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Response *Phaseolus vulgaris* L. Plant to *Glomus mosseae* and *Azotobacter chroococcum*

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Abstract

An experiment was performed to study the impact of Mycorrhizal fungus (*Glomus mosseae*) and *Azotobacter chroococcum* on the growth parameters of the common bean (Phaseolus vulgaris L.) plant. The experimental methodology employed in this study was a factorial design, specifically a randomized complete block design (RCBD), with three replications. The treatments consisted of four different biological fertilizers, namely: none-inoculated, inoculated with arbuscular mycorrhizal fungi (AMF), inoculated with Azotobacter, and a mixture of both (AMF+*Azotobacter*). The use of biofertilizers, whether used singly or in combination, resulted in improved germination rates, plant height, number of root nodes, as well as fresh and dry weights of both shoots and roots particularly (AMF + Azoto) treatment (96.33±1.52, 37.33±2.08, 50.00±2.00, 8.83±0.40, 3.90±0.20, 6.53±0.23, 2.30±0.20) respectively, compared to plants that were not infected. In addition, the dual treatment of the fungus AMF and the bacteria *A. chroococcum* was recorded a maximum mycorrhizal infection percentage of 90% compared with the control which recorded 20%. The results of this study suggest the existence of an additive or a synergistic relationship between *Glomus mosseae* and *A.chroococcum*.

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Introduction

The bean (*Phaseolus vulgaris* L.) is a significant crop globally and serves as a key source of proteins and carbohydrates in human diets in some regions (Qasim *et al*., 2020). In addition, it is important to remember that leguminous crops have the ability to meet their nitrogen needs either partially or entirely through self-sufficiency, achieved by the nodulation of their roots with highly efficient nitrogen-fixing strains of Rhizobia. Therefore, it serves a significant function in improving soil fertility through the process of biological nitrogen fixation (Loha *et al*., 2023). Then, the utilization of bio-fertilizers is considered as a promising alternative in particular for developing countries.

Arbuscular mycorrhizal fungi (AMF) have an extensive distribution, being present in the majority of soil environments. These organisms are classified within the phylum Glomeromycota, which encompasses 3 distinct classes: Glomeromycetes, Paraglomeromycetes and Archaeosporomycetes, (Sturmer, 2012; Redecker *et al*., 2013). It has been obviously shown that AM has the potential to enhance plant development by facilitating the enhanced absorption of phosphorus, particularly in soils with limited fertility (Dhiman *et al*., 2022). Mycorrhizal fungi have the ability to adapt, amass, and convey a substantial amount of phosphates through their hyphae, afterward delivering it to the root tissue cells. Previous research led by Hawkins *et al*. (2023) has demonstrated that mycorrhizal plants had the ability to absorb and accumulate a significantly higher amount of phosphate from the soil solution compared to a control plant.

Nitrogen fixation is the most significant microbial action (Hamilton *et al*., 2011) and biological processes (Hakeem *et al., 2016)* happening on the soil surface after photosynthesis. The function of biological nitrogen fixation is very important in conserving the fertility of the soil (Robson and Postgate, 1980). *Azotobacter* is utilized for the study of nitrogen fixation as well as for plant inoculation because of its fast growth and high levels of nitrogen fixation (Shokri and Emtiazi, 2010). Diazotrophic in nature, Azotobacter is categorized as a free-living, aerobic, non-symbiotic nitrogen fixer. According to Sumbul *et al*. (2020), it is acknowledged for its ability to add nitrogen to the soil through the process of biological nitrogen fixation. Furthermore, it triggers the production of phytohormones such as gibberellins and indole acetic acid, improves nitrate (NO_3), ammonium (NH_4), iron (Fe), and phosphate (H_2PO_4) absorption, and increases the enzyme's action of the nitrate reductase (Tolangi, 2022). It is generally acknowledged that co-inoculation with AM fungus and plant growth-promoting Rhizobacteria has more advantages for plant physiology and the uptake of essential nutrients (Silva *et al*., 2023). Additionally, it has been shown that this combination promotes root branching, inhibits plant pathogenic fungi (Wachowska and Rychcik, 2023), and aids in phytoremediation efforts (Al-Maliki, 2015).

The study's objective was to determine if the biofertilizer will reduce the need for chemical fertilizers and how well it will affect the yield of common bean plants grown in greenhouses.

Material and Methods

Soil sample

A soil sample (20 kg) was collected at a depth of 30 cm from the Abu Ghraib in Baghdad on 1/10/2022. The soil sample was placed in sterile polyethylene and the information regarding the place and time collection was recorded.

Isolates used in cultivation

The two isolates used in the study (*Glomus mosseae*) and *(Azotobacter chroococcum*) were obtained from the Directorate of Agriculture Research, Ministry of Science and Technology.

Physical and chemical properties of soil

The soil study findings indicated that the soil texture was classified as loam, with an electrical conductivity (EC) value of 0.463 and pH 7.2 (Table 1). Nitrogen (N), phosphorus (P), and potassium (K) values were determined to be 16.4, 27.5, and 290.08 $mgkg^{-1}$, respectively.

Table 1. The physical and chemical characteristics of the soil

Sand gkg^{-1} soil	Silt gkg^{-1} soil	Clay gkg^{-1} soil	Soil texture	Field Capacity %	pH	EC ds/m^{-1}	Available nutrients $mg\ kg^{-1}$		
							N	PO_4	K
325	420	240	Loam	35	7.2	0.463	16.4	27.5	290.08

Field experiment

A study was done from 2022 to 2023 at the Directorate of Agriculture Research in Al-Zafaraniya, Baghdad, Iraq. The aim of the research was to study the impact of Mycorrhizal fungus (*Glomus mosseae*) and *Azotobacter chroococcum* and on the growth parameters of the common bean (*Phaseolus vulgaris* L.) plant. The chemical and physical features of soil were conducted measurements (Table 1). The seeds underwent surface sterilization using a 1% solution of sodium hypochlorite, after which they were planted. Plastic pots were used containing 2 Kg sterilized soil. Four groups of pots were established: the first one was inoculated with *Glomus mosseae* (100 g) for each pot, the second was inoculated with *Azotobacter chroococcum* ($33*10^{-7}$cfu/ml), the third was inoculated with two isolates together and the last one remained without any inoculation (control). The plants were monitored as they were subjected to field conditions of appropriate lighting (10 hours), temperature (25°C) and humidity (65%). After 3 weeks growing plants were reduced to two plants for each pot by a thinned process.

The plants were harvested (60) days from the date of planting by cutting them from an area near the soil surface and drying the shoot after placing it in an electric oven, the temperature was set at 60°C for 48 hours until the weight stabilized (Lego, 2012). The roots were also extracted and washed well with water to get rid of the suspended dust were then kept in a preservative solution consisting of (ethanol: acetic acid: formalin at a ratio of 90: 5: 5) volume until laboratory tests were conducted.

Germination percentage

To determine the germination percentage, the number of seeds that germinated was counted (Mavi *et al*., 2010); it is calculated from the following equation:

Germination Rate = Number of seeds germinated / Number of seeds sown * 100

Staining root of mycorrhizae

The method of staining the root of mycorrhizae used was that of Phillips and Hayman (1970).

Assessment of mycorrhizal colonization in roots

The technique for measuring mycorrhizae in roots following staining was the slide method described by (Giovannetti and Mosse, 1980).

Statistical analysis

The experiment was conducted and analyzed as factorial experiments by using a randomized complete blocks design (RCBD) for three replicates. The mean values were compared by using the Tukey test at the probability of 5 % ($p \leq 0.05$) (Steel and Torrie, 1980).

Results and Discussion

The data reported in Table 2 demonstrates that germination, length of stem and root nodules were significantly affected by the inoculation of bio-fertilizers. It was markedly noticed that control treatment recorded the least numbers at all which recorded (75.00 %, 23.00 %, 20.00%), respectively. Biofertilizer inoculation affected the seeds germination to an appreciable level compared with control. The combination of the dual inoculation had the highest germination percentage (*Azotobacter* and AMF) (Pandey *et al*., 2003). According to (*Sharma et al*., 2020), the utilization of bio-fertilizers resulted in enhanced nutrient availability, hence positively influencing several production metrics. In their study, (Razmjooei *et al*., 2022) evaluated the response exhibited by Lettuce cultivars upon inoculation with *Azotobacter* biofertilizers. The efficacy of bio-fertilizers may be attributed to several factors, as elucidated by (Debnath and Acharya, 2021). These factors include the biological fixation of an adequate amount of atmospheric nitrogen, the synthesis of plant growth regulators, the reduction of ethylene production, and the dissolution of minerals like phosphorus.

Table 2. Impact of inoculation with *A. chrococcum* and *G. mosseae* in the germination, height and root nodes of the plant

Treatment	Germination%	Height	Root nodules
Control	75.00±3.00 C	23.00±2.00C	20.00±1.00 D
Azoto	87.66± 1.52 B	33.00±1.73 AB	50.00±2.00 C
AMF	84.33± 2.51 B	30.66±1.52B	77.66±2.51 B
Azoto + AMF	96.33±1.52 A	37.33±2.08 A	90.33±2.08 A
F value	36.03	24.20	35.58
(P<0.05)			

Figure 1. Root nodules (A): *A. chroococcum* and *G. mosseae* treatment

(B) Control treatment

The findings presented in Table 3 demonstrate that the use of the treatment of inoculation with *A. chroococcum* and AMF of the shoot and root system superiority, resulting in the greatest dry weight value among all treatments (8.83, 3.90, 6.53 and 2.30 g) respectively, in contrast to the control treatment, which gave the lowest average (5.66, 2, 3.60 and 1.23 g) respectively. The observed rise in weight and density of roots and shoot can be related to the symbiotic between mycorrhizal fungi and the plant, which causes an increase in root length after infection with mycorrhizal fungi occurs via the formation and explanation of hyphae and an increase in the surface area of the roots (Jakobsen and Legget, 2005) which enhanced an increase in the efficiency of absorption of nutrients such as nitrogen, phosphorus, sulphur, and some minor elements such as Zinc (Zn) and Copper (Cu) (Koltai, and Kapulnik, 2010). In addition, the function of *A. chrococcum* is in the secretion of plant hormones, like auxins, cytokinins, and gibberellins (Alsalim, 2019). These hormones have a significant impact on root development and density, ultimately leading to the observed increase in weight and density of roots and shoots. Furthermore, mycorrhizal fungi play a crucial function in enhancing the surface area of exposed roots inside the soil. The process enhances the uptake of water and nutrients, particularly phosphorus and trace elements like zinc and copper, even in low soil mobility conditions. Consequently, enhancing the growth of plants leads to a corresponding improvement in both root growth and vegetative growth of the plant. The microorganisms have a crucial role in facilitating nutrient absorption, enhancing water interactions, and augmenting the surface area of the roots (Jeber, and Khaeim, 2019; Smith. and Read, 2008). This result is consistent with (Saad *et al*., 2019).

Table 3. The impact of inoculation with *A.chrococcum* and *G. mosseae* in the shoot and root system

Treatment	Fresh weight shoot (g)	Dry weight shoot (g)	Fresh weight root (g)	Dry weight root (g)
Control	5.66± 0.11 C	2.00±0.10 D	3.60±0.20 C	1.23±0.30 C
Azoto	6.36±0.41 BC	2.66±0.15 C	4.86±0.30 B	1.50±0.20 BC
AMF	7.30± 0.36 B	3.03±0.15 B	5.00±0.20 B	1.86±0.15 AB
Azoto +AMF	8.83±0.40 A	3.90±0.20 A	6.53±0.23 A	2.30±0.20 A
F value	134.34	127.85	805.53	12.93
(P< 0.05)				

Colonization by Mycorrhizal fungi

Mycorrhizal plants exhibited significantly greater root colonization than non-mycorrhizal plants. The overall colonization of bean roots inoculated with *G. mosseae* ranged from 20% (control) (Fig. 2), to 80% (inoculated with AMF). However, the percentage of (AMF + *Azoto*) in root was 90% (Fig. 3). The significant increase in the percentage of mycorrhizal infection can be attributed to phosphorus deficiency conditions. In such conditions, the decrease in phospholipids within the root cell membranes results in the permeability of these membranes. Consequently, there is an increase in the secretion of reduced carbohydrates and amino acids by the roots, leading to a higher proportion of infected roots. Conversely, when phosphorus is readily available, the membranes of the root cells exhibit reduced permeability due to an increase in phospholipids. Consequently, a reduction in the quantity of carbohydrates and amino acids occurs, leading to a decline in the population of infected roots (Rubin and Görres, 2020).

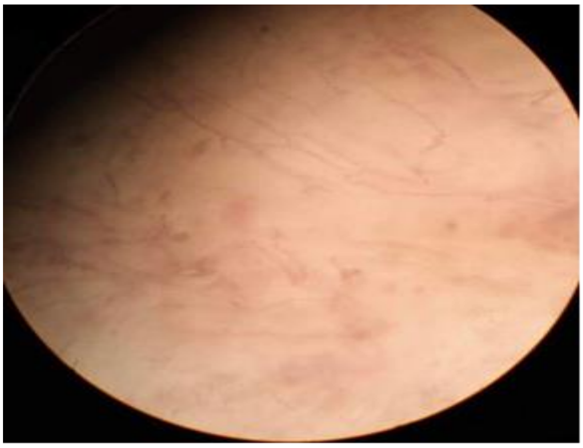

Figure 2. Root of *Phaseolus vulgaris* plant (Control)

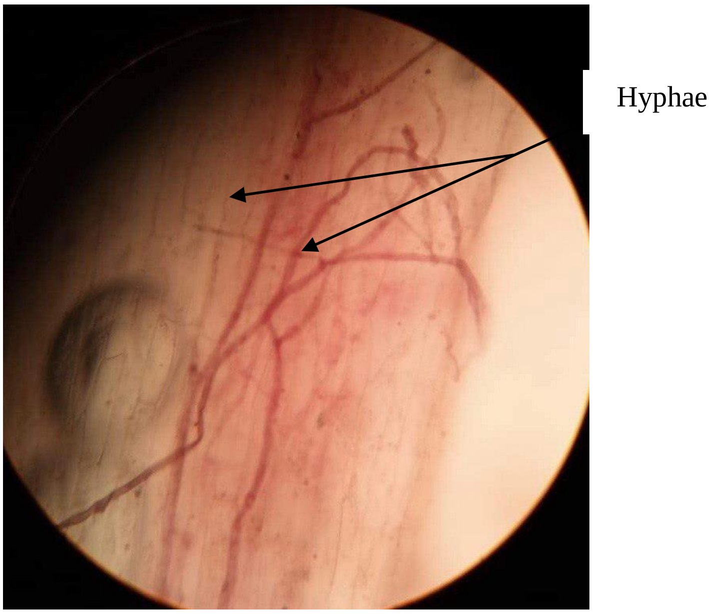

Figure 3. *AMF* + *Azoto* treatment

Conclusion

The findings of the study indicate that the application of *Glomus mosseae* and *A. chroococcum* to the common bean plant resulted in a considerable enhancement of numerous growth parameters, including germination rate, height of plant, number of root nodules, fresh and dry weight of shoot and root. The combination of AMF and *Azotobacter* shown significant favourable effects on various phases of plant development. using AMF and *Azotobacter*, either as a combined inoculation or individually, exhibited superior performance compared to the treatment without any inoculation across all observed features.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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