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Evaluating the efficiency of some chemical compounds and biotic agents in controlling the disease of root decay caused by *Fusarium Solani* on pepper

Entesar Marzoog Hussein
Al Furat Al-Awast Technical University, Babylon Technical Institute .Iraq.
entesar00001978@gmail.com

Abstract: The results of using isolation from the roots of pepper plants revealed symptoms of disease such as browning of the roots and rotting of a portion or the complete root caused by *Fusarium solani* fungus, as well as from all fields within the city of Babylon. The results of testing the effect of isolation on radish seeds revealed that the entire isolations testing of *F. solani* fungi resulted in a momentous reduction in the germination percentage under the probability level of 0.05, which ranged between 0% - 37% compared to the comparison treatment (with no pathogenic fungi), which was 100%. The findings showed that *P. fluorecsens* bacteria had a high inhibitory efficiency against an isolation of the pathogenic fungus *F.solani* (F.s2, Fs3 and Fs4) at a concentration of 5 × 107 (colony formation unit/ml), where the inhibition percentage was 82%, 67.77% and 75.22%, respectively compared to the comparison treatment in which the inhibition rate was 0. Beltanol pesticide achieved an inhibition rate of 100% with all isolations on PDA culture media. Also, all the treatments used, which include *Pseudomonas fluorescen*, *Penicillium corylyphilium* and salicylic acid, gave the best results in decreasing the infection with *F. solani* and with significant differences between them and the fungus treatment alone, where the treatment of bacteria *fluorescens.P + P. corylyphilium*, the infection severity of the fungus *F. solani* was less, as it reached 5% in comparison to the treatment of the pathogenic fungus alone, by 80%. As for the treatment of the bacteria *fluorescens.P + p.corylyphilium* together with the presence of the pathogenic fungus and in the absence of the pathogenic fungus, the highest value in length and fresh and dry weight of the vegetative and root groups, reached 28.50 and 48.50 cm, 52.50, 7.07, 44.00, and 5.03 g, correspondingly, in comparison to the treatment of pathogenic fungi alone. The average length and fresh and dry weight of the vegetative and root groups reached 18.75 and 26.00 cm 15.5, 2.26, 7.00 and 0.19 g respectively.
Keywords: *Pseudomonas fluorescen* , *Penicillium corylyphilium* , *Fusarium solani*

Introduction

Capsicum annum, a member of the Solanaceae family, is one of the most significant vegetable plants in numerous places across the world. The natural habitat of pepper plant is Central and South America, which was brought to Europe and Asia by the Spanish and Portuguese 400 years ago[1]. *Fusarium solani* Fungus is one of the most significant Fusarium species, spreading in a variety of soils and causing a variety of plant diseases. It is an indigenous fungus in the soil that may live for a long time in the form of Chlamydospores [2].Many issues have arisen as a result of the use of chemicals to control plant pathogens, including the emergence of some strains that are resistant to the effects of a number of pesticides, as well as negative environmental effects, prompting scientists to look for alternative ways to control plant pathogens while reducing the damage caused by chemical pesticides [3]. Biological control has been described as a method of reducing the density of the pathogen's vaccine or the density of the pathogen's parts in any case, whether the pathogen is active or dormant, by introducing one or more biological control agents into the soil and artificially introducing these organisms into the natural environments of pathogenic organisms .There are many biological control agents such as bacteria *Pseudomonas fluorescen* and fungus *Penicillium corylyphilium*, which have a significant role in resisting some pathogens in soil[4][5] .

Research objectives

1- Evaluating the efficacy of *Pseudomonas fluorescen* fungus *Penicillium corylyphilium* against the pathogenic fungus *F. solani*.

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2- Studying the role of salicylic acid in stimulating systemic resistance in plants.

Materials and Methods

1.2. Isolation and Identification of Fungus *Fusarium Solani*out of the roots of Pepper Plant

Some pepper planting sites in Babylon city have provided samples of diseased pepper plant roots. For 2-3 hours, the roots of the plants were cleaned with flowing water (tap water).The roots were cut into small pieces of about 0.5-1 cm and then superficially sterilized in sodium hypochlorite solution (1% free chlorine) by immersion for 3 min, and after washing with sterile water, they were dried on sterile blotting papers, 4 pieces were planted at a rate of 4 pieces in each **Petri** plate with a diameter of 9 cm, which contains 15-20 cm3 of sterile culture medium Potato Dextrose Agar (PDA). The plates were incubated in the incubator at 25 + 2 ° C for 72 hours. After the fungus get growing, the colonies were transferred to new **Petri** plates for the purpose of purification and diagnosis of the fungus, and characterization of the fungus, the characterization of the fungus *F. solani* is based on phenotypic and cult urological characteristics and microscopic examination by following some taxonomic keys for diagnosis [6][7].

2.2. Pathogen city Test of isolating *F. Solani* by radish seeds

The pathogen city of six isolations of *F. solani* fungus was tested (Table 2) according to the method of [8] and the findings have been taken after seven days through counting the rate of seed germination based upon the below equation:

$$\text{plant\%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Accordingly, the percentage of inhibition had been extracted through the following equation:

$$\text{inhibition\%} = \frac{\text{Rate of germinating seed in treatment} - \text{Rate of germinating seed number in comparison}}{\text{Rate of germinating seed number in comparison}} \times 100$$

2.3. Testing the antagonistic ability of *P. Fluorescens* against pathogenic Fungus *F.Solani* on PDA Medium

2.1.3 Determination of the effective concentration of the bacterial suspension for inhibiting the growth of *F.solani* fungus

A series of dilutions of the bacterial suspension *P. fluorescens* was prepared by taking one ml of liquid (NB) Nutrient Broth medium containing the bacterial cells using a sterile pipette and adding it to a test tube containing 9 ml of sterile water. The series of dilutions from 10^{-1} to 10^{-8} and then the containers that contain the culture medium (PDA) had been inoculated via taking 1 ml of every single dilution of the bacterial suspension and added in the form of spots (four spots). A disc of fungi with 0.5 cm diameter was planted in its core, taking out of the edges of the colony of *F. solani* in a rate of 4 plates per isolation and each dilution. For comparison, four inoculated plates with fungi had been with no bacteria inoculation, adding1 ml of sterile water and incubating the plates at a temperature of 25 ± 2 °C for 7 days. The amount of the rate of inhibition was computed via calculating the rate of diameter of the pathogenic colony fungi in the treatment of bacteria and comparing it with the diameter of the colony of the fungus in the comparison treatment. The percentage of inhibition of the fungus was calculated according to the equation [9].

$$\text{inhibition} = (R1-R2)/R1 \times 100\%$$

R1= maximum radial growth of pathogenic fungus colony only (control treatment).

R2=maximum radial growth of pathogenic fungus colony in the plates treated with bacterial inoculum.

2.3.2 Calculation of the population density of *P. fluorescens.*

After determining the lowest inhibitory dilution $10^{-6}$of the pathogen F bacterial inoculum *F. solani* in previous experience, four plates with a diameter of 9 cm containing PDA culture medium were prepared. The plates were inoculated with bacteria suspension 10^{-6}dilution at a rate of 1 ml/plate using a sterile pipette. 25 ± 2 °C for 48 hours was the duration for incubating the plates, after which the count of colonies in every single plate was rated and the bacterial colonies rate was multiplied in the reciprocal of effective dilution[10] and accordingly, the number of colonies is 5 x 107 (colony formation unit/ml).

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2.4. Testing the antagonistic ability of *p.corylyphilium* fungus against the pathogenic *F.solani* fungus on PDA culture medium

This experiment aimed at studying the antagonistic relationship among pathogenic *F.solani*fungi and biotic resistance fungi *p.corylyphilium* by the method of double cultivation technique between organic resistance and pathogenic fungi on PDA culture medium in Petri plates with a diameter of 9 cm, where each half of the plate with a disc diameter of 0.5 cm was inoculated by the colony of fungi while the other half of the plate was inoculated with a disc similar to *p.corylyphilium*fungus. The experiment was carried out with 4 replications. The inoculated plates had been positioned in the incubator at 25 ± 2 °C for 7 days, after calculating the rate of inhibition of fungal growth according to the scale of [11]. It consists of 5 degrees as follows:

Grade 1 - organic fungi covering the entire plate.
Grade 2 - organic fungi cover 2/3 of the plate area.
Grade 3 - organic and pathogenic fungi cover half of the plate.
Grade 4- organic fungi cover one third of the plate area and the pathogenic fungus covers two thirds of the plate.
Grade 5- The whole area is covered by pathogenic fungus covers.
The biotic agent is antagonistically active showing a level of antagonism of 2 or less with the isolation of the pathogenic fungi under research.

2.5. Testing the efficiency of the Pesticide Beltanol in inhibiting the growth of some isolations of pathogenic Fungus *F. solani* on PDA Culture Medium

The PDA culture medium was prepared in a glass beaker with a capacity of 500 ml. sterilized with an autoclave device at 121 °C with a pressure of 1.5 kg / cm2 for 20 min. The medium was cooled down to a temperature of 45 °C. Then, the pesticide Beltanol, produced by the Spanish company Probelte, was added, the active substance Chinosol 8-Hydroxy quinoline sulfate at a concentration of 1 ml / liter. The medium was poured into Petri plates with a diameter of 9 cm. As for the comparison, the plates contained PDA culture medium without adding the pesticide. The plates were inoculated with isolations of the fungus *F. solani* (FS5, FS4, FS3, FS2, FS1) with discs 5 mm diameter taken from the edges of the fungi colonies growing on the 7-day-old PDA medium, with 4 replicates for each isolation. The plates were incubated in the incubator at 25° C + 2. After 7 days, the results were recorded by calculating the average measurement of two perpendicular diameters from each colony and the percentage of fungal growth inhibition was calculated.

6.2. Evaluating the efficiency of some chemical compounds and biotic agents in decreasing the infection with *F.solan* Fungus and its effect on the vegetative and root system under the conditions of the wooden canopy.

This experiment was conducted at the beginning of March under the conditions of a wooden canopy. Sterilized soil was used with an Autoclave device. The soil was disseminated in plastic pots with a capacity of 2 kg, and homogeneous seedlings of pepper were planted with it. After 14 days of planting, the following treatments were carried out:

No	Type of treatment	No	Type of treatment
1	Control	10	Salycilc acid +*p.corylophilum*+watering
2	*F.solan*	11	Salycilc acid *F.solani* + *p.corylophilum* +watering
3	Salycilc acid+watering	12	*P.fluorescens*
4	*F.solani*+Salycilc acid	13	*F.solani*+*P.fluorescens*
5	Salycilc acid+sprinkling	14	*p.corylophilum* + *P.fluorescens*
6	Salycilc acid+sprinkling+*F.solani*	15	*F.solani*+*p.corylophilum* + *P.fluorescens*
7	*F.solani*+Beltanol	16	*p.corylophilum*+ *P.fluorescens* Salycilc acid+watering
8	*p.corylophilum*	17	*p.corylophilum*+ *P.fluorescens*

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			Salycilc acid+sprinkling
9	9 p.corylophilum + F.solani		

Each treatment was undergone four replications. In this experiment, a completely random design was adopted. Adding *P. fluorescens* inoculum at a rate of 20 ml of the bacterial suspension at a concentration of 5 x 7 10 (colony formation unit/ml) for each replicate. As for the inoculums of *P. corylophilum* fungus, it was added by 2% (w/w) a week before adding the vaccine of the pathogenic fungus *F.solani*, which was added by 1% (weight / weight) for the following treatments: 2, 4, 6, 7, 9, 11, 13, 15, except for the comparison treatment and the treatment of bacteria without the fungus. The chemical pesticide Bentanol was added at a concentration of (1 ml/L) and at a rate of 25 ml/refined, a day after adding the pathogenic fungus inoculums, adding salicylic acid at a concentration of 250 mg/L of water at a rate of 50 ml per pot. The results were reported after 30 days of planting in plastic pots. The intensity of root infection was rated through the pathological index consisting of five degrees, which are:

0 - The root system is healthy and the vegetative system is of normal growth and has a green color
1- More than 0-25% of the roots and root capillaries are of light browncolor.
2- More than 25-50% of the roots and root capillaries are of a dark brown color and the lower leaves are dry.
3- More than 50-75% of the roots and root capillaries are of a dark brown color with the lower leaves falling off.
4- More than 75-100% of the root total in a dark brown color
5- Plant death

The percentage of injury intensity and percentage of injury severity were calculated according to[12] Mchinney equation as follows:

$$\text{Severity of infection} = \frac{\text{Amount (number of infected plants x degree of its infection)}}{\text{Total number of tested plants x the maximum degree of infection}} \times 100$$

2.7. Statistical Analysis

The complete randomized design C.R.D was used in the design of all experiments. The least significant difference test (L.S.D) was adopted to ensure the significant differences between the rates of various treatments in the experiments under the 0.05 probability level to compare the results [13]. The statistical analysis was carried out byadopting the statistical program spss (2001).

3- Results and Discussion:

3.1.Testing the Pathogenicity of isolations of *F. solani*Fungus by radish seeds.

The results of testing the effect of isolations of the pathogenic fungus on radish seeds in (Table 1) showed that all tested isolations of *F. solani* caused a significant reduction in the percentage of seed germination under the probability level of 0.05. This percentage was ranged from 0% to 37% in comparison to the ratio of germination in the comparison treatment (without a pathogenic fungus), which had a germination rate of 100%. The isolations Fs2, Fs3 and Fs4 induced the highest reduction in germination in radish seeds, as the germination rate reached 0%, followed by isolation Fs5, which amounted to 18%, and isolation Fs1 showed less effect among the tested isolations in reducing the percentage of germination of radish seeds as the percentage of germination in its treatment was 37% compared

to the comparison treatment in which the percentage of germination of radish seeds was 100%. These results were similar to what[14] discovered in which he found that the difference of these tested fungal isolations in their effect on germination may be attributed to the quantity and quality of toxic substances they secrete, and their difference in their ability to secrete pectin-degrading enzymes, especially enzyme Polygalacturonase, as non-pathogenic isolations have low efficacy in producing this enzyme [15].

Table (1): Testing the pathogenicity of isolations of *F. solani* using radish seedson W.A. medium.

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No	Collection Area	Isolations	For Germination%
1	Bid'ah / Al-Mahaweel / Babylon	Fs1	37
2	Al Mahaweel / Babylon	Fs2	0.0
3	Al-Qasim/Babylon	Fs3	0.0
4	Tahmaziah/Babylon	Fs4	0.0
5	Nile / Babylon	Fs5	18
6	Comparison	Control	100
	L.S.D	-	16.56

* Each number stands for the rate of four duplicates.

2.3. Testing the antagonistic ability of *P. fluorescens*against the isolation of *F. solani* (F.s2, Fs3 and Fs4) on PDA Medium

The results of this experiment in Table (2) revealed that *P. fluorecsens* bacteria possess a high inhibitory efficiency in resisting the pathogenic fungus *F.solani* (F.s2) at a concentration of 5 x 107(colony formation unit/ml), where the percentage of inhibition was 82% if compared to the treatment of comparison in which the inhibition rate was zero. The reason for such a case may be due to the competition in some nutritional needs between these bacteria and the pathogenic fungus, and may be due to the similarity of the pattern of nutrient exploitation between bacterial spores and fungi, and the antagonistic ability of P., and its production of a number of enzymes that degrade the walls of fungus cells like Chitinolyticenzyme and Catalase[16] .

Table (2): Test the antagonistic ability of *p. fluorescens* bacteria against some isolation of *F. solani* on PDA medium

Treatment	Rate of F.solani fungus growth in plate (cm)	Inhibition Percentage
fluorescens.P+F.s2	1.62	82
fluorescens.P+F.s3	2.90	67.77
fluorescens.P+F.s4	2.23	75.22
F.solani fungus alone	9.00	0.00
L.S.D at level (0.05)	-	16.15

* Each number stands for the rate of four replicates.

3.3. Testing the Antagonistic Ability of *P.corylyphilium* against Isolation of *F.solani* (F.s2) on PDA Medium.

The results showed that the biotic resistance of fungus *p.corylyphilium* had achieved a high percentage of antagonism against isolations of the pathogenic fungus *F.solani* (Fs2,Fs3,Fs4), which reached the degree of (1) according to Bell measurement.

The antagonistic ability of *P. corylophilum*fungus may be due to the production of antibiotics in addition to other compounds, including alkaloids, alkanes, sesquiterpens compounds, fatty acids, essential oils 1, 3,8-P-2-menthatriene, 2-methylenecyclohexane, Anthracene and Neoisolongifolene, compound as well as having the ability to produce many organic acids such as Oxalic acid, Dibenzothiophene, Hexadecanoic, and alkane hydrocarbons [17][18][5]

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Figure (1): The picture on the right represents the antagonism between *p.corylyphilium* fungusand pathogenic fungus*F.solani* while the picture on the left represents pathogenic fungus *F.solani* alone

3.4. Testing the efficiency of the Pesticide Beltanol in inhibiting the growth of some isolations of the Pathogenic Fungus *F. solani* on PDA cultivatemedium

The results of this test in Table (3) illustrated that using the pesticide Beltanol at a concentration of 1 ml / liter led to the inhibition of the growth of the mycelium by 100% and for all isolates FS2, FS3 and FS4 on the culture medium compared to the comparison treatment in which the percentage of inhibition was 0%. This is consistent with what found [14], when he discovered that the use of this pesticide at the recommended concentration led to a complete inhibition of the growth of pathogenic fungi on PDA culture media.

Table (3): Efficiency test of Beltanol in inhibiting the growth of some isolates of the pathogenic fungus F. solani on PDA cultivate medium.

Type of treatment	Rate of diametric growth of pathogenic fungus F.solani (cm)			Rate of Inhibition %		
	FS2	FS3	FS4	FS2	FS3	FS4
Beltanol	0	0	0	100	100	100
Control	9	9	9	0	0	0

3.5. Evaluating the efficiency of some chemical compounds and biotic agents in decreasing the intensity of infection with *F. solani*Fungus and its effect on the vegetative and root system under greenhouse conditions

The results of the experiment of the greenhouse in Table (4) showed that all the treatments used, which include the biotic resistance agents p.fluorescens, *p.corylyphilium* and salicylic acid, gave the best results in decreasing the intensity of infection with *F. solani* fungus and with significant differences between them and the treatment of the fungus alone. The bacterial treatment of *p.fluorescens*+ *p.corylyphilium* fungus gave the highest inhibition of *F.solani* fungus, as the infection severity reached 5% in comparison to the treatment of the pathogenic fungus alone, reaching up to 80%. The reason for the superiority of the interaction coefficients in reducing the percentage of the severity of infection with the pathogenic fungus is that the use of the interaction between the biotic resistance agents achieves better results. This is because each biotic resistance may use different mechanisms to control the pathogen, and by combining these mechanisms from both agents of biotic resistance, there will be greater suppression of the pathogen, and the results will be better if the biotic resistance is used alone [19]. This result was identical to many studies that showed that the interaction between biotic resistance agents is more effective in reducing the severity of infection with plant pathogens when compared if a biotic agent was used alone [20]. Also, the treatment of *P. fluorescens* bacteria alone and *P. corylyphilium* alone reduced the infection severity to 10% for both treatments compared to the comparison treatment of pathogenic fungi. Salicylic acid treatment alone by

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watering and spraying reduced the infection severity to 40% and 45% compared to the comparison treatment for pathogenic fungi. Salicylic acid may result from the induction of resistance in plants against pathogenic fungi.[21][22] The results also showed the efficiency of the chemical pesticide Beltanol in reducing the severity of infection with pathogenic fungi, as it achieved a reduction in infection intensity of 0.00% in comparison to the comparison treatment (the pathogenic fungus alone), in which the infection intensity was 80.00.% . This corresponds to results found by [14] in the effectiveness of this pesticide in controlling F. Solani fungus, which causes the root decay disease of bean, apple and citrus fruits. The active effect of this chemical pesticide is due to the formation of chelating compounds with copper in the tissues of the host. This could facilitate passing into the cells of the pathogen and then is released and leads to killing the pathogen[23] . As for the studied growth criteria for pepper plants, the results indicated that the entire treatments marked a tremendous rise in growth criteria such as root and vegetative length, fresh and dry weight of the root and vegetative group, in comparison to treating pathogenic fungus *F. solani* alone and for all treatments. *fluorescens + p.corylyphilium* together with the presence of the pathogenic fungus had the highest value in height, fresh and dry weight of the shoot and root group, they reached 28.50 and 48.50 cm 52.50, 7.075, 44.00 and 5.033 g, correspondingly, in comparison to treating pathogenic fungi alone. The average length, fresh and dry weight of the vegetative and root groups in it reached 18.75 and 26.00 cm 15.5, 2.265, 7.00 and 0.195 g, respectively. The treatment of *p.corylyphilium* also gave the best results in the presence of the fungus, as the height and the fresh and dry weight of the shoots and roots reached 26.50 and 45.50 cm, 47.50, 4.458, 34.00 and 3.770 g. It reduced the severity of infection, reaching 10% compared to the treatment of pathogenic fungi alone. The reason may be due to the ability of these fungi to secrete plant growth regulators or their ability to dissolve some low-soluble nutrients such as zinc, copper, iron and manganese ions, and then make them more ready for plants[24][25] .This increase in the length of the vegetative and root systems may be due to the use of the fungus for its various mechanisms of parasitism, competition and the production of antibiotics in inhibiting the growth of pathogenic fungi, or the effectiveness of *Penicillium sp* belongs to protecting plants. It competes for food and place, as *Penicillium* fungus colonizes the roots of treated plants in addition to producing many antibiotics that reduce the density of plant pathogenic fungi [24][25][26].The rest of the treatments also showed significant differences represented by an increase in length and weight for the root and vegetative group in different proportions according to the type of bacterial treatment compared to the pathogenic fungus treatment.

Table (4) represents the efficiency of some chemical compounds and biological factors in decreasing the intensity of infection with F. solani fungus and their effect on the vegetative and root system under greenhouse conditions.

No	Treatment	Infection severity	Group weight (cm)		Total weight		Length(cm)	
			Soft	Dry	Soft	Dry	Stem	Root
1	By Plant Only	0.0	35.25	3.715		2.293	23.50	38.00
2	F.s	80	15.5	2.265		0.195	18.75	26.00
3	SA+ watering	0.0	45.5	4.528		2.358	24.25	42.25
4	F.s+ SA watering	40	35.5	4.208		2.220	24.75	42.50
5	SA sprinkling	0.0	42.75	4.508		2.078	26.25	42.50
6	F.s+SA sprinkling	45	34.75	3.295		2.378	26.50	41.50
7	F.s+Beltanol	0.0	33.00	3.838		2.223	24.50	42.75
8	p.cor	0.0	50.75	7.935		5.105	29.00	47.50
9	F.s+p.cor	10	47.50	6.183		5.060	28.00	45.00
10	SA watering +p.cor	0.0	42.25	5.515		2.510	25.50	44.00
11	F.s+SAwatering+p.cor	20	42.50	5.020		2.693	22.50	45.00
12	P.fl	0.0	51.25	5.272		4.063	28.00	46.25
13	F.s+P.fl	10	40.50	4.458		3.770	26.50	45.50

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14	p.cor+P.fl	0.0	64.25	8.946		6.530	29.50	65.50
15	F.s+p.cor+P.fl	5	52.50	7.075		5.033	28.50	48.50
16	SA+watering+p.cor+P.fl	0.0	44.25	4.225		3.596	24.50	45.50
17	F.s+SA+watering+p.cor+P.fl	20	39.25	3.990		2.715	24.50	45.25
	L.S.D	10.94	2.318	0.1557		0.1489	3.606	2.522

Each number represents an average of four replicates.
F.s = F.solani , P. fl = P. fluorescens ,p.cor= mushroom p.corylyphilium ,SA= salicylic acid.

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