International Journal of Aquatic Science
ISSN: 2008-8019
Vol 13, Issue 01, 2022

Study of the Effect of Rhizoctonia Solani (Kuhn) Root Rot Disease on the Growth and Yield of the Broad Bean Plant

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Abstract* :This study was conducted with the aim of evaluating the efficiency of some bioand chemical factors individually or in combination with each other in reducing the effect of Rhizoctonia root rot disease. The most frequent fungi genera appeared in most samples with a percentage of 88.94%, followed by Fusarium spp with an appearance rate of 71.82%. The results of the pot experiment showed that isolate Rhizoctonia solani led to a decrease in the growth indicators of broad bean plant, which included the weight of the total vegetative and root, fresh and dry and the plant length of the cultivar. Barcelona when the control treatment was infected, which amounted to 29.26 g, 6.13 g, 6.20 g, 0.66 g, 36.00 cm, and for the local cultivar, it reached 25.43 g, 6.50 g, 6.30 g, 0.83 g, 32.66 cm, compared to the healthy control treatment for the Barcelona cultivar, which reached 51.60 g, 12.40 g and 11 40 g, 3.06 g, 50.00 cm, and for the local cultivar, they were 46.70 g, 12.10 g, 10.67 g, 2.83 g, and 48.32 cm, respectively. The yield indicators, which included the average number of pods and seeds per plant when the control treatment was infected with the cultivar Barcelona, amounted to 7.31. and 24.67 for the local cultivar, which amounted to 6.00 and 21.63, respectively, compared to the control treatment of Saleem for the Barcelona cultivar, which amounted to 16.00 and 56.13, and for the local cultivar, which amounted to 16.66 and 59.34, respectively. Gibberellin + pesticide had the highest increase in the percentage of these indicators.

Keywords: broad bean root rot disease, Fusarium spp, Rhizoctonia solani, P. fluorescens

1. INTRODUCTION

The Vicia faba L plant, which belongs to the leguminous family Leguminaceae, is one of the most important and widespread economic plants in many countries of the world, which comes after the Grass family in terms of economic importance. It is used as good food for humans because its seeds contain a high protein content of 25-40%. (Natalia et al., 2008) The broad bean crop is grown in most of the agricultural regions of Iraq, as the cultivated area in it for the year 2012 is about 58,600 dunums, with a production of 114,600 tons (Mohammed and Wanted, 2015). Icarda (2003) Where the fungus infects the plant in all stages of growth, leading to the rotting of the seeds and thus the death of the seedlings before and after their flowering. It was found that extensive and wrong use has negative effects on the environment, human health and non-target organisms, so the thought of resorting to using other, safer alternatives, one of the most prominent of these alternatives is the use of microorganisms and some organic acids in bio-control programs to reduce the number of vaccines for pathogens as well as increase production, such as the use of bacteria Pseudomonas fluorescens and the fungus Trichoderma harzianum because these organisms have the important characteristics of surviving many pathogens such as improving plant growth and production as well as the antagonistic property towards the pathogen as well as ease of isolation and multiplication These organisms and the possibility of using them in a wide range of different conditions and growing them in many food media at low cost (Talla et al., 2015; Krupa et al. 2014). Because of the importance of this disease on the bean crop and the spread of the pathogen in Iraq in general and Salah al-Din provainc in particular, this study was conducted with the aim of:

1-Isolation of the pathogens associated with rotting of the roots and bases of stems of broad bean plant

2-The use of some organic acids, microorganisms and chemical pesticide s to reduce the negative impact of the disease in order to increase the growth and production of the crop.

2. MATERIALS AND METHODS

Isolation of fungi accompanying the roots and bases of the stems of broad bean plant

Samples of plants showing symptoms of infection were brought to the laboratory from different areas of the College of Agriculture,Tikrit University, Shirqat District, and Al-Alam sub-district of Salah El-Din province. Parts of the stems and roots of plants that showed symptoms of rotting and ulcers were taken, washed with water and sterilized with sodium hypochlorite (0.5%) solution.According to the recommended methods, they were dried using filter paper, transferred by sterile forceps, and planted with 3-4 plant pieces for each Petri dish with a diameter of 9 cm containing PDA Potato Dextrose Agar culture medium supplemented with antibiotic Tetracycline at a concentration of 250 mg/l sterilized by a thermal osmosis device at a temperature of 121°C and a pressure of 1.5 kg/cm^2 for 30 minutes, after which the dishes were placed in the incubator at a temperature of 25±2°C for three days.Colonies were purified by transferring a small piece of the ends of the fungal hyphae and placing it in the center of a Petri dish containing PDA culture medium.The fungal isolates were diagnosed after 4 days at the species level, depending on the colony shape, the medical branch of the mycelium, the composition of the carriers, spores and other structures formed by the fungus according to the approved taxonomic bases (Booth, 1971; Domasch and et al. 1980,(The percentage of appearance of each fungus was calculated by applying the following equation:

Percentage of appearance = (number of appearances of one fungus / total number of samples examined) x 100

Preparation of the inoculation for the fungus Rhizoctonia solani

The isolates of R. solani were grown on seeds of local millet Panicum miliaceum L after being thoroughly washed to remove dust and impurities attached to it, then dried at laboratory temperature. 100 g of millet seeds were placed in a 500 ml beaker and 10 ml of distilled water was added to it to moisten it.It was sterilized by autoclave at a temperature of 121 °C and a pressure of 1.5 kg/cm2 for one hour. After cooling, inoculate the beaker with the inoculation of isolates of the fungus R. solani at a rate of five tablets with a diameter of 0.5 cm. The duke was placed in the incubator at a temperature of 27 °C for twelve days, taking into account the shaking of the beakers every 3 - 2 days to ensure the distribution of the fungal vaccine inside the flask.

Study of the effect of bio and chemical control factors on growth and yield indicators of broad bean plant infected with rhizoctoni root rot disease in the pots.
The experiment was carried out in the fields of the College of Agriculture, Tikrit University, using a mixture of soil after sterilization with commercial formalin (5%).It was placed in plastic pots with a diameter (20 cm and a height of 25 cm) in equal quantities and then distributed to eleven treatments in three replicates (each replicate has three pots) contaminated the soil of 10 treatments with the fungus R. add pathogenic fungi,The pots soil was moistened and left for three days after covering it with nylon bags. The treatment of Pseudomonas fluorescens consisted of soaking the bean seeds in a suspension of bacteria grown on NB medium at a concentration of 107-108 CFU/ml for 90 minutes.Then it was left to dry on the filter paper in the sterile isolation room. After that, the seeds were planted at a rate of 20 seeds for each australia. As for the treatment of the elite pesticide (the active substance is aluminum phostel, the average of which is 80%). It included adding it to the soil at a concentration of 2 g/L irrigation to the soil at a rate of 0.5 liters of pesticide solution per aus. Also, the treatment of Pseudomonas fluorescens bacteria preceded the treatment with the pesticide in the interaction treatments, noting that all the seeds used were superficially sterilized with 5% sodium hypochloride solution for 2-3 minutes, As for the treatment of soil with humic acid, it included adding it to the soil at a concentration of 1000 mg / L and at an average of 10 ml / pot during the cultivation process.While the Gibberellin treatment included soaking the seeds for 30 minutes after which they were dried on filter paper before the planting process, the design used a Completely Randomized Design (CRD)and the results were statistically analyzed according to the L.S.D test at a probability level of 0.05,

The experiment included the following treatments:
1- Fostil aluminum treatment 2- Bacterial P. fluorescens treatment 3- Gibbrellin treatment 4- Humic treatment 5- Humic + P. fluorescens treatment 6- Bacteria + Gibbrellin 7- treatment with P. fluorescens 8- Bacterial treatment with Humec + Gibbrellin9 - Pesticide treatment + Humic + P. fluorescens + Gibbrellin
The pots were carefully watered until the pods were formed and the crop matured. The plants were carefully uprooted and for all treatments, and their roots were washed well and the following measurements were taken:-
A- Indicators of production
1- The average number of pods per plant
2- Average number of seeds per plant
B- Growth Indicators
1- The plant length (cm)
2- Average wet root and vegetative weight (gm)
3- Average dry root and vegetative weight (gm)

3. RESULTS AND DISCUSSION

Isolation and diagnosis
Several fungal genera were isolated and identified from the roots and bases of the stems of bean plants that showed symptoms of the disease (Table 1), and the most frequent was the genus Rhizoctonia, with an appearance rate of 88%.While the fungus Fusarium recorded an appearance rate of 71.88%, in addition to the lowest appearance rate of the genus Aspergillus spp, which amounted to 31.14. The presence of the two fungi Rhizoctonia spp and Fusarium spp with all plant samples and the highest percentage of appearance confirms that they are

one of the most important causes of root rot disease .This is consistent with what was recorded that these two sexes of fungi are endemic to the soil and attack different plant roots and families (Fournier, 2010; Jabr, 2001).

Table (1) Fungal species associated with broad bean root rot disease

fungus	% appearance
Alternaria spp	61.75
Aspergillus spp	31.14
Fusarium spp	71.82
Rhizoctonia solani Kühn	88.94

Effect of bio and chemical control agents on yield indicators of broad bean plants infected with rhizoctoniosis

All biological and chemical treatments affected the treatments contaminated with the pathogen Rhizoctonia solani when used alone or by interaction them in reducing the percentage of the pathogen's effect on broad bean plant, which led to an increase in the indicators of the yield of the bean plant compared to the control treatment contaminated with the pathogen Table (2)The interaction treatment Humic + bacteria + gibberellin + pesticide gave the highest average in the number of pods and seeds per plant for both cultivars, which amounted to 16.89 and 55.67 respectively for the local cultivar, while the Barcelona cultivar reached 15.89 and 53.66 respectively, while the averages of the number of pods and seeds per plant reached In the control treatment treated with pathogen only 6,00 and 21.63 respectively for the local cultivar. As for the Barcelona cultivar, it reached 7.31 and 24.67, respectively. The interaction treatment Humic + bacteria + gibberellin + pesticide excelled in all the studied traits compared to the control treatment can be explained by the role of humic acid in reducing the effect of pathogenic fungi on the yield traits by increasing the plant's resistance to infection with the pathogen by increasing the plant's content of nutrients such as iron, potassium, phosphorous, copper, zinc and manganese and the stimulation of plant defence enzymes, while the effect of bacteria may be due to their production of secondary metabolites that act as antibiotics such as Lipopeptide and growth regulators such as (IAA) Indole acetic acid (Meyer, 2010). In addition to the effect of the pesticide on the pathogen by increasing the activity of the peroxidase enzyme, which led to the stimulation of acquired resistance, as well as the effect of the hormone gibberellin in cell division and elongation, and this is consistent with what was reached (Tawil et al., 2019)

Table (2) The effect of biological and chemical treatments and their interactions on the different yield indicators of broad bean plants

Barcelona cultivar		local cultivar		cultivars / treatments
Average number of seeds per plant	Average number of pods per plant	Average number of seeds per plant	Average number of pods per plant	
40.00	11.23	42.00	14.43	pesticide
42.64	12.66	45.32	15.33	bacteria
42.86	12.46	40.00	14.37	gibberellin
43.25	12.86	47.00	15.78	humic

50.66	14.66	50.35	16.12	Humic + bacteria
50.00	13.45	48.56	15.00	pesticide + gibberellin
53.16	14.33	50.16	15.33	pesticide + bacteria
49.94	14.00	47.00	14.45	humic+ Gibberellin
53.66	15.89	55.67	16.89	Humic + bacteria + gibberellin + pesticide
24.67	7.31	21.63	6.00	infection (control)
56.13	16.00	59.34	16.66	non-infection (control)
5.3412	011.35	5.3412	1.3501	LSD

Effect of biological and chemical control factors on growth indicators of broad bean plants infected with rhizoctoniosis

It was shown from Table (3) that the cultivation of barley seeds in soils contaminated with the pathogenic fungus Rhizoctonia solani led to the infection of the resulting plants, which had a negative effect on all growth indicators such as the weight of the rootstock, dry and wet vegetative, plant height for both cultivars and the positive effect of all biological and chemical treatments. The interaction treatment between the treatments Humic + Bacteria + Gibberellin + chemical pesticide showed its ability to significantly increase the dry and fresh vegetative and root weight of the Barcelona cultivars, which amounted to 49.33, 12.30, 12.19 and 3.30, respectively, and the plant length was 52.00 cm While the local cultivars was 48.33, 10.96, 11.80 and 3.23, respectively, and the plant length was 49.33 cm, compared to the control treatment, which was 25.43, 6.50, 6.30 and 0.83 for Barcelona cultivar, respectively, and the plant height was 32.66, and the control treatment for the local variety was 29.26, 6.13, 6.2, 0.66 and 0.66 tall. The plant reached 36.00 .,The role of P. fluorescens and humic acid in increasing the percentage of growth indicators is due to the bacteria's ability to produce Lipopeptidocyclic, Amphisin and Endochitinase enzyme, which works to break down the walls of fungal cells and produce Siderophore, which makes iron not ready for the pathogenic fungus and then leads to its death and analysis As for humic acid, it works to protect the plant from infection by pathogenic fungi by raising the plant's systemic control by increasing the plant's content of nutrients as an element, P, K + 3, B + 2, Mn + 2, Zn and stimulating the defense enzymes in the plant as well as encouraging the growth and reproduction of beneficial and desirable microorganisms such as algae and yeasts in the soil solution, and this is consistent with what was mentioned (Kazempour, 2004; Al Jumairi et al., 2018; Hassan et al., 2019). As for the effect of the pesticide Fostil aluminum and gibberellin, the pesticide works to prevent the germination and growth of fungal hyphae as well as contribute to stimulating plants to produce phytotoxins that inhibit the growth of pathogenic fungi As for the gibberellin, it works to encourage the plant cell to elongate and divide below the meristem apex, and this is reflected in an increase in growth indicators.

Table (3) Effect of bio and chemical treatments and their interactions on growth indicators of broad bean plants

Barcelona cultivar					**local cultivar**					**cultivars**
plan	**Weig**	**Weig**	**Weigh**	**Weigh**	**plan**	**Weig**	**Weig**	**Weigh**	**Weigh**	

t length	**ht of dry root system**	**ht of fresh root system**	**t of dry vegetative growth**	**t of fresh vegetative growth**	**t length**	**ht of dry root system**	**ht of fresh root system**	**t of dry vegetative growth**	**t of fresh vegetative growth**	**treatments**
37.33	1.61	8.00	7.70	31.66	43.66	1.53	8.40	7.82	35.00	pesticide
38.86	1.33	7.91	8.00	32.50	45.56	1.30	7.66	6.73	32.56	bacteria
48.00	1.79	8.91	9.41	42.76	49.00	1.41	7.80	9.03	41.03	gibberellin
45.36	1.65	8.53	8.51	39.06	41.33	2.01	9.69	7.63	36.90	humic
45.96	2.50	10.56	9.66	45.13	44.00	2.34	10.29	10.36	45.36	Humic + bacteria
50.67	2.19	10.36	9.67	47.00	47.03	1.90	9.10	9.80	44.13	pesticide + gibberellin
40.33	2.56	10.61	8.70	39.43	45.66	2.96	10.33	9.62	42.40	pesticide + bacteria
49.96	2.00	9.98	9.43	44.00	47.86	2.17	9.89	10.10	43.99	humic+ Gibberellin
52.00	3.30	12.16	12.30	49.33	49.93	3.23	11.80	10.96	48.33	Humic + bacteria + gibberellin + pesticide
32.66	0.83	6.30	6.50	25.43	36.00	0.66	6.20	6.13	29.26	infection (control)
48.32	2.83	10.67	12.10	46.70	50.00	3.06	11.40	12.40	51.60	non-infection (control)
4.0442	0.5138	0.5138	1.3006	3.875	4.0442	0.5138	1.1565	1.3006	3.875	**LSD**

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