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Evaluation of the efficacy of some biological factors in suppressing Rhizoctonia solani root rot disease on broad Beans caused by the fungus Rhizoctonia solani

Khalaf A Mohammed*, Awf A Ahmed and Salah M Asmaiel
Plant Protection Department, College of Agriculture, Tikrit University
*Email: khalaf.attyih@tu.edu.iq

Abstract
This study was conducted to evaluate the efficacy of some types of biological and chemical agent, individually or in combination with each other, in reducing *Rhizoctonia* root rot disease on the bean, caused by the fungus *Rhizoctonia solani*. The laboratory results showed the efficiency of *Pseudomonas fluorescens* bacteria in inhibiting the fungus *Rhizoctonia solani* in the culture medium, and the results of the pot experiment showed the efficiency of all bio and chemical treatments, whether alone or with their interactions, in reducing the severity of infection with the fungus Rhizoctonia solani, where the lowest infection severity was recorded in the treatment of the interaction Humic + bacteria + Gibberellin + pesticide for the Shabella variety amounted to 18.33%. It also reduced the negative effect of the pathogenic fungus on the seeds, thus increasing the percentage of germination, as the highest percentage in the treatment of Humic + Bacteria + Gibberellin + pesticide for the local variety amounted to 97.33%. The treatments also led to a rise in the indicators of systemic resistance of the plant. Compared with the healthy control treatment, which included the activity of peroxidase enzyme.

Keywords: Rhizoctonia stem rot, Beans, Rhizoctonia solani

Introduction

The broad bean crop (Vicia fabae L) belongs to the Fabaceae family , which occupies the second place after the Poaceae family and is considered one of the most important food crops in the world, including green and grain production. Its importance lies in its nutritional value because its seeds contain a high percentage of protein estimated at (25-40) Its seeds also contain a good proportion of sugary, starchy and some vitamins, up to (56%), which play an important role in filling the shortage of animal protein, especially in poor countries that suffer from food crises. In addition to its other benefits represented in being useful in fixing nitrogen in the soil, and its dry grains are used in concentrated diets for animals (Hassan and Marib, 2017), This crop is exposed to many agricultural pests, such as insect pests, fungal and viral diseases, snake worms and parasitic flowering plants. Among the most important fungal diseases that affect this crop are ascochyta blight, broad bean rust, root and stem rot disease. The last disease caused by the fungus Rhizoctonia solani is considered the most important in affecting the crop in many regions of the world, including Iraq (Icarda 2003)), causing great economic losses on the crop, as the fungus attacks the plant in the growth stages, causing damping off of seedlings and the rotting of the seeds before and after the bud stage, as well as the rotting of the roots and the bases of the stems. The fungus is characterized by the formation of a fungus with a brown color, thick walls branching at semi-right angles with the presence of some shortenings near the branching areas and transverse

barriers,,another distinguishing characteristic of the fungus is the formation of sclerotia of different sizes (Blazir and Conway, 2004) enabling it to survive for several years under inappropriate conditions (Howard and Gent, 2007). Evaluation of the effectiveness of Pseudomonas fluorescens bacteria used in bio control and some newly used pesticides in combating the disease and the study of some factors affecting the development of the disease.

Materials and methods

This study was conducted in the laboratories of the Plant Protection Department - College of Agriculture - Tikrit University, and the field experiment were conducted in the fields of the College of Agriculture, Department of Plant Protection / Tikrit University during the planting season 2021-2022.

laboratory experiments

Isolation and identification of fungi from the roots and bases of plant stems infected with the disease

Random samples of plants showing symptoms of root and stem rot disease were brought from the fields of the College of Agriculture/University of Tikrit, Samples were taken from the affected plants. The stem area close to the surface of the soil and the root area was separated from the rest of the plant parts. The plant parts were washed with running water for 30 minutes and then left for a short period to dry. These parts were cut into small pieces 1-0.5 cm in length and sterilized with sodium hypochlorite solution at a concentration of 10% of the commercial preparation for ten minutes and then washed with sterile water three times. Then dried on a Whatman-N0.4 filter paper, 4-3 pieces were transferred to a Petri dish containing PDA medium prepared by dissolving 39 g of acar in a liter of water and adding the antibiotic Chloromphenicol at a rate of 250 mg/L and sterilized by autoclave at a temperature 121 m and a pressure of 1.5 kg/cm2 for 30 minutes. The plates were placed in the incubator at a temperature of 25 ± 2 C for three days, after that the plates were taken out and the fungal growths were transferred to a Petri dish containing potato medium, dextrose and PDA sterilized by the previous method. developing to the species level depending on the taxonomic traits adopted by Domsch et al. (1980).

Then calculate the percentage of appearance of each mushroom by applying the following equation:

Emergence percentage = number of mushroom appearance times in samples / total number of samples x 100

Purification and preservation of isolates of the fungus Rhizoctonia solani

The isolates of R. solani were purified by transferring parts of the tip of the fungal hyphae of a fresh colony of diagnosed mushrooms using a sterile needle and with the help of a microscope to a sterile Petri dish containing sterile PDA media, and incubating the plates at a temperature of 25 ± 2 °C for four days.

Preparation of the vaccine for the fungus Rhizoctonia solani

Isolates of R.solani were grown on seeds of local millet

Panicum miliaceum L. After it was thoroughly washed to remove dust and impurities attached to it, then dried at laboratory temperature, 50 g of millet seeds were placed in each 250 ml beaker

and a little water was added to it to moisten it.The flasks were sterilized with an autoclave at a temperature of 121 °C and a pressure of 1.5 kg/cm2 for one hour. After cooling, the flasks were inoculated with R. solani isolates, each individually at a rate of five 0.5 cm diameter discs for each isolate, and the flasks were placed in the incubator at a temperature of 27 °C for twelve days with Take care to shake the flasks every 2-3 days to ensure the distribution of the fungal inoculation inside

Preparation of the inoculum for the bacterial strain Pseudomonas fluorescens

A diagnosed isolate of P.fluorescens was obtained from the laboratories of the Plant Protection Department / College of Agriculture / Tikrit University. One liter of liquid NB medium was prepared and distributed in 100ml glass beakers. The medium was sterilized with an oxidizer at a temperature of 121°C and a pressure of 1.5 kg/cm2 for 30 minutes. After the sterilization process, the flasks were inoculated with bacteria and incubated at a temperature of 27±1°C for 48 hours.A series of dilutions (1-8) were prepared in sterile glass tubes. The diluted bacterial inoculum (10-8) was cultured in a petri dish by mixing with PDA nutrient medium to calculate the total number of colonies at a rate of 1 ml of inoculum / dish according to the average number of colonies growing in the dishes and multiplying In the inverted dilution (Clark, 1965).

(Number of bacteria in 1 ml = average number of colonies growing x dilution inverse)

Thus, the used concentration was 6.3 (x) 109 CFU/ml, which was adopted in subsequent studies

Liquid NB medium was also used to grow and multiply the isolate every 5-10 days.

Study of the antagonistic efficacy of Pseudomona flourescens against Rhizoctonia solani

This experiment was coundected in Petri dishes containing sterile PDA medium. The inoculum of the bacterial strain P.fluorescens grown on NB liquid medium was added at the age of 48 hours to the dishes in the form of spots near the edge of the dish and circularly around the center of the dish at an amount of 0.1 ml for each spot and at a rate of five spots for each A plate and four replicates, leaving four plates without adding the bacterial inoculum for a control treatment.All plates were incubated at 27±1°C for 48 hours, then the center of each plate was inoculated with a disc diameter (0.5 cm) taken from the edge of the colony of R. solani, 5-4 days old. The plots were observed again at the same temperature until the fungi colony in the control treatment reached the edge of the plate, according to the amount of growth inhibition according to Abbot's equation contained in Shaaban and Al-Mallah (1993).

$$\text{Inhibition } \% = \frac{R_1 - R_2}{R_1} \times 100$$

R1 = radial growth rate of the pathogenic fungus in the control treatment (cm)

R2 = radial growth rate of pathogenic fungi in dishes containing bacterial inoculum (cm).

Estimation of indicators of induced systemic resistance in the bean plant

Indicators of resistance of broad bean plant were estimated after 6 weeks of plant life, which was represented by the enzyme peroxidase

Prepare the enzyme extract

1 gm of the roots of each treatment was taken and washed with distilled water well, then dried using filter paper and then crushed using a ceramic mortar placed in an ice bath. Then 10 ml of phosphite buffer solution PH6 was added and filtered using Whatman no.1 filter paper. Then it was placed in 10 ml test tubes and centrifuged using a 4 C refrigerated centrifuge at 10,000 rpm for 20 minutes. and Marib, 2017)

Peroxidase enzyme determination

The peroxidase enzyme was estimated according to the method of Hammerschmidt et al. (1982) by taking 2.5 ml of quaycol solution and hydrogen peroxidase (H2O2) and 0.1 ml of the enzyme filtrate. For each treatment, the absorbance of the reaction mixture was measured using a UV-Spectrophotometer at a wavelength of 470 nm. Then the enzymatic unit was defined as the amount of change in absorbance at a rate of 0.01 per minute.

Pots experiments:-

Biological and chemical control of broad bean root and stem rot disease caused by the fungus Rhizoctonia solani.

Mixed soil was used in this experiment. The soil was sterilized using commercial formalin (40%). A 1/50 solution of formalin was prepared and used at a ratio of 3 liters / 1 m3 (Poultry, 1975) and after ten days of sterilization, they were placed in plastic pots (20 cm diameter and 25 cm high) in equal quantities. Then it was distributed to eleven plants in three replicates (three pots), all treatments were contaminated with R. days after being covered with nylon bags. As for the treatments of Pseudomonas fluorescens, it included soaking the seeds of a local cultivar in a suspension of bacteria grown on NB liquid at a concentration of 107-108 CFU/ml for 90 minutes and then left to dry in a sterile isolation room on a Whatman No.4 filter paper. Then they were stored at a temperature of 4°C for 8 hours, after which the seeds were sown at an average of 20 seeds per pot. Also, the treatment with P.fluorescens bacteria preceded the fumigation with the pesticide in the joint treatments in which the bacteria and the pesticide were involved. Note that all the seeds used were superficially sterilized. with 10% sodium hypochlorite solution for 2-3 minutes, then washed with sterile water. The experiment included the following treatments:

1- Previcur Energy 2- treatment with P. fluorescens bacteria 3- Gibbrellin treatment 4- Humic 5- treatment with Humic + P. fluorescens 6- Bacterial treatment with P. fluorescens 7- Gibbrellin treatment 8- Treatment with Humic + Gibbrellin 9 - Pesticide treatment + Humic + P. fluorescens + Gibbrellin

The pots were watered carefully, and after the germination of seeds and the emergence of seedlings, the percentage of germination was calculated as follows: -

$$\text{germination percentage}=\frac{The\ number\ of\ germinated\ seedlings}{Total\ number\ of\ seeds}$$

The experiment continued until the formation of the pods and the maturity of the crop, and the severity of the infection was calculated using the pathological evidence

= 0 a healthy plant, the root system is large and the roots are white.

= 1 slight brown discoloration on the roots and yellowing of a specified number of leaves.
= 2 The roots are completely discolored with a comprehensive yellowing of the leaves.
= 3 The coloration extends from the roots to the bases of the stems.
= 4 general death

The percentage of injury severity was calculated according to McKinney's equation (1923) as follows:

% infection severity=$\frac{(Number\ of\ plants\ degree\ 0*0)+(Number\ of\ plants\ degree\ 1*1)000+(Number\ of\ plants\ degree\ 4*4)}{Total\ plants\ tested\ x\ 4}$ ×100

Results and discussion

Isolation and diagnosis

Several genera of fungi were isolated and identified from the roots and stems of plants that showed symptoms of the disease (Table 1), and the most frequent was the genus Rhizoctonia, with an appearance rate of 100%. While the fungus Fusarium recorded an appearance rate of 70% and this is consistent with what was recorded that these two sexes are settlers in the soil and attack the roots of different plant families (Jabr, 2001).

Table (1) Fungi accompanying the roots and stems of infected broad bean plants

fungi	% appearance
Fusarium sp	70
Rhizoctonia solani	100

Rhizoctonia solani

The results in the test showed that P.fluorescens had a high antagonistic efficiency against R.solani in the medium P.D.A. The bacteria caused an inhibition of the pathogenic fungus by 85% (Table 2).The high antagonistic ability of P. fluorescens bacteria may be due to its production of many compounds that have the ability to inhibit the growth and activity of plant pathogens, the most important of which are Phenazyne, Pyluteorin, Pyrolintrin, Pyoverdin, Pyochelin, 2,4 diaceytelphloroglucinal Where these compounds inhibit the growth of the pathogen as well as the production of many enzymes such as β-glucanase, Protease, chitinase and for Pseudomonas bacteria the ability to produce lipopeptidocyclic and Amphisin. The enzyme Endochitinase, which works to break down the walls of fungal cells (Andersen et al., 2003) and produces the compound Siderophore, which works to make iron not ready for the pathogenic fungus, and then leads to its death and analysis, and this is consistent with what I mentioned (Al-Jubouri, 2022)

Table (2) represents the percentage of inhibition of bacteria P. Fluorescens for pathogenic fungi

%inhibition	treatments	**No.**
85	Bacteria P.fluorescens	**1**
100	non infected control treatment	**2**
0	Infected control treatment	**3**

pots experiment

Biological and Chemical Control of Root and Stem Rot Disease in Beans, Caused by Rhizoctonia solani

The effect of bio and chemical treatments and their interactions on the percentage of germination of broad bean seeds .The results of this experiment showed in Table (3) that the pathogenic fungus R. solani had a clear effect in reducing the percentage of germination compared to the control treatment, which amounted to 49.00% in the treatment of the pathogenic fungus of the cultivar Shabella, compared with 98.33% in the control treatment without pathogen, but the treatment of broad bean seeds with Bio control agents in combination with chemical factors led to reducing the negative effect of the pathogenic fungus R. solani in % of seed germination with significant differences from the treatment of pathogenic fungi, as the percentage germination increased from 49.00% for the Shabella cultivar and 53.33% for the local cultivar to 95.00% and 97.33%, respectively. In the interaction treatment Humic + bacteria + gibberellin + pesticide, followed by a pesticide + bacteria treatment, which reached 95.00 and 93.13% for the Shabella cultivar and the local cultivar, respectively, and the other treatments varied in their impact on the percentage of germination of broad bean seeds. The high percentage of germination in bio treatments in combination with chemical factors may be due to its direct effect on pathogenic fungi and reduce its effect on seed infection before germination. These results are in agreement with (Andersen et al., 2003) who confirmed that P. fluorescens bacteria have the ability to produce lipopeptidocyclic substance, Amphisin compound, and Endochitinase enzyme, which works to break down the walls of fungal cells, while the pesticide works to inhibit the germination of fungal spores and prevent their penetration into plant tissues and stop the development of hives And the mycelium of fungi, while the effect of humic acid is attributed to increasing the rate of germination by encouraging the growth and reproduction of microorganisms such as algae and yeasts in the soil solution and stimulating defense enzymes and an increase in cell division and widening resulting from the effect of gibberellic acid (Al-Shammari and Wael, 2016; Siddiqui, 2017).

Table (3) The effect of bio and chemical treatments and their interactions on the percentage of germination of broad bean seeds

average treatments	cultivar		treatments
	foreign cultivar	local cultivar	
93.33	91.66	95	pesticide
63.90	58.33	67.66	bacteria
95.33	60.00	58.66	gibberellin
63.16	66.66	59.66	humic
64.16	68.33	60.00	Humic + bacteria
89.41	85.00	93.83	pesticide + gibberellin
95.50	95.00	96.13	pesticide + bacteria
65.00	63.33	66.66	humic+ Gibberellin

96.16	95.00	97.33	Humic + bacteria + gibberellin + pesticide
51.16	49.00	53.33	infection (control)
97.50	98.33	96.66	non-infection (control)
Lsd = 6.006 at 0.05 . probability level	73.67	76.81	pesticide

Effect of bio and chemical treatments and their interactions on the percentage of infection severity of the pathogenic fungus R.solani on broad bean

The results in Table (4) showed the efficiency of all treatments in reducing the percentage of infection severity of broad bean plants with the disease R. The percentage of the severity of infection with the fungus R.solani on broad bean plant .It reached 18.33% and 22.20 for the Shabella and local culivars , respectively, compared to the percentage of the severity of infection with the two pathogenic fungi alone, which was 93.66% and 94.00% for the local and Shabella cultivars, respectively. fungal hyphae growth.The plant stimulates the production of phytoaloxins in the plant, which is considered an inhibitor of the growth of pathogenic fungi. As for the role of humic acid and gibberellin in reducing the severity of infection with pathogenic fungi, this is due to their effective role in stimulating self-resistance in the plant and the production of defense enzymes (Hassan et al., 2019). While the effect of P. fluorescens on the severity of infection is attributed to its ability to produce enzymes that degrade cell walls, such as Werra et al. (2006), as well as to Pseudomonas bacteria, the ability to produce lipopeptidocyclic substance, Amphisin, and Endochitinase enzyme, which works to break down the walls of fungal cells (Andersen et al., 2003).

Table (4) The effect of bio and chemical treatments and their interactions on the percentage of infection severity with the pathogenic fungus R.solani

averagetreatments	cultivar		treatments
	foreign cultivar	local cultivar	
35.56	36.66	34.33	pesticide
32.00	33.33	30.66	bacteria
38.02	38.48	38.03	gibberellin
33.70	35.66	31.73	humic
28.86	31.66	26.06	Humic + bacteria
34.50	36.66	32.33	pesticide + gibberellin
30.35	30.00	30.70	pesticide + bacteria
31.63	29.33	33.93	humic+ Gibberellin
20.25	18.33	22.20	Humic + bacteria + gibberellin + pesticide
93.83	94.00	93.66	infection (control)

0.00	0.00	0.00	pesticide
4.573lsd=	34.91	33.96	bacteria

Effects of chemical and bio treatments on the enzymatic activity of the enzyme Peroxidase of the bean plant of the Shabella cultivar and the local cultivar under the conditions of infection with the pathogenic fungus R.solani.

The results in Figure (1) showed the induction of peroxidase enzyme activity in the chemical and biological treatments of the bean plant of the two cultivars Shabella and local under the conditions of infection with the pathogenic fungus R.solani. The figure showed the efficiency of bio and chemical treatments and their interactions in increasing the activity of the enzyme Peroxidase. Also, the enzyme activity increased in the presence of the pathogenic fungus without treatments compared to the healthy control treatment. The highest enzymatic activity in the interaction treatment was Humic + Bacteria + Gibberellin + Shapella cultivar, amounting to 1.367 units/ml.It was followed by the treatment of Humic + Bacterium, which swallowed 1.183 units/ml, compared to the healthy control treatment of the Shapella cultivar, which amounted to 0.480 units/ml, which was the lowest value. The activity of the peroxidase enzyme is related to the plant's defense mechanisms and its resistance to pathogens.The peroxidase enzyme stimulates the plant's defense mechanisms through the polymerization of the cells' protein and the annealing of its walls, thus forming a defensive barrier against the penetration of pathogens (Dickerson et al., 1984); Young et al., 1995 Also, the peroxidase enzyme works on the oxidation of phenolic compounds and the formation of hydrogen peroxidase H2O2, which is one of the substances that inhibit the growth of pathogens (Zheng et al., 2005).

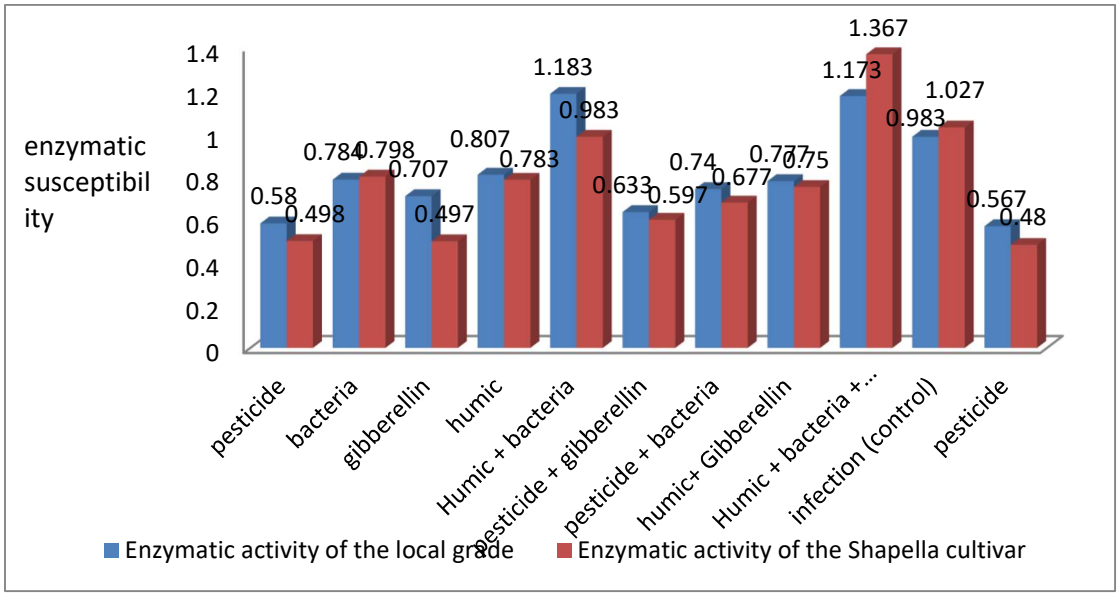

Figure (1) The effect of biological and chemical treatments on the enzymatic activity of peroxidase enzyme of LSD broad bean plant at the 0.05 probability level.

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