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Article

Biological control of the fungi causing root rot disease of Eggplant plants

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Abstract

The study aimed to identify some causes of eggplant root rot disease after isolation and diagnosis in some areas of Babylon Governorate and to evaluate the efficiency of the biological fungus Trichoderma harzianum and extract of some plants in control the pathogens of eggplant seedling death disease. The results of the field survey conducted in the fields of eggplant plants in the province of Babylon showed the presence of root rot in the eggplant in all areas covered by the field survey. Several types of fungi were isolated and identified from the roots of eggplant plants infected with root rot disease. The most frequent pathogenic fungi were Fusarium solani, Rhizoctonia solani, and Macrophomina phaseolina. The results showed that all tested fungi isolates were pathogenic and caused a significant reduction in the percentage of germination of cabbage and eggplant seeds. The results of the pot experiment showed that all the treatments that included the biological factor and aqueous Acacia extract had a significant effect in reducing the growth of pathogenic fungi, especially when the treatments were combined, as measured by the percentage of infection and the severity of infection in the treatment of pathogenic fungi F. solani, R. solani and M. phaseolina, Alone, the infection rate was 100.00%, and the severity of the infection was 76.67, 70.00 and 66.67%, respectively. The treatment of integration between the biological factor T. harzianum and the aqueous extract of Acacia achieved high superiority in reducing the infection rate, as it ranged between 11.11-24.33% and the severity of infection 4.44-15.00%.

Keywords: Plant extracts, Eggplant, fungi, root rot, Trichoderma harzianum.

Introduction

Eggplant, also known as brinjal (Solanum melongena L.), is the third largest crop after potatoes and tomatoes of the Solanaceae family. Global eggplant production is concentrated in seven countries: China, India, Egypt, Turkey and Japan. The eggplant spread from India to the western Islamic lands via Iran; It is native to India and central and southern China [1]. The areas cultivated with it have increased in Iraq, as the production of the eggplant crop in Iraq for the year 2020 was estimated at 207.2 thousand tons, while in Babylon Governorate, production reached 18,991 thousand tons [2]. In protected and open cultivation, eggplant yield is affected by many fungal, bacterial and viral pathogens that cause severe damage and reduce yield in quantity and quality [3, 4, 5]. Fusarium solani, F. oxysporum, Rhizoctonia solani and Macrophomina phaseolina are among the most critical and widespread

soil plant pathogens that attack the roots of the eggplant, causing seed rot, seedling death, root rot and wilt diseases [6]. The disease of seedling death and root rot is one of the most common diseases in nurseries and greenhouses and is widespread worldwide. [7]. Several methods, including chemical pesticides, have been used to control these pathogens. However, the extensive and ill-considered use of these materials has led to the emergence of many risks to human and animal health and the environment, as well as their high economic cost and the emergence of resistance by pathogens, which prompted the efforts of researchers in the field of plant protection to research Using less dangerous and safer methods, the most prominent of which is the use of microorganisms as vital agents to resist plant pathogens, reduce their inoculum and increase the quantity and quality of the crop [8, 9, 10]. One of the most important biological factors is the biological fungus Trichoderma spp because of its many mechanisms to control pathogens, reduce infection, and stimulate and defend the growth of the host plant and other mechanisms that prompted many researchers interested in the study [11]. Researchers are also increasingly interested in using plant extracts for pathogen control because they contain many effective and anti-pathogen compounds and have desirable environmental properties such as rapid decomposition, high specialization and low toxicity to the non-target organism [12]. Plants can synthesize secondary aromatic receptors, such as phenols, phenolic acids, quinones, flavonoids, tannins, and coumarins. It releases compounds that inhibit plant pathogens and increase the effectiveness of microorganisms with the inhibitory ability of these pathogens, which increases the control process well with the biological control agent [13]. This study was proposed, which aimed to identify some causes of the eggplant seedling death disease after isolating and diagnosing them in some areas of Babylon Governorate. An evaluation of some biological factors. Besides, some extracts control eggplant seedling death pathogens.

Materials and Methods

field survey

A field survey was carried out for 9 sites of eggplant cultivation fields located in Babylon Governorate from 6/10 to 10/10/2021. The number of infected plants was calculated based on the symptoms appearing on the plants, and the percentage of infestation was extracted for each field.

Isolation and identification of fungi accompanying the roots of infected eggplant plants

The isolation process was carried out on the eggplant samples that showed symptoms of infection represented by yellowing and wilting of leaves with the presence of rotting and ulceration of the roots and stems close to the soil's surface. Sterile forceps to Petri dishes with a diameter of 9 cm containing the medium (Potato Dextrose Agar PDA). The dishes were left in the incubator at 25 ± 1 °C for three days. The various fungi were purified and examined under the compound microscope, and the genera and species were identified based on the approved taxonomic keys with the help of A. Dr. Ahed Abd Ali [14, 15, 16, 17] and the percentage of appearance of the studied fungi was calculated as follows: repeat the fungus in the sample = (The number of fungi appeared in dishes\Total number of pieces used in the sample)x 100 [18].

Testing the pathogenicity by using cabbage seeds under laboratory conditions.

The pathogenicity of 9 isolates of *F. solani* was tested, 5 isolates from *R. solani* and 5 isolates from *M. phaseolin* according to the method of [19]. The plates were inoculated from their center with a 5.0 cm drop disc taken from near the edges of the colony of fungi at the age of five days. Then, the dishes were incubated at a

temperature of 25 ± 1 °C for three days, after which the cabbage seeds were sown in a circular motion near the edge of the dish at a rate of 10 seeds per dish for germination.

Effect of some pathogenic fungi on the germination of eggplant seeds under nursery conditions.

The experiment was carried out under the conditions of the net canopy (Siran) of the biological control techniques department, as the most pathogenic isolate in the previous test was selected from the tested fungi *R. solani* (Rh-4), *F. solani* (FS-2) and *M. phaseolina* (MP-6) and fungal inoculum loaded with seeds of local millet (*Panicum miliaceum* L.) were added to sterilized soil mixtures with an oxidizer that were distributed in 1 capacity plastic pots. Kg and the pathogenic fungus inoculum were added at 1% (weight/weight). Each pot was planted with ten local eggplant seeds superficially sterilized with sodium hypochlorite solution. Each treatment was repeated 3 times, and 3 replicates were left without adding the pathogenic fungus as a comparison. Under the conditions of the canopy for 8 days and with the emergence of seedlings in the control treatment, the bags were removed, and the percentage of germination was calculated after 15 days of sowing after the completion of the germination of the seeds of the control treatment. The percentage of germination was calculated.

Evaluation of the efficiency of aqueous extract of Acacia plant and biological factors in the percentage of severity of infection with the fungi R. solani, F. solani, and M. phaseolina. and some growth parameters of eggplant samples under greenhouse conditions

The field experiment was conducted in the greenhouse on 10/2/2022 using cork culture dishes, and in each well, sterilized sandy soil was placed in the purifier, planted with seeds of local eggplant, with six seeds for each eye in the cork. The experiment used a Randomized Complete Design (R. C. D) with three Replications. For each treatment, the inoculum of the pathogenic fungi R was added. *F. solani, R. solani and M. Phaseolina* loaded on local millet seeds to all treatments that require the addition of pathogenic fungi inoculum at a rate of 1% weight = weight, as for the biological control agent *T. harzianum* was added to millet seeds at a rate of one % weight = weight three days before adding the pathogenic fungus inoculum. As for treatment, aqueous Acacia extract was added at a concentration of five ml/culture eye directly after adding the pathogenic fungus inoculum. As for the control treatment, sterile millet seeds were added to it. Just. , was added according to the following transactions:

1-. *F.solani* (Fs-1) alone. 2- Fs-1+ *T .harzianum* 3- Fs-1+ Acacia Extract 4 - Fs-1 + the chemical pesticide Beltanol. 5- Fs-1+ Acacia extract + *T .harzianum*. 6-15, the treatment was repeated with R. solani (Rh-4) and *M . Phaseolina* (Mp-6) 16 - control treatment. 17- extract. 18- *T .harzianum*

The disease incidence was estimated by using the following equation:

% Disease incidence = (Number of infected seedlings\ Total number of total seedlings) × 100. after a month and a half of experimenting, by estimating the severity of the root rot disease of eggplant plants caused by the pathogenic fungi *R. solani, F. solani, and M. phaseolina.* using the following pathological evidence as following

0 - a healthy vegetative group with a white and healthy root system.

1- More than 0-25% of the root is colored in a light brown color and a specific number of root branches.

2- More than 25-50% of the root is discolored in a dark brown color, and a large number of root branches with Dry lower leaves.

3- More than 50-75% of the root is dark brown, with the lower leaves falling off.

4- More than 75-100% of the root is discolored in a dark color, or the plant has died.

The severity of the injury was calculated according to the Mckinney equation [21] and the pathological evidence.

Severity (%) = ((Plants in 1 degree ×1+… Plants in 5 degree ×5)/ all plants ×5) ×100%.

Also, the calculation of the wet and dry weight and the length of the shoot length of the eggplant.

Results

*Field survey of Eggplant root rot***.**

The results of the field survey listed Table 1, which was conducted in the fields of eggplant plants in the province of Babylon, showed the presence of eggplant root rot disease in all areas covered by the field survey, with an infection rate ranging between 30-70% and an infection severity ranging between 65-27%, and recorded the highest The percentage of infection in Al-Haswa and Al-Dabla fields reached 70%, followed by Al-Qurayya Al-Asriya and Al-Mussaib Al-Hamieh fields, the percentage of infection in both of them reached 60%. Furthermore, the results showed that the lowest infection rate was in the Al Mashroa district, reaching 30%, because it was the first time that it was planted with the eggplant crop, while the highest severity of infection was in the fields of Al-Haswa and Al-Dabla, which amounted to 65 %, followed by the Al-Qurayya Al-Asriya district with 56%, and then the Al-Mussaib Al-Hamieh district with 55%. While the infection was less severe in an Al Mashroa district, it amounted to 27%, and the reason for this is due to the different locations of the fields covered by the survey and the different environmental factors due to the difference in the date of the survey.

Region	Disease induce (%)	Severity (%)
Al-Haswa	70	65
Al-Qurayya Al-Asriya	60	56
Al-Mussaib Al-Hamieh	60	55
Al Mashroa	30	27
Al-Azraq	40	37
Al-Hamza	50	43
Al-Dabla	70	65
Al- Azawia	50	45
Al-Qasim	40	36

Table 1. Percentage of infection and severity of Eggplant root rot disease for some regions for the agricultural season 2021-2022.

Isolation and identification of fungi accompanying the roots of eggplant plants infected with root rot

Several types of fungi were isolated and identified from the roots of eggplant plants infected with root rot disease, table 2, figure 1, 2 and the most frequent pathogenic fungi was *Fusarium solani*, which was isolated from all districts covered by the survey. Its appearance rate was 65.22%, and the highest percentage of its appearance was 94 followed by the fungus *Rhizoctonia solani*, which was isolated from five regions, with a rate of 58.40%, and the highest percentage of its appearance was 80%, then the fungus *Macrophomina phaseolina*, which was isolated from and at a rate of 40.00%.

Fungus	Appearance rate (%)	Highest ratio of appearance
Fusarium solely	65.22	94
Rhizoctonia solani	58.40	80
Macrophomina phaseolina	40.00	93
Trichoderma spp.	20.00	20
Aspergillus Nigeria	17.88	34
Penicillium spp.	20.78	34
Alternaria alternate	14.67	22
Ceratobasidum	26.67	45
Chaetomium globosum	10.00	13
Mucor spp.	12.33	14

Table 2. Percentage of the appearance of fungi accompanying the roots of eggplant infected with root rot.

Testing the pathogenicity of isolates of pathogenic fungi using cabbage seeds on PDA

Table 3 showed that all tested isolates led to a significant reduction in germination percentage, compared to the comparison treatment in which the percentage of plant seeds was 100.00%. The isolate of the fungus *F.solani* (Fs2) was superior in reducing the percentage of germination, where the rate of germination percentage was 11.10%, where the germination percentage was 11.10%, followed by isolate Fs1, where the germination percentage reached 12.20%, while the germination percentage for the rest of the isolates ranged between 50.00-23.33%.

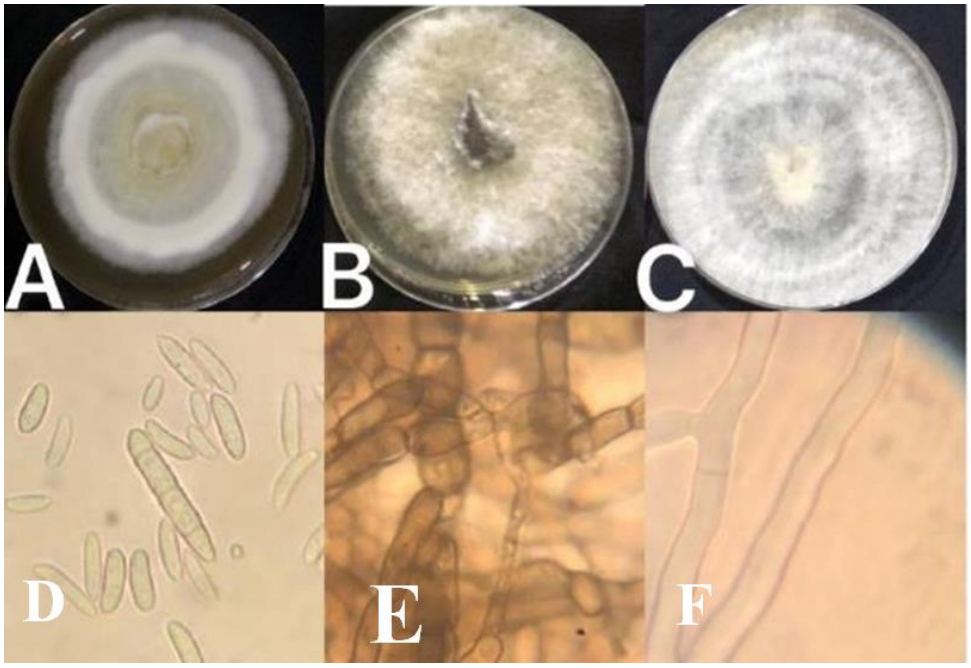

Figure 1. Some fungi isolated (most frequent) from the roots of eggplant plants infected with root rot disease, A = *F. solani* culture on PDA medium D = Macro and Micro conidia of *F. solani* under a light microscope with a magnification of X40. B = culture of *M. phaseolina* on PDA media, E = sclerotia of *M. phaseolina* R. solani X40. C = *R. solani* colony on PDA medium, F = the hyphae of *R. solani* under a light microscope at X40 magnification.

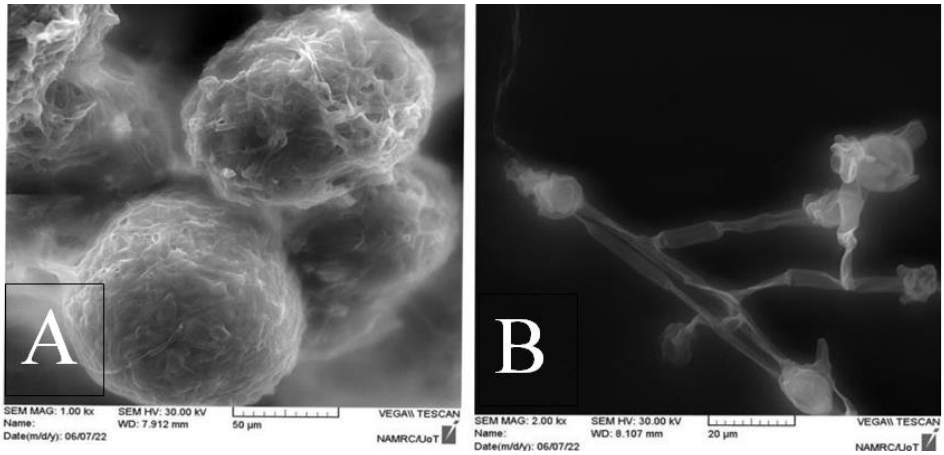

Figure 2. Using a scanning electron microscope, pathogenic fungi, A = sclerotia of *M. phaseolina*, B = Conidia of *F. solani*.

Region	Isolate symbol	No. germinated seeds	Germination (%)
-	control	10.00	**100.00**
Al-Haswa	Fs1	1.20	**12.20**
Al-Qasim	Fs2	1.10	**11.10**
Al-Qurayya Al-Asriya	Fs3	2.33	**23.33**
Al-Mussaib Al-Hamieh	Fs4	3.50	**35.50**
Al-Azraq	Fs5	4.40	**44.40**
Al Mashroa	Fs6	5.00	**50.00**
Al-Hamza	Fs7	3.00	**30.30**
Al-Dabla	Fs8	2.50	**25.50**
Al- Azawia	Fs9	4.33	**43.33**
Al Mashroa	Rh1	0.00	**0.00**
Al-Haswa	Rh2	3.67	**36.67**
Al-Hamza	Rh3	2.67	**26.67**
Al-Qurayya Al-Asriya	Rh4	5.00	**50.00**
Al-Mussaib Al-Hamieh	Rh5	3.33	**33.33**
Al-Haswa	Mp1	4.33	**43.33**
Al-Qurayya Al-Asriya	Mp2	5.50	**55.50**
Al-Hamza	Mp3	1.20	**12.22**
Al- Azawia	Mp4	2.25	**22.25**
LSD (0.05)	-	0.615	**8.567**

Table 3. Detection of the pathogenic isolates of *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina* using cabbage seeds on a PDA medium.

Each number in the table represents an average of three replicates: Fs = *Fusarium solani*, Rs = *Rhizoctonia solani*, and Mp = *Macrophomina phaseolina*. The number near the isolate symbol represents the isolate number.

Pathogenicity test to the pathogenic fungi Fusarium solani, Rhizoctonia solani and Macrophomina phaseolina in the germination of eggplant seeds.

The results indicated in Figure 3 that adding the pathogenic fungi *F. solani, R. solani* and *M. phaseolina* led to a reduction in seed germination compared to the

control treatment. The results showed that the tested isolate of the fungus *F. solani* (Fs2) caused a significant reduction in the percentage of seed germination, which amounted to 17.75% and significantly differed from the comparison treatment without addition, which had a germination rate of 100%. The isolate of the fungus *R. solani* (Rh1) prevented the germination of most of the eggplant seeds. It caused a severe reduction in the germination rate of 12.20%, as the isolate of *M. phaseolina* (Mp4) caused a significant reduction in the germination rate, which amounted to 20.00 compared to the comparison treatment.

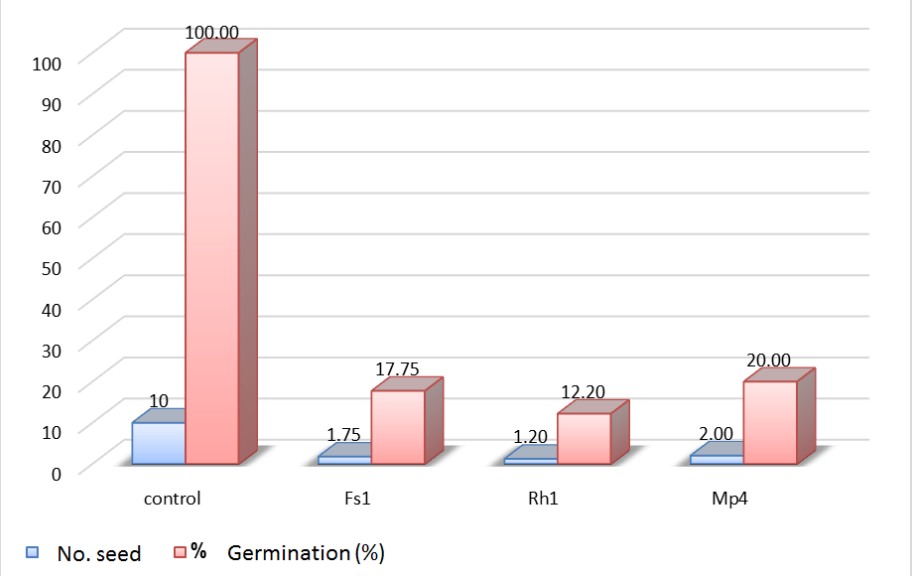

Figure 3. The effect of some isolates of pathogenic fungi on the germination of eggplant seeds.

Fusarium solani = Fs2*,1 Rh = Rhizoctonia solani, 4 Mp = Macrophomina phaseolina, the number next to the symbol represents the isolate number.

Evaluation of the efficacy and biological agent Trichoderma harzianum, aquatic acacia extract and Beltanol in the percentage and severity of infection with pathogenic fungi F. solani, R. Solana and M. phaseolina and some growth parameters of eggplant under greenhouse conditions.

The results of the experiment (Table 4) showed that all treatments that included the biological agent, aqueous acacia extract, had a significant effect on reducing the growth of pathogenic fungi, especially when the treatments were combined, compared to the percentage of infection and the severity of infection in the treatment of pathogenic fungi *F. solani and R. solani* and *M. phaseolina*, alone, where the infection rate was 100.00%. The infection severity reached 76.67, 70.00 and 66.67%, respectively. The treatment of integration between the biological factor T. harzianum and the aqueous extract of Acacia achieved high superiority in reducing the infection rate, as it ranged between 11.11-24.33% and infection severity 4.44-15.00%, respectively, for all pathogens *F. solani, R. solani* and *M. phaseolina*. This decrease indicates compatibility between the biological factor *T. harzianum* and aqueous acacia extract. The results of Table 4 also showed that all treatments used to control the root rot of eggplant pathogens, which included the biological agent *T. harzianum* and the aqueous extract of Acacia, led to a significant increase in the average plant height in soil contaminated with pathogenic fungi *F. solani, R. solani and M. phaseolina*. The highest length in the integration treatment between the pathogenic fungus, the biological factor *T. harzianum* and the aqueous acacia extract was 50.67, 52.00 and 51.00 cm, respectively, and an increase in the wet weight of 121.67, 132.67 and 123.00 g, as well as the dry weight of 30.00, 31.67 and 32.67

g, respectively. As for the treatment of the aqueous acacia extract and the pathogenic fungus, the vegetative length of the plant reached 44.00, 49.00 and 48.33 cm, respectively, and an increase in the wet weight of 120.00, 120.33 and 119.33 g, respectively, as well as the dry weight of 52.67, 28.00 and 27.67 g, respectively, compared to the treatment of pathogenic fungi F. *solani*, *R. solani* and *M. phaseolina* alone, which caused an apparent effect in reducing plant height and wet weight, which reached 21.33, 22.00, 20.67 cm, 57.67, 51.67 and 3.33 g. Jan 12.33, 10.33 and 6.67 g, respectively. As for the treatment of the fungicide with pathogenic fungi, the average plant length was 44.67, 45.00 and 43.33 cm, the soft color was 115.00, 116.00 and 114.33 g, and the dry weight was 21.33, 21.33 and 19.67 g, respectively.

Treatment	Disease incidence (%)	Severity (%)	Plant length (cm)	Wet weight (g)	Dry weight (g)
Fs-1	100.00	76.67	21.33	57.67	12.33
Fs-1+T.h	13.33	9.00	48.33	116.67	29.00
Fs-1 + A	20.00	10.00	44.00	120.00	25.67
Fs-1+T.h +A	11.11	4.44	50.67	121.67	30.00
Fs-1+ Beltanol	0.00	0.00	44.67	115.00	21.33
Rs-4	100.00	70.00	22.00	51.67	13.33
Rs-4+T.h	66.66	60.00	49.00	120.33	28.00
Rs-4+ A	36.00	28.33	47.33	118.67	27.00
Rs-4+T.h +A	24.33	15.00	52.00	123.67	31.67
Rs-4+ Beltanol	0.00	0.00	45.00	115.00	21.33
Mp-6	100.00	66.67	20.67	33.33	6.67
Mp-6+T.h	0.00	0.00	50.00	120.35	28.33
Mp-6+ A	31.67	27.00	48.33	119.33	27.67
Mp-6+T.h +A	13.33	8.33	51.00	123.00	32.67
Mp-6+ Beltanol	0.00	0.00	43.33	114.33	19.67
Control	0.00	0.00	47.00	115.33	25.67
T.h	0.00	0.00	53.33	124.33	32.67
A	0.00	0.00	44.00	120.67	30.67
T.h+A	0.00	0.00	55.50	126.00	34.33
LSD (0.05)	6.625	4.332	1.33	1.30	0.60

Table 4. Evaluation of the efficacy and biological agent Trichoderma harzianum, aqueous acacia extract in the percentage and severity of infection with pathogenic fungi of eggplant under greenhouse conditions. *Each number in the table represents an average of three replicates, Fs-1 = Fusarium solani, Rs-4 = Rhizoctonia solani, Mp-6 = Macrophomina phaseolina. T.h= Trichoderma harzianum, A= Acacia extract.

Discussion

The reason for the spread of the disease in these areas is attributed to the repeated cultivation of the eggplant crop or the cultivation of other crops belonging to the solanaceous family in the same fields. This led to the accumulation of the inoculum of pathogenic fungi, especially sclerotia, which remain in the soil for a long time and suit the environmental conditions, especially temperatures [22]. Alternatively, the reason may be due to the widespread and repeated use of chemical pesticides for pathogens, which developed a trait of resistance to pathogens, in addition to the processes of hoeing and weeding that lead to wounding the roots, which prepares them for the invasion of pathogenic fungi, as well as the difference in agricultural operations and in the type and method of adding fertilizers. These factors influence plants and make them more sensitive to response to plant diseases.

The pathogenicity of the isolates is attributed to their secretion of many toxic secondary metabolic compounds that kill the embryos and the ability to produce decomposing enzymes responsible for rotting in the seeds. The results of the diagnosis showed the presence of many fungi accompanying the roots of the eggplant, with less frequency, including the fungi *Alternaria alternata, Penicillium* spp, *Aspergillus nigeria, Trichoderma* spp., *Mucar* sp, *Chaetomium globosum* and the results agree with [23] that *F. solani, R. solani* and *M. phaseolina* are among the most frequent pathogenic fungi in samples of eggplant plants infected with the disease.
Root rot

The results of Table 3 indicated that all tested isolates of *R.solani* caused a significant reduction. It was evident in the germination of the cabbage seeds compared to the control treatment, and the isolates varied among themselves in reducing the germination percentage. The germination of seeds was 26.67%, while the germination percentage in the rest of the isolates ranged between 26.67-50.00%.

The pathogenic fungus *R. solani* attacks the seeds of the host and causes them to rot and prevent them from germination. It also attacks the seedlings before emergence, which causes a significant reduction in the percentage of germinated seeds by killing the seeds or weakening the seedling and delaying its emergence. Root rot, canker sores and shortening of the bases of the seedling stems occur near the surface of the soil, which causes the seedlings to fall off after emerging to the soil surface and die [6]. The results are consistent with what was shown by several studies of the emergence of soil pathogenic fungi and their spread on eggplant and its occurrence of seedling death disease on eggplant and other crops. And *R.solani* and *M. phaseolina* [24].

These results agree with what Koijam and Sinha [25] showed about the efficiency of *Trichoderma* isolates against the pathogenic *R. solani*, as it produced a high inhibition rate. Besides, it agrees with what [26] found that *T. harzianum* was among the most efficient biological agents tested against the pathogenic *R. solani* that causes eggplant seedling death disease. The treatment of the biological agent *T. harzianum* in the presence of the pathogenic fungus significantly reduced the infection rate with the pathogenic fungi *F. solani, R. solani* and *M. phaseolina* 13.33, 66.66 and 0.00%, respectively. The infection severity was 9.00, 60.00, and 0.00%, respectively, compared to treating pathogenic fungi alone. These results show that the biological factor can protect eggplant seedlings from infection with pathogenic fungi. Polyketides, Peptaibds, Diketo Piperzin [27].
The treatment of aquatic Acacia extract in the presence of the pathogenic fungi *F. solani, R. solani and M. phaseolina* achieved a significant and clear reduction, as the infection rate ranged from 20.00-36.00% and the infection severity ranged between 10.00-27.00%, respectively. The physiological action of plant aquatic extracts in the effect may be due to the nature of their content of active substances that can inhibit the growth of pathogenic fungi, as the results agree with what was observed by [28] that the inhibitory effect of these extracts may be due to their effect. Preventing spore germination and its effect in changing the permeability of cell walls or preventing the growth of the mycelial hypha in its early stages leads to the inhibition of the growth of these fungi.
The active inhibitory compounds found in plant extracts and antimicrobial microorganisms reduce the carbohydrate and total protein content and increase the activity of enzymes Saccnic, Fumaras, and Malik dehydrogenase. At the same time, dehydrogenase reduces the activity of catalase enzyme in both R.*solani* and *F. oxysporum*, which leads to an increase in toxicity and then their growth rates. Also,

some plants contain effective compounds capable of inhibiting the growth of pathogenic organisms, and these compounds have different chemical compositions than the traditional fungicides used to control the growth and survival of these organisms [29]. The results were in agreement with [30,31,32]. Acacia leaves have been shown to have antimicrobial, antioxidant and cytotoxic activities.

Conclusions

The spread of Eggplant root rot disease in all areas covered by the field survey. The pathogenic fungi *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina* were found to be the leading causes of eggplant root rot diseases in Babylon governorates. Efficiency of *Trichoderma harzianum* and Acacia plant extract alone or integrated in reducing the rate and severity of infection with pathogenic fungi *F. solani, R. solani* and *M. phaseolina* and provided plant protection and increased growth parameters of eggplant and its fruits under greenhouse conditions.

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