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Utilization The Fungus of Mycorrhriza to Control Bean Root Rot Caused by Pathogenic Fungi *Rhizoctonia Solani*

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Abstract

The study was conducted for the purpose of knowing the effect of the mycorrhizal fungus on controlling the root rot disease caused by the pathogenic fungus *Rhizoctonia solani*, the field experiment included four treatments and each treatment had five replicates, the treatments were as follows (Control, Mycorrhiza, Rhizoctonia, Mycorrhiza, Rhizoctonia), where the percentage of infection with the disease was (0.00, 0.00, 96.91, 63.58), respectively. while the growth indicators were studied, including plant height (106.24 , 112.58, 81.64 , 101,72) respectively , the average number of plant leaves for the above treatment , respectively (24.98 , 27.14 , 17.50 , 26.16) and the root length (23.40 , 30.32 , 10.32 , 28.42) , root wet weight (4.17 , 6.96 , 0.98 , 5.13) the mycorrhizal fungus treatment was superior in all studied indicators, followed by the treatment of mycorrhizal + rhizoctonia, while the pathogen treatment recorded the lowest percentage in all indicators , There were significant differences at a significant level of 0.05% between the treatments

key wards: - Mycorrhiza, Rhizoctonia solani, root rot

1. Introduction

One of the most important legume crops in Iraq is the faba bean (*Vicia faba* L.), also known as wide bean, horse bean, field bean, or tic bean [Singh et al. (2010)]. It is utilized in most animal diets as well as for human use. In addition to having a high percentage of carbohydrates, which in most cultivars reached 56% [Carmen et al. (2005)], it also has a high percentage of protein, which is estimated to be 25–40% [Natalia et al. (2008)]. If its roots have root nodes, it also enhances soil quality by fixing atmospheric nitrogen to the soil [Mona et al. (2011)].

An economically significant soil-borne pathogen is *Rhizoctonia solani* which causes damping-off disease in a number of agricultural plants, (Bartz et al., 2010; Saberi et al., 2013), R. *solani* is regarded as a pathogen that is challenging to control because of traits including high population variability, a diverse range of hosts, and long-term survival in soil (Thakur et al., 2018)

Soil solarization, cleanliness, and crop rotation are some cultural techniques that are not adequately effective ways of control. In the past, controlling R. solani using chemical pesticides—most often methyl bromide fumigation—has been effective. However, this practice poses substantial health hazards to people and pollutes the environment (Manganiello et al., 2018). In order to increase crop output and food safety, the biological control strategy therefore becomes a crucial part of disease management (Justyna et al., 2017).

Many academics in the fields of biological and agricultural sciences have turned their attention to the biological control as a key study area (Manganiello et al., 2018). In order to combat fungal diseases, bio-control agents employ a variety of tactics, including the synthesis of antimicrobial compounds, mycoparasitism or hyperparasitism, the activity of cell wall-lysing enzymes, and the induction of systemic resistance (ISR) activity (Vinale et al., 2006). Additionally, certain bio-control agents have the potential to enhance the germination rate, shoot and root weight, nutrient absorption, and yield of plants (Liu et al., 2018).

It has been established that 80% or more of vascular plants have a symbiotic connection with arbuscular mycorrhizal (AM) fungus. The symbiotic connection can provide the plant a variety of advantages, including as increased water and nutrient uptake, improved plant development, and increased germination rates (Jacott et al., 2017).

In exchange, the AM fungus fully rely on the nutrients released by a live root system (Jacott et al., 2017). The host's resistance to a variety of bacterial and fungal diseases, particularly those that cause rots, has also been reported to be increased by AM fungi (Hoeksema et al., 2010)

2. Materal and method

2.1 Cultivation of the bean plant Vicia faba

For the goal of germination of the seeds at room temperature, the bean plant's seeds were sterilized by 5% sodium hypochlorite for a period of 20 minutes, then thoroughly rinsed with sterile water, and finally put in a wet sterile cloth. A 30 cm diameter anvil (which had previously been steam sterilized) was filled with 2 kg of sterilized clay soil, 20 plastic containers, and 10 replications of each experimental treatment.

2.2 the application of mycorrhizal fungus to plants

To create fungal plants, the mycorrhizal fungal vaccine was injected into 10 plastic containers (two treatments only, five replications for each treatment). The vaccine was positioned at a distance of 4 cm from the soil surface.

2.3 Production of the pathogen vaccine Rhizoctonia solani

The pathogen inoculum was created using cultures that were grown for seven days at 25 °C and a 12-hour light-dark cycle in 100 ml Erlenmeyer flasks on a shaker (110 strokes per minute).

2.4 infection to the pathogen Rhizoctonia solani

Only 10 treatments—each with five replicates—were infected after the plants had been planted for four weeks, including the ten duplicates that had already received Mycorrhiza fungal inoculation. The plants were randomly dispersed under greenhouse circumstances with 16 photoperiods and 70% relative humidity for the control treatment, and the experiment was then released with the results being collected ten weeks following planting.

Calculated the percentage increase or decrease in the average readings of the criteria :The studied growth according to the following equation

$$\text{Percentage of increase or decrease} = \frac{\text{the average reading of the studied growth criterion} - \text{The average reading of the witness, for the same treatment}}{\text{average reading of the control for the same treatment}} \times 100\%$$

2.5 Effect of mycorrhizal fungi on different growth parameters

Monitoring the examined growth metrics in the various treatments on the experimental plants revealed that there were obvious differences in the values of these parameters depending on the kind of treatment. Through microscopic analysis and confirmation of the mycorrhizal fungus's dendritic vesicles and vesicles inside the tissues, which are expressed by mycorrhizal colonization, the existence of the fungus in plant roots may be determined. The host plant undergoes chemical, physiological, and morphological changes as a result of dendritic mycorrhiza colonization of the roots. These changes all contribute to an increase in the stimulation of plant growth components, such

as plant height, leaf count, and other growth components, as well as plant resistance to pathogenic fungi (23).

Where the following mesasurements were taken

1- Effect of mycorrhiza on plant height/cm,
2- number of leaves
3- Root length (cm)
4- root wet weight (g)

2.6 Calculation of the percentage of root sections colonized with mycorrhizal

Three plants were chosen at random from the bean plants; three roots, each measuring 10 cm in length, were then taken from each plant and cut into ten pieces, each measuring 1 cm. The Phillips and Hayman technique was used to stain root portions with trypan blue TB (26),

, ten root portions per slide, on glass slides, in a few drops of lactic acid, and then covered with a slide cover. It was examined using an optical microscope outfitted with a digital Olympus camera connected to a computer and a program to display the captured images to determine the extent of the dendritic mycorrhizal infection, as the presence of mycorrhizal infection is indicated by the presence of fungus hyphae, fungal vesicles, or dendritic branches inside the cells of the cortex of the root hairs, or both (20).

The percentage of root colonization with mycorrhiza was calculated by the following equation

percentage of roots colony mycorrhiza =

$$\frac{\text{Number of root sections colonized with mycorrhizal}}{\text{total number of root sections studied}} \times 100\%$$

2.7 Effect of arbuscular mycorrhizal fungi application on infection severity with Rhizoctonia solani

According to Abbasi and others (6), it was based on a reliable standardization scale, and a small modification was made to the standardization scale in terms of reading the symptom appearance. This included a reading for the symptom appearance of seedling fall disease during the first 30 days of the initiative's age and a reading for the symptom appearance during the second 30-day period (Exceeding the stage of the seedling's age) in terms of the scale.

Using the following pathological scale on the day the illness indicator was use

a score of one indicates a healthy tomato plant with no signs of illness; 2- Infection symptoms emerge but the seedlings do not wilt (dwarfing and yellowing in the plant, where a simple hydrolysis appears in the stalk where the stem is attached to the root, and this display stops and the seedling continues to grow during the first 30 days, but during the second 30 days does not appear hydrolysis in the stalk, and it appears yellowing symptoms from root rot after 30 days of age);

3-Seedlings die during the first 30 days of emergence from the soil's surface, or the plant totally withers after this time (hydrolysis in the stalk where the stem attaches to the root and narrows in this region within the first 30 days, or complete root rot beyond this time); 4- Fall of seedlings before to their emergence above the earth's surface (seeds rot or seedlings fall prior to emergence above the .(soil surface

The illness index (disease index) % was determined in

$R\%=\varepsilon\ (a \times b) \times 100/N \times K$

Where N is the total number of plants, K is the highest value of infestation severity on the scale, and R% is the percentage of disease index. Where an is the degree of infestation according to the assessment scale. Score equals 4. (24).

2.8 statistical analyses

The experiment was carried out in a randomized complete block design (BCRD). The experiment was statistically analyzed using the statistical program and the averages were compared by calculating the least significant difference (LSD) at the 5% level.

3. Results and Discussion

3.1 Effect of mycorrhizal fungi on different growth parameters

The statistical analysis showed that the use of this fungus produced significant results and changes in all of the growth indicators that were assessed compared to the control treatment, which led to the variations in the growth indicators of the bean plant in table (1).

Following the experimental plants' researched growth criteria under various treatments, it became obvious that the values of these criteria varied significantly depending on the kind of treatment (Table1). Through microscopic analysis and confirmation of the mycorrhizal fungus's dendritic vesicles and vesicles inside the tissues, which are expressed by mycorrhizal colonization, the existence of the fungus in plant roots may be determined. The host plant undergoes chemical, physiological, and morphological changes as a result of dendritic mycorrhiza colonization of the roots. These changes all contribute to an increase in the stimulation of growth components such as plant height, the number of leaves, and other growth components, as well as an increase in the plant's resistance to pathogenic fungi (22).

the effect of mycorrhiza on plant height – Comparatively to the control, the treatments with mycorrhiza and my+ rhizo increased the average plant height to 112.42 and 101.72 cm (106.42 cm). The two treatments, Mycorrhiza and My+ Rhizo, both showed a sizable rise in plant height. *Rhizoctonia solani* treatment caused the plant height to decrease to 81.64.

This was supported by earlier research, which demonstrated that soil inoculation with mycorrhizal fungi was essential in reducing seedling rot of the Indian agarwood tree Aquilaria and restricting the spread of the disease in root tissues. In addition, it increased the height of the host plant and the wet and dry weight of the root and vegetative groups by enhancing plant nutrition and thereby plant growth (34).

The effect of mycorrhiza on the number of leaves of the plant - The mean number of leaves in the treatments Mycorriza and My+Rhizo, respectively, was 27.98 and 26.26 leaves; this represents an increase in leaves, whereas the number of leaves dropped in the other two treatments. All of these alterations were significant when compared to the control C in the Rhizoctonia solani treatment with a percentage of the control 24.98. (Table1).

This effect is due to the fact that mycorrhiza increases the absorption surface at the roots by means of the extension of the hypha and its spread in the soil, and it also preserves the functions of the root cells, which is reflected on the plant by increasing its growth and the number of its leaves in addition to helping to increase its resistance to disease (**16**,27), and this What was confirmed by other studies that used mycorrhizal fungi in controlling tomato seedling disease (28), as well as in controlling tomato wilt disease caused by Fusarium oxysporum f. sp. lycopersici (23).

The effect of mycorrhiza on the length of roots - the length of plant roots reached 30.32 and 28.42 cm in the two treatments Mycorriza and My+Rhizo -, compared with the control C (23 cm), while the root length decreased to (10 cm) in the treatment *Rhizoctonia solani*

The effect of mycorrhiza on the wet weight of the root: -The results revealed a difference in the wet weight of the root total between the various treatments, which was 6.96 and 5.14 in the treatment of mycorrhiza and mycorrhiza + rhizoctonia, respectively, in comparison to the control, which was 4.17, where we notice an increase in the value of the wet weight of the two treatments.

While the wet weight value decreased to 0.98 in the treatment of rhizoctonia, we note that the mycorrhizal fungus led to an increase in the wet weight of the roots. This is because the fungus is able to improve the absorption of water and nutrients as well as the process of photosynthesis. These results concur with those of researcher (21) who found that the fresh weight of the root system increased by 50% after inoculation.

The superiority of mycorrhizal fungi in some of the research treatments may be attributable to the mycorrhizal's role in promoting the plant's secretion of growth materials and regulators, which work to promote systemic resistance by producing substances like jasmonic acid, chitinases, and

phenylalanine ammonia-lyase (PAL). The plant has greater access to nutrients like phosphorus, which promotes healthy vegetative development and increases its resistance to pathogens that cause illness (30, 33).

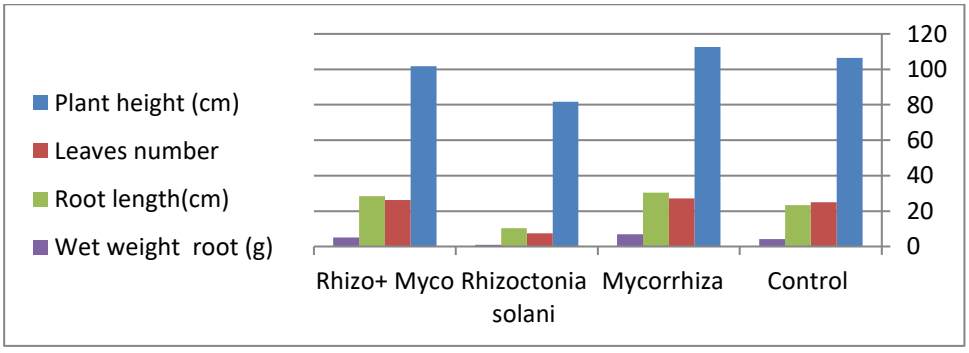
. Despite extensive research, it is still unclear how dendritic mycorrhizal fungi contribute to the development of induced systemic resistance (32), and the beneficial effects of the symbiotic relationship between mycorrhizae and plant roots are the result of intricate molecular interactions between the two symbiotic partners (17).

In one study on the topic, Glomus spp. and Pseudomonas spp. were shown to be physiologically able to manage the complex illnesses Rhizoctiona solani and Meloidogyne javanica on chickpea. Researchers were able to test and assess the efficacy of using Glomus spp. (G) and Pseudomonas sp. (P) as a biological control agent against the Rhizoctonia-Meloidogyne complex disease by contrasting the growth and disease features in infected and control plants.

Chickpea growth parameters are determined by measuring fresh and dry weight, shoot and root length, and shoot length. The use of (G) and (P) in a single treatment or in combination led to a decrease in the root gall index and in the severity of the root rot disease when compared to the infected and healthy control treatments. Additionally, the (M+ R+ G) and (M+ R+ G+ P) combination treatment increased the polyphenol oxidase (POD) and peroxidase (PPO) enzyme activity levels as well as the total phenol content in treated chickpea roots. Effective disease control has been suggested to entail the interaction of (G) and (P) on the spread of pathogens and the beneficial effects on chickpea development metrics (4)

Table (1). Effect of mycorrhiza on some growth indicators of bean plants

Treatment	Plant height (cm)	Leaves number	Root length(cm)	Wet weight root (g)
Control	106.42	24.98	23.40	4.17
Mycorrhiza	112.58	27.14	30.32	6.96
Rhizoctonia solani	81.64	7.50	10.32	0.98
***Rhizo*+ Myco**	101.72	26.16	28.42	5.13
LSD 5%=	5.054	0.944	1.96	0.97



3.2 Calculation of the percentage of root sections colonized with mycorrhizal

Table 2. The effect of different treatments on the rate of mycorrhizal colonization in the root sections of the bean plant

Treatment	Total number of examined root segment	Mycorrhiza root number	Mycorrhization %
Control	25	0	0
Mycorrhiza	25	18	72
Rhizoctonia solani	25	2	8

***Rhizo*+ Myco**	25	16.25	65

The highest percentage of mycorrhizal colonization of the roots of the bean plant was in the treatment of Mycorrhiza, which amounted to 70%, followed by the treatment of Mycorrhiza + Rhizoctonia, which amounted to 65%, while the lowest percentage of mycorrhizal colonization was 8% in the treatment of Rhizoctonia, as shown in Table 2. In the roots and the degree of coexistence with the root, and this deficiency is greater when the appearance of the pathogenic fungus is the presence of mycorrhizae.

mycorrhizal fungus (AMF) may be able to lessen the harmful effects of soil-borne diseases, although this depends on the genotype of the plant, the pathogen, and the interaction with the AMF. The dual inoculation's total phenolic content increased along with a 68% reduction in disease severity due to the AMF biofertilization. Additionally, it was shown that both pathogen treatment alone and AMF combination generated variations in the individual phenolic profiles. Vanillic acid considerably varied across treatments in dual inoculations, indicating that it may help to increase the resilience of mycorrhizal roots to soil-borne pathogens. (**9**)

3.3 Effect of arbuscular mycorrhizal fungi application on infection severity with Rhizoctonia solani

The effect of the mycorrhizal fungus on the disease index The plants of the different treatments were followed up and observed and compared with the comparison treatment in terms of the value of the disease index in each of the treatments where the value of the disease index in the rhizoctonia treatment was 96.91, while the disease index in the comparison and Rhizoctonia treatments was 0%, and the disease index value was 63.58 % in the treatment of Rhizoctonia + Mycorrhiza

It was shown from Table 3 that the treatment to which the mycorrhizal fungus was added with the presence of the pathogenic fungus showed a significant effect in reducing the disease index and its percentage reached 63.58%, where the roots colonized with mycorrhiza made resistance against the pathogen (23)

In one study, scophyllum nodosum extract and mycorrhizal eolonization were examined. Rhizoctonia Root Rot immune responses in pea plants are induced in a coordinated manner, and plant growth and productivity are improved.

This study looked at the biocontrol potential of seaweed (Ascophyllum nodosum) extract at concentrations of 1, 2, and 3%, as well as mycorrhization of pea roots, against Rhizoctonia root rot in a greenhouse setting. Additionally, their impacts on the physiological, ultrastructural, transcriptional, and growth status of pea plants were investigated. The outcomes demonstrated a synergistic overexpression of the defense-related genes peroxidase (23.2-fold) and chitinase II, as well as the response factor (JERF3) recording 18.2-fold, during mycorrhizal colonization of pea roots and the administration of seaweed extract at 3%. (31.8-fold)

Additionally, this therapy enhanced the phenolic content of pea roots, increased the activity of the antioxidant enzymes POD and PPO, and sparked many hypersensitive responses at the ultrastructural level of the cell, resulting in a 73.1% decrease in disease severity. Also noted was a synergistic growth-promoting impact on pea plants. This dual treatment greatly increased the photosynthetic pigments in pea leaves, increasing their yield (24 g/plant). Numerous studies have been done on how mycorrhizal colonization affects plant development and resistance. However, it is vital for safety and food security to create enhanced and synergistically acting biological agents for plant disease management and growth promotion as alternatives to the chemical fungicides. These findings lead to the conclusion that

Table 3. Effect of arbuscular mycorrhizal fungi application on infection severity with Rhizoctonia solani

Treatment	**Disease index**	**Disease severity (1-4scale**
Control	0.00	1.00a

Mycorrhiza	0.00	1.00a
Rhizoctonia solani	96.91	4.00b
***Rhizo*+ Myco**	63.58	2.58c

the values printed with the same letter in the same column have no significant difference between them according to Duncan's Multiple Range Test at a probability of 5%

4. Conclusion

1. It is concluded that it is possible to use mycorrhizal to control root rot disease
2. The addition of mycorrhizal to protect the seeds and improve the different growth indicators
3. Soil treatment with mycorrhizal fungus against the pathogen Rhizoctonia solani showed that the mycorrhizal infection reduced the infection rate to 65.6%

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