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The Effect of Azotobacter, Mycrorrhizae and Humic Acid on the Vegetative Traits of *vigna radiata l.*

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Abstract

A study was conducted in the summer season of 2022 in the village of Aday in Baquba District - Diyala Governorate on the mung plant *Vigna radaita* L. (a local green variety) in clay soil ,in order to study the effect of bio-fertilization with *Azotobacter chroococcum* and the Mycorhiza fungi (*Glomus mosseae*) and organic fertilization using humic acid on the vegetative characteristics of the mung bean *(V. radaita* L.). The Experiment were carried out according to the randomized complete block design (R.C.B.D) with three replications using two fertilization, the first was biological fertilization, which included four treatments, namely (without addition, the addition of Azotobacter, the addition of Mycorrhizae, and the addition of Azotobacter + Mycorrhizae) and the second was organic fertilization, which included four concentrations of humic acid 0, 3, 6 and 9 gm.l^{-1} the result findings were:The treatment of biofertilization by adding Azotobacter and Mycorrhizae in all the studied characteristics of plant height, root length, number of branches, leaf area of the vegetative total reached 76.87 cm, 43.01 cm,15 branch plants^{-1} , 660.77 cm^2. The treatment of organic fertilization with humic acid at a concentration of 9 gm.l^{-1} was superior giving it the highest mean of plant height, root length, number of branches, leaf area of the vegetative total 77.6 cm, 16cm, 15.39 branch plant^{-1} , 660.83 cm^2 .

Keywords: azotobacter, mycorrhiza, humic acid, *Vigna radiata*, biofertilization, organic fertilization.

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تأثير الازوتوباكتر,المايكورايزاوحامض الهيوميك على الصفات الخضرية لنبات الماش *Vigna radiata* L.

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الخلاصة

أُجريت تجربة حقلية في الموسم الخريفي لسنة 2022 في قرية عداي التابعة الى قضاء بعقوبة –محافظة ديالى على نبات الماش .*Vigna radaita* L (صنف محلي خضراوي) في تربة طينية بهدف دراسة تأثير التسميد الحيوي ببكتريا الازوتوباكتر *Azotobacter chroococcum* وفطريات المايكورايزا Mycorhiza (*Glomus mosseae*) والتسميد العضوي بحامض الهيوميك والتداخل بينهما في نمو وحاصل نبات الماش . نفذت التجربة وفق تصميم القطاعات العشوائية الكاملة (R.C.B.D) وبثلاثة مكررات و تضمنت عاملين الاول التسميد الحيوي الذي تضمن اربعة معاملات وهي(بدون اضافة و اضافة الازوتوباكتر و اضافة المايكورايزا وأضافة الازوتوباكتر + المايكورايزا)والعامل الثاني التسميد العضوي الذي تضمن اربعة تراكيز من حامض الهيومك 0 و3 و6 و9 غم.لتر$^{-1}$.وفيما يأتي أهم النتائج التي تم التوصل اليها: تفوق معاملة التسميد الحيوي بأضافة الازوتوباكتر و المايكورايزا في جميع متوسطات الصفات المدروسة من ارتفاع النبات وطول الجذر و عدد التفرعات و المساحة الورقية ، اذ بلغت 76.87 سم ، 43.01 سم, 15فرع.نبات$^{-1}$, 660.77 سم^{2}. تفوقت معاملة التسميد العضوي بحامض الهيوميك بتركيز 9 غم.لتر$^{-1}$ بأعطائها اعلى متوسط لأرتفاع النبات وطول الجذر و عدد التفرعات و المساحة الورقية اذ بلغت 77.6سم ، 40.24 سم ،15.39 فرع.نبات$^{-1}$ ، 660.83 سم^{2} .

الكلمات المفتاحية: الازوتوباكتر،المايكورايزا،حامض الهيوميك،نبات الماش،التسميد الحيوي ،التسميد العضوي

Introduction

The *Vigna radiate L.*(Mung plant) is one of the summer crops belonging to the Fabaceae. The plant tolerates high temperatures and drought. *V. radiate L.*cultivation in Iraq takes place in most of the governorates, as the area cultivated with mash was estimated at 25308 dunums, with a production rate of 8944 ton per dunums.$^{-1}$ [1]. Mung bean seeds are used as a cheap source of protein, as the percentage of protein in mung bean seeds ranges from 19-29% and is native to the Indian subcontinent. It is rich in the amino acid lysine. Mash is rich in carbohydrates, vitamins, zinc, calcium and iron, and also contains isoflavoines that are antioxidants, cancerous and microbial diseases. Also mung bean flour is used in the manufacture of bread and sweets, and plant residues are used as feed for animals. In addition, it is considered as a green fertilizer for the soil due to its ability to fix nitrogen; It is low for water needs and also has a good economic return [2] [3].

Biofertilizer is a substance that contains living materials for microorganisms that are placed on seeds, in the soil, or with roots to enhance plant growth through primary nutrients that activate important plant processes such as nitrogen fixation and phosphorus facilitation and it is It is environmentally friendly, such as Rhizobium and Azotobacter [4]. It depends on a variety of factors including physical and chemical for soil (eg organic matter, pH, and temperature and soil moisture). Abundance varies with soil depth. Azotobacter is an N-fixing bacterium[5].

Biofertilizers secrete antibiotics for the growth of fungi, which in turn reduces plant disease, and this helps reduce the use of chemical pesticides. The world has moved towards clean agricultural techniques in order to reduce the use of mineral fertilizers, where natural materials such as biofertilizers and organic fertilizers are used, which are complementary to mineral fertilizers [6][7]. The relationship between mycorrhiza fungi and some plants has been described as a mutualistic symbiosis, as it benefits both the plant and the fungus, and also plays a major role in supplying the plant with phosphorus and many other elements, as well as protecting plants from infection with pathogens present in the soil[8], and also contribute to increase plant tolerance to environmental stress conditions and others

Organic fertilizers are one of the modern agricultural trends that use organic natural resources to grow crops and improve their production away from industrial chemicals that cause environmental and health damage to humans [9], and humic acid is one of the components of organic matter that has multiple benefits when added to the soil, including increasing the efficiency of chemical use, reducing its quantity, maintaining the balance of nutrients, reducing stress, and maintaining soil health and environmental safety [10]. Humic acid is considered one of the most important humic organic acids because it contains many nutrients important for plant growth, which prompted specialists to add it to agricultural crops [11].

The aim of the study: To study the effect of fertilization with azotobacter bacteria and mycorrhiza fungi and the effect of fertilization with humic acid on the vegetative characteristics of the mung bean plant and the interaction between them.

Materials and methods

A field experiment were carried out in one of the agricultural fields in Diyala Governorate - Baquba district - Aday village, in the summer season 15/8/ 2022, on the mung bean plant, in order to study the effect of biofertilization and organic fertilization and the interaction between them on the vegetative characteristics of the mung plant. Random samples were taken from different locations of the soil of the field before planting to conduct the analysis to find out some of the chemical and physical characteristics of the soil of the study, which was conducted in the laboratories of the Directorate of Agriculture in Baqubah, as shown in Table (1).

Table 1: some physical and chemical characteristics of the study soil before planting

T	analysis type	The result		Notes
1	Salinity Ec	2.51 ds m^{-1}		
2	Soil pH reaction	7.45		
3	Organic matter O.M	19.9 gm. kg^{-1}		
4	Calcium carbonate	270 gm. kg^{-1}		
5	potassium	170 mg. kg^{-1}		
6	phosphorous	8.6mg.kg^{-1}		
7	tissue	Clay 445.6	gm. kg^{-1}	The tixture is clayey
		Silt 318.4		
		Sand 236.0		
8	nitrogen	15.07mg. kg^{-1}		

A factorial experiment with two factors was carried out according to the Randomized Complete Block Design (R.C.B.D.), with three replications, to study the effect of organic and biological fertilization and the interaction between them on the growth and yield of the mung bean plant. The distance between one line and another is 50 cm, at a rate of 4 lines in each experimental unit, and four plants in each line, and the distance is 1 m between experimental units and 2 m between replicates. The number of experimental units in each replicate = 16 experimental units, and thus the total number of experimental units equals 48 experimental units, with a plant density of 80,000 plants.h^{-1}. The experimental treatments included two factors, the first factor is biological fertilization, which included four treatments: without addition, the addition of Azotobacter, the addition of Mycorrhiza, the addition of Azotobacter + Mycorrhizae. The second factor was the organic fertilization, which included four concentrations of humic acid 0, 3, 6 and 9 $gm.L.^{-1}$.

The study used a locally grown species of seeds called Vigna radiate L. Azotobacter chroococcum was collected from the Department of Agricultural Research of the Ministry of Al-Zafaraniya's science and technology section as a local isolate of the bacterial inoculum. Additionally, the Mycorrhizal fungus vaccine was used. Mosseae Glomus The inoculum, which was used in the study, was acquired from the Department of Agricultural Research, the Al-Zafaraniya and the Ministry of Science and Technology. It was composed of (spores + infected roots + soil with moss).

Studied traits

1. **Plant height (cm)** Five plants were randomly taken at the flowering stage from the two middle lines, and the plant height was measured by means of a measuring tape from the position of the plant's contact with the soil surface to the highest growing peak in the plant, and then the average height of the plants was extracted.
2. **root length (cm)** The root length was measured after it was carefully extracted from the soil and washed with light running water, using a measuring tape, as the average length of five plants was taken from the two medians of each experimental unit.
3. **The number of branches per plant (branch. $plant^{-1}$)** It was done by measuring the average number of branches for five plants from the two middle lines for each experimental unit.
4. **leaf area (cm^2)**The leaf area was measured based on the method reported by [12] by using the disc method, as a certain number of discs were taken, their weights were measured, and the surface area of the leaves was calculated according to the following equation:

$$\text{paperarea} \quad = The\ known\ disk\ space\ is\ x\ space \frac{For\ tender\ leaves\ weight}{For\ soft\ tablets\ weight}$$

statistical analysis : The statistical analysis of the data was carried out using the SAS system as a factorial experiment within the Randomized Complete Block Design (R.C.B.D), and the averages were compared under the probability level of 0.05 [13].

Results and Discussion

The effect of bio and organic fertilization and the interaction between them on the vegetative characteristics of mung bean

1. **Plant height (cm):** The results of Table (2) indicate that there are significant differences in plant height when biofertilizing, as the biofertilization treatment with Azotobacter and Mycorrhizae gave the highest average plant height of 76.87 cm compared to the non-fertilization treatment, which amounted to 71.49 cm, an increase rate of 7.52%, and the reason for this is that pollination Azotobacter bacteria led to an increase in their numbers in the soil and the formation of root nodes that fix the nitrogen that the plant exploits and thus leads to an increase in plant height as well as its important role in increasing the manufacture of chlorophyll, which leads to an increase in the process of photosynthesis and the production of proteins, and all of these lead to stimulating the growth of the plant, which It works to increase the height of the plant [14]. As for the mycorrhiza fungi, it supplies the plant with nitrogen, which is fixed by root nodule bacteria, as well as phosphorus, which the fungus transfers to the plant, as well as other elements such as zinc and iron [4], as well. On the beneficial effect of infection of the roots with mycorrhiza fungi in improving plant intake of nutrients and bacterial activity on the other hand, it leads to a state of balanced nutrition and then shows its positive effect in improving plant characteristics, especially plant height [15][16]. For organic fertilization, humic acid fertilization led to an increase in plant height, as it gave the treatment of adding humic acid at a concentration of 9 gm.l.$^{-1}$ The highest plant height reached 77.60 cm compared to the non-fertilization treatment, which reached 71.30 cm, with an increase of 8.83%. The reason for this is that humic acid is one of the encouraging and stimulating factors to improve the physical and chemical properties of

the soil, which works to increase the availability of major and minor elements important in vital processes to build plant cells and increase their elongation [17]. And also the role it plays in increasing the speed of cell division and growth of plant cells and thus increasing plant height [18]. As well as its direct effect on the various vital processes of the plant, such as respiration, photosynthesis, protein building and other enzymatic activities, as its effect is similar to the effect of plant hormones that increase the rate of plant growth and create the best conditions for increased cell division and an increase in the length of the phalanx, and this leads to an increase in plant height [19], in addition to that humic acid increases the formation of proteins because it contains amino acids that activate the growth process in the plant [20] These results are consistent with the findings of [16][21].

Table 2: Effect of organic and biological fertilization and the interaction between them on the height of the mung bean plant (cm)

Biofertilization	**Organic fertilization (humic) gm.l^{-1}**				**average**
	0	3	6	9	
control	64.33	72.37	73.41	75.87	71.49
Azotobacter	71.46	74.00	75.40	75.46	74.08
Mycorrhiza	73.50	74.93	75.93	77.92	75.57
Z+M	75.93	73.20	77.18	81.16	76.87
average	71.30	73.62	75.48	77.60	
LSD	**biofertilization 2.408**	**organic fertilization 2.408**		**Interaction 4.817**	

2. **Root length (cm):** The results of Table (3) indicate that there are significant differences in the root length of the mash plant when biofertilizing, as the biofertilization treatment with Azotobacter and Mycorrhizae gave the highest mean, reaching 43.01 cm, compared to the non-fertilization treatment, which amounted to 31.74 cm, with a significant increase of 36.66%. The reason for this is due to the positive role of mycorrhiza fungi and azotobacter bacteria after infection, which leads to an increase in the efficiency of absorption of elements through the extension of the hyphae, an increase in the surface area of the roots, an increase in the absorption area, and nitrogen fixation, which was reflected in the metabolic activities within the plant that led to an increase in

the root length and making the plant more resilient. To grow and provide the needs of the plant, in addition to that the biofertilization increases the weight of the root system of the plant, due to its ability to dissolve phosphate and produce indole acetic acid and some microelements such as iron[22]. As for the organic fertilization, humic acid fertilization led to an increase in root length, as it gave the treatment of adding humic acid at a concentration of 9 gm.l.$^{-1}$ The highest average root length reached 40.24 cm, compared to 33.14 cm without fertilization, an increase of 21.42%. The reason is that organic fertilization works to supply the plant with organic acids and nutrients, including phosphorous and nitrogen, which increases the manufacture of nutrients and stimulates cell division, thus increasing the root total. [23][24].

Table 3: The effect of organic and biological fertilization and the interaction between them on the root length of mung bean plant, cm

Biofertilization	Organic fertilization (humic acid) gm.l^{-1}				average
	0	3	6	9	
control	29.97	30.95	31.82	34.23	31.74
Azotobacter	32.73	35.95	37.56	38.87	35.61
Mycorrhiza	33.69	43.32	35.71	38.72	36.28
Z+M	36.18	41.38	45.37	49.13	43.01
average	33.14	35.65	37.61	40.24	
LSD	**biofertilization 3.160**	**organic fertilization 3.160**		**Interaction 6,320**	

3. **Number of branches (branch. plant^{-1})** The results of Table (4) indicate that there are significant differences in the number of branches of the mung bean plant when biofertilizing, as the treatment of biofertilization with Azotobacter and Mycorrhiza gave the highest average, as it reached 15.00 branches.$^{-1}$ Compared to the non-fertilization treatment, which amounted to 12.25 branches^{-1} With an increase of 22.44%, the reason for this is due to the role of biofertilization in increasing the concentration of the nitrogen element, which plays a role in stimulating growth hormones, including the auxin hormone, which has an important role in the division and expansion of cells. The nitrogen element also contributes to the production of cytokines, which work to encourage the growth of lateral shoots and increased branching [25]. As for organic

fertilization, humic acid fertilization led to an increase in the number of branching, as the fertilization treatment was given by adding humic acid at a concentration of 9 gm.l.$^{-1}$ The highest average number of branches of the mung bean plant, which amounted to 15.39 branches.plant^{-1} Compared to the non-fertilization treatment, which amounted to 13.08 branches.plant^{-1} With an increase of 17.66%, the reason for this is that humic acid works to stimulate cell division and elongation due to its direct effect on the process of respiration, photosynthesis, other enzymatic reactions and protein building, as well as its effect similar to the effects of plant hormones and works to increase the absorption of nutrients, which leads to an increase in synthetic materials that It accumulates in the plant [26]and [21]. Humic acid also increases the vegetative growth of the plant, as it increases the permeability of plant cell membranes to materials, which leads to an increase in branching, leaves, and leaf area [27][28].

Table 4: The effect of biological and organic fertilization and the interaction between them on the number of branches of the mung bean plant (branch.plant^{-1})

Biofertilization	**Organic fertilization (humic acid) gm.l^{-1}**				**average**
	0	3	6	9	
control	10.76	12.02	12.90	14.33	12.25
Azotobacter	13.60	14.80	14.40	15.36	14.54
Mycorrhiza	13.36	13.60	14.30	15.50	14.19
Z+M	14.60	14.16	14.90	16.36	15.00
average	13.08	13.39	14.12	15.39	
LSD	**biofertilization 0.463**		**organic fertilization0.463**		**Interaction 4.817**

4. **Paper area (cm^2):** The results of table (5) indicate that there are significant differences in the average leaf area of the mung bean plant, as the average leaf area increased with the addition of biological fertilizers, as the treatment of biofertilization with Azotobacter and Mycorrhizal plants gave the highest average leaf area of 660.77 cm.^{2} cmpared to a non-composting 603.68cm^2 . With an increase of 9.45%, the increase in the leaf area is attributed to the positive role of Mycorrhiza and Azotobacter fungi after infection, which led to an increase in the absorption efficiency of elements, especially nitrogen and phosphorus, through the expansion of hyphae, and an increase in the absorption

area, as well as the efficiency of hyphae in absorption. It is more efficient than the absorption of root hairs, which is reflected in the metabolic activities within the plant, and increases the characteristics of plant growth, including the leaf area of the mung crop [29]. Also, biofertilization causes an increase in the leaf area, which increases the efficiency of the photosynthesis process, and this is reflected in an increase in the output of the process itself [30][31][16]. Also, the results in Table (5) indicate the superiority of the treatment of adding acid Humic at a concentration of 9 gm.L^{-1} with an average of 660.83 cm^2 ,Compared to the non-control treatment, which amounted to 600.77 cm^2 . With an increase of 9.99%, the reason for this is due to the fact that humic acid works on the readiness to absorb microelements that have a direct effect on plant growth as it increases cell division, especially in meristematic cells [32]. Likewise, humic acid works like plant hormones such as gibberellins, which work to elongate and widen the leaves, which results in an increase in leafy area [33], as well as the role of organic matter in increasing the levels of nutrients absorbed and promoting the growth of the root system, which is reflected in the leafy area [34], and these results are consistent with each of [35][16].

Table 5: The effect of biological and organic fertilization and the interaction between them on the leaf area of mung bean (cm^2)

Biofertilization	**Organic fertilization (humic acid) gm.l-1**				**average**
	0	3	6	9	
control	518.89	582.55	650.06	663.21	603.68
Azotobacter	614.15	617.48	639.38	646.66	629.42
Mycorrhiza	629.10	637.20	652.34	643.17	640.45
Z+M	640.95	648.30	663.55	690.29	660.77
average	600.77	621.38	651.33	660.83	
LSD	**biofertilization 31.44**	**organic fertilization31.443**			**Interaction 62.885**

Conclusion

The effect of biofertilization with Azotobacter and Mycorrhizal fungi had a significant effect on all studied traits. -The organic fertilization with humic acid at a concentration of 9 gm.l^{-1} led to an improvement on vegetative characteristics of mung bean plant. -Increasing the effectiveness and activity of Azotobacter and Mycorrhizal fungi when mixed together, as they work more efficiently than the individual treatments, which led to an increase on vegetative characteristics of mung bean.

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