2020, Velusamy et al., 2021). This superiority shows the importance of bio- and organic fertilization in dispensing with an amount of nano-fertilizer to preserve the environment and human health by improving the quality of the agricultural product, as well as reducing production costs and reducing environmental pollution resulting from the addition of large amounts of nano-fertilizer.

Vegetative growth characteristics of stevia plant

Plant height rate (cm/plant)

Appendix 2 and 6, and Figure 2 show that the addition of the bio-inoculant B1 was significantly superior and recorded the highest rate of plant height during the two harvests, reaching 84.23 and 74.59 cm/plant, the lowest being recorded by the B0 comparison treatment at 72.26 and 63.43 cm/plant rates, respectively. It is noted that *P.aeruginosa* bacteria can dissolve phosphate, thus further enhancing the growth of plant roots and, consequently, the absorption of minerals by the plant. It also produces the siderophore compound that increases the development of plant tissues and thus increases its height while increasing the plant's resistance to pathogens by dissolving insoluble iron, in addition to its ability to produce an essential bioactive compound IAA that works to increase the rate of plant height (Yasmin et al., 2014; Uzair, 2018; Al-Khalidi Al-Taweel, 2024). The results showed the significant superiority of the study factor that includes adding white mushroom waste at three levels (Ab0, Ab1, and Ab2), as the Ab2 level outperformed by giving the highest rate of stevia plant height, which reached 85.05 and 75.70 cm plant^{-1}, respectively compared to Ab0 where 68.69 and 60.22 cm plant^{-1} were recorded, respectively. Mushroom waste is characterized by high amounts of organic material and its chemical, physical, and biological properties. These helps enhance its ability to improve soil properties and bioremediation (Jonathan et al., 2012).

Mushroom waste contributed to improving soil structure, increasing microbial activity, and providing nutrients, which led to an increase in nitrogen and phosphorus concentrations due to biological activity, thus increasing the plant's content of absorbed nutrients, which is positively reflected in the course of the vital and physiological processes of plant cells and the process of carbon construction, which increases the manufactured carbohydrate materials and the occurrence of division, elongation and development of plant cells, which is reflected in growth indicators (Taha et al., 2018). The addition of nano fertilizer at its four levels, which included (N0: without addition, N1: addition of nano zinc, N2: addition of nano boron, N3: joint addition of half the recommendation of zinc and nano boron), showed a significant superiority between the levels. The results demonstrated in Appendices 2 and 6 show that the N3 level was significantly superior in the rate of stevia plant height during the first and second harvests. It recorded 83.30 and 73.88 cm/plant, respectively, compared with the control treatment N0, which was 71.15 and 63.28 cm/plant, respectively.

The combined influences of nano-zinc and boron on plant growth and development were reflected in biomass of stevia plant since its height depends on the ability of the plant to increase photosynthesis, while the significant improvement rate of the height of the plant could be due to the positive effects of nano-size nutrients that enhance rates of photosynthesis and other metabolisms that are vital for the development of several metabolisms of Stevia required for cell division and elongation (Gorzi & Omidi, 2020; Sanjeev & Sanjay, 2020). Results from Appendices 2 and 6 also show that there were substantial variations in average plant height under binary interaction treatments between *P. aeruginosa* bacteria and white fungus waste; the binary treatment B1Ab2 performed better and yielded the maximum average plant height for both vines, with values of 89.08 and 79.86 cm/plant, respectively, as opposed to the comparison treatment B0Ab0, which resulted in 62.57 and 53.94 cm/plant for the same vines. Increased plant height.

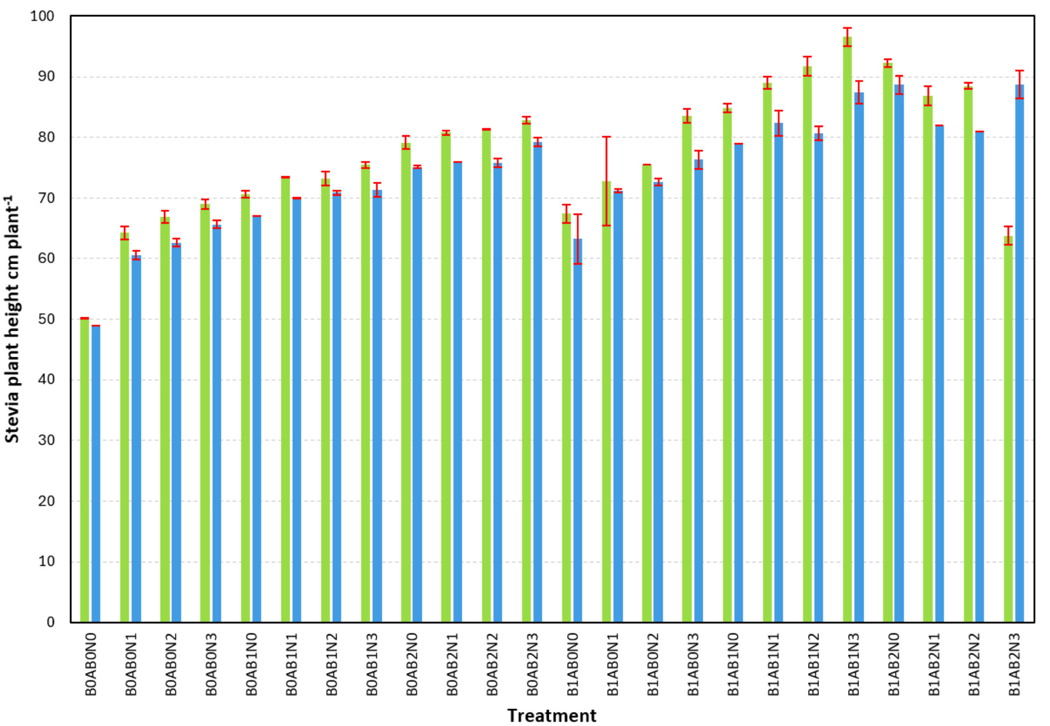

Figure 2. The combined effect of the three study factors on the average height of stevia plants in the first and second vines.
■ Stevia plant height in first harvest. ■ Stevia plant height in the second harvest.

The white mushroom waste fertilizer increases plant height because it can provide nutrients and organic acids. Its nitrogen content increases the mass of protoplasm, division of cells, and volume of growth more than that provided by the other macrophages (Tavarini & Angelini 2013; Al-Taweel and Al-budairy, 2024), besides the contribution of *P. aeruginosa* bacteria in the aspect of multiple growth regulators and amino acids that helped in improving plant nutrition (Wang et al., 2021). On the other hand, the interaction between *P. aeruginosa* bacteria and nanofertilizer significantly influenced the stevia plant height. Dual treatment B1N3 was the best concerning the highest height reading for both plants, 90.84 and 80.70 cm/plant, respectively, compared to the control treatment B0N0, which gave 66.65 and 58.04 cm/plant, respectively. Height increments in stevia plants can be ascribed to the positive association of *P. aeruginosa* bacteria, which play a part in improving nutrient availability and add up to a multiplication of growth regulators and organic acids; because of these, there is also increased phosphorus solubility, which in turn fosters better plant nutritional conditions (Adesemoye & Ugoji, 2009), besides its role as a micronutrient in the areas of importance for root growth, cell division, and elongation of the plant, its nano form becomes more efficient for its role of elements in plant growth and development.

On the other hand, is a micronutrient that took part in the production of tryptophan, which is part of indole acetic acid (IAA) participating in stimulation where growth takes a main role in the synthesis of the carbonic enzyme where it helps in the transfer of carbon dioxide in the process of photosynthesis (Marzouk et al., 2019). Appendices 2 and 6 show that the binary interaction between white mushroom waste and nano fertilizer gave a significant superiority in the rate of stevia plant height, as the Ab2N3 treatment outperformed the two plants and recorded 87.57 and 78.33 cm/plant compared to the comparison treatment, which recorded the lowest height of 58.78 and 50.12 cm/plant respectively. The superiority of the combination of mushroom waste, zinc, and nano-boron is that adding white mushroom waste has a vital effect on the soil, as it is an important source of nutrients, many organic acids, and various hormones. It is also a reformer of many biological, chemical, and physical properties by improving the soil composition, increasing aeration and permeability to water and roots, and increasing the soil's ability to hold water and nutrients. All these factors provided optimal growth conditions for the plant, which led to an increase in the plant height rate. The combined effects of nano-boron and zinc improved plant growth and development. The marked increase in the rate of plant height can be attributed to favorable impacts from zinc and nano-boron in enhancing photosynthesis, which further led to the development of many plants metabolic products involved in cell division and elongation (Sanjeev & Sanjay, 2020). Results in Appendices 2 and 6 reasoned statistically show that there was a superiority in the triple intervention among the study factors regarding increases in both periods, as B1Ab1N3 manifested superiority over the two periods, achieving 90.84 and 80.70 cm/plant, respectively, as opposed to the control treatment which produced the least values for the two periods, which presented 50.14 and 42.00 cm/plant, respectively. The reason why the triple combination is superior is that the addition of all types of biofertilizer influenced nitrogen fixation and stimulated the growth of the root system. It also aided in the secretion of some plant hormones, such as gibberellin and cytokinin, further influencing cell division and elongation. The absorption rate of necessary nutrients increased, affecting the increase in the average height of the plant (Uzair et al., 2018). Mushroom residues also affect the height of the plant through their role in forming energy-rich compounds, as they are rich in phosphorus. Energy compounds such as ATP act as cofactors for enzymes in the plant and work to strengthen the roots of the plant and increase its branches, which helps in increasing the absorption of nutrients from the soil solution, which is reflected in increasing the height of the plant (Shi et al., 2023). Zinc and nano-boron also have a positive effect in increasing the height of the plant due to the biological cycle by stimulating the process of photosynthesis and activating the division of meristematic cells and the occurrence of elongation through ideal expansion in the cell wall, which is necessary (Marzouk et al., 2019).

Effect of *P. aeruginosa* bacteria, white fungus waste, and nano fertilizer on the dry weight rate of stevia leaves (g/plant) in the first and second harvests

The statistical analysis results are presented in Appendices 3 and 7, which indicate the significant effect of *P. aeruginosa* bacteria on the rate of dry weight of stevia leaves in the first and second harvests. The application of B1 bio-inoculum also showed excellent results. Improvement was recorded, and the highest dry weight rate of stevia leaves was at 57.6 and 49.5 g/plant, respectively, compared to the comparison treatment B0 at 42.8 and 36.6 g/plant, respectively, as shown in Figure 3. In this study, the supposed higher response by Stevia plants to bio-inoculation of the *P. aeruginosa* bacteria is defined as it played its effect directly through stimulating plant growth and, simultaneously, resistance against pathogens and pollutants existing in soil as well as stimulating plant defenses which therefore made nutrients undergo the unavailable invasive form converted into available nutritional forms therein estimating better plant growth (Villamarín-Gallegos et al., 2020). The statistical analysis results revealed a significant influence of the different levels of white fungus waste addition. It gave the best treatment means in the first and second genes for dry weights of stevia leaves. The best treatment means were obtained at the Ab2 level, providing 60.1 and 51.6 g/plant compared to the mean value of the comparison treatment, Ab0, which was 38.7 and 33.0 g/plant in the first and second genes, respectively. White mushroom waste contains large percentages of mineral nitrogen and many other plant foods. It can improve the organic matter content of the soil, enhance moisture retention, control pH, and, most importantly, positively affect the availability of potassium, calcium, and magnesium in the soil towards the plant uptake of nutrients (Medina et al., 2009) which is reflected relatively positively in Appendices 3 and 7 by increasing plant height toward a significant increase in the dry weight of the leaves.

The application of nano-fertilizer at four levels, which includes (N0, N1, N2, and N3), reflected a very high influence on stevia leaf dry weights since the N3 treatment was significantly higher at both harvests, with plant recording 57.8 and 49.8 g, respectively, over control N0 with 41.1, 35.1 g/plant. The increase in dry weight of stevia leaves may be explained. Thus, increased improvement in vegetative growth resulted, perhaps attributed to the beneficial effects of zinc and boron toward enhancement of photosynthesis. Since the addition of nano zinc caused the above chemicals in the plant with subsequent antioxidant activity, it must have enhanced the dry weight of leaves (El-Hoseiny et al., 2020; El-sheery et al., 2020). As for the interaction between *P. aeruginosa* bacteria and white fungus waste, it resulted in significant superiority in increasing the dry weight rate of stevia leaves in the two harvests, as the dual treatment B1Ab2 recorded the highest rate of 67.8 and 58.2 g/plant, respectively, compared to the comparison treatment B0Ab0, which gave 34.0 and 28.8 g/plant, respectively. Using fungus waste with biofertilizers together was more effective in enhancing plant growth and increasing yield because the interaction led to the accumulation of nutrients in the plant (Yousef et al., 2020). The interaction of *Pseudomonas aeruginosa* bacteria with the nano-fertilizer was marked in its effect since the dual treatment, B1N3, yielded the highest dry leaf weight of Stevia plants, 66.3 and 57.3 g/plant, in the two gardens, respectively, compared to the check treatment, B0N0, which gave 34.3 and 29.0 g/plant, in the respective gardens.

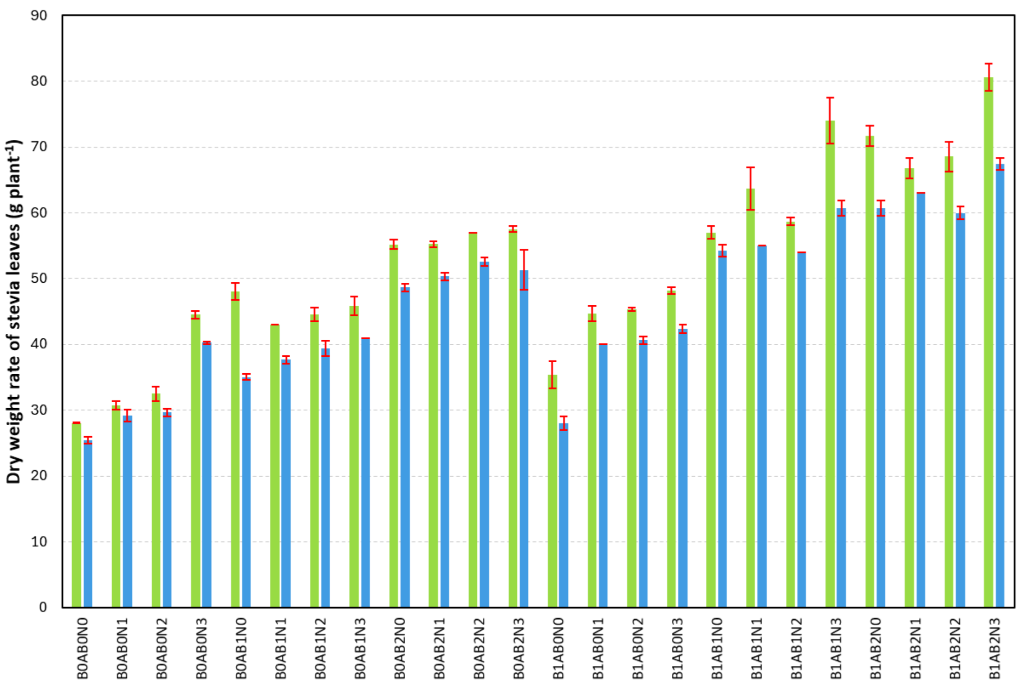

Figure 3. The combined effect of the three study factors on the dry weight of stevia leaves (g/plant) in the first and second harvests. ■ Weight of stevia leaves in the first pound. ■ Weight of stevia leaves in the second pound.

This may relate to the increase in the dry weight of Stevia leaves due to the function of zinc and boron in indispensable elements in the vital activities of the plant, such as the division and growth of the cells of the plant, sugar, and carbohydrate translocation, and added nutrient absorption by the plant (Javed, 2018), in addition to the role of *P. aeruginosa* bacteria, which plays an essential role in plant life through various mechanisms, including fixing atmospheric nitrogen, dissolving phosphate, secreting growth-stimulating substances, producing organic acids, protecting against pathogens, and increasing the absorption of nutrients, thus increasing the plant's vegetative mass (Miladinova-Georgieva et al., 2023). Appendices 3 and 7 binary effects between white fungus waste and the nano fertilizer showed significant differences regarding the dry weight rate of leaves. Ab2N3 treatment excelled in both periods, with 65.8 and 56.6 g/plant against the check treatment, which recorded 31.5 and 26.5 g/plant, respectively. This confirms the interaction between the two factors, as the white fungus waste compost compensated for most of that supplied by the nano-fertilizers. Accordingly, it confirms the role of nano-fertilizers in the vegetative group. This could be attributed to the sufficient and balanced supply of major and minor nutrients for good vegetative growth. Adding mushroom waste and nano-fertilizers enhanced this effect (Owaid et al., 2017; Martín et al., 2023). Appendices 3 and 7 data revealed that the triple interaction among the study factors significantly improved the rate of dry-weight leaves. The triple combination B1Ab1N3 proved to be better in both periods, with a value of 76.7 and 66.9 g/plant, respectively, compared to the comparison treatment, which gave 27.6 and 22.6 g/plant, respectively (Figure 3). The results emphasize the value of fertilization using fungal waste and bio-inoculum to lower the effects of added nano-fertilization, therefore negating any environmental damage due to increased levels of nano-fertilization while productivity is maintained at optimum levels; additional organic matter enhances the nutrient pool availability and further amelioration of other soil properties for the enhancement of the thermal system of the soil. Also, it helps the added bacteria enhance the ability to release stimulants and growth regulators that strengthen the growth of the roots and improve them to facilitate the uptake of nutrient elements by the plant. Besides, there was an interaction effect between the nano-elements and (Zn+B), which indicates the additional effect of each of them when they interact with each other and the difference between their effect alone, in addition to the balanced preparation of nutrients that works to increase the activity and effectiveness of vital processes in the plant and thus increase the absorption of nutrients by the roots, which leads to increased growth and increased dry weight of the plant (Momivand et al., 2021; Gaber et al., 2021; Al-Jubouri and Al-Taweel, 2024).

Effect of *P. aeruginosa* bacteria, white fungus waste, and nano fertilizer on the total yield of stevia leaves (kg/h) in the first and second harvests

Figure 4 shows an increase in the total yield when *P. aeruginosa* bio-inoculation is added because the treatment of adding bio-inoculation B1 was significantly better and achieved the highest total yield of dry leaves during the two harvests, reaching 4604 and 3960 kg/h, respectively, compared to the comparison treatment B0, which recorded the lowest yield, reaching 3426 and 2929 kg/h, respectively. Bio-inoculation increased the total leaf yield compared to no fertilization. This increase can be attributed to the role of the bio-inoculation mentioned above, which affected growth and increased the availability of nutrients (Yasmin et al., 2017), thus leading to an increase in plant height, which was positively reflected in the dry leaf weight (Appendices 4 and 8), and then an increase in the total yield.

The statistical analysis results indicated the significant superiority of the study factor, which involved the addition of white fungus waste at three levels: Ab0, Ab1, and Ab2. It should, therefore, be noted that level Ab2 proved to be better as it gave the highest total leaf yield in the two gardens, which were 4807 and 4126 kg/ha, respectively, compared to the check treatment, Ab0, which gave 3093 and 2638 kg/ha, respectively. The factor for the betterment of white fungus waste is ascribed to a good deal of key actions of living things and some essential enzymes for them and the biochemical processes of the plant that supply the plant roots with energy and food; the white mushroom waste led to bettering the traits of vegetative growth of the plant because it has organic acids that bring about an increase in the availability of nutrients in the soil, and thus their absorption by the roots; hence, the dry weight rate of the leaves and the yield increases. Regarding the application of nano fertilizer at its four levels (N0, N1, N2, N3), the results depicted in Appendices 4 and 8 indicate that level N3, which comprises half of the recommended dosage of nano zinc along with half of the recommended dosage of nano boron proved vastly better throughout the two harvests, with 4624 and 3983 kg/h, respectively, compared to the base treatment N0, which had 3291 and 2808 kg/h for the same respective harvests. The reason for the increase in the total yield is the function of zinc and nano-boron; that is, zinc and nano-boron have a vital function in protein formation since they extensively participate in the nitrogen cycle and transformation of the same into indispensable amino acids as well as the synthesis of DNA and RNA with some involvement in their control. Moreover, zinc presents itself as a vital component for many compounds of enzymes and ribosomes and has a significant role in the process of forming carbohydrates and chlorophyll; therefore, it increases the vegetative growth, the increase of dry leaves by weight, and the general yield (Salem at el., 2020).

The interaction of bacteria, *P.aeruginosa*, with white fungus waste significantly manifested a better increment in the combined yield of the leaves of Stevia in both the first and second harvests as the dual treatment B1Ab2 proved to be the best among all treatments with the highest rate in two harvests amounting to 5422 and 4657 kg/ha, respectively, compared to the comparison treatment B0Ab0 with 2717 and 2300 kg/ha, respectively. The increase in total plant yield results from organic fertilizer added to the plant body composition of total nitrogen and its ready-made forms that get released into the soil once they are biodegraded along with the bioinoculant added to the soil, as nitrogen fixed by the bioinoculant added to the soil is working out to be amino acids and also compounds that are useful to the plant in tissue-building effects will, therefore, improve the growth of the plant increasing its vegetative mass and total leaf yield (Kumar et al., 2013; Leong et al., 2022).

One of the most popular interactions was between the bacteria *P. aeruginosa* and nanofertilizer. The dual treatment B1N3 resulted in total leaf yield amounts for the two cultivars at 5304 and 4582 kg/ha for cultivars A and B, respectively. In the comparison treatment, B0N0 values were pegged at a mean of 2742 and 2318 kg/ha, respectively. This interaction confirms the possibility of obtaining high productivity and providing quantities of up to half or more of nano fertilizer when adopting alternative natural biofertilizers and that the addition of bioinoculum led to an increase in the absorbed amount of nitrogen, phosphorus, iron, zinc and boron in the vegetative system, which led to the formation of a strong root system that is efficient in absorbing nutrients from the soil, thus increasing the absorbed amount of elements in the vegetative system, which led to an increase in the total yield (Pandey et al., 2012; Pramanik et al., 2020). Appendices 4 and 8 demonstrate that the binary interplay between white mushroom waste and nano fertilizer led to a marked superiority in the total yield rate of stevia leaves since the Ab2N3 treatment topped the performance of the two plants with 5264 and 4524 kg/ha against 2205 and 2117 kg/ha of the comparison treatment, respectively.

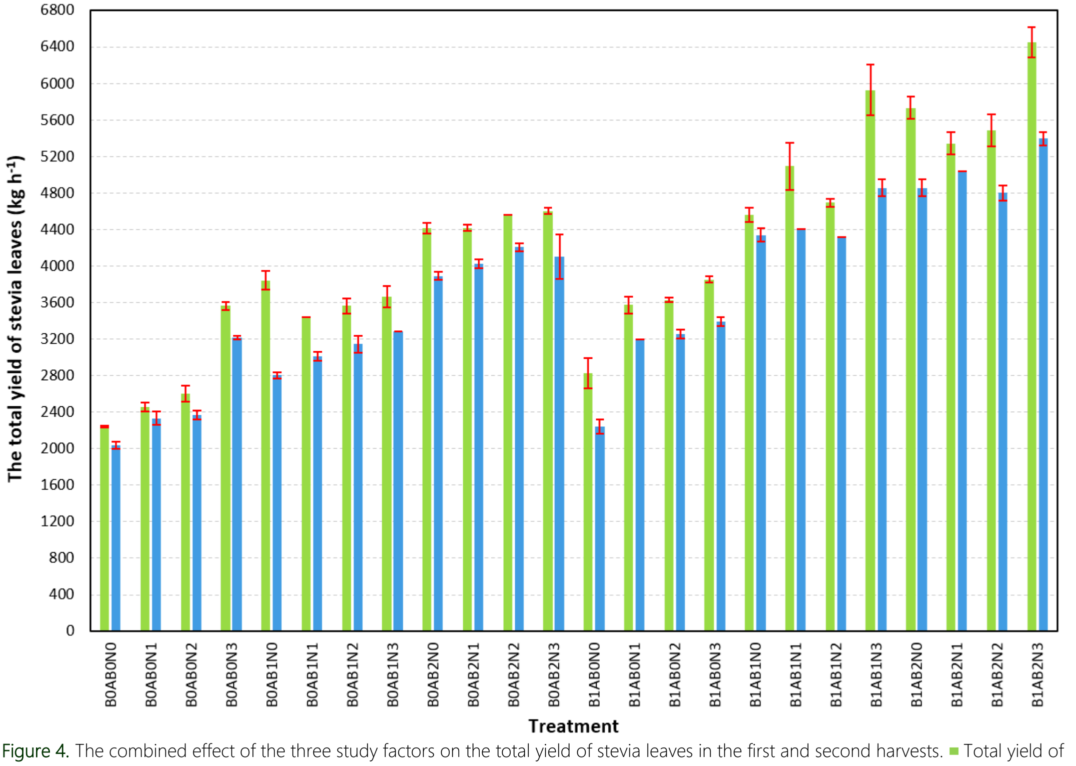

Figure 4. The combined effect of the three study factors on the total yield of stevia leaves in the first and second harvests. ■ Total yield of stevia leaves in the first harvest. ■ Total yield of stevia leaves in the second harvest.

The addition of white mushroom waste increased nutrient concentrations in the soil, hence increasing nutrient availability in the soil solution and the uptake of nutrients by the plant's roots. These, in turn, affected plant growth indicators by increasing plant height and dry leaf weight (Baptista et al., 2023). It further improved zinc and nano-boron effects on carbon metabolism, respiration, protoplasmic construction, protein synthesis, and assorted enzymatic reactions, thus increasing plant growth rate. This consequently increased plant growth indicators and yield (Gorzi & Omidi, 2021). The data in Appendices 4 and 8 show that the triple interaction between the study factors indicated a significant superiority in increasing the total yield of stevia leaves; the triple combination B1Ab1N3 outperformed the two crops and registered 6133 and 5355 kg/h, respectively, compared to the comparison treatment, which resulted in 2205 and 1808 kg/h, respectively. The reason for the superiority of the triple combination B1Ab1N3 is due to bio-pollination; zinc and nano-boron increase available nitrogen and phosphorus in the soil, and with microelements and the secretion of organic acids, there is also the secretion of growth regulators like auxin, which enable rooting and gibberellin, which increases the growth rate of roots as well as stems, and finally cytokines play an essential function in most fundamental processes that have an important role in the enhancement of growth as well as the formation of the total yield of plants (Sanjeev & Sanjay, 2020). Mushroom waste is added to the mixture in better physical and chemical properties in the soil, better fertility, moisture content in the soil, and thus raising the plant and improving the availability of the nutrients required for essential work inside; added waste improves vital processes inside because the decay products of applied mushroom waste are in the form of nitrogenous and phosphatic compounds among others. The mushroom waste provides essential nutrients and contains hormones and vitamins used by the plant for its growth and for increasing the height of the plant. This is positively reflected in the dry weight of the leaves as well as the total yield (Spago et al., 2014; Paula et al., 2018; Al-Hasnawi & Jarallah, 2024).

4. Conclusions

The triple combination B1Ab1N3 proved its superiority in the enzyme activity of the pyrophosphatase enzyme in the first and second harvests, while the triple combination B1Ab2N3 achieved growth and yield characteristics of the stevia plant by which it recorded at the highest rate of plant height of dry weight of leaves and, total yield. The results would, therefore, open an avenue to proper management of agro-waste, including spent mushroom waste, thus working to increase soil fertility in line with sustainable development provisions.

We recommend from this study to increase the cultivation of economic medicinal plants, which are considered an alternative to white sugar, as they are healthy, in addition to the use of purely organic fertilizers rich in major and minor nutrients to meet the needs of the plant, as it also needs minor elements, as organic fertilizers and organic waste encourage environmental sustainability and protect it from pollution

Conflicts of Interest

There are no conflicts of interest.

Authors' Contribution

Zahraa J. Al-budairy: conceptualization, data curation, research, methodology, writing the initial draft, and review, Research, data curation, software, and review, Conceptualization, research, formal analysis, and reviee, Formal analysis, data curation, and review, Formal analysis, review. Luma S. Al-Taweel: supervision and Conceptualization, research, formal analysis, review, and supervision.

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Appendix

Effect of *P. aeruginosa*, white mushroom waste, and nano-fertilizer on the effectiveness of inorganic pyrophosphatase enzyme (μg PO_4^{-3}-P g^{-1} soil $5h^{-1}$) after the first harvest and the second. It includes studying the effect of the same factors on plant height, leaf yield and total yield in the two gardens (Period 1: Appendices 1, 2, 3 y 4; Period 2: Appendices 5, 6, 7 y 8)

Appendix 1
Pyrophosphatase enzyme in the first and second vines

Pseudomonas aeruginosa inoculation (B)	*B0*		*B1*	
	98.92		177.39	
LSD 0.05	1.81			
White mushroom waste (Tones h^{-1})	Ab0	Ab1	Ab2	
	86.33	151.08	177.04	
LSD 0.05	2.22			
Nano fertilizer (kg/h)	N0	N1	N2	N3
	124.28	133.61	135.72	159.00
LSD 0.05	2.56			
Bilateral interaction between inoculation with P. aeruginosa and white fungus waste				
	Ab0	Ab1	Ab2	
B0	63.25	102.00	131.50	
B1	109.42	200.17	222.58	
LSD 0.05	3.14			
Bilateral interaction between P. aeruginosa inoculation and nano fertilizer				
	N0	N1	N2	N3
B0	91.89	96.56	98.56	108.67
B1	156.67	170.67	172.89	209.33
LSD 0.05	3.62			
The dual interaction between white mushroom waste and nano fertilizer				
	N0	N1	N2	N3
Ab0	76.67	84.17	85.83	98.67
Ab1	127.83	146.17	146.33	184.00
Ab2	168.33	170.50	175.00	194.33
LSD 0.05	4.44			

Appendix 2
Average height of stevia plants in the first and second vines

Pseudomonas aeruginosa inoculation (B)	*B0*		*B1*	
	72.26		84.23	
LSD 0.05	1.31			
White mushroom waste (Tones h^{-1})	Ab0	Ab1	Ab2	
	68.69	80.98	85.05	
LSD 0.05	1.60			
Nano fertilizer (kg/h)	N0	N1	N2	N3
	71.15	78.11	80.05	83.30
LSD 0.05	1.85			
Bilateral interaction between inoculation with P. aeruginosa and white fungus waste				
	Ab0	Ab1	Ab2	
B0	62.57	73.18	81.02	
B1	74.82	88.78	89.08	
LSD 0.05	2.26			
Bilateral interaction between P. aeruginosa inoculation and nano fertilizer				
	N0	N1	N2	N3
B0	66.65	72.82	73.80	75.76
B1	76.38	83.40	86.30	90.84
LSD 0.05	2.61			
The dual interaction between white mushroom waste and nano fertilizer				
	N0	N1	N2	N3
Ab0	58.78	68.51	71.20	76.29
Ab1	74.22	81.20	82.46	86.04
Ab2	81.53	84.62	86.50	87.57
LSD 0.05	3.20			

Appendix 3
Dry weight of stevia leaves in the first and second harvests

Pseudomonas aeruginosa inoculation (B)	*B0*		*B1*	
	42.8		57.6	
LSD 0.05	1.1			
White mushroom waste (Tones h^{-1})	Ab0	Ab1	Ab2	
	38.7	51.8	60.1	
LSD 0.05	1.27			
Nano fertilizer (kg/h)	N0	N1	N2	N3
	41.1	50.7	51.1	57.8
LSD 0.05	1.5			
Bilateral interaction between inoculation with P. aeruginosa and white fungus waste				
	Ab0	Ab1	Ab2	
B0	34.0	42.1	52.4	
B1	43.4	61.5	67.8	
LSD 0.05	1.8			
Bilateral interaction between P. aeruginosa inoculation and nano fertilizer				
	N0	N1	N2	N3
B0	34.3	43.0	44.7	49.3
B1	48.0	58.4	57.5	66.3
LSD 0.05	2.1			
The dual interaction between white mushroom waste and nano fertilizer				
	N0	N1	N2	N3
Ab0	31.5	37.9	38.9	46.4
Ab1	41.0	53.3	51.6	61.3
Ab2	50.9	60.9	62.8	65.8
LSD 0.05	2.5			

Appendix 4
Total yield of stevia leaves in the first and second harvests

Pseudomonas aeruginosa inoculation (B)	*B0*		*B1*	
	3426		4604	
LSD 0.05	83			
White mushroom waste (Tones h^{-1})	Ab0	Ab1	Ab2	
	3093	4144	4807	
LSD 0.05	101			
Nano fertilizer (kg/h)	N0	N1	N2	N3
	3291	4056	4089	4624
LSD 0.05	117			
Bilateral interaction between inoculation with P. aeruginosa and white fungus waste				
	Ab0	Ab1	Ab2	
B0	2717	3369	4193	
B1	3470	4920	5422	
LSD 0.05	143			
Bilateral interaction between P. aeruginosa inoculation and nano fertilizer				
	N0	N1	N2	N3
B0	2742	3443	3575	3944
B1	3840	4670	4602	5304
LSD 0.05	165			
The dual interaction between white mushroom waste and nano fertilizer				
	N0	N1	N2	N3
Ab0	2516	3035	3115	3708
Ab1	3282	4267	4129	4900
Ab2	4075	4868	5023	5264
LSD 0.05	202			

Appendix 5

Pyrophosphatase enzyme in the first and second vines

Pseudomonas aeruginosa inoculation (B)	*B0*		*B1*	
	77.94		129.44	
LSD 0.05	1.25			
White mushroom waste (Tones h^-1)	Ab0	Ab1	Ab2	
	72.42	110.46	128.21	
LSD 0.05	1.53			
Nano fertilizer (kg/h)	N0	N1	N2	N3
	93.72	102.94	103.89	114.22
LSD 0.05	1.77			
Bilateral interaction between inoculation with P. aeruginosa and white fungus waste				
	Ab0	Ab1	Ab2	
B0	53.58	77.83	102.42	
B1	91.25	143.08	154.00	
LSD 0.05	2.17			
Bilateral interaction between P. aeruginosa inoculation and nano fertilizer				
	N0	N1	N2	N3
B0	72.89	76.00	77.89	85.00
B1	114.56	129.89	129.89	143.44
LSD 0.05	2.50			
The dual interaction between white mushroom waste and nano fertilizer				
	N0	N1	N2	N3
Ab0	60.17	73.50	73.33	82.67
Ab1	98.00	109.83	111.33	122.67
Ab2	123.00	125.50	127.00	137.33
LSD 0.05	3.07			

Appendix 6

Average height of stevia plants in the first and second vines

Pseudomonas aeruginosa inoculation (B)	*B0*		*B1*	
	63.43		74.59	
LSD 0.05	1.03			
White mushroom waste (Tones h^-1)	Ab0	Ab1	Ab2	
	60.22	71.12	75.70	
LSD 0.05	1.27			
Nano fertilizer (kg/h)	N0	N1	N2	N3
	63.28	68.68	70.21	73.88
LSD 0.05	1.46			
Bilateral interaction between inoculation with P. aeruginosa and white fungus waste				
	Ab0	Ab1	Ab2	
B0	53.94	64.82	71.54	
B1	66.50	77.42	79.86	
LSD 0.05	1.79			
Bilateral interaction between P. aeruginosa inoculation and nano fertilizer				
	N0	N1	N2	N3
B0	58.04	63.86	64.77	67.07
B1	68.52	73.50	75.65	80.70
LSD 0.05	2.07			
The dual interaction between white mushroom waste and nano fertilizer				
	N0	N1	N2	N3
Ab0	50.12	60.85	62.61	67.32
Ab1	66.67	71.02	70.78	76.00
Ab2	73.07	74.17	77.23	78.33
LSD 0.05	2.53			

Appendix 7

Dry weight of stevia leaves in the first and second harvests

Pseudomonas aeruginosa inoculation (B)	*B0*		*B1*	
	36.6		49.5	
LSD 0.05	1.0			
White mushroom waste (Tones h^-1)	Ab0	Ab1	Ab2	
	33.0	44.6	51.6	
LSD 0.05	1.2			
Nano fertilizer (kg/h)	N0	N1	N2	N3
	35.1	43.5	43.9	49.8
LSD 0.05	1.4			
Bilateral interaction between inoculation with P. aeruginosa and white fungus waste				
	Ab0	Ab1	Ab2	
B0	28.8	36.2	44.9	
B1	37.2	53.1	58.2	
LSD 0.05	1.7			
Bilateral interaction between P. aeruginosa inoculation and nano fertilizer				
	N0	N1	N2	N3
B0	29.0	36.8	38.4	42.3
B1	41.2	50.1	49.4	57.3
LSD 0.05	2.0			
The dual interaction between white mushroom waste and nano fertilizer				
	N0	N1	N2	N3
Ab0	26.5	32.4	33.4	39.7
Ab1	35.2	45.8	44.3	53.2
Ab2	43.7	52.1	54.0	56.6
LSD 0.05	2.4			

Appendix 8

Total yield of stevia leaves in the first and second harvests

Pseudomonas aeruginosa inoculation (B)	*B0*		*B1*	
	3426		4604	
LSD 0.05	83			
White mushroom waste (Tones h^-1)	Ab0	Ab1	Ab2	
	3093	4144	4807	
LSD 0.05	101			
Nano fertilizer (kg/h)	N0	N1	N2	N3
	3291	4056	4089	4624
LSD 0.05	117			
Bilateral interaction between inoculation with P. aeruginosa and white fungus waste				
	Ab0	Ab1	Ab2	
B0	2717	3369	4193	
B1	3470	4920	5422	
LSD 0.05	143			
Bilateral interaction between P. aeruginosa inoculation and nano fertilizer				
	N0	N1	N2	N3
B0	2742	3443	3575	3944
B1	3840	4670	4602	5304
LSD 0.05	165			
The dual interaction between white mushroom waste and nano fertilizer				
	N0	N1	N2	N3
Ab0	2516	3035	3115	3708
Ab1	3282	4267	4129	4900
Ab2	4075	4868	5023	5264
LSD 0.05	202			