The Role of Biological Growth Stimulant in Reducing Fertilizer Recommendation and Increasing Some Systemic Resistance Indicators in broad bean Plants

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Abstract:

The study was carried out in the laboratory of the College of Agriculture, Anbar University, to evaluate some growth-stimulating organism *A. chroococcum*, *Rhizobium* sp., *G. mosseae* with different fertilization rates 0%., 50%.,100% In inhibiting pathogenic *F.equiseti* which causes root rot in broad beans, and there is an interaction between them in some biochemical measurements phenols., Phenylalanine Ammonia Layse., Prolinee The results showed superior treatment *A. chroococcum* In increase phenols., Phenylalanine Ammonia Layse, Prolinee which gave 55.93,28.34,5.73 mg g-1 respectively. Followed by *G. mosseae* treatment, which gave 54.13, 26.64, 5.26 mg g-1, sequentially Then treat *Rhizobium* sp 52.50, 23.28.,5.28 mg g-1 sequentially. While the best fertilizer recommendation was 100%, as the percentage of Phenols, Phenylalanine Ammonia Layse, Proline reached 51.48, 27.10, 5.54, mg g-1 respectively. While the best treatment was the interaction between *A. chroococcum* bacteria and a 100% fertilizer recommendation, which was 66.60, 35.43,7.19 mg g-1, respectively. Compared to the pathogenic fungus treatment only, the percentage of Phenols, Phenylalanine Ammonia Lyse, Proline reached 32.34, 16.60, 3.36 mg g-1 respectively. The results showed that the treatment with *A. chroococcum* bacteria gave the highest dry weight of the shoot, reaching 176.97 g Followed by *G. mosseae* treatment, the average dry weight of the shoot reached 172.11g. The results showed that the treatment *A. chroococcum* bacteria gave the highest dry and fresh weight of the root system, as the dry weight reached 386.25g and the fresh weight reached 625 .00g.

Keywords: *Azotobacter chroococcum*, *Fusarium equiseti*, phenols, Phenylalanine Ammonia Layse.

دور محفزات النمو الحيوية في تقليل التوصية السمادية وزيادة بعض مؤشرات المقاومة الجهازية في نبات الباقلاء

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الملخص:

نفذت الدراسة في مختبر كلية الزراعة جامعة الانبار لتقييم بعض الاحياء المحفزة للنمو *A. chroococcum* و *Rhizobium sp* و *G. mosseae* والتوصيات السمادية بنسبة 0% و 50% 100 % في تثبيط الفطر الممرض *F. equiseti* المسبب لمرض تعفن جذور الباقلاء وتداخل بينهما في بعض مقايس الكيمو حيوية phenols وPhenylalanine Ammonia Layse وبينت النتائج تفوق معاملة *A. chroococcum* في زيادة phenols وPhenylalanine Ammonia Layse و Proline واعطت 55.93 و28.34 و5.73 ملغم غم على التوالي تلتها معاملة *G. mosseae* اعطت 54.13 و26.64 و5.26 ملغم غم على التوالي ثم معاملة *Rhizobium* sp 52.50 و23.28 و5.28 بتتابع. بينما كانت أفضل توصية هي التوصية السمادية 100%اذ بلغت نسبة Phenols وPhenylalanine Ammonia Layse وProline 51.48 و27.10 و 5.54 ملغم غم على التوالي. في حين كان أفضل معاملة تداخل بين بكتريا *A. chroococcum* وتوصية سمادية 100 % اذ كانت 66.60 و 7.19, 35.43ملغم غم على التوالي مقارنة بمعاملة الفطر الممرض فقط اذ بلغت نسبة Phenols و Phenylalanine Ammonia Layse وProline 32.34، 16.60، 3.36 ملغم غم على التوالي. وضحت النتائج ان معاملة بكتريا *A. chroococcum* اعطت اعلى وزن جاف للمجموع الخضري اذ بلغت 176.97غم تلتها معاملة *G. mosseae* اذ بلغ معدل الوزن جاف للمجموع الخضري 172.11 غم وبينت النتائج ان معاملة بكتريا *A. chroococcum* اعطت اعلى وزن الجاف والطري للمجموع الجذري اذ بلغ الوزن الجاف فيها 386.25 غم والوزن الطري بلغ 625.00 غم.

الكلمات المفتاحية: *Fusarium equiseti*, Phenylalanine Ammonia Layse, , *Azotobacter chroococcum*, *phenols*

Introduction

The researches in the field of pest control have employed several sustainable methods with lasting effects against theseto maintain a pollution-free environment One of the most important methods is biological control, which is one of the oldest methods Recently, due to the benefits of environmental biological control compared to the chemical pesticides studies and research have increased and received great attention from the biological control (Lahlali et al., 2022). To combat pathogens, biological means are used to eliminate or reduce their effects by using

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microorganisms present in the root zone Since plants are resistant to these various pathogens, the most effective area in determining nutritional needs is the root zone, and this is due to the activity of microorganisms in the soil, which is at a high level. Therefore, increasing the efficiency of plants in nutrient absorption largely depends on the presence of symbiotic free-living soil microorganisms (Ozdemir *et al.,* 2020). There has been an increasing interest in controlling plant diseases through beneficial microorganisms due to the global need for environmentally friendly alternatives to chemical pesticides and fertilizers A large number of bacterial and fungal strains, viruses, nematodes, and insects have been used as biological control agents They have indicated that there are many applications for the biological control of numerous soil-borne plant pathogens, including those causing seed and root rot, seedling damping-off, and stem base diseases (Ptaszek *et al.,* 2023; Abed and Khdhum, 2020).

Broad bean (*Vicia faba L.*) is a good source of protein through biological nitrogen fixation (Jensen et al., 2010). It ranks sixth among legume crops, with a global production reaching 4.56 million tons annually (FAOSTAT, 2022). The fungus *F. solani* is one of the most widespread fungi. When isolated from soil and roots, the fungus can live as a saprophyte or facultative parasite, infecting plants. It tends to parasitize living tissues that are more susceptible to living in a saprophytic state, causing a range of pathological conditions, infecting the roots of mature plants, leading to root rot and seedling damping-off (Li and Li, 2022; Abed *et al.,* 2019). Therefore, this study aims to evaluate the use of *A. chroococcum*, *Rhizobium sp.*, and *G. mosseae* as bio-fertilizers to reduce fertilizer usage and to test their effectiveness as growth promoters in enhancing plant growth and resistance to root rot disease in broad beans.

Materials and Methods:

the bacteria samples *A. chroococcum*, *Rhizobium* sp., and the pathogenic fungus *F. equiseti* were obtained from the Soil Microbiology Laboratory, and the growth-stimulating fungus *G. mosseae* was obtained from the greenhouses of the Department of Soil and Water Resources, College of Agriculture, Anbar University. The growth-stimulating fungus *G. mosseae* was obtained from greenhouses for previous studies. The experiment was conducted in the greenhouse of the Department of Soil and Water Resources on November 24, 2023, following a completely randomized design (CRD) with a factorial experiment system and three replicates. Each replicate included 4 black pots with a capacity of 15 kg. Surface-sterilized broad bean (Spanish type) seeds were planted at a rate of 5 seeds per pot. Each pot was filled with 15 kg of soil (Loam type) sterilized with formalin (10:1 liter).

The study aimed to evaluate the impact of *A. chroococcum*, *Rhizobium sp.*, and *G. mosseae*, along with fertilization recommendations at rates of 0.0% and 50% (0.815 g of urea nitrogen fertilizer per pot, 2.25 g of triple superphosphate fertilizer per pot, and 0.275 g of potassium sulfate fertilizer per pot). The 100% fertilization recommendation included 1.63 g of urea nitrogen fertilizer per pot, 4.5 g of triple superphosphate fertilizer per pot, and 0.55 g of potassium sulfate fertilizer per pot. The study focused on their effects on the growth parameters of broad bean plants and their resistance to root rot disease caused by the fungus *F. equiseti*. The pathogenic fungus *F. equiseti* was added at a rate of 10 g per kilogram of soil. The treatments were implemented as follows:

1. Control treatment without any addition of biological growth promoters, with fertilization recommendations at rates of 0%, 50%, and 100%.
2. Treatment with the pathogenic fungus *F. equiseti*, with fertilization recommendations at rates of 0%, 50%, and 100%.
3. Treatment involving soaking seeds in a suspension of *A. chroococcum* (2.4×10^7) for two hours before planting, against the pathogenic fungus *F. equiseti*, with fertilization recommendations at rates of 0%, 50%, and 100%.
4. Treatment involving soaking seeds in a suspension of *Rhizobium spp.* (1.2×10^7) for two hours before planting, against the pathogenic fungus *F. equiseti*, with fertilization recommendations at rates of 0%, 50%, and 100%.
5. Treatment with the fungus *G. mosseae* at a rate of 10 g per kilogram of soil, against the pathogenic fungus *F. equiseti*, with fertilization recommendations at rates of 0%, 50%, and 100%.

Certain Enzymes: Total Phenols

The phenol content in broad bean leaves was measured for all plants from each replicate for each treatment using the Arnow method (1937).

1. **Sample Preparation**:
    - 1 gram of broad bean leaves was taken and ground using a ceramic mortar with 80% methanol.
    - The mixture was placed in a water bath with continuous stirring for 10 minutes at a temperature of 95°C.
2. **Extraction and Reaction**:
    - In a sterile glass tube, a sample of 1 ml of the extract was taken and mixed in sterile distilled water to an amount of 5 ml, in addition to that, it was mixed with 250 µl of Folin-Ciocalteu reagent.
    - The solution was incubated for 30 minutes at a temperature of 25 °C.
3. **Spectrophotometric Measurement**:
    - An appropriate volume of the solution was placed in the spectrophotometer cell, and the absorbance was recorded at a wavelength of 515 nm.
    - The phenol content was quantified utilizing catechol as a standard reference, reported in mg of phenol per gram of fresh plant tissue (Meena et al., 2008).

Phenylalanine Ammonia-Lyase (PAL) Activity

In 1984, an experiment was conducted following the method of Dickerson et al. to measure the activity of the enzyme phenylalanine ammonia lyase (PAL) in bean leaves. The plant material, previously stored in the freezer and amounting to 0.5 g, was washed. After drying and chopping, it was mixed with 3 ml of sodium borate solution (0.1 M, pH 7). The mixture was then ground under refrigerated conditions and centrifuged for 15 minutes at 16,000 rpm. The supernatant was collected and used as the enzyme source. A 0.4 ml aliquot of the enzyme extract was incubated with 0.5 ml of sodium borate solution and 0.5 ml of phenylalanine for 30 minutes at 30 °C. The conversion of phenylalanine to trans-cinnamic acid was measured by determining the absorbance at 290 nm using a spectrophotometer. Enzyme activity was calculated as the amount of enzyme needed to produce one microgram of cinnamic acid per minute per gram of fresh plant tissue. This process was repeated for all plant samples.

Proline Content in Plants

The proline content in plants was estimated using the method by Bates (1973). 0.1 ml sample of plant extract was mixed with 2 ml of ninhydrin reagent and glacial acetic acid. The absorbance of the mixture was read at a wavelength of 520 nm. The amount of proline was determined from a standard curve and calculated using the formula:

$$\mu\text{moles Proline/g} = \left(\frac{\mu\text{g proline/ml} \times \text{toluene}}{115.5\mu\text{g/}\mu\text{mole}}\right) / \frac{\text{g sample}}{5}$$

Where mg Proline/ml is the proline concentration from the standard curve, toluene (ml) is the volume of toluene used (1 ml), 115.5 is a constant, and g of sample is the weight of the plant sample.

Vegetative dry weight (g plant^{-1})

The process involved collecting the biomass, which included stems, branches, and leaves, by cutting the above-ground parts at soil level. After collection, the biomass was washed with regular water to the soil. It was then placed in paper bags and dried in an electric oven at 65°C until a constant weight was achieved. Once dried, the biomass was weighed using a sensitive electronic balance. Finally, the average dry weight per plant was calculated in grams based on these measurements.

Dry and fresh weight of root system (g plant^{-1})

After harvesting the plants from the soil, the roots were separated from the shoot biomass and thoroughly cleaned. The fresh weight of the roots was measured using a Sartorius balance, with readings recorded in grams per plant. Subsequently, the roots were dried in an electric oven at 70°C until a constant dry weight was achieved. The dry weight of the roots was then recorded.

Results and Discussion

The results of (Table1) showed that the treatment with the bacterium *A. chroococcum* gave the highest phenol content at 55.93 mg g-1, followed by the treatment with the fungus *G. mosseae* at 54.13 mg g-1, and then the treatment with the bacterium *Rhizobium* sp., 52.50 mg g-1. This was compared to the control treatment contaminated with the pathogenic fungus *F. equiseti*, 32.34 mg g-1. The results also indicated that the levels of fertilization 0%, 50%, 100% had phenol contents of 32.60, 46.54, and 51.48 mg g-1, respectively. The best interaction was between the bacterium *A. chroococcum* and the 100% recommended fertilization level, 66.6 mg g-1, followed by the interaction between the fungus *G. mosseae* and the 100% recommended fertilization level, 65.20 mg g-1.These findings are consistent with those of Trivedi *et al.*, (2020), who indicated that increased activity of defence-related enzymes in plants, including peroxidase, and higher phenol content are positively correlated with the induction of systemic resistance in plants against pathogens. The accumulation of phenolic compounds secreted by plants and effectively in infected cells contributes to enhancing their defense against plant diseases caused by harmful fungi (Hassan et al., 2021).

Table 1: Interaction between biological determinants and fertilization endorsements based on the concentration of phenols in broad bean leaves (mg g^{-1})

Treatment Name	Fertilization Recommendation 0.0%	Fertilization Recommendation 50%	Fertilization Recommendation 100%	Effect of Treatments (A)
Control	18.00	24.30	26.10	**22.80**
F. equiseti	27.70	32.63	36.70	**32.34**
F. equiseti + G. mosseae	37.80	59.40	65.20	**54.13**
F. equiseti + A. chroococcum.	40.10	61.10	66.60	**55.93**
***F. equiseti + Rhizibium* sp**	39.40	55.30	62.80	**52.50**
Mean B	**32.60**	**46.54**	**51.48**	
$LSD_{0.05}$	**A**= 2.474	**B**= 1.916	**A*B**= 4.285	

(Table2) shows the significant difference in treatments through different interactions. The *A. chroococcum* treatment was found at 28.34 mg/g which is considered the highest PAL content, followed directly by *G. mosseae* and *Rhizobium* sp. treatments, which recorded 26.64 mg/g and 23.28 mg/g, respectively. And, since PAL was 16.60 mg/g, it was considered the most effective and influential on the plant compared to the control treatment contaminated with the pathogenic fungus *F. equiseti*. The results examined showed that the PAL levels at 0%, 50% and 100% fertilization rates were 17.44 mg/g, 20.84 mg/g and 27.10 mg/g, respectively. The best interaction was observed between *A. chroococcum* treatment and the 100% fertilization recommendation, which achieved a significant difference from the other treatments, with a PAL content of 35.43 mg/g. This effect can be attributed to the stimulation of plant cells by the biological agents used, which resulted in an increase in the concentration of PAL enzyme. The resistance of plants to biotic and abiotic stresses has an important and required effect, so the enzyme can include the process of preventing stresses or reducing their effects. The increased activity of the PAL enzyme in plants is one of the indicators of induced resistance. With the increase in the availability of this enzyme, the activation of other defense mechanisms in the plant increases. It contributes to the production of trans-cinnamic acid, which acts as a promoter of salicylic acid synthesis, a signal for the formation of systemic acquired resistance (SAR). SAR is involved in the biosynthesis of phenolics and phytoalexin (Jun et al.,2018). What prevents the growth or germination of pathogen spores is their release outside the plant tissues so that they do not reactivate. In addition, this enzyme has an important effect as it is responsible for converting the amino acid phenylalanine to trans-cinnamic acid. For many organic acids, this enzyme is considered an intermediate in their biosynthesis. These organic acids are then converted to phenolic compounds such as caffeic acid and p-coumaric acid. In addition, in many reactions leading to the synthesis of lignin and salicylic acid, which are necessary for the induction of systemic acquired resistance (SAR) in plants at the site of

infection, where this enzyme plays a prominent role in the synthesis process, they are essential. (Deng and Lu,2017)

Table 2: Interaction between biological factors and fertilization recommendation on PAL concentration in broad bean leaves (mg g-1)

Treatment Name	Fertilization Recommendation 0.0%	Fertilization Recommendation 50%	Fertilization Recommendation 100%	Effect of Treatments (A)
Control	13.93	12.96	15.43	**14.11**
F. equiseti	16.40	16.13	17.26	**16.60**
F. equiseti + G. mosseae	18.76	27.53	33.63	**26.64**
F. equiseti + A. chroococcum.	20.26	29.33	35.43	**28.34**
***F. equiseti + Rhizibium* sp**	17.86	18.23	33.76	**23.28**
Mean B	**17.44**	**20.84**	**27.10**	
$LSD_{0.05}$	**A**= 0.652	**B**= 0.505	**A*B**= 1.130	

From (Table 3), we can notice that the results indicate that proline enzyme has a significant effect. Its percentage increased with *A. chroococcum* treatment, which showed the highest value for proline 5.730 mg L-1, followed by *Rhizobium* sp. treatment. As for the proline enzyme content, its percentage was 5.28 mg L-1, followed by *G. mosseae* treatment, where the proline enzyme content was 5.26 mg L-1. On the other hand, the lowest value was 3.360 mg L-1, which represented the treatment with the pathogenic fungus *F. equiseti*. Three fertilization levels were adopted for measurement (0%, 50%, and 100%), where the results showed its significant effect with proline enzyme concentrations of 3.27 mg L-1, 4.23 mg L-1, and 5.54 mg L-1, respectively. It was found that with the highest fertilization rate (100%), the best possible effect and interaction in proline concentration between *A. chroococcum* was shown. Compared to other treatments, and due to the role of proline in protecting the plant from reactive oxygen species (ROS), a high and effective effect was achieved with a proline concentration of 7.19 mg L-1. These results enhance the elimination of hydroxyl radicals and singlet oxygen in the plant through the ability of proline in the plant to adapt and interact with oxidative stress. In addition, it works to prevent oxidation of cell membranes, which enhances and improves the overall resilience of the plant against oxidative damage (Abd El-Hai & El-Saidy 2016)

Table 3: The effect of the interaction between biological factors and fertilization recommendation on proline concentration in broad bean leaves (g plant^{-1})

Treatment Name	Fertilization Recommendation 0.0%	Fertilization Recommendation 50%	Fertilization Recommendation 100%	Effect of Treatments (A)
Control	2.03	2.29	2.02	**2.11**
F. equiseti	3.18	2.73	4.16	**3.36**
F. equiseti + G. mosseae	3.31	5.30	7.18	**5.26**
F. equiseti + A. chroococcum.	4.36	5.64	7.19	**5.73**
***F. equiseti + Rhizibium* sp.**	3.46	5.19	7.19	**5.28**
Mean B	**3.27**	**4.23**	**5.54**	
$LSD_{0.05}$	**A**= 0.648	**B**= 0.502	**A*B**= 1.123	

The results in (Table 4) indicate that the treatment with the bacterium *A. chroococcum* significantly outperformed the other treatments, with dry weight of 176.93 g plant^{-1}. This was followed by the treatments with *G. mosseae* and *Rhizobium* sp., 172.11 g and 169.66 g per plant, respectively. Compared to the control treatment contaminated with *F. solani*, 140.94 g plant-1, all treatments showed a significant superiority over their

counterparts. It was also shown that 100% full fertilization significantly outperformed 0% and 50% fertilization, which had dry weights of 172.22 g plant-1. The results showed a significant interaction with other treatments when *A. chroococcum* and the 100% fertilization recommendation were interacted with 183.13 g plant-1, followed by *G. mosseae* treatment and the recommended fertilization level of 100%, which resulted in 176.66 g per plant. The secretion of siderophores in the bud system, which can be used to control fungal diseases, caused a significant increase in the dry weight of the *A. chroococcum* bacteria. Here, the plants need for the basic nutrient, which is to fix atmospheric nitrogen freely, appears. It is a very important source for the creation or synthesis of chlorophyll molecules and nucleic acids (RNA and DNA) (Alkobaisy, 2020). Also, due to its role in the synthesis of amino acids and proteins, we find an increase in the dry matter weight of the plant and a clear improvement in the growth of the buds. In addition, when infected with the *G. mosseae* fungus, there can be a clear increase in plant growth, both in buds and roots, due to several factors. One significant factor is the role of mycorrhiza in enhancing nutrient absorption, especially phosphorus, by exploring areas beyond the root's reach. The fungal hyphae extend far outside the plant's root system, increasing the volume of soil exploited, and thereby enhancing the absorption of water and essential macro and micronutrients necessary for plant growth (Khadhum et al., 2020; Awad and Farhan, 2012).

Table 4: The effect of the interaction between biological factors and fertilization recommendation on the dry weight of the shoot system (g)

Treatment Name	Fertilization Recommendation 0.0%	Fertilization Recommendation 50%	Fertilization Recommendation 100%	Effect of Treatments (A)
Control	154.23	161.76	176.40	**164.13**
F. equiseti	133.40	139.03	150.40	**140.94**
F. equiseti + G. mosseae	164.40	175.26	176.66	**172.11**
F. equiseti + A. chroococcum.	172.40	175.40	183.13	**176.97**
***F. equiseti + Rhizibium* sp**	165.73	168.73	174.53	**169.66**
Mean B	**158.03**	**164.04**	**172.22**	
$LSD_{0.05}$	**A=** 2.545	**B=** 1.971	**A*B=** 4.408	

The results of (Table 5) indicated that the treatment with the bacterium *A. chroococcum* significantly outperformed the other treatments in terms of the dry weight of the root system, reaching 386.25 g plant^{-1}. This was followed by the treatment with the fungus *G. mosseae*, at 382.36 g plant^{-1}, and then the treatment with the bacteria *Rhizobium* sp., with an average dry weight of the root system at 372.64 g plant^{-1}. All treatments significantly outperformed the control treatment contaminated with the fungus *F. solani*, 265.88 g plant^{-1}. The results in this paper confirmed that the recommendation of 100% fertilization is the best, followed by the recommendation of 50%, where the dry weight of the total roots reached 367.73 g and 350.96 g plant-1 respectively. The results also showed that the best interaction was between A. chroococcum bacteria at the recommended fertilization of 100%, which achieved a significant difference compared to other treatments, which reached 397.66 g plant-1, followed by *G. mosseae* fungus and the recommended fertilization level was 100%, which reached 391.00 g plant-1, compared to the treatment with the pathogenic fungus and the fertilization recommendation of 0.0%, which reached 250.33 g plant-1. The increase in the dry weight of the root system is attributed to the role of bio-growth promoters in secreting growth regulators that play a role in cell division, activating plant growth, secreting some organic acids, photosynthesis, and transporting manufactured materials to their storage sites (Kumar *et al*., 2012). It also secretes plant hormones and some growth regulators responsible for cell division and elongation, which contribute to the growth, development, and increase of the root system and the surface area of the absorption zone. This, in turn, enhances the rate of water and nutrient absorption and protects the plant from biotic and abiotic stresses (Rahma *et al.*, 2022).

Table 5: The effect of the interaction between biological factors and fertilization recommendation on the dry weight of the root system (g)

Treatment Name	Fertilization Recommendation 0.0%	Fertilization Recommendation 50%	Fertilization Recommendation 100%	Effect of Treatments (A)
control	321.66	341.00	389.66	**350.77**
F. equiseti	250.33	268.00	279.33	**265.88**
F. equiseti + G. mosseae	371.00	385.10	391.00	**382.36**
F. equiseti + A. chroococcum.	373.43	387.66	397.66	**386.25**
***F. equiseti + Rhizibium* sp**	363.86	373.06	381.00	**372.64**
Mean B	**336.06**	**350.96**	**367.73**	
$LSD_{0.05}$	**A=** 4.76	**B=** 3.69	**A*B=** 8.26	

The effect of bio-growth stimulants and the recommended fertilization rate on the fresh weight of the root system**.**

(Table 6) results indicate that all treatments achieved a significant increase in the fresh weight of the root system for broad bean plants compared to the control treatment with the presence of the pathogenic fungus *F. solani.* The fresh weight in the control was 570.85 g plant^{-1}. The treatment with the bacterium *A. chroococcum* achieved a significant difference, reaching 625.00 g plant^{-1}, followed by the treatments with the fungus *G. mosseae* and the bacterium *Rhizobium sp*., which reached 618.89 g plant^{-1} and 588.54 g plant^{-1}, respectively. The results showed that the best fertilization recommendation was 100%, with the fresh weight of the broad bean roots reaching 615.86 g plant^{-1}, followed by the 50% fertilization recommendation, with a fresh root system weight of 607.09 g plant^{-1}. The results showed that the best interaction was between *A. chroococcum* and the recommended fertilizer level of 100%, which amounted to 641.26 g plant-1, followed by *G. mosseae* and the fertilizer level of 100%, which amounted to 634.66 g plant-1, which was compared with the treatment with the pathogenic fungus and the recommended fertilizer level of 0.0%, which amounted to 561.99 g plant-1. *A.* bacteria contribute effectively to the increase in the fresh weight of the roots, as they are directly responsible for the secretion of active substances such as vitamins B1, B2, B4, B12, gibberellin, indole acetic acid (IAA), and folic acid. In addition, it works to increase the size of the root system and root hairs, which in turn will generate an increase in the nitrogen content in the plant (Rajarman**,** et al., 2013). Additionally, bio-growth stimulants enhance the plant's disease tolerance by compensating for root mass and function loss due to disease damage, effectively increasing the root absorption surface area through hyphae extension and spread, thus promoting plant growth and disease resistance. Additionally, the reason for the increased growth of the plant and its root system when infected with the fungus *G. mosseae* is attributed to several factors, including the role of *G. mosseae* in promoting the absorption of nutrients, especially phosphorus, by exploring areas beyond the root's reach. The hyphae of the fungus extend far outside the plant's root system, thus increasing the volume of soil exploited and consequently enhancing the absorption of water and essential nutrients, both major and minor, for plant growth. This is consistent with the findings of Al-Asafi et al., 2018.

Table 6: The effect of the interaction between biological factors and fertilization recommendation on the dry weight of the root system (g)

Treatment Name	Fertilization Recommendation 0.0%	Fertilization Recommendation 50%	Fertilization Recommendation 100%	Effect of Treatments (A)
control	606.66	618.66	637.06	**620.80**
F. equiseti	561.99	573.93	576.63	**570.85**
F. equiseti + G. mosseae	597.63	624.66	634.66	**618.98**
F. equiseti + A. chroococcum.	609.86	623.86	641.26	**625.00**
***F. equiseti + Rhizibium* sp**	581.33	594.33	589.66	**588.44**
Mean B	**591.49**	**607.09**	**615.86**	
$LSD_{0.05}$	**A=** 5.596	**B=** 4.335	**A*B=** 9.693	

Conclusions

The study concluded that growth-promoting organisms, particularly *A. chroococcum*, significantly enhance the biochemical parameters and biomass of broad beans when used alongside different fertilization rates. *A. chroococcum* was the most effective treatment, significantly increasing levels of phenols, phenylalanine ammonia lyase, and proline. The interaction of *A. chroococcum* with 100% fertilization rate was particularly effective, resulting in the highest levels of these biochemical parameters and biomass measurements. This treatment notably outperformed the control with the pathogenic fungus alone, demonstrating its potential in mitigating root rot caused by *F. equiseti* and promoting overall plant health and growth.

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