International Journal of Applied Sciences: Current and Future Research Trends

(IJASCFRT)

ISSN: 2790-3990

https://ijascfrtjournal.isrra.org/index.php/Applied_Sciences_Journal

Impact of Biochare and Mycorrhizal Fungus *Glomus* sp. as Biofertilizer on *Phaseolus vulgaris* Plant Growth

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Abstract

The study aimed to determine the effect of different concentrations of biochare and there interaction with the mycorrhizal fungus *Glomus mosseae* growth of *Phaseolus vulgaris* plant cultivated in plastic pots. The results showed a significant increase in the vegetative growth parameters of green bean plants ,The interaction treatment between mycorrhizal fungi and the addition of 3% of biochare achieved the highest average plant height which was 39.13 cm compared control treatment which was 30.90 cm . The highest average number of leaves was in the treatment of mycorrhizal fungus interaction with 1% biochare, which was 17.33 leaves.plant-1, The treatment of mycorrhizal fungi interacting with 3% biochare significantly outperformed the rest of the treatments and recorded 14.94 gm in fresh shoot weight ,The interaction treatment of mycorrhizal fungus with 3% biochare significantly outperformed the rest of the treatments, as it recorded a dry weight of 2.540 gm ,as for the control treatment, it recorded the lowest dry weight of the green mass, which was 1.487 gm.Treatment of mycorrhizal fungus and 1% biochare interaction achieved the highest increase in root length, which was 63.8 cm. The treatment of mycorrhizal fungus interaction with 3% Biochare significantly outperformed the rest of the treatments, as it recorded a root fresh weight of 28.36 gm, treatments of mycorrhizal fungus with 3% biochare recorded a significant increase in the average dry weight of the root compared to the rest of the treatment and was 2.257 gm compared to the control treatment which recorded the lowest average dry weight of the root and was 0.897 gm.

Keywords: Mycorrhizal; Biochare; Phaseolus vulgaris;Glomus mosseae ;growth.

Received: 3/27/2025
Accepted: 5/10/2025
Published: 5/20/2025

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1. Introduction

Phaseolus vulgaris L. is a member of the Fabaceae family of legume plants. It is a crop high in protein, carbs, and minerals including vitamins, calcium, phosphorus, iron and amino acid niacin [1]. In Iraq, beans are grown for their dry seeds and green seeds. When planted in the fall, they only produce green pods. As winter season plants, they can also be grown in protected conditions in winter. Although it is known that legumes can fix atmospheric nitrogen and enter the crop rotation, beans are not thought to be effective legumes in doing so as a result, fertilization whether chemical, organic or biological is one of the most important crop service operations and a means of enhancing production because it controls the physiological processes in plants, especially nutrition [2]. Biofertilizers is one of the ways to reduce the excessive use of chemical fertilizers because they are of economic importance in agriculture by improving the absorption of nutrients such as phosphorus, nitrogen and microelements or in the secretion of some growth regulators, as well as their importance in biological control and the low cost of these organisms , Mycorrhizae fungi one of these biohertilizers , as inoculation with mycorrhizae leads to improving the growth condition of the plant and increasing the yield and reducing nutritional requirements and reducing fungal diseases [3,4]. Mycorrhizal fungi also improve the soil structure, acting as a framework that holds soil particles together and creates suitable conditions for the formation of soil aggregates by fungal threads and their extension within the soil, In addition they produce Glomalin, which is a sticky glycoprotein that is characterized by its high ability to stick soil particles together ,It also contributes to the formation of aggregates and increases their stability, which leads to improving soil properties [5]. Mycorrhizal fungi also contribute to improving water relations by extending their hyphae to distances far from the roots, which facilitates increased water absorption and the absorption of many slow-moving elements, including phosphorus [6] .

Biochare is produced from plant materials such as grasses, agricultural and forest residues that decompose at high temperatures, During this process the physical and chemical properties of the plant material change into a highly porous, stable, carbon-rich material [7]. This process of producing Biochare from plant residues can be considered one of the means of waste disposal and recycling [8]. Biochare is characterized by its high surface area and ability to adsorb nutrients and organic and inorganic molecules [9], its neutral acidity, as well as its low conductivity and its high content of carbon and other nutrients [10]. Adding biochare to soil can increase water holding capacity, thereby reducing water and nutrient leaching. Minimizing nutrient losses through leaching can improve farmers' profits and sustainability by increasing fertilizer use efficiency, reducing fertilizer costs, and avoiding the need for leaching, Biochare can reduce irrigation requirements and make it possible to expand production on limited water supplies [11]. When combined with the above benefits, it modifies the root zone habitat of plants and the surrounding microbial community, often resulting in greater microbial abundance and activity, and can also increase crop yields. Some studies suggest that biochare has improved plant growth and some report no or negative effects of biochare on plant growth [12]. Recent research suggests that it can be used as a soil improver and plant growth stimulant, as biochare improves many physical, chemical, and biological soil properties [13,14,15] . the current study aimed to study the effect of inoculation with the mycorrhizal fungus *Glomus mosseae* and its interaction with the addition of biochare on the growth of green bean plant *Phaseolus vulgaris* L**.**, and mycorrhizal characteristics of the mycorrhizal fungus *Glomus mosseae*.

2. Materials and Methods

An experiment was conducted in plastic pots during the 2022 agricultural season to study the effect of the interaction between biofertilization with *Glomus mosseae* mycorrhizal fungus and the addition of biochare on the growth of green bean *Phaseolus vulgaris* L. In this study, a sandy-mixed soil was used and placed in 2 kg plastic pots. The chemical and physical properties of the soil were examined before planting in the laboratories of the Agricultural Research Department in Baqubah, as shown in Table 1. The experiment was conducted according to the following steps:

Table 1: Some physical and chemical properties of soil before planting.

Analysis type	Unit of measurement	Analysis result
Electrical conductivity E.C.	Dsm^{-1}	2.5
Soil pH		7.1
Organic Matter O.M.	%	2.1
soil texture	Sandy	
	Sand	92 %
	Silt	5.10 %
	Clay	2.90 %

*Green bean seeds *Phaseolus vulgaris* L. Local variety were obtained from the local market in Baqubah city - Diyala Governorate. The seeds were superficially sterilized by soaking them in 2% sodium hypochlorite solution for 3 minutes, after which the seeds were washed several times with heat-sterilized distilled water.

**Glomus mosseae* mycorrhizal fungus was obtained from the Agricultural Research Department in Zafaraniya, affiliated with the Ministry of Science and Technology. The fungal inoculation consists of soil containing fungal spores and pieces of white corn roots infected with the mycorrhizal fungus. The mycorrhizal fungus vaccine was added to the soil at a rate of 10 $g.kg^{-1}$ soil.

*Biochare prepared from eucalyptus wood was used according to the method described in [16]. It was prepared by exposing the wood to a high temperature of 500°C in a special oven in the absence of oxygen for 3 hours. After that, the samples were left in the oven to cool and were removed from the oven after 24 hours, crushed using a metal mortar, passed through a sieve with a hole diameter of 1 mm, and stored the carbon in bags until the experiment was carried out. Biochare was added to the potting soil at two concentrations: 1% and 3% of the soil weight.

*Plastic pots were filled with soil and fungal inoculum and biochare were added before planting. Then, green bean seeds were planted at a rate of 3 seeds per pot in March 2022. After that, the plants were thinned to one plant per pot. The experiment included 18 experimental units consisting of 6 treatments and 3 replicates for each treatment. After one month of planting, the mycorrhizal characteristics were studied, while the vegetative and root growth characteristics were taken after two months of planting. The studied characteristics included all of the following:

*Percentage and severity of root colonization by mycorrhizal fungi, the ink and vinegar staining method, known as Ink and Vinegar, was used as described in [17]**.** The percentage of root infection by mycorrhizal fungi was calculated using the equation described by [18]. The severity of mycorrhizal infection was calculated according to the equation [19].

* Plant height (cm) measurement was taken with a metric tape measure from the point of contact of the plant with the soil to the highest terminal tip. Root length (cm) measurement was taken with a metric tape measure after extracting the root from the soil and washing it with running water. The fresh weight of the vegetative group was calculated at the end of the experiment, where a sensitive balance was used to measure the weight, the samples were placed in perforated paper envelopes and placed in an electric oven at a temperature of 65°C until the weight stabilized. The fresh weight of the root system was calculated at the end of the experiment after washing the roots with running water to remove the dirt stuck to the root, then the fresh weight was taken using a sensitive balance. To take the dry weight, the roots were placed in perforated paper envelopes and the samples were dried using an electric oven at a temperature of 65°C until the weight was stable.The number of leaves per plant was calculated by taking the average number of leaves per plant.

*The results of the experiment were statistically analyzed according to the analysis of variance method. The significant differences between the means were compared at the probability level of 0.05 using Duncan's multiple range test. The Genstat 2012 program was used in the statistical analysis.

3. Results and Discussion

3.1. mycorrhizal characteristics

Figure (1) shows the effect of adding Biochare to the soil on the percentage and severity of infection of green bean roots with the mycorrhizal fungus *Glomus mosseae* ,The results show a significant decrease in the percentage and severity of infection with the mycorrhizal fungus compared to the treatment of inoculation with the mycorrhizal fungus only, also the percentage and severity of infection decreased with increasing the concentration of Biochare to reach 50% and 36.66% respectively at a concentration of 3% Biochare and 70% and 46.66% respectively at a concentration of 1% Biochare. As for the treatment of the mycorrhizal fungus only, the percentage and severity of infection recorded 100% and 60% respectively. The presence of Biochare in the soil enhances the nutritional status of the plant through its ability to absorb important nutrients for the plant on its surface [20] (Gao, 2016). Consequently, the plant's need for mycorrhizal fungi decreased, which was reflected in the percentage and severity of root infection with mycorrhizal fungi. This was shown by the study of [21] Sun and his colleagues (2022), which showed that the percentage of maize roots infection with mycorrhizal fungi decreased when the soil was treated with Biochare.

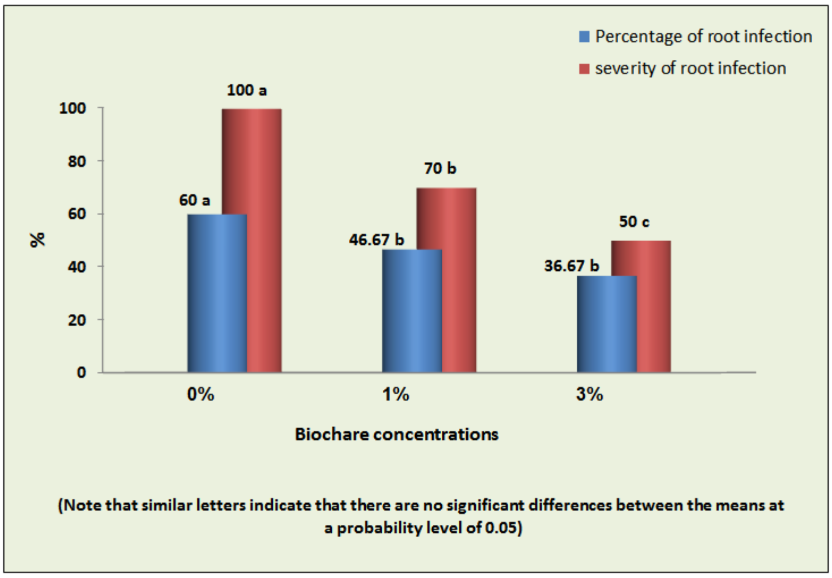

Figure 1: Effect of biochare on the percentage and severity of green bean roots infection with the mycorrhizal fungus *Glomus mosseae*. Note that the probability value p-values ≤ 0.00.

3.2. Shoot growth characteristics

The results shown in Table (2) The results showed that the addition of mycorrhizal fungi led to a significant increase in plant height, and the average plant height increased with the addition of biochare. The interaction treatment between mycorrhizal fungi and the addition of 3% of biochare achieved the highest average plant height, followed by the interaction treatment with the concentration of 1%, which were 39.13 cm and 38.5 cm respectively. Compared to the treatment of adding mycorrhizal fungi only, which was 36.20 cm and the control treatment, which was 30.90 cm . The results in Table (2) also show a significant increase in all treatments in the number of leaves compared to the control treatment without addition. The highest average number of leaves was in the treatment of mycorrhizal fungus interaction with 1% biochare, which was 17.33 leaves.plant^{-1}, with an increase rate of 57.55.

Table (2) also shows a significant increase in the fresh weight of the green bean plant growing in plastic pots in the treatment of interaction between mycorrhizal fungi and Biochare, which also significantly outperformed the treatment of adding biochare only. The results show a significant increase in the fresh green weight of green beans when biochare was added and interacted with mycorrhizal fungi. The treatment of mycorrhizal fungi interacting with 3% biochare significantly outperformed the rest of the treatments and recorded 14.94 gm. As for the treatments of adding mycorrhizal fungi and control, they recorded the lowest average fresh green weight and were 10.51 gm and 9.93 gm, respectively.

Table (2) also shows a significant increase in the green dry weight of the bean plant. The interaction treatment of mycorrhizal fungus with 3% biochare significantly outperformed the rest of the treatments, as it recorded a dry weight of 2.540 gm, followed by the two treatments of 3% biochare, which was 2.127 gm. As for the control treatment, it recorded the lowest dry weight of the green mass, which was 1.487 gm.

The increase in plant height when treated with mycorrhizal fungi or Biochare confirms their positive effect on plant growth, as mycorrhizal fungi have the ability to increase the availability of nutrients in the soil, especially phosphorus, which is included in the composition of many metabolic compounds important in plant growth [22] , [23] . The results of the current study on the ability of mycorrhizal fungi to improve the growth of green beans agree with a number of studies, including [24]. The results of the current study also agree on the positive effect of adding Biochare in improving the growth characteristics of the vegetative group, which can be attributed to the increase in the availability of nutrients in the soil and providing a suitable environment for the growth of soil organisms beneficial to the plant [13].

Table 1: Effect of inoculation with biochare and mycorrhizal fungus *Glomus mosseae* on plant height and leaves number for green bean plant .

plant height (cm)					leaves number leaf . plant^{-1}			
Biofertilizer	Biochare concentrations			Average	Biochare concentrations			Average
	0%	1%	3%		0%	1%	3%	
Without mycorrhiza	30.90 c	34.80 b	36.33 ab	34.01 b	11.00 b	15.67 a	16.33 a	14.33 b
With mycorrhiza	36.20 ab	38.5 a	39.13 a	37.94 a	16.00 a	17.33 a	16.67 a	16.67 a
Average	33.55 b	36.65 a	37.73 a		13.50 b	16.50 a	16.50 a	

*Note :Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00 .

Table 2: Effect of inoculation with biochare and mycorrhizal fungus *Glomus mosseae* on fresh and dry weights for shoot part of green bean plant .

Fresh weight (gm)					dry weight (gm)			
Biofertilizer	**Biochare concentrations**			**Average**	**Biochare concentrations**			**Average**
	0%	**1%**	**3%**		**0%**	**1%**	**3%**	
Without mycorrhiza	9.93 c	11.24 bc	13.16 ab	11.44 a	1.487 c	1.820 bc	2.127 ab	1.811 a
With mycorrhiza	10.51 c	13.14 ab	14.94 a	12.87 a	1.610 bc	1.953 abc	2.540 a	2.030 a
Average	14.05 a	12.19 b	10.22 c		1.548 b	1.887 b	2.333 a	

*Note :Similar letters indicate that there are no significant differences between the means at a probability level of 0.05. Note that the probability value p-values ≤ 0.00

3.3. Root growth characteristics

Table (3) shows that all treatments were significantly superior to the control treatment without any significant differences between the remaining treatments. The table shows that the treatment of mycorrhizal fungus and 1% Biochare interaction achieved the highest increase in root length, which was 63.8 cm, with an increase rate of 50.47%, followed by the treatment of mycorrhizal fungus interaction with 3% Biochare, which was 61.4 cm, with an increase rate of 44.81%. All treatments of adding Biochare also achieved a significant increase in root lengths, as the concentration treatments of 3% and 1% recorded lengths of 60.9 cm and 55.5 cm, respectively, with an increase rate of 43.63% and 30.89%, respectively. As for the treatment of adding mycorrhizal fungus alone, it recorded 55.3 cm, with an increase rate of 30.42%. While the control treatment recorded the lowest root length rate, which was 42.4 cm.

Table (4) shows a significant increase in the fresh weight of the root system of green beans, as the treatment of mycorrhizal fungus interaction with 3% Biochare significantly outperformed the rest of the treatments, as it recorded a fresh weight of 28.36 gm, with an increase rate of 96.80%. We also note from the table that there were no significant differences between the treatments of 3% Biochare and the treatment of interaction between mycorrhizal fungus and 1% Biochare, which were 24.73 gm. and 24.02 gm., respectively, with an increase rate of 71.61% and 66.68%, respectively, followed by the treatments of 1% Biochare and mycorrhizal fungus, which recorded 18.33 gm and 17.48 gm , respectively, with an increase rate of 27.20% and 21.30%, respectively. As for the control treatment, it recorded the lowest weight rate. The fresh root mass was 14.41 gm . Table (5) also shows that the two treatments of mycorrhizal fungus with 3% Biochare and 1% Biochare recorded a significant increase in the average dry weight of the root mass compared to the rest of the treatment and was 2.257 gm and 1.933 gm respectively, with an increase rate of 151.61% and 115.49% respectively, compared to the control treatment which recorded the lowest average dry weight of the root and was 0.897 gm.

Table 3: Effect of inoculation with biochare and mycorrhizal fungus *Glomus mosseae* on Root length for green bean plant .

Root length (cm)				
Biofertilizer	**Biochare concentrations**			**Average**
	0%	**1%**	**3%**	
Without mycorrhiza	42.4 b	55.5 a	60.9 a	52.9 b
With mycorrhiza	55.3 a	63.8 a	61.4 a	60.2 a
Average	48.8 b	59.6 a	61.2 a	

*Note :Similar letters indicate that there are no significant differences between the means at a probability level of 0.05. Note that the probability value p-values ≤ 0.00

Table 4: Effect of inoculation with biochare and mycorrhizal fungus *Glomus mosseae* on fresh and dry weights for root part of green bean plant.

Fresh weight (gm)					dry weight (gm)			
Biofertilizer	Biochare concentrations			Average	Biochare concentrations			Average
	0%	1%	3%		0%	1%	3%	
Without mycorrhiza	14.41 d	18.33 c	24.73 b	19.16 b	0.897 b	1.081 b	1.304 b	1.094 a
With mycorrhiza	17.48 c	24.02 b	28.36 a	23.29 a	1.320 b	1.933 a	2.257 a	1.837 a
Average	15.95 c	21.18 b	26.54 a		1.108 b	1.507 a	1.781 a	

Note :Similar letters indicate that there are no significant differences between the means at a probability level of 0.05. Note that the probability value p-values ≤ 0.00

The increase in plant growth, including root system growth characteristics, is attributed to the ability of mycorrhizal fungi to dissolve the precipitated phosphate compounds and release them into the soil solution in the form of H2PO4-1 and HPO4-2 through the soil pH [25]. The plant needs phosphorus in large quantities as the second element after nitrogen, and it is important in the physiological processes within the plant and the production of energy compounds [26]. Increasing the concentration of phosphorus within the plant leads to the formation of a strong root system in the soil and thus leads to increased absorption of nutrients. On the other hand, mycorrhizal fungi have contributed to improving water relations by infecting plant roots to wide distances far from the root extension, which facilitates the preparation of nutrients, especially phosphorus and nitrogen, which enter into the composition of the chlorophyll molecule, proteins, amino acids, DNA and RNA, which contributes to increasing the chlorophyll content of the leaves [22] .

The positive role of mycorrhizal fungi after infection has led to an increase in the efficiency of absorbing nutrients through the extension of hyphae and increasing the absorption area, which is greater than the absorption of root hairs, which was reflected in the metabolic activities within the plant and also positively reflected on the characteristics of vegetative growth and the plant's content of nutrients, or perhaps the reason is that mycorrhizal fungi work to improve soil properties by causing biological, physical and chemical changes by secreting calomalin, which is considered one of the protein compounds that work to stick soil particles together and 80% of them work to stick the fungal hyphae to the soil particles, and also improve the soil composition such as the Polysaccharides compound that helps stick the soil together, which leads to increasing its ability to retain water. Also, the effect of mycorrhizae in improving vegetative growth indicators is due to its secretion of some biochemical compounds in the root growth zone Rhizospher, which work to expand and grow root hairs, which increases the surface area of the roots, which in turn affects the process of photosynthesis and the manufacture and accumulation of nutrients in plant tissues, and thus increases the dry weight of the plant [27].

The role of organic carbon (Biochare) is to improve the physical and chemical properties of the soil and increase the activity of microorganisms. It also encourages the formation of chelating compounds with micronutrients and prevents them from fixing and increases the chance of their absorption by the plant, thus its positive role in increasing the vegetative growth characteristics of the plant. It also increases the cation exchange capacity (CEC), increases the surface area, affects the degree of soil interaction, increases the availability of plant nutrients such as nitrogen and phosphorus, and enhances the soil's ability to retain water [11]. It also activates many vital and physiological processes, which encourages many enzymes that play a major role in the production of food inside the plant by encouraging the process of cell division and cell elongation, and thus increasing the height of the plant, the number of leaves, and the process of opening and closing the stomata. It regulates the osmotic potential of the plant cells, and the nutrients nitrogen, phosphorus and potassium are ready for absorption after being mineralized in the soil by microorganisms, which play an important role in the process of photosynthesis by increasing the effectiveness of the plant to carry out this process, which leads to an increase [28].

4. Conclusions

The results of this study showed that The addition of mycorrhizal fungi to the green bean plant significantly outperformed all the studied vegetative and root indicators compared to the control treatment.The addition of Biochare to the soil achieved a significant superiority in most of the vegetative and root growth indicators of green bean plants.The results of the interaction showed a significant effect on all vegetative and root growth indicators.The interaction of mycorrhizal fungi with the addition of Biochare did not have a negative effect on plant growth, on the contrary, all interaction treatments achieved a significant increase in the studied growth characteristics.

Acknowledgements

After completing the research, I would like to thank everyone who contributed to its completion and follow-up, especially those who conducted the statistical analysis.

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