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Research Article

Role of Adding Bio-Inoculation, Mushroom Farm Waste, and Nano-fertilizers on The Content of Stevia Leaves of Total Carbohydrate Active Compounds

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Abstract: The experiment investigated the impact of *P. aeruginosa* bacteria, mushroom farm waste, and nano-fertilizers on carbohydrates, gibberellins, Stevioside, and Rebaudioside A. sweeteners in stevia plants. The study involved three factors: bacterial inoculation with two levels of *P. aeruginosa, three levels* of white mushroom waste, and four levels of nano-fertilizer, including options like nano-zinc and nano-boron. The research was conducted in the field using a randomized complete block design (RCBD) to ensure reliable results. GenStat statistical data analysis is performed using specialized statistical software commonly used in the fields of agriculture and biology, particularly in the analysis of field and laboratory experimental data. *P. aeruginosa* plays an essential role in bioremediation, and producing antipathyogenic compounds. White button mushroom waste helps improve soil fertility. Nanofertilizers enhance nutrient uptake. Combining these treatments can increase the accumulation of natural sweeteners in stevia plants and improve their quality. The triple combination B1Ab1N3 achieved significant superiority in most of the studied traits, which included total carbohydrates during the first and second harvests, which recorded (77.5 and 70.8)%, respectively, compared to the control treatment B0Ab0N0, which recorded (40.7 and 30.29)%, respectively. In contrast, the triple combination B1Ab2N3 achieved the highest content of gibberellins and sweeteners Stevioside and Rebaudioside A. In the leaves of the stevia plant in the First and second harvest of stevia crop in sequence, it reached (20.88 and 22.86)%, (9.925 and 10.964)%, and (7.637 and 8.386)%, respectively, compared to the comparison treatment B0Ab0N0, which recorded (8.79 and 10.13)%, (5.335 and 5.716)% and (1.534 and 1.686)%.

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1. Introduction

The current importance of bioremediation stems from its alignment with the environmental and agricultural challenges facing Iraq. The country is suffering from an increasing scarcity of water resources and deteriorating soil fertility due to high salinity and chemical pollution. This underscores the need to adopt sustainable agricultural technologies that increase water use efficiency and restore soil vitality. Combining biofertilization with mushroom farm waste and nanofertilizers represents a promising solution, as it contributes to improving soil structure and increasing its ability to retain moisture and nutrients, reducing the need for irrigation and chemical fertilizers (Al-Kubaisi et al., 2013; Çığ and Sönmez, 2020; The National News, 2023).

The research gains additional significance as it examines the stevia plant, a native plant with potential adaptation to Iraq's environmental conditions. It is characterised by the production of locally produced healthy sweeteners, opening up promising economic prospects for both the local market and export markets. Furthermore, recycling mushroom farm waste and converting it into a food source for the soil achieves added environmental value by reducing pollution and decreasing reliance on imported agricultural inputs. This aligns with global trends toward sustainable agriculture and the use of modern technologies such as nanotechnology to enhance productivity and quality.

The types of bacteria added as biofertilizers vary, and their uses vary in terms of being phosphorus solubilisers, such as Pseudomonas bacteria, in addition to the presence of some species that analyse organic materials and secrete some growth stimulants, such as Xanthomonas bacteria (Karpiński et al., 2025). Mandal et al. (2013) found that bioinoculation improved the production of secondary compounds in Stevia. Vafadar et al. (2014) reported significantly higher values of stevioside content in the plant leaves after bioinoculation, 63.01 mg g^{-1}, compared to that of the control, 48.05 mg g^{-1}. The researchers attributed the increase in the stevioside compound, which is one of the secondary metabolism compounds, to the relationship this compound shares with the stimulants produced by the bioinoculation, which may be due to the release of hormones and some biologically active molecules produced by other microorganisms. As a result, the soil works as a stimulator that stimulates stevioside production, in addition to the role of fertilizers as stimulants influenced by vaccination bacteria, such as nutritional value mushroom waste, especially NPK, containing high cation exchange capacity. Some mushroom waste is also used to treat water contaminated with nickel and copper. Adding white mushrooms to soils intended for agriculture is among the natural directions. Due to its large quantities of waste, it differs from chemical fertilizers because it supplies nutrients slowly.

The white mushroom waste fertilizer can replace or supplement the use of growing media to cultivate crops of economic value. The research conducted by Taha (2018) found that adding extra mushroom waste supplementation to watercress plants significantly affected all the parameters related to growth and yield compared with the control treatment. Meanwhile, nano zinc influences the synthesis of fats, the metabolism of carbohydrates, proteins, and nucleic acids, and the activity of plant hormones (Zafar et al., 2022; Shin et al., 2025). Boron is a microelement essential for good plant growth but poorly defended in the soil. Nano boron fertilizer corrects this shortcoming, supplying the plant with boron at a higher concentration. The ability of nano boron fertilizer to enter the plant cells and provide them with the required microelements will help transport sugar. The lack of this element provokes the local death of the plant tissues. It is a critical element for protein formation because it influences nitrate reduction and amino acid formation (Gehlout et al., 2022; Fanin et al., 2025).

The current progress in the agricultural sector, encompassing various aspects, including the cultivation of new field crops with economic returns and high nutritional value, such as Stevia, requires more effort and continuous pursuit in developing appropriate plans to expand these crops' cultivation areas. (Stevia rebaudiana Bertoni) It is a perennial herbaceous plant belonging to the Asteraceae family. It is a multi-cut plant, giving 3-4 cuts annually. It is characterised by its low water requirements (Al-Khafaji, 2014). It is grown in various types of soil. It is one of the most profitable crops, with significant demand in global markets. Its importance lies in the fact that its leaves contain a group of local compounds from the group of steviol glycosides, which are 200-300 times sweeter than table sugar without harming or raising blood sugar levels because they contain no calories. Therefore, this plant is a safe alternative to refined table sugar. Stevia leaves contain Steviol glycosides, which can benefit human health. Stevia leaves have functional and sensory properties superior to many other highly effective sweeteners and are likely to become a significant source of highly effective sweeteners. The sugars in Stevia can be used in more than 600 food products, such as nutritional supplements, doughs, baked goods, medicines, etc. (Mohamed and Al-Said, 2014; Maisuria et al., 2022).

Stevia is also a good source of carbohydrates, proteins, crude fibres, and minerals (Abou-Arab et al., 2010). Rebaudioside A. is a compound that belongs to Steviol glycosides, which is more effective than Stevioside and is the most stable among the glycosides. It has solubility and does not taste bitter due to its additional glucose unit at the C-13 position, giving it a higher sweetening power than

Stevioside. Thus, it is the most interesting component in stevia extracts (Lemus-Monica et al., 2012), given the above-mentioned importance of the stevia plant from a medical and economic perspective, which requires increasing its cultivation in several ways, in addition to following a specific fertilization system to achieve better production. According to Taipei (2005), the deficiency of boron affects sugar crops; therefore, it is necessary to include this element because these crops are depleted and, at high production rates, need high levels of fertilizer application to achieve maximum yields.

This study aims to evaluate the effect of adding *Pseudomonas aeruginosa*, mushroom farm waste, and nanofertilizers on the active carbohydrate content of stevia leaves. The aim is to improve the nutritional and functional value of the plant, increase its productivity and quality, and utilise sustainable, environmentally friendly agricultural methods. This approach utilises agricultural waste and reduces reliance on traditional chemical fertilisers. The study also seeks to explore the feasibility of applying these treatments under Iraq's environmental conditions, characterised by water scarcity and soil degradation. This will contribute to finding practical solutions to increase agricultural production efficiency and conserve natural resources.

2. Materials and Methods

Diwaniyah Agricultural Research Station (affiliated with the Ministry of Higher Education) Location: Located in Al-Qadisiyah Governorate (Diwaniyah), central Iraq, at approximately 31.99°N and 44.92°E. The area is located near the Euphrates River and on the fertile floodplain of the Tigris River, making it an important agricultural region. Diwaniyah's current urban population is approximately 610 000.

Climate and Geographic Diversity: The area has a hot desert climate (BWh according to the Köppen classification), with long, dry summers with maximum temperatures reaching 52 °C and mild to cool winters (minimum temperatures ranging from 13 °C to 15 °C). Annual rainfall is scarce (less than 120 mm), and the surrounding area consists of wet agricultural plains, ponds, and semi-desert areas. The importance of Stevia in the region: Due to the high population density, Stevia is considered a healthy alternative to sugar, especially for diabetics or those looking to reduce their calorie intake. It is naturally extracted and regulates blood sugar levels, helping to reduce the risk of oxidative stress and obesity. Stevia is widely grown locally, meeting the growing demand for low-calorie sweeteners. Given the significant population growth in Diwaniyah, it is a promising and profitable agricultural option (Figure 1).

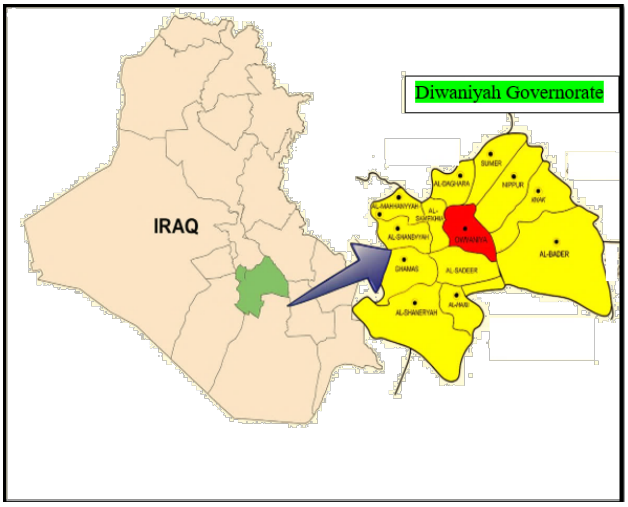

Figure 1. Map of Iraq showing the administrative division of Diwaniyah Governorate, where Stevia was first cultivated.

The trial was conducted at Diwaniyah Research Station during the Winter 2024 agri-season to prepare the field for cultivating stevia plants. The field was partitioned into three 'sectors, with each sector containing 24 experimental units, and various fertilizers were applied, including nitrogen, phosphate, and potassium, along with bio-inoculation and mushroom waste treatments.

The stevia seedlings were planted on February 15, 2024. They were irrigated through a drip system, with the first harvest occurring on June 15, 2024, followed by a second harvest on October 15, 2024.

The following treatments were used in the experiment. Each treatment was replicated three times, bringing the total number of experimental units to 72. The experiment included the following factors:

1. Factor 1: Biological inoculum at two levels: B0 = without adding the biological inoculum, and B1 = adding the biological inoculum of *Pseudomonas aeruginosa*.

2. Factor 2: Adding white mushroom waste (spent compost), at three levels: Ab0 = without adding white mushroom waste, Ab1 = adding white mushroom waste at the level of 5 tons h^{-1}, and Ab2 = adding white mushroom waste at the level of 10 tons h^{-1}.

3. The third factor: adding nano fertilizer at four levels: N0 = without adding nano fertilizer, N1 = adding 2 kg h^{-1} of nano boron, N2 = adding 4 kg h^{-1} of nano zinc, and N3 = adding a mixture of 1 kg h^{-1} of nano boron + 2 kg h^{-1} of nano zinc.

Stevia seedlings were planted on February 15, 2024, in experimental units, each containing three rows 50 cm apart and 25 cm between plants. The plant density reached 80 000 plants per row. The number of plants per row was 5, i.e., 15 plants per experimental unit. Irrigation was performed by calculating the available water value as the difference between the field capacity and the permanent wilting point. A volumetric moisture level was maintained at 50-55% of the available water consumption throughout the growing season. Irrigation was performed via a network of drippers placed at 25 cm and 50 cm intervals, respectively. Manual weddings were held once a week. The bio-inoculant was added to the soil after planting at a rate of 2 ml per plant, according to the treatments. White Mushroom residues were added two weeks before planting, according to the treatments. Nano-fertilizers were added all at once at planting, according to the treatments. The crop was harvested for the first time on 6/15/2024, and then again on 10/15/2024.

Table 1. Some chemical analyses of the studied soil were done before planting

Parameter	Value	Unit
pH $_{1:1}$	7.23	-
EC $_{1:1}$	4.78	ds m^{-1}
CEC	15.18	Centi mole $^{+}$ kg^{-1}
Available Nitrogen	23.8	mg Kg^{-1} soil
Available phosphorus	4.6	
Available Potassium	258.91	
$CaCO_3$	201.00	g Kg^{-1} soil

2.1. Studied characteristics

2.1.1. Soluble and insoluble carbohydrates (%)

The determination of soluble or insoluble carbohydrates was conducted following the method outlined by Joslyn (1970). 0.2 g sample was mixed with 8.8 ml of 80% perchloric acid and incubated in a water bath at 60 °C for 30 minutes. After incubation, the mixture was filtered, centrifuged, and subjected to additional extractions. The final filtrate was then diluted to 50 ml, and a specific chemical reaction was performed to measure the optical absorbance at 490 nm, allowing for the calculation of reducing sugars based on a glucose standard calibration curve.

2.1.2. Estimation of the content of gibberellic acid (%)

To analyse the gibberellic content in leaves, a sample of 3 g of ground leaves is mixed with 20 ml of zinc acetate and allowed to sit for 5 minutes. Then, 20 ml of potassium ferrocyanide is added, and the mixture is centrifuged at 8000 rpm for 15 minutes. Afterwards, 5 ml of the supernatant is combined with 15 ml of 30% hydrochloric acid and incubated for 90 minutes, after which the absorbance is measured at 254 nm using a standard solution prepared from 10-50 μg mL^{-1} (Kaufman et al., 1976).

2.1.3. Estimation of medically active compounds in Stevia leaves

Estimation of the content of stevioside and rebaudioside A. in the leaves. The Percentage of each of the Stevioside and rebaudioside A. compounds was estimated in one of the laboratories of the Ministry of Science and Technology, using a high-performance liquid chromatography (HPLC) device, Agilent Model 1200, according to the method mentioned by Vaněk et al. (2001). The extraction and separation of stevioside and rebaudioside A. involved collecting and drying leaf samples at room temperature until a constant weight was achieved. The dried samples were ground and stored in sealed bags in a refrigerator until analysis. For the study, standard solutions of Stevioside and rebaudioside A. were used, and qualitative and quantitative detections were performed by comparing the retention times of the samples with those of the standard compounds (Table 2).

Table 2. The retention time of standard stevioside and rebaudioside A. compound under chromatographic separation conditions

Standard compound	Vessel detention time (min)
Stevioside	6.02
Rebaudioside A.	8.08

2.2. Statistical analysis

The measured data in three repetitions for the study indicators were taken and statistically analysed according to the design above using the Genstat program. The averages were compared using the least significant difference (LSD) test at a probability level of 0.05 (Al-Rawi and Khalaf Allah, 1980).

3. Results and Discussion

3.1. Carbohydrate content of leaves (%) in the first and second harvests

The results shown in Table 3 and Table 4 indicate the combined effect of *P. aeruginosa* bacteria, white Mushroom waste, and nano fertilizer on the total carbohydrate content of stevia leaves during the first and second harvests the addition of the bio-inoculant B1 was significantly superior giving the highest total carbohydrates value over the two harvests at 67.5% and 61.2 %, respectively, compared B0 control or reference treatment which gave values of 54 % and 45.9% over the same harvest periods. These bacteria stimulate an increase in the size of the root system, which is positively reflected in an increase in the biomass of the vegetative system. This is because the root system's ability to absorb water and nutrients includes nitrogen, phosphorus, potassium, magnesium, iron, copper, and manganese. Simultaneously, an increase in total carbohydrates in the vegetative system was also noted. An increase in the concentration of total carbohydrates is related to the improvement of Steviol glycoside group synthesis (Xu et al., 2023).

Results of the statistical analysis show that the study factor, which involves adding white mushroom waste at three levels (Ab0, Ab1, and Ab2), is significantly superior as the Ab2 level proves best by providing the highest total carbohydrate content in the leaves of the Stevia plant at both stages; it attained 67.0% and 61.2%, respectively, compared to the control treatment Ab0, which registered 52.5% and 44.4% in that order. This came as a result of adding white Mushroom waste and its role in improving the physical, chemical, and biological properties of the soil, and the resulting release of the most significant amount of nutrients ready for absorption by the roots and then affecting the physiological processes of the plant and the subsequent positive effect on the characteristics of vegetative growth. The ready nitrogen resulting from the Mushroom waste also enters into the composition of several organic compounds essential in the vital processes in the plant, as it enters into the composition of nucleic acids such as DNA and RNA and enters into the composition of enzymes and cytochromes necessary in the process of respiration and photosynthesis (Alves et al., 2022), while the ready phosphorus also resulting from white Mushroom waste is essential for several vital processes such as photosynthesis and carbohydrate building in plant leaves (Huang et al., 2011).

The statistical analysis data showed that the study factor included the addition of nano fertilizer at four levels (N0, N1, N2, N3), as the results shown in Table 5 and Table 6. It was during the two periods that the N3 level was proven to be significantly superior and that the highest rate of total carbohydrates in the leaves was recorded 65.2% and 57.7%, respectively, in comparison to N0, and the composition of other organic compounds. All these activities improve the plant's vegetative growth, represented by increasing the plant height, dry weight of the leaves, and the total yield, which is positively reflected in increasing the Percentage of carbohydrates in plant leaves (Zewail et al., 2021; Demir and Akıncı, 2022). The reason is that the elements of zinc and nano boron enter into the composition of the group of enzymes that participate in the construction process.

The results showed that the interaction between *P. aeruginosa* bacteria and white Mushroom waste resulted in a significant superiority in the total carbohydrate content of the leaves in the first and second harvests; both the dual treatment B1Ab2 registered the highest rate of total carbohydrates during the two periods at 71.6% and 65.6%, respectively compared to the comparison treatment B0Ab0 which gave 44.20% and 34.7%, respectively. The marked increase in percentage carbohydrate content of stevia plant leaves under the bacterial inoculation treatment supplemented with a part of the fertilizer recommendation for white mushroom waste is attributed to bacterial inoculation added with mushroom waste that establishes highly efficient and organized mineral element distribution both in the plant and the soil, further enhancing the availability of these nutrients in the soil and driving increased consumption of nutrients by the plant, positively influencing the total carbohydrate content of the plant leaves (Allu et al., 2014; Li et al., 2020). On the other hand, the interaction between *P. aeruginosa* bacteria and nano-fertilizer had a marked effect on the total carbohydrate rate because the dual treatment B1N3 reported the highest total rate of carbohydrates during the two harvests, which was 72.4% and 65.1%, respectively, as compared to the comparison treatment B0N0, which showed 48.4% and 40.2%, respectively. Physiological plant characteristics due to increased photosynthesis components resulting from the interaction of nano and bio fertilizers stimulate interest in enhancing dry weight, resulting from enhanced nutrient absorption by the plant from the soil, positively related to the plant's total carbohydrate contents (Allavi and Padmanabh, 2019).

Table 3 and Table 4 indicate that the dual interaction between white Mushroom waste and nano fertilizer exhibited significant superiority in the rate of total carbohydrates in the leaves since the Ab2N3 treatment even outperformed in the two harvests, whereby it recorded 70.2% and 64.6%, in comparison to the comparison treatment, which happened to record 48.9% and 40.2%, respectively. It is attributed to the favorable function of white mold residues in fostering the plant's green mass, and for equilibrium, there is an increase in root mass due to heightened ramifying of roots as well as root hair development; this becomes an increase in the root surface area whereby positive reflection is to be affected in increasing mineral element absorption by the plant and their accumulation in the leaves, positively affecting leaf contents of total carbohydrates (Li et al., 2023; Al-Hasnawi and Jarallah, 2024).

In addition to the function of zinc and nano-boron, the nutrient uptake plays a role in direct impact on the nutrient permeability and stability of the cell wall; it reflects positively on growth features and yield of Stevia, hence an increase in the content of carbohydrates in the leaves (Rabia et al., 2017). Results of the statistical analysis show that the triple interaction between the study factors displayed a significant superiority in leaf content of total carbohydrates, as the triple combination B1Ab1N3 proved to be superior during the two seasons and recorded 77.5% and 70.8%, respectively, compared to the check treatment, which recorded 40.7% and 30.2%, respectively.

This superiority resulted from adding the bacterial vaccine, which led to an increase in the readiness of these elements in the soil and its effect on the decomposition of the white Mushroom waste used which works to improve its physical, chemical, and fertility properties, such as increasing the cation exchange capacity and its work as a chelating substance that limits the loss and deposition of nutrients. In addition, it reduces the degree of soil reaction in the root zone while releasing ions. Hydrogen, various organic acids, and CO_2 gas, when the decomposed yield from (Kaya et al., 2019; Yu et al., 2021), which leads to an increase in the availability of elements in the soil or through the role of zinc and nano-boron in increasing the activation of the enzyme H+_ATPase in the cell membrane of cells as this enzyme increases the absorption of nutrients and their transfer and amino acids through wood and bark (Tahir et al., 2009), and the effect of bacteria used in increasing the activity of the root system and increasing the speed of root respiration and providing the energy necessary for the roots to actively absorb elements and thus increase the Percentage of carbohydrates in plant leaves. (Durairaj et al., 2017).

Table 3. The effect of *P. aeruginosa* bacteria, white Mushroom waste, and nano-fertilizer on the plant content of the Percentage of total carbohydrates in the leaves in the first harvest

		B0			**B1**
***P.aeruginosa* inoculation (B)**		54.0			67.5
LSD 0.05		0.9			
		Ab0		**Ab1**	**Ab2**
White mushroom waste (Tones h^{-1})		52.5		62.8	67.0
LSD 0.05		1.1			
		N0	**N1**	**N2**	**N3**
Nano fertilizer (kg h^{-1})		55.8	61.2	60.8	65.2
LSD 0.05		1.2			
Bilateral interaction between inoculation with *P.aeruginosa* and white Mushroom waste					
		Ab0		**Ab1**	**Ab2**
B0		44.20		55.6	62.3
B1		60.8		69.9	71.6
LSD 0.05		1.5			
Bilateral interaction between *P.aeruginosa* inoculation and nano fertilizer					
		N0	**N1**	**N2**	**N3**
B0		48.4	54.8	55.0	58.0
B1		63.2	67.5	66.6	72.4
LSD 0.05		1.8			
The dual interaction between white mushroom waste and nano fertilizer					
		N0	**N1**	**N2**	**N3**
Ab0		48.9	52.5	52.3	56.3
Ab1		56.5	63.0	62.5	69.2
Ab2		62.0	68.0	67.6	70.2
LSD 0.05		2.1			
Triple interaction between study factors					
		N0	**N1**	**N2**	**N3**
B0	**Ab0**	40.7	43.8	44.8	47.5
	Ab1	49.3	57.2	55.2	60.8
	Ab2	55.2	63.4	65.0	65.6
B1	**Ab0**	57.2	61.1	59.7	65.0
	Ab1	63.7	68.8	69.7	77.5
	Ab2	68.9	72.6	70.3	74.8
LSD 0.05		least significant difference 3.0			

Table 4. Effect of *P. aeruginosa* bacteria, white Mushroom waste, and nano fertilizer on the plant content of total carbohydrate percentage in leaves in the second harvest

		B0			**B1**
***P.aeruginosa* inoculation (B)**		45.9			61.2
LSD 0.05		1.0			
		Ab0		**Ab1**	**Ab2**
White mushroom waste (Tones h^{-1})		44.4		54.9	61.2
LSD 0.05		1.2			
		N0	**N1**	**N2**	**N3**
Nano fertilizer (kg h^{-1})		48.2	54.0	54.2	57.7
LSD 0.05		1.4			
Bilateral interaction between inoculation with *P.aeruginosa* and white Mushroom waste					
		Ab0		**Ab1**	**Ab2**
B0		34.7		46.1	56.8
B1		54.2		63.6	65.6
LSD 0.05		1.7			
Bilateral interaction between *P.aeruginosa* inoculation and nano fertilizer					
		N0	**N1**	**N2**	**N3**
B0		39.3	46.7	47.2	50.2
B1		57.1	61.3	61.2	65.1
LSD 0.05		2.0			
The dual interaction between white mushroom waste and nano fertilizer					
		N0	**N1**	**N2**	**N3**
Ab0		40.2	44.7	45.5	47.4
Ab1		48.4	55.8	54.4	61.0
Ab2		56.1	61.4	62.7	64.6
LSD 0.05		2.5			
Triple interaction between study factors					
		N0	**N1**	**N2**	**N3**
B0	**Ab0**	30.2	34.4	36.5	37.7
	Ab1	39.2	48.8	45.4	51.2
	Ab2	48.5	56.9	59.9	61.8
B1	**Ab0**	50.1	55.0	54.5	57.1
	Ab1	57.5	62.7	63.5	70.8
	Ab2	63.6	66.0	65.5	67.4
LSD 0.05		least significant difference 3.5			

3.2. Effect of *P. aeruginosa* bacteria, white mushroom waste, and nano fertilizer on gibberellins in leaves (%) in the first and second harvests

The results in Table 5 and Table 6 showed the study of the combined effect of *P. aeruginosa* bacteria, white Mushroom waste, and nano fertilizer on the gibberellins content of stevia leaves (%) in the first and second harvests, as the two tables show that the addition of the bio-inoculum B1 was significantly superior and recorded the highest Percentage of gibberellins, Attaining 16.82% and 18.96%, respectively, as opposed to the comparison treatment B0, which marked 13.02% and 16.43%, respectively. The soil-inoculated bacteria *P. aeruginosa* are instrumental in enhancing nitrogen levels and conversion to amino acids and compounds aiding tissue formation in the plant; subsequently, it enhances plant growth and boosts the vegetative mass, positively manifested by growth features, yield, and leaf content of gibberellin (Stover et al., 2000; Dimkić et al., 2022).

White Mushroom waste addition at its three levels, Ab0, Ab1, and Ab2, caused a significantly superior gibberellin leaf content in the two gardens, with level Ab2 being the best by giving the highest Percentage of gibberellin, reaching 17.43% and 20.49%, respectively, compared to the comparison treatment Ab0, which showed 11.77% and 13.95%, respectively. This is because of the function of white mushroom waste in reducing pH since it comprises organic acids responsible for increasing nutrient availability in the soil; this works to enhance their absorption by the roots, hence leading to superiority in both growth and yield traits of the plant, and lessening the element deficiency stress on which related to an increase in the content of gibberellin on the leaves (Tay et al., 2011; Marques et al., 2014).

The statistical analysis data also indicated a significant effect of applying the nano-fertilizer that contains four levels expressing the content of stevia leaves' gibberellin (%) in the two vines. The best superiority was for level N3 during the two vines. The percentages were 16.47% and 19.26%, respectively, compared to the control treatment N0, which gave 12.68% and 15.39%, respectively. This is because Zinc and Nano-boron help make photosynthesis more efficient and increase the output stored as dry matter in plant parts. All of this will have a good effect on how well photosynthesis works and will help raise the production of this process, along with dry matter, total yield, and sweetener concentration. It will also boost the Percentage of gibberellin in stevia plant leaves. (Sarma and Sarma, 2017).

The gibberellin content in leaves was significantly increased due to the interaction between *P. aeruginosa and white Mushroom waste, resulting in a high percentage of superiority* in leaves. The double treatment B1Ab2 gave the maximum value for the first and second cuts, 19.33% and 21.29%, respectively, compared to the check treatment B0Ab0, which showed 10.02% and 12.41%, respectively. This is because the white Mushroom waste is rich in many major and minor nutrients, and the addition of *P. aeruginosa* into the soil helps in decomposing the white Mushroom waste and also contributes to nutrient decomposition; hence, leaf dry weight increased, and carbon metabolism was encouraged, leading to more synthesis from photosynthesis and their subsequent accumulation in the plant. This promoted increased gibberellin content in plant leaves (Medina et al., 2009).

On the contrary, in the interaction between *P. aeruginosa* bacteria and nano-fertilizer, a significant contribution was shown in the content of gibberellin in the leaves of Stevia. Dual treatment B1N3 brought about the maximum Percentage in two plants, which was 18.53 and 20.62%, respectively, compared to the check treatment B0N0, which gave 11.65% and 14.43%, respectively. This could be attributed to the impact of zinc and nano-boron on many of the essential activities and their principal roles in stimulating vegetative growth. One of the most critical processes is the biosynthesis of auxins, including the hormone (IAA) that directs the development of seedlings and growing plant tips by its contribution to the synthesis of (Tryptophan), and then the effect of zinc on nitrogen metabolism and photosynthesis (Cierech et al., 2018).

In addition to the essential role played by added *P. aeruginosa* bacteria in promoting soil properties via nutrient solubilization, this became clear, especially with the increased vegetative growth where it resulted in more increase both in the length of the plant and in the Percentage of carbohydrates in the leaves as well as the Percentage of dry matter in the leaves, also with an increase in the gibberellin content in the leaves of the plant (Sarma and Saikia, 2014). Results further revealed that dual interactions of white Mushroom waste x nano fertilizer caused significant superiority in the percentage rate of gibberellin in the leaves, as Ab2N3 treatment turned out better in the two harvests with percentages of 19.23% and 21.90%, as against the comparison treatment that gave 10.07% and 12.00%, respectively.

This is because white mushroom waste improves the soil composition, balances its aeration, reduces the pH, and increases the available amount of water and mineral elements that the plant can obtain from the appropriate amount of nutrients necessary for its growth (Moon et al., 2012).

In addition to the significant role played by nano zinc and boron in many vital functions, including stimulating several enzymes, including the enzyme found in chloroplasts and acting as a regulator of the cell's pH, thus preventing the change like proteins, encouraging the work of the growth regulator, stimulating the formation of cytochrome, and the role of zinc in building proteins, vitamins, and some enzymes, and its significant association with the element nitrogen. These critical roles of zinc and boron were positively reflected in the gibberellin content in the leaves (Zhang et al., 2011).

The statistically analysed data in the tables show that the triple interaction between the study factors indicated a significant superiority in the Percentage of gibberellin in the leaves, as the triple combination B1Ab2N3 was superior in the two genotypes. It was 20.88% and 22.86%, respectively, compared to the check treatment, which was 8.79% and 10.13%, respectively. This is because, through their roles, nano zinc and boron enhance the efficiency of bioinoculation with the bacterium *P.aeruginosa*. This process enhances nitrogen fixation, major and minor nutrient availability, and chelating compounds. It also boosts plant hormones for growth regulation and the maintenance of soil fertility while safeguarding the plant against environmental stress conditions. This also includes white Mushroom waste decomposition, further increasing the plant's availability and uptake of major and minor elements. Such activities directly enhance the gibberellin content of the plant. (Goldbach and Wimmer, 2007; Cierech et al., 2018; Khan et al., 2022; Al-Khalidi and Al-Taweel, 2024;).

Table 5. The effect of *P. aeruginosa* bacteria, white Mushroom waste, and nano fertilizer on the plant's gibberellin content in the first harvest

		B0		**B1**	
***P.aeruginosa* inoculation (B)**		13.02		16.82	
LSD 0.05		0.19			
White mushroom waste		**Ab0**	**Ab1**		**Ab2**
(Tones h^{-1})		11.77	15.56		17.43
LSD 0.05		0.23			
		N0	**N1**	**N2**	**N3**
Nano fertilizer (kg h^{-1})		12.98	15.02	15.22	16.47
LSD 0.05		0.26			
Bilateral interaction between inoculation with *P.aeruginosa* and white Mushroom waste					
		Ab0	**Ab1**		**Ab2**
B0		10.02	13.52		15.53
B1		13.53	17.60		19.33
LSD 0.05		0.32			
Bilateral interaction between *P.aeruginosa* inoculation and nano fertilizer					
		N0	**N1**	**N2**	**N3**
B0		11.65	13.07	12.98	14.41
B1		14.32	16.97	17.47	18.53
LSD 0.05		0.37			
The dual interaction between white mushroom waste and nano fertilizer					
		N0	**N1**	**N2**	**N3**
Ab0		10.07	11.89	12.09	13.05
Ab1		13.32	15.77	16.03	17.13
Ab2		15.56	17.39	17.55	19.23
LSD 0.05		0.45			
Triple interaction between study factors					
		N0	**N1**	**N2**	**N3**
B0	**Ab0**	8.79	9.73	10.39	11.17
	Ab1	12.14	14.04	13.42	14.47
	Ab2	14.00	15.45	15.12	17.57
B1	**Ab0**	11.34	14.06	13.80	14.92
	Ab1	14.49	17.50	18.63	19.79
	Ab2	17.12	19.34	19.99	20.88
LSD 0.05		least significant difference 0.64			

Table 6. Effect of *P. aeruginosa*, white Mushroom waste, and nanofertilizer on plant gibberellin content in the second harvest

		B0		**B1**	
***P.aeruginosa* inoculation (B)**		16.43		18.96	
LSD 0.05			0.30		
White mushroom waste (Tones h^{-1})		**Ab0**	**Ab1**		**Ab2**
		13.95	18.65		20.49
LSD 0.05			0.37		
Nano fertilizer (kg h^{-1})		**N0**	**N1**	**N2**	**N3**
		15.39	18.12	18.02	19.26
LSD 0.05			0.42		
Bilateral interaction between inoculation with *P.aeruginosa* and white Mushroom waste					
		Ab0	**Ab1**		**Ab2**
B0		12.41	17.21		19.68
B1		15.49	20.10		21.29
LSD 0.05			0.52		
Bilateral interaction between *P.aeruginosa* inoculation and nano fertilizer					
		N0	**N1**	**N2**	**N3**
B0		14.43	16.92	16.47	17.90
B1		16.34	19.32	19.57	20.62
LSD 0.05			0.60		
The dual interaction between white mushroom waste and nano fertilizer					
		N0	**N1**	**N2**	**N3**
Ab0		12.00	14.42	13.93	15.45
Ab1		15.55	19.21	19.41	20.43
Ab2		18.61	20.72	20.72	21.90
LSD 0.05			0.73		
Triple interaction between study factors					
		N0	**N1**	**N2**	**N3**
B0	**Ab0**	10.13	13.01	12.67	13.81
	Ab1	14.70	17.78	17.39	18.96
	Ab2	18.47	19.95	19.35	20.93
B1	**Ab0**	13.86	15.83	15.18	17.09
	Ab1	16.39	20.64	21.43	21.91
	Ab2	18.75	21.48	22.08	22.86
LSD 0.05			least significant difference 1.03		

3.3. Effect of *P. aeruginosa* bacteria, white Mushroom waste, and nano fertilizer on the content of Stevioside sweeteners (%) in the leaves in the first and second harvests

The results of statistical analysis in Table 7 and Table 8 showed a study of the joint effect of *P. aeruginosa, white Mushroom waste, and nano fertilis*er on stevioside content of leaves in the first and second harvest. The results inferred that the application of bio-inoculate B1 was significantly better in both the harvests and expressed the highest Percentage of stevioside sweetness, 7.894% and 8.783%, respectively, as compared to the reference treatment B0, giving 7.212% and 7.996%, respectively. A marked increase in the Stevioside compound secondary metabolites is probably due to the relationship of this compound with the exudates released by the bacteria used, which may release hormones and some biologically active molecules produced by other microorganisms in the soil, which act as stimulants that stimulate the production of the Stevioside compound (Jha et al., 2009).

The statistical analysis results revealed the marked superiority of the study factor, which is composed of white mushroom waste addition at three levels (Ab0, Ab1, Ab2), with the highest Percentage of Stevioside sweeteners throughout the two seasons. The levels reached 8.359% and 9.294%, respectively, as opposed to the control Ab0, which was 6.571% and 7.269%, respectively. White mushroom waste positively affects plant growth-promoting bacteria and enhances anti-pathogens in soil. As for the plant, the impact of white mushroom waste is mainly nutritional since it contains micro- and macronutrients; its action is visible as that of a simple fertiliser, besides various functions represented by its hormonal effects, which constitute one of the primary reasons for the efficiency of biostimulation in the plant group. This leads to more nitrogen, an essential element that goes into the composition of proteins, enzymes, enzyme cofactors, nucleic acids, and phytochromes. It, therefore, plays a critical role in the biochemical pathways needed by the Stevia plant for the synthesis of the Stevioside compound (Ma et al., 2021).

The statistical analysis data indicated that the study factor comprised the addition of nano fertilizer at four levels (N0, N1, N3, N4), as presented by the results in Tables 13 and 14, which illustrate that the N3 level manifested remarkable superiority throughout the two harvests and noted 8.088% and 8.985%, respectively, compared to the comparison treatment N0, which reflected 6.964% and 7.696%, respectively. This is due to the role of zinc and boron in the photosynthesis process through their ability to increase membrane permeability and facilitate the absorption of nitrogen and iron. These two compounds enhance plastid formation and photosynthesis. Thus, the stevia leaves' content of carbohydrates increases total carbohydrates, thus increasing the content of Stevia leaves in Stevioside sweeteners (Ghasem et al., 2018).

The interaction between the *P. aeruginosa* bacteria and the white Mushroom residues resulted in markedly higher percentages of Stevioside sweeteners in the leaves of the stevia plant in the first and second harvests since the dual treatment B1Ab2 attained the maximum Percentage of Stevioside sweeteners in the two harvests, which were 8.785% and 9.773%, respectively, as compared to the check treatment B0Ab0, which gave 6.294% and 6.940%, respectively. The reason for the superiority is the white Mushroom residues. They play a significant role in many physiological processes in the plant due to their provision of some amino compounds for plant growth, which ultimately leads to enhanced growth of root hairs and the spread of the root system in the soil (Joniec et al., 2022). In addition, bacteria play a significant role in the increased effectiveness of carbon metabolism and the building of carbohydrates and sugars. Their contribution to the accumulation of nutrients in the leaves and transporting them to the other parts of the plant, the plant will excel in all growth and yield characteristics, which is positively reflected in the content of plant leaves from sweeteners like Stevioside (Gupta et al., 2006).

Conversely, the interaction between *P .aeruginosa* bacteria and nano-fertilizer showed a notable influence on the Percentage of Stevioside sweeteners in the leaves of the stevia plant, where the dual treatment B1N3 attained the maximum Percentage of sweeteners during the two harvests, which were 8.622% and 9.569%, respectively, as opposed to the comparison treatment B0N0, which yielded 6.654% and 7.335%, respectively. This is because *P. aeruginosa* bacteria increased the availability of primary elements as well as the use of micro-nanoparticles such as zinc and boron, which are involved in the process of food production, respiration, and protoplasmic construction, as they are involved in the composition of DNA and RNA, which are necessary for cell division, in addition to the manufacture of hormones and auxins, which are essential in the division and elongation of cells in the interstitial tissues of the stem, thus increasing the height of the plant and increasing the vegetative mass and the plant's content of total carbohydrates, which was positively reflected in increasing the Percentage of Stevioside sweeteners in the leaves (Silva et al., 2010; Javid et al., 2017). Table 7 and Table 8 show that the binary interaction between white Mushroom waste and nano fertilizer resulted in a significant increase in the Percentage of Stevioside sweeteners in Stevia plant leaves, specifically in the Ab2N3 treatment. Outperformed. The two plants. 9.076% and 10.069%. Comparison treatment. 5.880% and 6.369%. This is the fact that white Mushroom waste, Zinc nano boron. Important in the plant life cycle. Zinc-responsible hormone. Indole Acetic Acid (IAA). Necessary for cell elongation. This works total plant carbohydrate content Reflected Percentage. Stevioside sweeteners Stevia Leaves (Agrawal et al., 2017). The data in Table 7 and Table 8 show that the triple interaction between the study factors revealed superiority significantly in the percent of Stevioside in Stevia leaves since the triple combination B1Ab1N3 proved to be superior with the two genes having 9.925 and 10.964%, respectively compared to the check treatment, which resulted in 5.335 and 5.716%, respectively since Zn is essential in the amino acid Tryptophan synthesis (source of the hormone Indoleacetic Acid for cell elongation). Apart from their function in synthesising many metabolic and storage compounds, they relate to cell growth and division and the formation of new cells, thereby increasing Stevioside content in the leaves (Zafar et al., 2016). It can also be noted the role of white Mushroom waste in stimulation the bacteria added into the soil to supply the plant with its needs, which was reflected by the plant vegetative, where this led to an increase in photosynthesis, hence an increase in Stevioside content in the leaves due to the movement of nutrients to the leaves (Hood et al., 2011; Rajavat et al., 2022).

Table 7. The effect of *P. aeruginosa* bacteria, white Mushroom waste, and nano fertilizer on the content of Stevioside in the leaves in the first harvest

		B0			**B1**
***P.aeruginosa* inoculation (B)**		7.212			7.894
LSD 0.05			0.005		
		Ab0		**Ab1**	**Ab2**
White mushroom waste (Tones h^{-1})		6.571		7.729	8.359
LSD 0.05			0.006		
		N0	**N1**	**N2**	**N3**
Nano fertilizer (kg h^{-1})		6.964	7.562	7.598	8.088
LSD 0.05			0.007		
Bilateral interaction between inoculation with *P.aeruginosa* and white Mushroom waste					
		Ab0		**Ab1**	**Ab2**
B0		6.294		7.408	7.933
B1		6.848		8.051	8.785
LSD 0.05			0.009		
Bilateral interaction between *P.aeruginosa* inoculation and nano fertilizer					
		N0	**N1**	**N2**	**N3**
B0		6.654	7.367	7.272	7.555
B1		7.274	7.757	7.925	8.622
LSD 0.05			0.010		
The dual interaction between white mushroom waste and nano fertilizer					
		N0	**N1**	**N2**	**N3**
Ab0		5.880	6.790	6.672	6.943
Ab1		7.174	7.650	7.847	8.246
Ab2		7.840	8.246	8.276	9.076
LSD 0.05			0.013		
Triple interaction between study factors					
		N0	**N1**	**N2**	**N3**
B0	**Ab0**	5.335	6.608	6.514	6.721
	Ab1	7.066	7.477	7.373	7.716
	Ab2	7.563	8.016	7.928	8.226
B1	**Ab0**	6.425	6.972	6.829	7.164
	Ab1	7.281	7.824	8.321	8.776
	Ab2	8.116	8.476	8.624	9.925
LSD 0.05			least significant difference 0.018		

Table 8. Effect of *P. aeruginosa*, white Mushroom waste, and nano fertilizer on leaf content of Stevioside in the second harvest

***P.aeruginosa* inoculation (B)**		**B0**			**B1**
		7.996			8.783
LSD 0.05			0.005		
White mushroom waste (Tones h^{-1})		**Ab0**		**Ab1**	**Ab2**
		7.269		8.606	9.294
LSD 0.05			0.006		
Nano fertilizer (kg h^{-1})		**N0**	**N1**	**N2**	**N3**
		7.696	8.419	8.458	8.985
LSD 0.05			0.007		
Bilateral interaction between inoculation with *P.aeruginosa* and white Mushroom waste					
		Ab0		**Ab1**	**Ab2**
B0		6.940		8.234	8.815
B1		7.597		8.978	9.773
LSD 0.05			0.009		
Bilateral interaction between *P.aeruginosa* inoculation and nano fertilizer					
		N0	**N1**	**N2**	**N3**
B0		7.335	8.193	8.056	8.401
B1		8.058	8.644	8.859	9.569
LSD 0.05			0.010		
The dual interaction between white mushroom waste and nano fertilizer					
		N0	**N1**	**N2**	**N3**
Ab0		6.369	7.569	7.397	7.740
Ab1		7.994	8.509	8.774	9.147
Ab2		8.726	9.178	9.203	10.069
LSD 0.05			0.012		
Triple interaction between study factors					
		N0	**N1**	**N2**	**N3**
B0	**Ab0**	5.716	7.366	7.167	7.513
	Ab1	7.868	8.333	8.220	8.517
	Ab2	8.421	8.881	8.783	9.173
B1	**Ab0**	7.021	7.773	7.628	7.967
	Ab1	8.121	8.686	9.327	9.777
	Ab2	9.031	9.475	9.623	10.964
LSD 0.05			least significant difference 0.017		

3.4. Effect of *P. aeruginosa* bacteria, white mushroom waste, and nano fertilizer on the content of Rebaudioside A. sweeteners in the leaves in the first and second harvests

The Table 9 and Table 10 show a study of the interaction between *P. aeruginosa*, white Mushroom waste, and nano fertilizer on the content of Rebaudioside A. sweeteners in the leaves of the Stevia plant (%) for the first and second harvests, as the results illustrate that the addition of the bio-inoculate B1 was significantly better and gave the highest Percentage of Rebaudioside A. sweeteners in the leaves of the Stevia plant, 4.699% and 5.180%, respectively. These were compared with the control treatment B0, which gave 3.918% and 4.649%, respectively. The addition of *P. aeruginosa* bacteria contributed to the formation of a large and strong root system through the bio-exchange processes of nutrients, thus increasing the absorption of nutrients by the plant, which led to an increase in the plant height and improved growth and yield characteristics, which was positively reflected in the content of Rebaudioside A. in the leaves(Wu et al., 2011). The results of the statistical analysis showed the significant superiority of the study factor that includes the addition of white Mushroom waste at three levels (Ab0, Ab1, and Ab2), as the level Ab2 outperformed by giving the highest Percentage of Rebaudioside A. during the two periods, reaching 5.156 and 6.218%, respectively, compared to the comparison treatment Ab0, which recorded 3.303 and 3.582%, respectively. This may be attributed to the white Mushroom residues' effect in increasing the nutrient concentration in the leaves, primarily N and iron, and reducing both chlorine and sodium, since they play an effective role in pushing the plant towards vegetative growth, especially nitrogen, and increasing the photosynthesis process. This, in turn, leads to an increase in the resulting organic compounds and thus increases the plant's Rebaudioside A. content (Wu et al., 2020).

Data analysis showed that it included the study factor to add nano-fertilizer at four levels, which are (N0, N1, N2, and N3); as demonstrated by the results in Table 9 and Table 10, level N3 was significantly higher during the two harvests with percentage recording 4.680% and 5.427%, respectively compared to the comparison treatment N0, which recorded 3.852% and 4.312%, respectively. Perhaps this happens due to the role of nano zinc in activating the physiological functions of the plant and working to increase the biomass of the plant through cell division, elongation, and increasing its size, thus increasing the primary and secondary metabolic compounds, including the local Rebaudioside A., in addition to the positive effect of nano boron, as it works to improve plant growth and increase the yield through its impact on the mechanics of many critical vital processes in the plant such as cell respiration, water and nutrient absorption, photosynthesis, and protein building (Moezzi et al., 2012).

The interaction among the *P.aeruginosa* bacteria and white Mushroom residues resulted in a considerable enhancement of the amount of the Rebaudioside A. sweeteners in the leaves of the stevia plant for the first and second harvests since the dual treatment B1Ab2 had the highest Percentage at 6.139 and 6.909%, respectively, as compared to the check treatment B0Ab0 which gave 2.868% and 3.126%, respectively. The increased content of Rebaudioside A. in the leaves attributed to the impact of white Mushroom residues on activating many essential activities and stimulating the bacteria provided with the soil in addition to that attributed to increasing nutrient availability due to its role and increasing the ability of the plant to absorb the nutrients, thus increasing the accumulated synthesized materials by the plant represented in carbohydrates and metabolic compounds with positive reflection on the content of Rebaudioside A. in the leaves of the stevia plant (Pandey et al., 2012; Kumar et al., 2013; Lal et al., 2021).

On the other hand, the interaction between *P. aeruginosa* bacteria and nano-fertilizer had a significant effect on the content of Rebaudioside A. in the leaves, as the dual treatment B1N3 recorded the highest Percentage of Rebaudioside A. in the leaves, reaching 5.338 and 5.828%, respectively, compared to the comparison treatment B0N0, which recorded 3.462 and 4.067%, respectively. The reason may be that the white Mushroom waste increases the development and accumulation of sugars, enzymes, and amino acids, as its effect is indirect through the contribution of microorganisms present in the soil to its decomposition, in addition to the role of bacteria added as a bio-inoculum in increasing the decomposition of these wastes and the release of major and minor elements and increasing the possibility of their absorption by the plant, which is reflected in growing the leaf content of Rebaudioside A. (Adesemoye and Vgoji, 2009; Nair et al., 2009; Uzair et al., 2018).

Results obtained proved that the dual interaction between white Mushroom waste and nano-fertilizer led to a significantly higher percentage rate of Rebaudioside A in the leaves, wherein the

Ab2N3 treatment outperformed the two plants, with percentage content values of 5.964 and 7.028 as compared to the comparison treatment, recording 2.552 and 2.747%, respectively. It is attributed to zinc and nano-boron impacts on vital activities due to their influence on the activity of numerous enzymes and the contribution of meristematic cells and their division to raise the height of the plant and improve its growth and yield characteristics. These seem to be positively reflected in De Beradis et al. (2010) leaf contents of Rebaudioside A. The probable mode of action of white Mushroom waste is giving the plant primary and secondary nutrients that have an apparent effect on many vital and physiological processes and on which many enzymes act in the enhancement of production of food, so they activate cell division and elongation, and thus the increased content of Rebaudioside A. in the plant (Prakash et al., 2014; Dai et al., 2017).

Data from Table 9 and Table 10 show that there was a highly significant triple interaction of the study factors in both trials, indicating superiority in increasing the Percentage of Rebaudioside A, where the triple combination B1Ab1N3 outperformed, giving 7.637 and 8.386%, respectively, as compared to the check treatment giving 1.534 and 1.686%. The reason may be due to the role of the nano zinc and boron elements in the movement and division of cells, as a result of the ability of its molecules to enter the cellular channel, and make the cell membrane more permeable, which contributes to the plant's ability to absorb nutrients (Baek and An, 2011), in addition to the role of white Mushroom waste in stimulating the bacteria added to the soil to biodegrade them, which contributed to providing essential elements, improving cell division, cell elongation, and increasing the permeability of cell membranes, which facilitates the movement of nutrients that may have a direct effect on various vital processes. These include the growth of the vegetative group and the increase in the accumulation of carbohydrates and processed nutrients, thus increasing the leaf content of Rebaudioside A. (Jonathan et al., 2011; Wang et al., 2021; Al-Jubouri and Al-Taweel, 2024).

Table 9. The effect of *P. aeruginosa* bacteria, white Mushroom waste, and nano fertilizer on the leaf content of Rebaudioside A. in the first harvest

		B0			**B1**
***P. aeruginosa* inoculation (B)**		3.918			4.699
LSD 0.05			0.094		
White mushroom waste (Tones h^{-1})		**Ab0**	**Ab1**		**Ab2**
		3.303	4.467		5.156
LSD 0.05			0.115		
		N0	**N1**	**N2**	**N3**
Nano fertilizer (kg h^{-1})		3.852	4.191	4.511	4.680
LSD 0.05			0.133		
Bilateral interaction between inoculation with *P.aeruginosa* and white Mushroom waste					
		Ab0	**Ab1**		**Ab2**
B0		2.868	4.713		4.173
B1		3.739	4.220		6.139
LSD 0.05			0.163		
Bilateral interaction between *P.aeruginosa* inoculation and nano fertilizer					
		N0	**N1**	**N2**	**N3**
B0		3.462	3.947	4.242	4.022
B1		4.243	4.436	4.781	5.338
LSD 0.05			0.188		
The dual interaction between white mushroom waste and nano fertilizer					
		N0	**N1**	**N2**	**N3**
Ab0		2.552	3.216	3.669	3.777
Ab1		4.369	4.560	4.639	4.298
Ab2		4.636	4.798	5.227	5.964
LSD 0.05			0.231		
Triple interaction between study factors					
		N0	**N1**	**N2**	**N3**
	Ab0	1.534	2.643	3.606	3.688
B0	**Ab1**	4.826	4.991	4.949	4.087
	Ab2	4.025	4.206	4.171	4.290
	Ab0	3.570	3.789	3.732	3.866
B1	**Ab1**	3.913	4.130	4.328	4.510
	Ab2	5.247	5.390	6.283	7.637
LSD 0.05			least significant difference 0.326		

Table 10. Effect of *P. aeruginosa*, white Mushroom waste, and nano fertilizer on leaf content of Rebaudioside A. in the second harvest

		B0		**B1**	
***P.aeruginosa* inoculation (B)**		4.649		5.180	
LSD 0.05			0.004		
White mushroom waste (Tones h^{-1})		**Ab0**	**Ab1**		**Ab2**
		3.582	4.942		6.218
LSD 0.05			0.005		
		N0	**N1**	**N2**	**N3**
Nano fertilizer (kg h^{-1})		4.312	4.868	5.050	5.427
LSD 0.05			0.006		
Bilateral interaction between inoculation with *P.aeruginosa* and white Mushroom waste					
		Ab0	**Ab1**		**Ab2**
B0		3.126	5.294		5.526
B1		4.039	4.591		6.909
LSD 0.05			0.007		
Bilateral interaction between *P.aeruginosa* inoculation and nano fertilizer					
		N0	N1	N2	N3
B0		4.067	4.611	4.889	5.027
B1		4.556	5.124	5.210	5.828
LSD 0.05			0.008		
The dual interaction between white mushroom waste and nano fertilizer					
		N0	**N1**	**N2**	**N3**
Ab0		2.747	3.528	3.959	4.096
Ab1		4.698	4.904	5.009	5.159
Ab2		5.490	6.171	6.181	7.028
LSD 0.05			0.010		
Triple interaction between study factors					
		N0	**N1**	**N2**	**N3**
B0	**Ab0**	1.686	2.946	3.867	4.005
	Ab1	5.147	5.333	5.288	5.407
	Ab2	5.368	5.553	5.513	5.669
B1	**Ab0**	3.808	4.109	4.051	4.187
	Ab1	4.248	4.474	4.731	4.911
	Ab2	5.612	6.790	6.849	8.386
LSD 0.05			least significant difference 0.014		

Conclusion

We conclude from the study that the triple combination B1Ab1N3 had a significant superiority in most of the studied traits, which included the average total carbohydrate content of leaves during the first and second harvests. In contrast, the triple combination B1Ab2N3 had the highest content of gibberellins and sweeteners Stevioside and Rebaudioside A. in the leaves of the stevia plant in the two harvests.

Based on the scientific evidence derived from the study results, the triple treatment B1Ab1N3 can be recommended as an effective strategy for increasing the total carbohydrate content in stevia leaves during the first and second harvests, which positively impacts the nutritional and functional value of the plant. The data also show that the triple treatment B1Ab2N3 contributes to an increase in the accumulation of gibberellins and natural sweeteners such as stevioside and rebaudioside A, which are critical bioactive compounds used in the manufacture of low-calorie sweeteners. This opens up promising economic prospects in light of the growing global demand for natural sugar substitutes.

In addition, the results emphasise the importance of integrating these treatments with sustainable agricultural techniques, such as drip irrigation and the use of agricultural wastes, such as mushroom farm residues, as soil conditioners, to enhance crop productivity and reduce reliance on harmful chemical fertilizers. It is also recommended to conduct large-scale field experiments in different environments and seasons to verify the effectiveness of these treatments under varying climatic and agricultural conditions. Finally, it is proposed to intensify future studies to investigate the physiological and biochemical mechanisms that link the increase in carbohydrates in leaves and the stimulation of the production of steviol glycosides, which will contribute to the development of programs to improve stevia production at the local and global levels, especially in countries suffering from water scarcity and deteriorating soil fertility, such as Iraq.

Ethical Statement

I PhD student Zahraa Jassim Al-Budairy, from Al-Qadisiyah University, Faculty of Agriculture, Department of Soil and Water Sciences, Iraq, confirm that this research was conducted through my own personal efforts, adhering to the principles of integrity and scientific honesty, and in accordance with the ethical standards followed in scientific research.

All steps of the study from collecting soil samples, to fieldwork in all its stages, to collecting plant samples for two consecutive seasons, then laboratory work, to collecting, analyzing, and interpreting data were carried out with the utmost precision. There was no tampering or distortion of the results in any aspect of the study. The sources and references used were scientifically documented. This research did not use any materials, organisms, or data in violation of laws or ethical principles. It also did not violate the rights of any entity or individual. I certify that this work is original and has not been previously submitted to any academic or research institution for publication or evaluation, and that all the results and conclusions contained therein reflect my own scientific efforts and research.

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It is acknowledged that all costs involved in conducting the research, analysing the data, and preparing the results were borne solely by the researcher, ensuring complete independence and academic impartiality at all stages of the research process.

Author Contributions

PhD student Zahraa Jassim Al-Budairy worked on this research at all stages, from experimental design, sample collection, field and laboratory work, data analysis, writing and discussing results, and finally preparing the final draft. Professor Luma Salih Al-Taweel provided general academic supervision, providing academic and methodological guidance throughout the various research phases.

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References

Abou-Arab, A. E., Abou-Arab, A. A., & Abu-Salem, M. F. (2010). Physico-chemical assessment of the natural sweeteners stevioside produced from Stevia rebaudiana Bertoni plant. *African Journal of Food Science*, *4*(5), 269–281. https://doi.org/10.21608/jfds.2009.115819.

Adesemoye, A. O., & Ugoji, E. O. (2009). Evaluating *P. aeruginosa* as plant growth-promoting rhizobacteria in West Africa. *Archives of Phytopathology and Plant Protection*, *42*, 188–200. https://doi.org/10.1080/03235400601014791

Agarwal, H., Kumar, S. V., & Rajeshkumar, S. (2017). A review on green synthesis of zinc oxide nanoparticles–An eco-friendly approach. Resource-*Efficient Technologies*, *3*(4), 406–413. https://doi.org/10.1016/j.reffit.2017.03.002.

Agrawal, S., Rathore, P., & Singh, R. P. (2017). Effect of zinc and boron application on growth, hormone regulation (IAA), carbohydrate metabolism and stevioside content of Stevia rebaudiana Bertoni. *Journal of Plant Nutrition, 40*(18), 2565–2576. https://doi.org/10.1080/01904167. 2017.1346685

Al-Hasnawi, A. H., & Jarallah, R. Sh. (2024). Effect of sorghum and sunflower rhizosphere soil and the fertilization type on the total and active carbonate minerals percentage. *AIP Conference Proceedings*. https://doi.org/10.1063/5.0202219.

Al-Jubouri, E. A. K., & Al-Taweel, L. S. J. (2024). Zeolite, mineral fertilizer and humic acid impact on biomass carbon in soil. *AIP Conference Proceedings*, 3079, 020026. https://doi.org/10.1063/5.0207604.

Al-Khafaji, M. A. (2014). Plant Growth Regulators: *Application and Utilizations in Horticulture*. Bookstore for Printing, Publishing and Translating, University of Baghdad. https://raqaoaj.net/iasj/download/c1b1b798883aa919.

Al-Khalidi, R. J. H., & Al-Taweel, L. S. (2024). Effect of plant extracts and humic acid on soil ammonium content and *Nitrosomonas* numbers in potato-grown soil. *IOP Conference Series: Earth and Environmental Science*. https://doi.org/10.1088/1755-1315/1371/8/082009.

Al-Kubaisi, Q., Al-Dabbas, M., & Al-Khafaji, B. (2013). Assessment of water quality in Iraq: Current status and future perspectives. *Journal of Environmental and Public Health, 2013*, 1–12. https://pmc.ncbi.nlm.nih.gov/articles/PMC3698740

Allavi, S., & Padmanabh, D. (2019). Micronutrients zinc and boron enhance stevioside content in *Stevia rebaudiana* plants while maintaining genetic fidelity. *Industrial Crops and Products*, *140*, 111646. https://doi.org/10.1016/j.indcrop.2019.111646

Allu, S., Kumar, N.P., Audipudi, A.V. (2014). Isolation, Biochemical and PGP characterization of endophytic Pseudomonas aeruginosa isolated from chilli red fruit antagonistic against chilli anthracnose disease. *Int. J. Microb. Appl. Sci. 2014,* 3, 318–329.

Al-Rawi, Khashe' Mahmoud and Abdul Aziz Mohammed Khalaf Allah. (1980). Design and Analysis of Agricultural Experiments. Ministry of Higher Education and Scientific Research. Dar Al-Kutub for Printing and Publishing. University of Mosul.

Alves, L.D.S., Caitano, C.E.C., Ferrari, S., Vieira Júnior, W.G., Heinrichs, R., De Almeida Moreira, B.R., Pardo-Giménez, A., (2022). Zied, D.C. Application of Spent Sun Mushroom Substrate in Substitution of Synthetic Fertilizers at Maize Topdressing. *Agronomy, 12*, 2884.

Baek, Y. W., & An, Y. J. (2011). Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO) to Escherichia coli, Bacillus subtilis, and Streptococcus aureus. *Science of the Total Environment, 409*(8), 1603–1608. https://doi.org/10.1016/j.scitotenv.2011.01.014

Cierech, M., Kolenda, A., Wojnarowicz, J., Szmigiel, D., Koltsov, I., Tyszkiewicz, R., & Kurzydłowski, K. J. (2018). Zinc and boron nanoparticles as factors improving the growth and physiological activity of plants. *Nanoscale Research Letters, 13*(1), 178. https://doi.org/10.1186/s11671-018-2589-8.

Çığ, F., & Sönmez, İ. (2020). Utilization of mushroom compost waste as an organic fertilizer and its effects on soil fertility and plant nutrient content. *Yuzuncu Yil University Journal of Agricultural Sciences*, *30*(2), 312–320. https://dergipark.org.tr/tr/pub/yyutbd

Dai, J., Chen, B., & Xu, W. (2017). Recycling of spent mushroom substrate as organic fertilizer enhances soil fertility and plant growth. *Waste and Biomass Valorization, 8*(5), 1495–1503. https://doi.org/10.1007/s12649-016-9664-8.

De Berardis, F., Marconi, G., & Iannucci, A. (2010). Effects of micronutrients on growth, enzymatic activity, and rebaudioside A accumulation in Stevia rebaudiana Bertoni. *Journal of Plant Nutrition*, *33*(10), 1501–1514.https://doi.org/10.1080/01904167.2010.489586

Demir, Z., & Akıncı, Ş. (2022). The impact of nano-fertilizers on growth parameters and biochemical constituents of medicinal and aromatic plants. *Yuzuncu Yil University Journal of Agricultural Sciences*, *32*(1), 85–96. https://dergipark.org.tr/tr/pub/yyutbd

Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., & Fira, D. (2022). Plant growth-promoting rhizobacteria (PGPR): Mechanisms of action and application in sustainable agriculture. *Microorganisms*, *10*(6), 1173. https://doi.org/10.3390/microorganisms10061173

Durairaj, C., Velmurugan, P., Dhamodharan, R., Rajalakshmi, G., Kalaichelvan, P. T., & Lee, K. J. (2017). Effect of plant growth-promoting rhizobacteria on growth, physiology, and biochemistry of plants under stress conditions. *Journal of Applied and Natural Science, 9*(1), 294–303. https://doi.org/10.31018/jans.v9i1.1158.

Fanin N., Mooshammer M., Sauvadet M., Meng C., Alvarez G., Bernard L., Bertrand I., Blagodatskaya E., Bon L., Fontaine S., et al. (2022).Soil enzymes in response to climate warming: Mechanisms and feedbacks. *Funct. Ecol., 36*, 1378–1395.

Gehlout, S., Priyam, A., Afonso, L., Schultze, G.A., & Singh, P.P. (2022). Application of metallic nanoparticles as agri inputs: Modulation in nanoparticle design and application dosage needed. In Nanotechnology in Agriculture and Environmental Science, Deshmukh, S.K., Kochar, M., Kaur, P., Singh, P.P., Eds., *Taylor & Francis Group*: Boca Raton, FL, USA,, pp. 16–54.

Ghasem, A., Abdolamir, H., & Saeid, M. (2018). Effect of zinc and boron on growth, chlorophyll content, and stevioside accumulation in Stevia rebaudiana Bertoni. *Journal of Plant Nutrition, 41*(14), 1843–1851. https://doi.org/10.1080/01904167.2018.1463382.

Goldbach, H. E., & Wimmer, M. A. (2007). Boron in plants and animals: Is there a role beyond cell-wall structure? *Journal of Plant Nutrition and Soil Science*, *170*(1), 39–48. https://doi.org/10.1002/jpln.200625161

Gupta, R., Sharma, S., & Sood, A. (2006). Plant growth-promoting rhizobacteria influence carbon metabolism, carbohydrate accumulation, and stevioside content in Stevia rebaudiana Bertoni. *Plant Growth Regulation*, *48*(1), 61–69. https://doi.org/10.1007/s10725-005-5134-2

Hood, R. C., Bruns, M. A., & Lentz, R. D. (2011). Utilization of mushroom compost and its effect on soil microbial activity and plant growth. *Compost Science & Utilization, 19*(3), 152–160. https://doi.org/10.1080/1065657X.2011.10737002.

Huang, W., Liu, J., Zhou, G., Zhang, D., & Deng, Q. (2011). Effects of precipitation on soil acid phosphatase activity in three successional forests in southern China. *Biogeosciences, 8*, 1901–1910.

Javid, M. G., Sorooshzadeh, A., Moradi, F., Modarres Sanavy, S. A. M., & Allahdadi, I. (2017). The role of zinc and boron in plant growth, carbohydrate accumulation and yield formation. *Journal of Plant Nutrition*, *40*(1), 1–13.

Jha, P. N., Gupta, G., Jha, A. K., & Mehrotra, R. (2009). Association of plant growth-promoting rhizobacteria with Stevia rebaudiana Bertoni and their effect on plant growth and stevioside production. *Applied Soil Ecology*, *42*(3), 222–230.https://doi.org/10.1016/j.apsoil.2009.03.007

Jonathan, S. G., Lawal, M. M., & Oyetunji, O. J. (2011). Effect of spent mushroom compost of *Pleurotus pulmonarius* on growth performance of four Nigerian vegetables. *Mycobiology*, *39*(3), 164–169. https://doi.org/10.5941/MYCO.2011.39.3.164

Joniec, J., Kwiatkowska, E., & Kwiatkowski, C. A. (2022). Assessment of the effects of soil fertilization with spent mushroom substrate in the context of microbial nitrogen transformations and the potential risk of exacerbating the greenhouse effect. *Agriculture*, *12*(8), 1190. https://doi.org/10.3390/agriculture12081190

Joslyn, M. A. (1970). *Physical, chemical, and instrumental methods of analysis*. In Methods in Food Analysis.

Karas, P., Metsoviti, A., Zisis, V., et al. (2015). Dissipation, metabolism and sorption of pesticides used in fruit-packaging plants: Towards an optimized depuration of their pesticide-contaminated agro-industrial effluents. *Science of the Total Environment*, *531*, 129–139. https://doi.org/10.1016/j.jhazmat.2016.07.071

Karpiński, T. M., Kłósek-Popielarczyk, M., Szkaradkiewicz-Karpińska, A., Mrozikiewicz, D. W., & Chudzińska-Popiel, J. (2025). Activity of antiseptics against *Pseudomonas aeruginosa* and its adaptation potential. *Antibiotics*, *14*(1), 30. https://doi.org/10.3390/antibiotics14010030

Kaufman, P. B., Ghosheh, N. S., & Nakosteen, L. (1976). Analysis of native gibberellins in the internode, nodes, leaves, and inflorescence of developing Avena plants. *Plant Physiology*, *58*(2), 131–134. https://doi.org/10.1104/pp.58.2.131

Kaya, M., Yıldırım, E., & Güvenç, İ. (2019). Effects of bio-fertilizers and organic amendments on growth, yield and nutrient uptake of medicinal plants. *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi*, 29(3), 462–471. https://dergipark.org.tr/tr/pub/yyutbd

Khan, M. S., Gao, J., Zhang, M., Xue, J., & Zhang, X. (2022). *Pseudomonas aeruginosa* Ld-08 isolated from *Lilium davidii* exhibits antifungal and growth-promoting properties. *PLOS ONE*, *17*(6), e0269640. https://doi.org/10.1371/journal.pone.0269640

Kumar, A., Munder, A., Aravind, R., Eapen, S. J., Tümmler, B., & Raaijmakers, J. M. (2013). Friend or foe: Genetic and functional characterization of plant endophytic *Pseudomonas aeruginosa*. *Environmental Microbiology*, *15*(3), 764–779. https://doi.org/10.1111/1462-2920.12031

Lal, R., Bouma, J., Brevik, E., Dawson, L., Field, D. J., Glaser, B., Hatano, R., Hartemink, A. E., Kosaki, T., Lascelles, B., et al. (2021). Soils and sustainable development goals of the United Nations: An International Union of Soil Sciences perspective. *Geoderma Regional*, *25*, e00398. https://doi.org/10.1016/j.geodrs.2021.e00398

Lemus-Mondaca, R., Vega-Gálvez, A., Zura-Bravo, L., & Ah-Hen, K. (2012). Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on biochemical, nutritional and functional aspects. *Food Chemistry, 132*(3), 1121–1132. https://doi.org/10.1016/j.foodchem.2011.11.140

Li, C., Lan, W., Jin, Z., Lu, S., Du, J., Wang, X., Chen, Y., & Hu, X. (2023). Risk of heavy metal contamination in vegetables fertilized with mushroom residues and swine manure. *Sustainability*, *15*(10), 10984. https://doi.org/10.3390/su151410984

Li, F., Kong, Q., Zhang, Q., Wang, H., Wang, L., & Luo, T. (2020). Spent mushroom substrates affect soil humus composition, microbial biomass and functional diversity in paddy fields. *Applied Soil Ecology*, *149*, 103489. https://doi.org/10.1016/j.apsoil.2019.103489

Ma, Z., Zhang, Y.-Q., Wang, L.-J., Hu, G.-L., Gong, X.-Q., Bai, Q., Su, S.-C., & Qi, J.-X. (2021). Short-term effects of spent mushroom substrate mulching thickness on the soil environment, weed suppression, leaf nutrients, and nut characteristics in a hazelnut orchard. *Agronomy*, *11*(6), 1122. https://doi.org/10.3390/agronomy11061122

Maisuria, H. J., Dhaduk, H. L., Kumar, S., Sakure, A. A., & Thounaojam, A. S. (2022). Teak population structure and genetic diversity in Gujarat, India. *Current Plant Biology, 32*, 100267. https://doi.org/10.1016/j.cpb.2022.100267.

Mandal, S., Evelin, H., Giri, B., Singh, V. P., & Kapoor, R. (2013). Arbuscular mycorrhiza enhances the production of stevioside and Rebaudioside A.-A in *Stevia rebaudiana* via nutritional and non-nutritional mechanisms. *Applied Soil Ecology*, *72*, 187–194. https://doi.org/10.1016/j.apsoil.2013.07.003

Marques, E. L. S., Martos, E. T., Souza, R. J., Silva, R., Zied, D. C., & Souza Dias, E. (2014). Spent mushroom compost as a substrate for the production of lettuce seedlings. *Journal of Agricultural Science*, *6*(7), 138–143. https://doi.org/10.5539/jas.v6n7p138

Medina, E., Paredes, C., Pérez-Murcia, M. D., Bustamante, M. A., & Moral, R. (2009). Spent mushroom substrates as component of growing media for germination and growth of horticultural plants. *Bioresource Technology*, *100*(18), 4227–4232. https://doi.org/10.1016/j.biortech.2009.03.055

Moezzi, A., McDonagh, A. M., & Cortie, M. B. (2012). Zinc oxide particles: synthesis, properties and applications. *Chemical Engineering Journal*, 185, 1–22. https://doi.org/10.1016/j.cej.2012.01.076

Mohamed, K., & El-Sayed, Y. (2014). Nutritional and health value of natural and artificial sweeteners used as sugar substitutes. *Assiut Journal of Environmental Studies*, *39*. https://doi.org/10.1002/efd2.70028

Moon, Y. H., Shin, P. G., & Cho, S. J. (2012). Feeding value of spent mushroom (Pleurotus eryngii) substrate. *Journal of Mushroom Science, 10*(4), 236–243. https://doi.org/10.1002/9781119149446.ch20

Nair, S., Sasidharan, A., Rani, V. D., Menon, D., Nair, S., Manzoor, K., & Raina, S. (2009). Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. *Journal of Materials Science*: *Materials in Medicine, 20*(1), 235–241. https://doi.org/10.1007/s10856-008-3548-5

Pandey, P. K., Yadav, S. K., Singh, A., Sarma, B. K., Mishra, A., & Singh, H. B. (2012). Cross-species alleviation of biotic and abiotic stresses by the endophyte *Pseudomonas aeruginosa* PW09. *Journal of Phytopathology*, *160*(10), 532–539. https://doi.org/10.1111/j.1439-0434.2012.01941.x

Prakash, I., Markosyan, A., & Bunders, C. (2014). Development of next generation stevia sweetener: Rebaudioside A. M. *Foods*, *3*(1), 162–175. https://doi.org/10.3390/foods3010162

Rabia, J., Muhammad, U., Buhara, Y., Muhammad, Z., & Ekrem, G. (2017). Effect of zinc oxide (ZnO) nanoparticles on physiology and steviol glycosides production in micropropagated shoots of Stevia rebaudiana Bertoni. *Plant Physiology and Biochemistry*, *110*, 94–99. https://doi.org/10.1016/j.plaphy.2016.05.032

Rajavat, A. S., Mageshwaran, V., Bharadwaj, A., Tripathi, S., & Pandiyan, K. (2022). Spent mushroom waste: An emerging bio-fertilizer for improving soil health and plant productivity. *In New and Future Developments in Microbial Biotechnology and Bioengineering* (pp. 345–354). Elsevier. https://doi.org/10.1016/B978-0-323-85579-2.00010-1

Sarma, B., & Sarma, B. K. (2017). Fabrication of Ag/ZnO heterostructure and the role of surface coverage of ZnO microrods by Ag nanoparticles on the photophysical and photocatalytic properties of the metal-semiconductor system. *Applied Surface Science*, *410,* 557–565. https://doi.org/10.1016/j.apsusc.2017.03.154

Sarma, R. K., & Saikia, R. (2014). Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. *Plant and Soil*, *377*(1), 111–126. https://doi.org/10.1007/s11104-013-1981-9

Shin, H. J., Ro, H. S., Kawauchi, M., et al. (2025). Review on mushroom mycelium-based products and their production process: From upstream to downstream. *Bioresources and Bioprocessing*, *12*, 3. https://doi.org/10.1186/s40643-024-00836-7

Silva, S. N. R. L., Farias, C. B. B., Rufino, R. D., Luna, J. M., & Sarubbo, L. A. (2010). Glycerol as substrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP0992. *Colloids and Surfaces B: Biointerfaces*, *79*(1), 174–183. https://doi.org/10.1016/j.colsurfb.2010.03.050

Stover, C. K., Pham, X. Q., Erwin, A., Mizoguchi, S., Warrener, P., Hickey, M., Brinkman, F., Hufnagle, W., Kowalik, D., & Lagrou, M. (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature*, *406*(6799), 959–964. https://doi.org/10.1038/35023079

Taha, S.A. (2018). Effect of organic fertilizer addition and spraying seaweed extract on some growth characters, yield and active ingredient of arugula plant (*Eruca sativa* Mill). *Journal Tikrit Unv. For Agri. Sci.*, *18*(1), 2018.

Tahir, S. T., Hussain, M., Usman, S. K., Hasanain, A., & Mumtaz, A. (2009). Effect of vanadium doping on structural, magnetic and optical properties of ZnO nanoparticles. *Applied Surface Science*, *255*(20), 8506–8510. https://doi.org/10.1016/j.apsusc.2009.06.003

Taipei, R. O. C. (2005). Symptoms of micronutrient deficiency in crops, and how to remedy them. *AGNET*, Taipei, Taiwan. E-mail: ffic@agnet.org.

Tay, C. C., Liew, H. H., Redzwan, G., Yong, S. K., Surif, S., & Abdul-Talib, S. (2011). Pleurotus ostreatus spent mushroom compost as green biosorbent for nickel (II) biosorption. *Water Science and Technology*, *64*(12), 2425–2432. https://doi.org/10.4028/www.scientific.net/amr.781-784.636

The National News. (2023). Iraq will only be able to meet 15% of its water needs by 2035, UN official warns. https://www.thenationalnews.com/mena/iraq/2023/05/18/iraq-will-only-be-able-to-meet-15-of-its-water-needs-by-2035-un-official-warns

Uzair, B., Kausar, R., Bano, S. A., Fatima, S., Badshah, M., Habiba, U., & Fasim, F. (2018). Isolation and molecular characterization of a model antagonistic Pseudomonas aeruginosa divulging in vitro plant growth promoting characteristics. *BioMed Research International*, *2018*, 6147380. https://doi.org/10.1155/2018/6147380

Vafadar, F., Amooaghaie, R., & Otroshy, M. (2014). Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *Journal of Plant Interactions*, *9*(1), 128–136. https://doi.org/10.1080/17429145.2013.779035

Vaněk, T., Nepovim, A., & Valicek, P. (2001). Determination of stevioside in plant material and fruit teas. *Journal of Food Composition and Analysis*, *14*(4), 383–388. https://doi.org/10.1006/jfca.2000.0974

Wang, X., Zhou, X., Cai, Z., Guo, L., Chen, X., Chen, X., Liu, J., Feng, M., Qiu, Y., Zhang, Y., et al. (2021). A biocontrol strain of *Pseudomonas aeruginosa* CQ-40 promotes growth and controls Botrytis cinerea in tomato. *Pathogens*, *10*(1), 22. https://doi.org/10.3390/pathogens10010022

Wu, C. Y., Liang, C. H., & Liang, Z. C. (2020). Evaluation of using spent mushroom sawdust wastes for cultivation of *Auricularia polytricha. Agronomy*, *10*(12), 1892. https://doi.org/10.3390/agronomy10121892

Wu, D., Ye, J., Ou, H., Wei, X., Huang, X., He, Y., & Xu, Y. (2011). Genomic analysis and temperature-dependent transcriptome profiles of the rhizosphere originating strain *Pseudomonas aeruginosa* M18. *BMC Genomics*, *12*, 438. https://doi.org/10.1186/1471-2164-12-438

Xu, J., Yu, W., Luo, H., Shi, H., & Gu, L. (2023). Effects of chitosan and alginate oligosaccharides on growth and steviol glycosides biosynthesis in Stevia rebaudiana. *International Journal of Biological Macromolecules, 240,* 124376. https://doi.org/10.1016/j.ijbiomac.2023.124376.

Yu, H., Liu, P., Shan, W., Teng, Y., Rao, D., & Zou, L. (2021). Remediation potential of spent mushroom substrate on Cd pollution in a paddy soil. *Environmental Science and Pollution Research*, *28*(30), 36850–36860. https://doi.org/10.1007/s11356-021-13266-1

Zafar, H., Ali, A., Ali, J. S., Haq, I. U., & Zia, M. (2016). Effect of ZnO nanoparticles on Brassica nigra seedlings and stem explants: Growth dynamics and antioxidative response. *Frontiers in Plant Science*, *7*, 535. https://doi.org/10.3389/fpls.2016.00535

Zafar, S., Perveen, S., Khan, M. K., Shaheen, M. R., Hussain, R., Sarwar, N., et al. (2022). Effect of zinc nanoparticles seed priming and foliar application on the growth and physio-biochemical indices of spinach (*Spinacia oleracea* L.) under salt stress. *PLOS ONE*, *17*(1), e0263194. https://doi.org/10.1371/journal.pone.0263194

Zewail, R. M. Y., Ali, M., El-Gamal, I. S. H., Al-Maracy, S. H. A., Islam, K. R., Elsadek, M., Azab, E., Gobouri, A. A., ElNahhas, N., & Mohamed, M. H. M. (2021). Interactive effects of arbuscular mycorrhizal inoculation with nano boron, zinc, and molybdenum fertilization on stevioside contents of Stevia (*Stevia rebaudiana* L.) *plants. Horticulturae*, *7*(8), 260. https://doi.org/10.3390/horticulturae7080260

Zhang, J. (2011). Silver-coated zinc oxide nanoantibacterial synthesis and antibacterial activity characterization. In 2011 *International Conference on Electronics and Optoelectronics (ICEOE)* (Vol. 3, pp. V3-94–V3-97). IEEE. https://doi.org/10.1109/ICEOE.2011.6013309