Enhancing Sunflower Plant Growth with Biofertilizer, Triple Superphosphate, and Alfalfa Straw

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Abstract

The experiment was conduct in one of the private agricultural fields in Diwaniyah Governorate - Al-Daghara District during the autumn season of 2024. The study aimed to investigate the response of sunflower plant to Bacillus subtilis inoculum, alfalfa straw, triple superphosphate fertilizer and their interaction on dry plant weight, dry root weight, bacterial population density, growth hormone B1 and phosphorus element. The experiment conducted applied according to the split-plot system with an R.C.B.D. design, arranged in a factorial manner with three replicates. The results showed that the single biological bacterial inoculation, alfalfa straw, and triple superphosphate at levels of 100 kg ha-1 were effective. Achieved a significant increase in the following characteristics: plant phosphorus content (1.784, 2.137, and 1.821) mg P kg-1, as well as the bacterial population density (11.390, 11.482, and 11.461) CFU 10^6 g -1 dry soil. Dry plant weight (122.28, 119.66, 125.03) g plant -1, dry root weight (28.44, 29.71, 29.29) g plant-1. as well as increasing the concentration of growth hormone B1 (73.89, 75.09, 75.33) µg g -1 The combination of bacterial bio-inoculation with alfalfa straw and the combination of both with triple superphosphate showed a significant increase in the characteristics of plant phosphorus content (1.896, 1.858, 1.867)mg P kg -1 . The number density of bacteria (11.475, 11.580, 11.539) CFU 10^6 g-1 dry soil, dry plant weight (131.02, 127.57, 129.61) g plant-1. Dry root weight (30.68, 31.69, 32.14) g plant-1, as well as increased concentration of growth hormone B1 (76.18, 77.05, 76.30) µg g -1. The results of the triple interaction between bio-inoculation, alfalfa straw, and triple superphosphate showed an increase in the following characteristics: plant phosphorus content (1.954) mg P kg-1. number density of bacteria (11.710) CFU 10^6 g-1 dry soil, dry plant weight (135.75) g plant-1, dry root weight (35.75) g plant-1, as well as increased concentration of growth hormone B1 (78.75) µg g -1.

Keywords: alfalfa straw, Bacillus subtilis, Triple superphosphate fertilizer, sunflower crop.

1. Introduction

Bioinocula are a natural source for regulating crop growth and increasing leaf area and chlorophyll content (1). Phosphate-dissolving bacteria are highly energetic when added as a bio-inoculum, especially in soils with low phosphorus content. They also can reduce and control plant diseases and produce antibiotics (2). Bacillus subtilis have developed

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mechanisms to stimulate plant growth, as they increase the availability of nutrients. The most important of these mechanisms is responsible for increasing phosphorus in a form ready for absorption by the plant, by secreting organic acids in the soil that dissolve its inorganic compounds and thus increase the effectiveness of phosphate acids (organophosphate mineralization) (3). The balanced and readily available availability of phosphorus for plants, as explained by (4), occurs through several organisms in the soil, the most important of which are Bacillus subtilis, the breakdown of the bonds between calcium and precipitated phosphate results in an increase is the amount of liberated and available phosphorus by lowering the soil pH. Phosphorus plays a crucial role in processes like cell division plant growth, seed formation, as well as the transfer of genetic traits and the regulation of biological activities, in addition to the formation of energy-rich compounds, proteins, plant fats, phospholipids, phytin, cell proteins, mitochondrial membranes, chloroplasts, and transport compounds. Sunflowers are a major and important crop in Iraq due to their diverse uses, the most important of which is oil extraction for oil-bearing varieties, as well as their use as animal feed (5). The study aimed to investigate the effects of Bacillus subtilis inoculum, jet straw feedand the effect of triple superphosphate fertilizer on the population density of Bacillus subtilis inoculum and plant growth.

.2Material and Methods

The experiment carried out in a private agricultural field located in the Diwaniyah Governorate/Al-Daghara District, sunflowers planted during the fall season of 2024. The field is at approximately latitude 32.20 north and longitude 44.85 east. The field soil was prepared by carrying out the necessary field operations, which included plowing it well twice, smoothing and leveling the land and removing bushes and weeds to prepare it for planting sunflowers. Then, three main irrigation ditches dug along the field, including sub-irrigations for each experimental unit. There were 21 experimental units located within the three

main sections of the field. Each experimental unit consisted of five lines, with a spacing of 25 cm between Plants and 75 cm between rows. It is important to note that the experimental setup was (at least initially) carefully designed, with c. 36 × units (4 × 4 m² in size), each with a 1 m space between the units.

The experimental factors were as follows:

.1Factor 1: Bacillus subtilis, at two levels:

-B0. No inoculation with Bacillus subtilis.

-B1. Inoculation with Bacillus subtilis.

.2Factor 2: Alfalfa Straw, at two levels:

-M0. Level 1 (0 t Ha.(^{1} ◌̄

-M1. Level 2 (1 t Ha.(^{1} ◌̄

.3Factor 3: Triple superphosphate fertilizer, at three levels:

-P0. Level 1 (0 kg ha.(^{1} ◌̄

-P1. Level 2 (50 kg ha.(^{1} ◌̄

-P2. Level 3 (100 kg ha.(^{1} ◌̄

According to the Randomized Complete Block Design (R.C.B.D). Then random samples of the experimental soil taken before planting to conduct some physical, chemical, and biological analyses (Table No. 1). The seeds (variety ZAHRA F1) planted on 8/3/2024, and agricultural operations carried out to serve the crops by irrigating and weeding whenever necessary. The harvest carried out after the plants reached the stage of full maturity, with the appearance of signs of maturity on the plants represented by the falling of leaves and yellowing of the plant on 11/12/2024.

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Table 1. Chemical, physical and biological properties of the soil (before planting(

Traits	Values		Units
soil components	Clay	500	g kg soil^{-1}
	Sand	160	
	Silt	340	
Soil Texture	Clay soil		-
Bulk Density	1.31		g cm^{-3}
Organic Matter	2.5		g kg soil^{-1}
Soil pH	7.4		-
Electrical Conductivity (ECe)	2.6		Ds m^{-1}
CEC	12.70		Centimole charge kg^{-1} soil
Positive dissolved Ions	Na+	12.3	mmol L^{-1}
	Ca+	14.1	
	Mg+	12	
Negative dissolved Ions	CO_3^{-2}	Nill	mmol L^{-1}
	HCO^{3-}	53.6	
	SO_4^{-2}	19.01	
Available Ions	N	10.20	mg kg^{-1} soil
	P	9.01	
	K	114.03	
Biometric Estimates	Total bacteria	$1.4*10^6$	CfU g^{-1} dry soil
Microbial Density	R ieguminosarum	Nill	

.1-2Traits Studied

.1-1-2Phosphorus absorbed by plants: It were measured in plants by the soft digestion method using ammonium molybdate and ascorbic acid, and then its percentage is measured using a spectrophotometer at a wavelength of 882 nanometers according to the Olsen method and as stated in (6.(

.2-1-2Sampling Method: Ten sunflower discs randomly cut from the midribs of each experimental unit after reaching full maturity the discs were air-dried, and the seeds separated by hand. We then measured the seed number density, along with the dry weight of the vegetative and root systems. In addition, phosphorus concentration studied.

.3-1-2Total bacterial count CFU/gm-1 dry soil: It estimated according to (7) and the method of dilutions and plate counting used by taking 10 g of soil and dissolving it in 90 ml of distilled water and shaking it well. Then a series of dilutions of the soil suspension were prepared from 10-1 to 10-6 and Nutrient Agar used. The nutrient medium poured into prepared and sterilized Petri dishes and 1 ml of the 10-6 dilution taken in three replicates. The Petri dishes placed in the incubator for 5 days at 28°C. After incubation, the Colony Counter device, which used for counting bacteria, used and in this way, the total number of bacteria in 1 g of dry soil was calculated.

.4-1-2Dry weight of shoot and root system (g plant-1): It was dried in the oven at a temperature of 65°C until the weight was constant, then the weight of the dry green mass was calculated using a sensitive balance. After cutting the green mass, the root mass of the plant was taken and placed in paper bags, and the transaction codes were written on them, and Then it was dried in the oven at a temperature of 65°C until the weight was constant, then the weight of the dry root mass was calculated using a sensitive balance.

.5-1-2Extraction and determination of vitamins B1 (µg/g) isolated by taking 20g of each sample and drying it with a horn at 35°C. Then 10ml of 1% sodium hydroxide and 25ml of buffer solution (phosphate buffer) the samples were added and left in the dark for 24 hours. The sample filtered using filter paper and 50ml taken and placed in a centrifuge at 3000 rpm. The filtrate taken and stored in the refrigerator until analysis. Vitamin B1 (µg/g) determined by taking 3g of the sample and grinding it well using a ceramic mortar. Then it placed in a volumetric flask and 20ml of methanol added to it and placed in a shaker for 4 hours. After that, the sample was transfer to a centrifuge at 5000 rpm for 15 minutes. The filtrate was transferred to a new tube and the pH was adjusted to 9 using 1 M KH_2PO_4. The retention time used to diagnose the vehicles .

Table 2. Detection conditions for measured vitamins B1.

Name	Description
Transport Phase	Ethyl acetone: distilled water, formic acid 3, 47, 50
Separation Column	(4.6 mm×25 cm) C18:ODC
Detector	215UV
Flow Rate	1 ml/min

.2-2Statistical Analysis

The statistical analysis software Genestate utilized to analyze variance and used the randomized complete block design (RCBD) and the means of the treatments compared using the least significant difference (LSD) at a probability level of 0.05 (8 .(

.3Results and Discussion

.1-3The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on Phosphorus Concentration in the Vegetative Part (mgP kg-1(

The table (3) shows the application of bacterial inoculum B1 led to an increase in the concentration of available phosphorus in the plant, as it reached the highest average 1.784 mg P kg-1. In the flowering stage compared to the comparison treatment B0, which achieved the lowest average as it reached 1.538 mg P kg-1. This attributed to the role of phosphate-dissolving bacteria, as they dissolve and increase the availability of phosphorus through the mineralization processes of organic phosphorus in the soil by the plant. As well as the effect of microorganisms added to the soil, as they secrete organic acids and produce the phosphatase enzyme. Which works to increase the availability of phosphorus and its absorption by the plant (9). The concentration of available phosphorus in the plant significantly increased by the application of M1 as it reached the highest average 1.742 mg P kg-1 compared to the comparison treatment M0, Which achieved the lowest average. As reached 1.621 mg P kg-1 as time passes and nutrients consumed, phosphate considered a source to compensate for the current deficiency in elements, including available phosphorus in the plant, and it affects the conditions of nutrient preparation. The phosphorus concentration was improved significantly by the application of different levels of triple superphosphate (P) compared to the control. As it reached the highest average 1.692 and 1.821 mg P kg-1 compared with the control treatment, which achieved the lowest average, which reached 1.531 mg P kg-

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1, This is attributed to the fact that it increases the phosphorus concentration in the plant and the release of phosphorus. This led to an increase in plant growth, indicating the possibility of using it as a primary source of phosphorus, as it is available and its cost is suitable as an alternative to traditional fertilizers with high costs. This is consistent with (10). The binary interaction (BM) achieved the highest concentration of phosphorus in the plant, reaching the highest average 1.867 mg P kg-1. Due to the role of the bacterial inoculum as a factor that increases the absorption of nutrients, including phosphorus. The binary interaction (PB) achieved the highest concentration of available phosphorus in the plant, reaching 1.802 and 18.96 mg P kg-1. This were attributed to the positive role of the bacteria used, as they are characterized by a wide range of positive properties that stimulate plant growth and their ability to increase the solubility of phosphorus because of their secretion of organic and inorganic acids and converting unavailable forms into more available forms. Which

leads to an increase in the rate of phosphorus in the plant (11). The binary interaction (PM) achieved significant differences in the concentration of available phosphorus in the plant, reaching 1.784 and 1.858 mg P kg-1 due to the good interaction between jet and triple superphosphate. The results of the table confirmed that the triple interaction B1M1P1 led to a significant increase in the concentration of available phosphorus in the plant at the level of 100 kg/hectare, as it reached the highest average of available phosphorus in the plant, 1.954 mg P kg-1.

Table 3. The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on Phosphorus Concentration in the Vegetative Part (mgP kg-1(

M*B Interaction	**Mineral fertilizer levels (P)**			**Alfalfa residue (M)**	**Bio-fertilizer(B)**
	2	**1**	**0**		
1.540	1.704	1.551	1.365	M0	B0
1.617	1.789	1.614	1.448	M1	
1.701	1.865	1.650	1.588	M0	B1
1.867	1.927	1.954	1.721	M1	
0.043	0.075			Lsd 0.05	
P*B Interaction					
B Means	P			B	
1.578	1.746	1.583	1.407	B0	
1.784	1.896	1.802	1.655	B1	
0.031	0.053			Lsd 0.05	
P*M Interaction					
M Means	P			M	
1.621	1.785	1.600	1.477	M0	
1.742	1.858	1.784	1.584	M1	
0.031	0.053			Lsd 0.05	
	1.821	1.692	1.531	P means	
	0.038			Lsd 0.05	

.2-3

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The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on Bacterial population density per unit (CFU/10^6 g -1 dry soil(

The table (4) shows the application of bacterial inoculum B1 led to an increase in the population density of bacteria in the soil, as it reached the highest average 11.390 CFU 10^6 g -1 dry soil. Compared to the control treatment B0, which achieved the lowest average, as it reached 11.323 CFU 10^6 g -1 dry soil the reason for the increase were attributed to the organic and mineral root secretions, which are considered energy sources for the added bacteria, which contribute to increasing their reproduction numbers (12). The population density of bacteria in the soil was significantly increased. by the application of M1, as it reached the highest average 11.482 CFU 10^6 g -1 dry soil compared to the control treatment M0, which achieved the lowest average, as it reached 11.231 CFU 10^6 g -1 dry soil. This is due to providing suitable conditions for bacterial growth. And their numbers increased, the population density of bacteria in the soil was improved significantly by the application of different levels of triple superphosphate (P) compared to the control, as it reached 11.395 and 11.61 CFU 10^6 g -1 dry soil compared to the control treatment that achieved the lowest average of 11.214 CFU 10^6 g -1 dry soil. This is due to the phosphorus released from triple superphosphate over time, with the formation of a root group that contributes with its secretions to the adsorption of bacteria, in addition to being a source of energy in the soil. The binary interaction (BM) achieved the highest population density of bacteria in the soil, reaching 11.539 CFU 10^6 g -1 dry soil, as biofertilizers increase the plant's ability to absorb nutrients present in the soil. As explained by (13), it secretes types of enzymes and growth-stimulating hormones, and overall the number of bacteria of this type increases. The binary interaction (PB) achieved the highest number density of bacteria in the soil, reaching 11.475 and 11.467 CFU 10^6 g -1 dry soil. The reason for this were attributed to the role of phosphorus released from triple superphosphate in increasing the root system and its branches, which gives a larger surface area for adsorption of bacteria and the start of their biological activity. The binary interaction (PM) achieved significant differences in the number density of bacteria in the soil, reaching 11,580 and 11,570 CFU 10^6 g -1 dry soil. This is due to providing suitable conditions and a surface area for adsorption of bacteria, which reflected in enriching the soil content of bacteria. The results of the table confirmed that the triple interaction B1M1P1 led to a significant increase in the number density of bacteria in the soil when Level (100) kg/hectare, as it reached the highest numerical density of bacteria in the soil at the flowering stage, 11.710 CFU 10^6 g -1 dry soil.

Table 4. The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on Bacterial population density per unit (CFU/10^6 g -1 dry soil(

M*B Interaction	Mineral fertilizer levels (P)			Alfalfa residue (M)	Bio-fertilizer(B)
	2	1	0		
11.221	11.363	11.180	11.120	M0	B0
11.426	11.547	11.450	11.280	M1	
11.241	11.340	11.240	11.143	M0	B1
11.539	11.593	11.710	11.313	M1	
0.011	0.019			Lsd 0.05	
P*B Interaction					
B Means	P			B	

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11.323	11.455	11.315	11.200	B0
11.390	11.467	11.475	11.228	B1
0.008	0.013			Lsd 0.05
P*M Interaction				
M Means	P			M
11.231	11.352	11.210	11.132	M0
11.482	11.570	11.580	11.297	M1
0.008	0.013			Lsd 0.05
	11.461	11.395	11.214	P means
	0.010			Lsd 0.05

.3-3

The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on Dry Weight of the Vegetative Part (g/plant -1(

The table (5) shows that there was a significant effect of the study factors, as the highest average weight was reach with factor b1 was reach at the flowering stage 122.28 g/plant ,^{1} ◌ compared to the comparison factor 106.08 g/plant .^{1} ◌ This is due to the vital role of bacteria in nitrogen fixation and thus increasing the availability of nutrients that enter into the process of photosynthesis as well as entering into the formation of nucleic acids and amino acids, which increases the dry weight of the plant, as explained by (14). The net weight of the plant significantly increased by the application of M1, as it reached 119.66 g/plant ^{1} ◌ at the flowering stage, compared to the comparison factor, which reached 108.70 g/plant .^{1} ◌ This attributed to the general content of nutrients that help in plant growth and increase the overall weight after the drying process. the dry weight of the plant was improved significantly by the application of different levels of triple superphosphate (P) compared to the comparison coefficient, as it achieved averages of 113.63 and 125.03 g plant .^{1} ◌ For the addition levels (P1, P2) respectively, compared to the comparison coefficient (P0), which achieved the lowest average of 103.87 g/plant .^{1} ◌ The reason for the increase attributed to the high content of phosphorus that is releas by the bacterial vaccine and is consider one of the growth stimulants that increase the overall average of dry weight. Compared to the comparison factor, as it achieved averages, the increase was attributed to the high content of phosphorus released by the bacterial inoculum and is considered one of the growth stimulants that increases the overall average of dry weight. The binary interaction (BM) achieved a significant increase in the dry weight of the plant, as it reached the highest average of 129.61 g/plant ,^{1} ◌ compared to the comparison interaction that achieved the lowest average of 102.45 g/plant .^{1} ◌ The binary interaction between the bacterial inoculum and the residues considered a distinctive model in terms of preparing the activating elements for the bacterial organisms, then the organisms prepare the nutritional elements, including phosphorus, and thus it were positively reflected on the overall average of dry weight, the binary interaction (PB) achieved the highest average increase. Significant difference in the dry plant weight trait at (P2) as it reached 132.02 g/plant ^{1} ◌ because microorganisms are responsible for the formation of growth stimulating substances such as (Indol acetic acid) which follows (Phytohormones) in addition to enzymes and hormones such as (Auxin, Cytokinins) which work to increase growth as explained by (15). And it is noted that the interaction of the two factors has a prominent relationship in stimulating growth, as explained by (16). The binary interaction (PM) achieved the highest average significant

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increase in the dry plant weight trait at (P2) as it reached 127.57 g/plant .^{1} ¯ And is attributed to the fact that the phosphorus released in a ready form with the additional rate of the second factor is considered an essential element in accelerating growth and thus increasing the general percentage of dry weight. The results of the table confirmed that the triple interaction B1M1P1 led to a significant increase in the dry plant weight trait at the level of (100) kg ^{1} ¯ as it reached The highest average was 135.75 g/plant ,^{1} ¯ compared to the comparison factor B0M0P0, which achieved the lowest average, which reached (90.59) g/ plant.^{1} ¯

Table 5. The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on Dry weight of the vegetative part (g/plant - 1(

M*B Interaction	Mineral fertilizer levels (P)			Alfalfa residue (M)	Bio-fertilizer (B)
	2	1	0		
102.45	115.27	101.48	90.59	M0	B0
109.72	122.83	107.02	99.29	M1	
114.95	129.72	110.27	104.86	M0	B1
129.61	132.32	135.75	120.75	M1	
3.16	5.48			Lsd 0.05	
P*B Interaction					
B Means	P			B	
106.08	119.05	104.25	94.94	B0	
122.28	131.02	123.01	112.81	B1	
2.24	3.87			Lsd 0.05	
P*M Interaction					
M Means	P			M	
108.70	122.49	105.88	97.73	M0	
119.66	127.57	121.39	110.02	M1	
2.24	3.87			Lsd 0.05	
	125.03	113.63	103.87	P means	
	2.74			Lsd 0.05	

.4-3

The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on Dry root Weight (g/plant -1(

The table (6) shows that there was a significant effect of the study factors. The highest average weight with b1 factor was at the flowering stage 28.44 g/plant ,^{1} ¯ Compared to the comparison factor 24.88 g/plant .^{1} ¯ This increase is due to the high content of phosphorus that released by the bacterial inoculum from the used wastes and is consider one of the growth stimulants, that increases the overall average of dry root weight and reflected in the continuous root growth, and ends with an increase in its overall mass. Dry root weight significantly increased by the application of M1, as it reached 29.71 g/plant ,^{1} ¯ at the flowering stage compared to the comparison factor, which reached 23.82 g/plant .^{1} ¯ the dry root weight was improved significantly by the application of different levels of triple superphosphate (P) compared to the

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comparison factor, as it achieved averages of 27.60 and 29.29 g/plant .[1] For the addition levels (P1, P2), respectively, compared to the comparison factor. (P0) which achieved the lowest average as it reached 23.09 g/plant .[1] The reason for the increase were attributed to the release of ready phosphorus, which supports the plant with a main source for growth with other major elements whose direct effect on increasing the volume masses was observed. The binary interaction (BM) achieved a significant increase in the dry root weight trait as it reached the highest average 32.14 g/plant .[1] Compared to the comparison factor, which achieved the lowest average as it reached 22.48 g/plant .[1] This increase is due to the high content of phosphorus, which were releas by the bacterial vaccine from the used waste and is consider one of the growth stimulants, which increases the general average of the root weight. The binary interaction (PB) achieved the highest average significant increase in the dry root weight trait at (P2) as it reached 30.68 g/plant .[1] The microorganisms are responsible for the formation of growth stimulating materials such as (Indol acetic acid) which follows (Phytohormones) in addition to enzymes and hormones such as (Auxin, Cytokinins) that work to increase growth as explained by (15) and it is also noted that the interaction of the two factors has a significant relationship in stimulating growth as in (16). The binary interaction (PM) achieved the highest average significant increase in the dry root weight trait at (P1) as it reached 31.69 g/plant .[1] In addition, attributed to the fixed effect of the released phosphorus on plant growth and thus an increase in the size of the leaves, stem and roots of the plant and an increase in the dry weight of the roots. The results of the table confirmed that the triple interaction B1M1P1 led to a significant increase in the dry root weight trait at the level of (100) kg ,[1] as it reached the highest average 35.75 g/plant ,[1] compared to the comparison factor B0M0P0 as it achieved the lowest average as it reached 19.73 g/plant.[1]

Table 6. The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on dry root weight (g/plant - 1(

M*B Interaction	**Mineral fertilizer levels (P)**			**Alfalfa residue (M)**	**Bio-fertilizer (B)**
	2	**1**	**0**		
22.48	25.53	22.19	19.73	M0	B0
27.28	30.28	27.63	23.94	M1	
24.75	29.33	24.83	20.09	M0	B1
32.14	32.03	35.75	28.62	M1	
1.43	2.47			Lsd 0.05	
P*B Interaction					
B Means	P			B	
24.88	27.90	24.91	21.83	B0	
28.44	30.68	30.29	24.36	B1	
1.01	1.75			Lsd 0.05	
P*M Interaction					
M Means	P			M	
23.62	27.43	23.51	19.91	M0	
29.71	31.15	31.69	26.28	M1	
1.01	1.75			Lsd 0.05	
	29.29	27.60	23.09	P means	
	1.23			Lsd 0.05	

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.5-3

the Effect of Biofertilization and Triple Superphosphate and alfalfa straw on Growth Hormone B1 Concentrations

The table (7) shows the application of bacterial inoculum increased the vitamin B1 concentration. The highest concentration was 73.89 µg g-1, compared to the control treatment B0, which had the lowest average of 70.73 µg g-1. These bacterial species produce hormones and secrete vitamins directly or indirectly, thereby affecting crop growth and production (17). The application of alfalfa straw increased the vitamin B1 concentration. The highest concentration was 75.09 µg g-1, compared to the control treatment, with the lowest average of 69.53 µg g-1. This attributed to the fact that the alfalfa straw contains nutrients that stimulate the regeneration of the rhizosphere, thus producing significant amounts of growth hormones (18). Application of triple superphosphate increased the concentration of absorbed vitamin B1. The concentration of vitamin B1 increased at the P1 and P2 levels by 72.62 and 75.33 µg g-1, compared to the control, which produced the lowest average, 68.99 µg g-1. This is because it stimulates an increase for vitamin B1 absorbed by stimulating plant growth and increasing vitamin content (19). The binary interaction (BM) resulted in the highest nitrogen concentration in the soil. The vitamin B1 increased to 76.30 µg g-1. Bacterial inocula play a direct role in stimulating growth hormones and directly influence the process of preparing vitamin B1 for absorption and stimulation of the preparation of secondary nutrients (18). The double intervention (PB) achieved the highest concentration of absorbed vitamin B1 in the soil, 76.18 and 74.41 µg g-1. This were attributed to the stimulation of organisms by released phosphorus, which helps activate organisms, thus interacting with them to produce various vitamins (20) and (21). Double-intervention (PM) significantly increased vitamin B1 concentration values, 77.05 and 76.48 µg g-1. This is due to the beneficial interaction

between the alfalfa stow and triple superphosphate in the role of nutrients, including phosphorus released from the two factors, and its role in plant growth, photosynthesis, and subsequent renewal of binary elements (22). The results of the table confirmed that the triple interaction B1M1P1 led to a significant increase in the concentration of available vitamin B1 in the soil at the level of 100 kg/hectare, as it reached the highest average 78.75 µg g-1.

Table 7. The Effect of Biofertilizer, Alfalfa Straw and Triple Superphosphate on Growth Hormone B1 Concentrations

M*B Interaction	Mineral fertilizer levels (P)			Alfalfa residue (M)	Bio-fertilizer (B)
	2	1	0		
67.59	72.43	67.42	62.92	M0	B0
73.88	76.53	74.21	70.89	M1	
71.47	74.79	70.08	69.55	M0	B1
76.30	77.57	78.75	72.59	M1	
1.02	1.76			Lsd 0.05	
P*B Interaction					

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B Means	P			B
70.73	74.48	70.81	66.91	B0
73.89	76.18	74.41	71.07	B1
0.72	1.25			Lsd 0.05
P*M Interaction				
M Means	P			M
69.53	73.61	68.75	66.23	M0
75.09	77.05	76.48	71.74	M1
0.72	1.25			Lsd 0.05
	75.33	72.62	68.99	P means
	0.88			Lsd 0.05

Conclusion

The combination of bacterial bio-inoculation with alfalfa straw and the combination of both with triple superphosphate showed a significant increase in the characteristics of plant phosphorus content (1.896, 1.858, and 1.867) mg P kg -1. The number density of bacteria (11.475, 11.580, 11.539) CFU 10^{6} g -1 dry soil, dry plant weight (131.02, 127.57, 129.61) g plant -1, dry root weight (30.68, 31.69, 32.14) g plant -1. As well as increased concentration of growth hormone B1 (76.18, 77.05, 76.30) µg g -1. The results of the triple interaction between bio-inoculation, alfalfa straw and triple superphosphate showed an increase in the following characteristics: plant phosphorus content (1.954) mg P kg -1, number density of bacteria (11.710) CFU 10^{6} g -1 dry soil. Dry plant weight (135.75) g plant -1, dry root weight (35.75) g plant -1, Also increased concentration of growth hormone B1 (78.75) µg g -1.

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