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Efficacy of NPK nano fertilizer and bio-bacterial fertilizer on weight and chlorophyll of two cultivars of fenugreek (Trigonella foenum-graceum L.)

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***Abstract*---**A field experiment was conducted in accordance with Completely Random Block Design (CRBD) and three repeats at the University of Baghdad/ College of Education for Pure Sciences (Ibn Al-Haitham) during 2020-2021 to evaluate the effect of nano NPK nano-fertilizer with concentrations (0.5, 0.7, 1 $ml.L^{-1}$) with control treatment (without any addition) and second treatment a combination of bacterial fertilizer; *Bacillus mucilaginosus*, *Bacillus megaterium*, *Rhizobium* and *Azotobacter chroococcum* with two concentrations (10^7,10^9) C.F.U gm^{-1} dry soil with control treatment (without any addition) with two varieties of fenugreek plant (Indian and local). Results showed that the addition of the nanofertiliser treatment mixture exceeded the concentration of 0.75 $ml.L^{-1}$ and the combination of the biofertilizer 10^9 C.F.U gm^{-1} dry soil with the local variety fenugreek plant significantlly in the wet weight of the vegetative total (38.526 $gm.plant^{-1}$) compared to the control treatment (without addition). The results also showed that the treatment of adding a nanofertiliser treatment mixture exceeded the concentration of 0.75 $ml.L^{-1}$ and the combination of the biofertilizer 10^9 C.F.U gm^{-1} dry soil with the fenugreek plant (local) significantlly in the dry weight of the vegetative total (15.102 $gm.plant^{-1}$) compared to the control treatment (i.e. without addition). The addition of the nanofertiliser treatment mixture at concentration of 0.75 $ml.L^{-1}$ and C.F.U gm^{-1} dry soil 10^9 with the Indian fenugreek plant also significantlly exceeds the

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overall chlorophyll content (46.712 spad) compared to the control treatment (without addition).

***Keywords*---**biofertilizer, nanofertiliser (NPK), fenugreek plant, chlorophyll.

Introduction

Nanofertilizer is defined as nanomaterials or nanoparticles for certain essential or beneficial nutrients through which they can be transmitted to plants in order to support plant growth and improve production, and can be divided into three macro-nanofertilizer, micro-nanofertilizer and nano-particulate fertilizer based on plant requirements for nutrients [1]. It has a significant role in losing nutrients to soil and thus reducing soil pollution with excess mineral fertilizer. Also, nanofertilizers avoid nutrient interaction with air, water and microorganisms in the soil 2]. Many studies have also taken care of the positive role of nanofertilizers in improving biomass, plant length, root size, chlorophyll and protein content.

The continuous and excessive use of chemicals causes health and environmental risks, degradation of soil characteristics and thus a decrease in crop production, so the use of different bacterial strains as biofertilizers reduces the use of chemical fertilizers and provides high-quality crops free of harmful chemicals and safe for human consumption [4]. *Trigonella foenum-graecum* L. belongs to Fabaceae family is a prominent spice crop used in the human diet [5]. Fresh fenugreek leaves contain ascorbic acid (220.97 mg /100 gm) and beta-carotene (19 mg/100 g) and are a rich source of calcium, iron and zinc [6]. Fenugreek plant seeds also contain a high percentage of carbohydrates, proteins, flavonoids, alkalids, saponins glycosides, gels, free amino acids and minerals [7]. The current study aims to determine the effect of spraying nanofertiles (NPK) and bio-fertilizers on the plant's green part of the fresh, dry weight and chlorophylle in two varieties of fenugreek plant. Fresh weight and dry plant weight are studied to know the effect of the experimental factors whether they are osmosis or structural, if the significance between fresh and dry weight are far away, the effect is osmosis and in the case where the significance between fresh and dry weight are close, the effect is structural.

Materials and Methods

Isolation and diagnosis of Bacillus megaterium

Bacillus megaterium was isolated from the soil of the field, serial dilutions were cultured on the solid medium of Pikovskaya and then cultural, microscopic and biochemical tests were used to diagnose these bacterial colonies that showed a transparent area around them due to phosphate solubility [8].

Rhizobium isolation and diagnosis

Rhizobium was isolated from the soil of the field, serial dilutions were cultured on the solid medium of yeast Extract Mannitol Agar and then cultural, microscopic and biochemical tests were used to diagnose these bacterial colonies that showed convex, semi-transparent mucous circular colonies of a creamy color graded in white [9].

Isolation and diagnosis of Azotobacter chroococcum

Azotobacter chroococcum was isolated from the soil of the field, serial dilutions were cultured on the solid medium of Azotobacter Agar Mannitol and then cultural, microscopic and biochemical tests were used to diagnose these bacterial colonies that showed rod colonies and their cells are usually in pairs or chains, some non-motile and others motile by peripheral or polar flagellum, negative for gram stain [10].

Rhizobium isolation and diagnosis

Bacillus mucilaginosus was isolated from the soil of the field, serial dilutions were cultured on the solid medium of Aleksanderove Agar Medium and then cultural, microscopic and biochemical tests were used to diagnose these bacterial colonies that showed rod circular, white milky colonies transparent on the Aleksanderove Agar Medium [11].

Field experiment

A field experiment was conducted according to the Completely Random Block Design (CRBD) and three repeats at the University of Baghdad, College of Education for Pure Sciences Ibn Al-Haitham during the growth season 2020-2021. The following factors included; NPK nanofertilizer with three concentrations (0.5, 0.7, 1 ml.L^{-1}) with control treatment (without any addition), bacterial fertilizer of Bacillus *mucilaginosus*, *Bacillus megaterium*, *Rhizobium*, *Azotobacter chroococcum* with Two concentrations (10^7, 10^9) C.F.U gm-1 dry soil with control treatment (without any addition) and two categories of fenugreek plant (Indian and local).

Table (1): Shows some of the physical, chemical and biological characteristics of field soil before cultivation

<table>
<tr><td colspan="2">Characteristics</td><td>Value</td><td>Unite</td></tr>
<tr><td colspan="2">Soil texture</td><td>Loam</td><td>-</td></tr>
<tr><td colspan="2">Soil interaction</td><td>6.89</td><td>pH</td></tr>
<tr><td colspan="2">Electrical conductivity</td><td>5.8</td><td>Decisiemens.m^{-1}</td></tr>
<tr><td rowspan="3">Soil separators</td><td>Silt</td><td>455</td><td>gm.kg^{-1}</td></tr>
<tr><td>Mud</td><td>234</td><td>gm.kg^{-1}</td></tr>
<tr><td>Sand</td><td>311</td><td>gm.kg^{-1}</td></tr>
<tr><td colspan="2">Available nitrogen</td><td>51</td><td>mg.kg^{-1} soil</td></tr>
<tr><td colspan="2">Available potassium</td><td>574.5</td><td>mg.kg^{-1} soil</td></tr>
<tr><td colspan="2">Available phosphours</td><td>33.12</td><td>mg.kg^{-1} soil</td></tr>
<tr><td rowspan="3">Dissolved ions in soil</td><td>Calcium</td><td>402</td><td>mg.kg^{-1} soil</td></tr>
<tr><td>Ferric</td><td>23</td><td>mg.kg^{-1} soil</td></tr>
<tr><td>Copper</td><td>0.4</td><td>mg.kg^{-1} soil</td></tr>
</table>

solution	Zinc	0.3	$gm.kg^{-1}$ soil
Total number of bacteria	*Rhizobium*	2.0231×10^{4}	C.F.U gm^{-1} dry soil
	Bacillus mucilaginosus	1.5454×10^{5}	C.F.U gm^{-1} dry soil
	Bacillus megaterium	1.7654×10^{6}	C.F.U gm^{-1} dry soil
	Azotobacte	1.0653×10^{5}	C.F.U gm^{-1} dry soil

Preparation of bacterial inoculum from Bacillus mucilaginosus, Bacillus megaterium, Rhizobium, Azotobacter chroococcum

Nutrient broth media preperated 100 ml and sterilized at 121°C and pressured 15 pounds/inch^2 for 15 minutes, after the sterilization ended leave the medium to cool and inoclum by the loop by filled with bacterial isolations that were isolated and previously diagnosed. The inoclum medium was transferred to a 150 rpm vibrator incubator for 48 hours, and the microbiome density was counted to the inoclum [12]. A 2 litre conical flask with 1,000 ml nutrient broth and the flask was sterilized by autoclave at temperature 121°C; the pressure 15 pounds/inch^2 for 15 minutes and left to cool, the flask was inoclumed by adding 1 ml of broth colonies equipped with sterile pipette and incubation of the flask in the incubator 28°C and for 48 hours [13].

Statistical analysis

The results were statistically analyzed using Genstat and Excel according to the design of the working experiment in three factors and three repeates according to the Completely Random Block Design (CRBD) and the mean were tested with the lowest significant difference (LSD) at a probability level (0.05) according to [14].

Results

Fresh weight of vegetative total (gm. plant $^{-1}$)

Table 2 shows a significant increase in the mean fresh weight of the first cutting gm.Plant^{-1}, which surpasses the local variety, which recorded 30.721 gm. Plant^{-1} compared to Indian variety with a record of 28.443 gm. Plant^{-1}, an increase of 8.011% compared to the Indian variety. The characteristic of mean nanoconcentration increased significantally, with a concentration of 0.75 ml. L^{-1} higher mean 32.111 gm.Plant^{-1}, an increase of 21.173% compared to plants not treated with nanofertilizer. The mean concentration of the bacterial inoclum increased significantally, with the concentration of 10^9 C.F.U gm^{-1} dry soil at the highest mean of 32.427 gm. Plant^{-1} is an increase of 27.169% compared to plants not treated with bacterial inoclum.

The results of the bilateral interaction between the two plant varieties and the mean concentration of nanofertiliser were observed to increase significantally, with the Indian variety registering at the concentration of nanofertiliser 0.75 ml. L^{-1} recorded the highest mean binary interaction of 34.174 gm.Plant^{-1}, an increase of 29.2796% compared to plants not treated with bilateral interaction. While the bilateral interaction between the two plant varieties and the mean concentration of the bacterial inoclum increased significantly, the local variety and the concentration of the bacterial inoclum 10^9 C.F.U gm^{-1} dry soil recorded the highest mean bilateral interaction of 33.702 gm. $Plant^{-1}$, an increase of 34.592% compared to plants not treated with bilateral interaction.

The bilateral interaction between the mean concentration of nanofertiliser and the mean concentration of the bacterial inoclum showed a significant increase, with the nanofertiliser concentration recorded at 0.75 $ml.L^{-1}$ and the concentration of the bacterial inoclum 10^9 C.F.U gm^{-1} dry soil the highest mean bilateral inteaction is 36.121 g,. $Plant^{-1}$ and an increase of 57.163% compared to plants not treated with bilateral interaction. The results of the triplete interaction of the treatments studied were also significant, and it was observed a significant increase as the local variety recorded at the concentration of the nanofertiliser 0.75 $ml.L^{-1}$ and the concentration of bacterial inoclum 10^9 C.F.U gm-1 dry soil the highest mean bilateral interaction is 38.526 $gm.Plant^{-1}$ is an increase of 69.471% compared to plants not treated with triplete interaction.

Table (2): The effect of bacterial fertilizer and nano-fertilizer and their interaction in the mean fresh weight of the first cutting (gm. $Plant^{-1}$) of the two fenugreek plant varieties

Effect of mean variety interaction × concentration of nanofertilizer ($ml.L^{-1}$)	Concentration of bacteria C.F.U gm^{-1} dry soil			Concentrations of nanofertilizer ($ml.L^{-1}$)	Varieties
	10^9	10^7	0		
26.565	29.347	27.115	23.233	0	Indian
28.403	31.222	29.376	24.610	0.5	
30.047	33.716	30.111	26.313	0.75	
28.757	30.325	29.942	26.003	1	
26.434	29.860	26.710	22.733	0	Local
29.996	33.655	31.211	25.122	0.5	
34.174	38.526	36.382	27.615	0.75	
32.281	32.765	35.716	28.362	1	
0.0108**	0.0187**				L.S.D =0.05
Effect of mean variety					
28.443	31.153	29.136	25.039	Indian	Effect of mean variety interaction × concentration of bacteria C.F.U gm^{-1} dry soil
30.721	33.702	32.505	25.958	Local	

0.0054**	0.0093**			L.S.D =0.05	
Effect of mean concentration of nanofertilizer (ml.L^{-1})					
26.500	29.604	26.913	22.983	0	Effect of mean concentration of nanofertilizer (ml.L^{-1})× concentration of bacteria C.F.U gm^{-1} dry soi
29.199	32.439	30.294	24.866	0.5	
32.111	36.121	33.247	26.964	0.75	
30.519	31.545	32.829	27.183	1	
0.008**	0.013**			L.S.D =0.05	
	32.427	30.820	25.499	Effect of mean concentration of bacteria C.F.U gm^{-1} dry soil	
	0.007**			L.S.D =0.05	

Dry weight of vegetative total (gm.Plant^{-1})

Table (3) shows a significant increase in the mean dry weight of the first cutting gm. Plant^{-1} for the fenugreek plant where it exceeds the local variety which recorded 10.685 gm.Plant^{-1} compared to Indian variety with a record of 8.160 gm.Plant^{-1}, an increase of 30.939% compared to the Indian variety. The characteristic of mean nanoconcentration increased significantly, with a concentration of 0.75 ml.L^{-1} higher mean 10.680 gm. Plant^{-1}, an increase of 35.224% compared to plants not treated with nanofertilizer. The mean concentration of the bacterial inoclum increased significantly, with the concentration of 10^9 C.F.U gm^{-1} dry soil the highest mean of 11.799 gm. Plant^{-1} and the increase rate is 74.618% compared to plants not treated with bacterial inoclum.

The results of the bilateral interaction between the two plant varieties and the mean concentration of nanofertiliser indicated a significant increase as the Indian variety recorded at the concentration of nanofertiliser 0.75 ml.L^{-1} recorded the highest mean binary interaction of 12.250 gm. Plant^{-1}, an increase of 78.090% compared to plants not treated with bilateral interaction. While the bilateral interaction between the two plant varieties and the mean concentration of the bacterial inoclum increased significantly, the local variety and the concentration of the bacterial inoclum 10^9 C.F.U gm-1 dry soil recorded the highest mean bilateral interaction of 13.384 gm.Plant^{-1}, an increase of 102.788% compared to plants not treated with bilateral interaction.

The bilateral interaction between the mean concentration of nanofertiliser and the mean concentration of the bacterial inoclum is shown to increase significantly, with the nanofertiliser concentration recorded at 0.75 ml.L^{-1} when the bacterial inoclum 10^9 C.F.U gm^{-1} dry soil has the highest mean bilateral interaction of 13.357 gm.Plant^{-1}, an increase of 131.410% compared to plants not treated with bilateral interaction. The results of the triplete interaction between the treatments studied were also significant, with the local variety and the concentration of nanofertilized recorded 0.75 ml.L^{-1} and the concentration of the bacterial inoclum 10^9 C.F.U gm^{-1} dry soil the highest mean bilateral interaction is 15.102 gm.Plant^{-1}

is an increase of 168.336% compared to plants not treated with triplete interaction.

Table 3: The effect of bacterial fertilizer and nanofertilizer and their interaction in the mean dry weight of the first cutting (gm. plant^{-1}) of the two fenugreek plant varieties

Effect of mean variety interaction × concentration of nanofertilizer (ml.L^{-1})	Concentration of bacteria C.F.U gm^{-1} dry soil			Concentrations of nanofertilizer (ml.L^{-1})	Varieties
	10^9	10^7	0		
6.879	8.643	6.365	5.628	0	Indian
8.241	10.149	7.861	6.712	0.5	
9.110	11.611	8.322	7.398	0.75	
8.410	10.452	8.116	6.662	1	
8.918	11.712	9.127	5.915	0	Local
10.252	12.111	11.722	6.923	0.5	
12.250	15.102	13.656	7.993	0.75	
11.318	14.612	12.518	6.823	1	
0.0040**	0.0069**				L.S.D =0.05
Effect of mean variety					
8.160	10.214	7.666	6.600	Indian	Effect of mean variety interaction × concentration of bacteria C.F.U gm^{-1} dry soil
10.685	13.384	11.756	6.913	Local	
0.0020**	0.0035**			L.S.D =0.05	
Effect of mean concentration of nanofertilizer (ml.L^{-1})					
7.898	10.178	7.746	5.772	0	Effect of mean concentration of nanofertilizer (ml.L^{-1})× concentration of bacteria C.F.U gm^{-1} dry soil
9.246	11.130	9.792	6.818	0.5	
10.680	13.357	10.989	7.696	0.75	
9.864	12.532	10.317	6.743	1	
0.003**	0.005**			L.S.D =0.05	
	11.799	9.711	6.757	Effect of mean concentration of bacteria C.F.U gm^{-1} dry soil	
	0.002**			L.S.D =0.05	

Total chlorophyll content (Spad)

Table 4 shows a significant increase in the mean total Spad content of the fenugreek plant, with the Indian variety exceeding 38.098 Spad compared to the local variety of 35.318 Spad and an increase of 7.872% compared to the local variety. The characteristic of mean nanoconcentration increased significantly, with a concentration of 0.75 $ml.L^{-1}$ is the highest mean of 39.380 Spads and an increase of 23.98% compared to plants not treated with nanofertilizer. The characteristic of the mean concentration of the bacterial inoclum increased significantly, with the concentration of 10^9 C.F.U gm^{-1} dry soil the highest mean of 39.358 Spad and an increase of 20.578% compared to plants not treated with bacterial inoclum. The results of the bilateral interaction between the two plant varieties and the mean concentration of nanofertiliser were observed to increase significantly, with the Indian variety registering at the concentration of nanofertiliser 0.75 ml. L^{-1} recorded the highest mean binary interaction of 41.695 Spad and an increase of 32.6300% compared to plants not treated with bilateral interaction.

While the bilateral interaction between the two plant varieties and the mean concentration of the bacterial inoclum increased significantly, the Indian class at the concentration of the bacterial inoclum 10^9 C.F.U gm^{-1} dry soil recorded the highest mean bilateral interaction of 41.953 Spad and an increase of 29.129% compared to plants not treated with bilateral interaction. The bilateral interaction between the mean concentration of nanofertiliser and the mean concentration of the bacterial inoclum is shown to increase significantly, with the nanofertiliser concentration recorded at 0.75 $ml.L^{-1}$ at the concentration of the bacterial inoclum 10^9 C.F.U gm^{-1} dry soil the highest mean spad 42.459 and an increase of 41.98% compared to plants not treated with bilateral interaction. The results of the triplete interaction between the treatment studied were also significant, as we observed a significant increase as the Indian variety and the concentration of nanofertilized 0.75 $ml.L^{-1}$ were recorded at the concentration of the bacterial inoclum 10^9 C.F.U gm^{-1} dry soil the highest mean spad 46.712 and an increase of 56.814% compared to plants not treated with triplete interaction.

Table 4: The effect of bacterial fertilizer and nanofertilizer and their interaction in the mean total chlorophyll content (Spad) of two fenugreek plant varieties

Effect of mean variety interaction × concentration of nanofertilizer ($ml.L^{-1}$)	Concentration of bacteria C.F.U gm^{-1} dry soil			Concentrations of nanofertilizer ($ml.L^{-1}$)	Varieties
	10^9	10^7	0		
31.437	33.813	30.711	29.788	0	Indian
38.438	42.361	41.321	31.633	0.5	
41.695	46.712	43.663	34.711	0.75	
40.820	44.924	43.713	33.823	1	

32.084	33.823	32.413	30.017	0	Local
35.335	36.191	37.313	32.501	0.5	
37.065	38.206	38.617	34.371	0.75	
36.786	38.836	37.241	34.281	1	
0.0031**	0.0053**				L.S.D =0.05
Effect of mean variety					
38.097	41.952	39.852	32.488	Indian	Effect of mean variety interaction × concentration of bacteria C.F.U gm^{-1} dry soil
35.317	36.764	36.396	32.792	Local	
0.0015**	0.0026**			L.S.D =0.05	
Effect of mean concentration of nanofertilizer (ml.L^{-1})					
31.761	33.818	31.562	29.903	0	Effect of mean concentration of nanofertilizer (ml.L^{-1})× concentration of bacteria C.F.U gm^{-1} dry soil
36.887	39.276	39.317	32.067	0.5	
39.380	42.459	41.140	34.541	0.75	
38.803	41.880	40.477	34.052	1	
0.002**	0.004**			L.S.D =0.05	
	39.358	38.124	32.641	Effect of mean concentration of bacteria C.F.U gm^{-1} dry soil	
	0.002**			L.S.D =0.05	

Discussion

Fresh weight results for the vegetative total in table (2) showed that the addition of the nanofertilizer treatment mixture exceeded the concentration of 0.75 ml^{-1} and the combination of the biofertilizer of 10^9 C.F.U gm^{-1} dry soil with the (local) variety fenugreek plant significantly in the fresh weight of the vegetative total (38.526 gm.Plant^{-1}) compared to the control treatment (i.e. without addition). This superiority is due to the role of nanofertiliser NPK, one of whose components is nitrogen, as it is characterized by its importance in increasing and sustaining vegetative growth processes as well as its role in the formation of proteins and chlorophyll and encouraging the formation of growth regulators, which is reflected in increased vegetative growth and thus the increase of the fresh weight of the plant, and we can note this through its effect on plant height or it may be due to the positive and effective role of nitrogen in the formation of a strong root total with the ability to absorb nutrients and elements from the soil and thus increase the efficiency of carbon representation and then increase plant growth [15]. The results in agreement with *Mathiola incana* L. Nanofertilizer also have a high surface area and slow release that helps quickly absorb nutrients and speed of penetration, representation and movement, leading to increased growth speed and quality (e.g. protein and starch) by stimulating photosynthesis [18] leading to fresh weight gain in the end. The effect of the quadruple bacterial inoclum in the fresh weight gain of the vegetative total is also due to its role in dissolving low-dissolving organic acids and dissolving non-dissolved mineral phosphorus, producing certain hormones such as auxins, gibberellic and vitamins and stimulating plant resistance against viruses, leading to improved growth conditions and fresh weight gain [19, 20]. The results are also consistent with [21] on the fenugreek plant.

Dry weight results in table (3) showed that the addition of the nanofertilized treatment mixture exceeded the concentration of 0.75 ml^{-1} and the combination of the biofertilizer 10^9 C.F.U gm^{-1} dry soil with the (local) variety fenugreek plant significantally in the dry weight of the vegetative total (15.102 $gm.plant^{-1}$) compared to the control treatment (i.e. without addition). The results of the total chlorophyll content also showed table (3) the treatment of adding the nanofertilizer treatment mixture at concentration of 0.75 ml^{-1} and the combination of the biofertilizer 10^9 C.F.U gm^{-1} dry soil with the variety fenugreek plant (Indian) significantally in the form of total chlorophyll content (spad 46.712) compared to the control treatment (i.e. without addition). The use of NPK nanofertilizer, which increases the dry weight of leaf (3), chlorophyll (table 4) compared to the control treatment, these results are consistent with what he found [22] when NPK nano was used on the beans plant, the results could be due to the physiological role of nitrogen in a bi-molecule compound such as porphyrin found in metabolism such as cytochrome stain and chlorophyll.

It is essential for respiration, photosynthesis and phosphorous-promoted co-enzymes and essential for most of the enzymes and amino acids produced that are used to produce protein [23], however, potassium is responsible for enzyme activity and protein stability [24]. Nitrogen found within the nano-fertilizer structure increases the activity of biophysical and synthesizing processes within the plant, such as carbon representation, respiration, energy production, growth hormones, and stimulating plant enzymes responsible for the synthesis of various plant tissue synthesing materials such as proteins, vitamins, sugars and other important substances, thus reflecting weight gain and size [25]. The effect of nanofertiliser, which makes nutrients available to plants that increase the formation of chlorophyll pigment and photosynthesis, is also due to the promotion of parameters of vegetable growth in general [26]. The bacterial isolated used in this study increases the amount of dry weight of the vegetative total [3], due to the increase in the amount of nitrogen, phosphorus and potassium processed through the effectiveness of the four bacteria used in the soil and the conversion of these elements into amino acids and compounds used by the plant in tissue formation and thus the formation of plant growth [27]. It is also consistent with the results [28] on flax and results [29] on the fenugreek plant.

The use of the bacterial inoclum consisting of *Bacillus mucilaginosus*, *Bacillus megaterium*, *Rhizobium*, *Azotobacter chroococcum* acts as it increases the chlorophyll (table 4) and the results are consistent with [30]. This may be due to the use of co-bioinoclum, which has led to increased nutrient availability, photosynthesis activity, chlorophyll formation, and nitrogen metabolism in plants, which eventually improves plant height leading to strong plant growth [31, 32]. Nitrogen absorption leads to increased total chlorphyll in the plant, leading to increased representation and biosynthesis and metabolism processes [33] as consistent with the results [34] on cowpea lobea (*Vigna unguiculata* L. Walp). It also corresponds to the results [35] the use of isobacter on two varities of pea plant.

The difference in varieties in their response to the transactions used is due to genetic variation, which is reflected in their behaviour in the absorption of nutrients from the soil as well as their geographical origin and their response to environmental damage [36]. [37] also noted that the varieties of the fenugreek differ significantlly in the number of seeds, pods and total product, and the difference in the content of their seeds from trigonelline is due to the genetic composition of the varieties under the circumstances of the studied experiment.

References

1. Chhipa, H. (2017). Nanofertilizers and nanopesticides for agriculture," Environ. Chem. Lett., vol. 15, no. 1, pp. 15–22.
2. DeRosa, M.C.; Monreal, C.; Schnitzer, M.; Walsh, R. and Sultan, Y. (2010). Nanotechnology in fertilizers. Nat. Nanotechnol., 5(2): 91.
3. Raliya, R. and Tarafdar, J.C. (2013).ZnO nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in Clusterbean (*Cyamopsis tetragonoloba* L.). Agric. Res., 2(1): 48–57.
4. El Naim, A. M.; Ahmed, A. I.; Ibrahim, K. A.; Suliman, A. M. and Babikir, E. S. N. (2017). Effects of nitrogen and bio-fertilizers on growth and yield of roselle (*Hibiscus sabdariffa* var *sabdariffa* L.). Int. J. Agric. For., 7(6): 145–150.
5. Aasim, M.; Baloch, F. S.; Nadeem, M. A.; Bakhsh, A.; Sameeullah, M. and Day, S. (2018). Fenugreek (*Trigonella foenum-graecum* L.): An underutilized edible plant of modern world. In Global perspectives on underutilized crops, Springer: 381–408.
6. Baliga, M. S. et al. (2017). Anti-Diabetic Effects of Leaves of Trigonella foenum-graecum L.(Fenugreek): Leads from Preclinical Studies. J Food Chem Nanotechnol, 3(2): 67–71.
7. Jhajhria, A. and Kumar, K. (2016). Fenugreek with its medicinal applications," Int. J. Pharm. Sci. Rev. Res, 41(1):194–201.
8. Al Rubaye, A. T.; Abdul-Ratha, H. A. and Hadown, H. A. (2019). Effect of Local and Imported Biofertilizers on Growth and Yield of Potato. Iraqi J. Agric. Sci., 50(1): 431–445.
9. Vincent, J. M. (1970). A manual for the practical study of the root-nodule bacteria. A Man. Pract. study root-nodule Bact.
10. Becking, J.-H. (1981). The family Azotobacteraceae. In The prokaryotes, Springer: 795–817.
11. Atlas, R. M.; Parks, L. C. and Brown, A. E. (1995). Laboratory manual of experimental microbiology. Mosby-Year Book. Inc., USA.
12. Elkoca, E.; Turan, M. and Donmez, M.F. (2010). Effects of single, dual and triple inoculations with Bacillus subtilis, Bacillus megaterium and Rhizobium leguminosarum bv. Phaseoli on nodulation, nutrient uptake, yield and yield

parameters of common bean (Phaseolus vulgaris l. cv.'elkoca-05'). J. Plant Nutr., 33(14): 2104–2119.

13. Black, C. A. (1965). Methods of soil analysis part 1 physical properties Am. Soc. Agron. Inc. Publ. Madison Wisconsin, USA.
14. Steel, R.G.D. and Torrie, J. H. (1960). Principles and procedures of statistics. Princ. Proced. Stat.
15. Ayub, M.; Naeem, M.; Nadeem, M.A.; Tanveer, A.; Tahir, M. and Alam, R. (2011). Effect of nitrogen application on growth, yield and oil contents of Fennel (*Foenoculum vulgare* Mill.). J. Med. Plants Res., 5(11): 2274–2277.
16. AL-Zurfi, M.T.H. and Al-kaaby, H.N.H. (2016). EFFECT OF SPRAYING ORGANIC FERTILIZERS Optimus Plus AND Azotobacter ON GROWTH AND FLOWERS OF PLANT *Mathiola incana* L. Euphrates J. Agric. Sci., 8(1).
17. Sabh, A.Z. and Shallan, M.A. (2008). Effect of organic fertilization of broad bean (*Vicia faba* L.) by using different marine macroalgae in relation to the morphological, anatomical characteristics and chemical constituents of the plant. Aust J Basic Appl Sci, 2: 1076–1091.
18. Lal, R. (2008). Promise and limitations of soils to minimize climate change. J. soil water Conserv., 63(4):113A-118A.
19. Murphy, J. F.; Reddy, M.S.; Ryu, C.-M.; Kloepper, J.W. and Li, R. (2003). Rhizobacteria-mediated growth promotion of tomato leads to protection against Cucumber mosaic virus," Phytopathology, 93(10): 1301–1307.
20. Zaki, M.F.; Fawzy, Z.F.; Ahmed, A.A. and Tantawy, A. S. (2012). Application of phosphate dissolving bacteria for improving growth and productivity of two sweet pepper (*Capsicum annuum* L.) cultivars under newly reclaimed soil. Aust. J. Basic Appl. Sci., 6(3): 826–839.
21. Sahu, P. K.; Naruka, I. S.; Haldar, A.; Chundawat, R.S. and Kumar, H. (2020). Study the effect of integrated nutrient management on vegetative growth of fenugreek (*Trigonella foenum-graecum*) L. J. Pharmacogn. Phytochem., 9(3): 1389–1394.
22. Aziz, B.R. (2021). Effect of Foliar Application of Nano-NPK Fertilizer on Growth and Yield of Broad bean (*Vicia Faba* L.). Zanco J. Pure Appl. Sci., 33(4): 90–99.
23. Espinosa, M.; Turner, B.L. and Haygarth, P.M. (1999). Preconcentration and separation of trace phosphorus compounds in soil leachate. Wiley Online Library.
24. Hänsch R. and Mendel, R. R. (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Curr. Opin. Plant Biol., 12(3): 259–266.
25. Ali, F.M.; Alhadithi, H.J.M. and Sharqi, H. S. (2017). EFFECT OF DIFFERENT COMBINATION OF MACRO AND MICRONUTRIENTS ON THE GROWTH AND YIELD OF GARLIC *Allium sativum* L. Iraqi J. Agric. Sci., 48(1): 192.
26. Al-Juthery, H.W.A.; Ali, N. S.; Al-Taey, D.K.A. and Ali, E. (2018). The impact of foliar application of nanaofertilizer, seaweed and hypertonic on yield of potato. Plant Arch., 18(2): 2207–2212.
27. Bashan, Y.; Holguin, G. and De-Bashan, L. E. (2004). Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol., 50(8): 521–577.
28. AL-Zuhery, G.F.H. and Radha, H.A.A. (2019). Effect of Biofertilizer Comibination, Vermicompost and Chemical Fertilization in the Availability of

Nitrogen, Phosphorus and Potassium in the soil and its Concentrations in *Linum usitatissimum* L. Iraqi J. Soil Sci., 19(1).
29. Rugheim, A.; Taha, K.E.M. and Ali, S.E.A. (2014). Influence of nitrogen fixing and phosphorus solubilizing bacteria inoculation on fenugreek symbiotic properties, growth and yield. International journal of Horticulture. Agric. Food Sci., 42–47.
30. Husain, N.; Nair, R. and Yadav, R. K. S. (2021). Crop productivity and nutrient uptake of fenugreek (*Trigonella foenum-graecum* L.) as influenced by inorganic fertilizers and bio-inoculant (Rhizobium, PSB and KSB). 2021.
31. Meena, S. S.; Mehta, R.S.; Bairwa, M. and Meena, R. D. (2014). Productivity and profitability of fenugreek (*Trigonella foenum-graecum* L.) as influenced by bio-fertilizers and plant growth regulators," Legum. Res. Int. J., 37(6): 646–650.
32. Raiyani, V. N.; Kathiriya, R. K.; Thummer, V. M. and Rupareliya, V. V. (2018). Effect of FYM and biofertilizers on growth, yield attributes and yield of fenugreek (*Trigonella foenum-graecum* L.). IJCS, 6(4): 746–748.
33. Ali, N. S.; Rahi, H. S. and Al-Jumaili, A. A. (2014). Soil Fertility Book, Arab Soc. Libr. Sci. Books House Printing, Publ. Distrib. Coll. Agric. Baghdad.
34. Jha, B. and Trivedi, J. (2021). Effect of different bio-fertilizer and fertility level on growth, quality and yield attributes of cowpea (*Vigna unguiculata* L. Walp). 2021.
35. Yousif, S.H. and Yousif, K.H. (2020). Effect of inoculation with Azotobacter and organic fertilizer on growth and root nodule of Pea (*Pisum sativum* L.)," Kufa J. Agric. Sci., 12(1).
36. Alam, S.M. (2001). Genetic variations in nutrient contents by wheat and its substitution lines. Pakistan J. Biol. Sci.,. 4(6): 642–644.
37. Dash S.R. and Kole, P.C. (2000). Association analysis of seed yield and its components in fenugreek (*Trigonella foenum-graecum* L.). Crop Res., 20(3): 449–452.