Research Article

Evaluation of the Effective Microorganisms® (EM-1™) and Biocontrol Fungi *Trichoderma* against Pathogenic Fungi (*Rhizoctonia solani* and *Fusarium solani*) Isolated from Pepper Roots

Baidaa R. Jasim and Ahed A.H. Matloob*

Biological Control Department, Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University, 51006 Al-Mussaib, Babylon, Iraq.

Abstract | This *in-vitro* study aimed to isolate the pathogenic fungi associated with the pepper root rot disease and to evaluate the antagonistic potential of the effective microorganisms preparation (EM-1™) and *Trichoderma* fungal strains against these pathogenic fungal isolates under laboratory conditions. Initial field surveys showed the widespread incidence of the pepper root rot disease in all areas of the Babylon Governorate, Iraq. Isolation and diagnosis studies showed different pathogenic fungal species associated with the infected pepper roots. The most dominant pathogenic fungi included *Fusarium solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. All isolates of *R. solani* and *F. solani* caused a significant reduction in pepper seed germination. Further bioassays regarding the bioefficacy of effective microorganisms and *Trichoderma* fungal strains showed that these biocontrol agents caused a significant inhibition of both pathogenic fungi. Maximum inhibition (100%) of the pathogenetic fungi was exhibited by 10 and 15% concentrations of EM-1™ as compared to control treatments. The results showed that the inhibition percentage increases as the concentration of the EM-1 preparation increases compared to the comparison treatment. Similarly, *Trichoderma* species including *T. viride*, *T. reesei*, *T. harzianum*, and *T. koningiopsis*, exhibited high antagonistic ability against both pathogenic fungi *F. solani* and *R. solani*, suggesting the implication of these biocontrol microbes combating pepper root rot and other diseases caused by *F. solani* and *R. solani*.

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***Correspondence** | Ahed A.H. Matloob, Biological Control Department, Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University, 51006 Al-Mussaib, Babylon, Iraq. **Email:** com.ahd1@atu.edu.iq
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Introduction

Pepper, *Capsicum annuum* L., is a widely cultivated solanaceous vegetable, and is mainly used as a spice, vegetable, and for other purposes. Peppers contain large amounts of vitamin C, many times higher than citrus fruits, and contain many nutrients and vitamins (Lu and Cui, 2019; Parisi *et al*., 2020; Papathanasiou *et al*., 2021). It is grown in Iraq in early autumn in protected agriculture facilities and in early spring in open cultivation. The area under pepper cultivation in general reached about 2,119 hectares in 2020, and the total production was estimated to be about 46,498 tons (Central Statistical Organization of Iraq, 2021).

There has been a decline in the pepper per unit area production due to several reasons, the most important of which is the infection of pepper roots by many pathogenic fungi, including *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium solani*, and *Pythium* spp. (Hyder *et al*., 2020; Matloob and Al-Baldawy, 2020; Nikitin *et al*., 2023). Wide use of synthetic fungicides against pepper diseases has created many ecological consequences, including the development of pathogenic resistance against fungicides, effect on non-target organism, including beneficial microbes, and environmental contaminations (Bozdogan, 2014; Jorge-Escudero *et al*., 2022; McLaughlin *et al*., 2023; Islam *et al*., 2024; Wan *et al*., 2025).

This has encouraged those working in the field of agricultural pest control to find alternative methods to harmful pesticides, including the use of biological control agents such as the use of beneficial microorganisms, such as rhizobacteria plant growth-promoting (PGPR) bacteria such as *Azotobacter* spp. and the fungus *Trichoderma* spp. (Al-Daghari, 2020; Wang and Ji, 2021; Kredics *et al*., 2024). The fungus *Trichoderma* also has multiple antagonistic capabilities against different plant pathogens, in addition to its importance in improving the plant production and growth and inducing systemic resistance in plants against diseases (Harman and Uphoff, 2019; Matloob, 2019; Abdullahi *et al*., 2021; Guzmán-Guzmán *et al*., 2023).

In addition, the effective microorganism technology® (EM-1™) has also been demonstrated to improve the growth and production of pepper plants under salt stress conditions, and to increase the efficiency of photosynthesis, as well as facilitate the absorption of many nutrients (Abd El-Mageed *et al*., 2020; Liriano González *et al*., 2021; Abdelkhalik *et al*., 2025). Due to prevailing incidence and damage inferred by pepper root rot disease and because of the ecological consequences of synthetic fungicides, this study aimed to determine the pathogenic diversity associated with the infected pepper roots on one hand, and to evaluate, on the other hand, the effectiveness of some environment-friendly disease management strategies *i.e.* the effective microorganisms (EM-1) and different species of biocontrol fungus *Trichoderma* against the pathogenic fungal isolates under laboratory conditions.

Materials and Methods

Field survey

A field survey was conducted in 11 sites in the Babylon Governorate during the period from 3/22 to 6/30 during the agricultural season 2023-2024. Random samples were collected from the fields and the infection rate was calculated according to the following equation:

$$Disease\ incidence\ (\%) = \left(\frac{numer\ of\ infected\ pepper\ seedlings}{Total\ number\ of\ seedlings\ sampled}\right) \times 100$$

The percentage of infection severity for the root system was calculated according to the 6-degree pathological index as follows:

0 = healthy plant, 1 = more than 0-25% of the roots are infected, 2 = more than 25-50% of the roots are infected, 3 = more than 50-75% of the roots are infected, 4 = more than 75-100% of the roots are infected.

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

$$Disease\ severity\ (\%) = \left(\frac{(plants\ in\ 1\ degree\ \times 1 + \cdots plants\ in 5\ degree\ \times 5)}{All\ plants\ sampled\ \times 5}\right) \times 100$$

Isolation and diagnosis of pathogenic fungi associated with pepper plant roots

The isolation process was carried out from the pepper plant samples that showed symptoms (represented by root rot, poor plant growth, and yellowing and wilting of leaves). In brief, sterile pieces of infected roots were transferred in the form of 4 pieces in each glass Petri plate (9 cm in diameter) containing autoclaved sterile potato dextrose agar (PDA) medium, and the plates were incubated in an incubator at a temperature of 25±1 °C for three days. The fungi were purified and identified at the genus and species level using taxonomic criteria based on the characteristics of the fungal colonies and the nature of the fungal mycelium, as well as the spores and structures they formed (Ellis, 1971; Booth, 1977; Summerell and Lesile, 2006). The percentage of the fungi that appeared was calculated according to the following equation:

$$\text{Disease severity }(\%) = \left(\frac{\text{number of root pieces in plate on which fungi appeared}}{\text{total number of root pieces used for each sample}}\right) \times 100$$

Detection of pathogenic fungal isolates

The pathogenicity of the 8 isolates of the fungus *F. solani*, 8 isolates of the fungus *R. solani*, and 5 isolates of the fungus *M. phaseolina* was tested according to the method of Bolkan and Butler (1974). After PDA solidification, the Petri plates were inoculated from the fungal strains with a 0.5 cm disk taken from the edges of the fungal colony of *R. solani*, *F. solani*, and *M. phaseolina*. Then, the Petri plates were incubated in an incubator at a temperature of 25±1°C for three days. The seeds of the cabbage were planted after sterilization with 1.0% chlorine-free sodium hypochlorite solution in a circular manner near the edge of the Petri plate at a rate of 10 seeds per plate. The plates were then again incubated in the incubator at a temperature of 25±1°C, and after seven days, the data was recorded regarding the germination of cabbage seeds, and the percentage of seed germination was calculated.

Preparation of R. solani and F. solani inoculum

Local millet seeds were used to prepare the fungal inoculums according to the method of Dewan (1989). Millet seeds were washed with water to remove the impurities and dust, soaked in water for six hours, and then left on a piece of gauze for 30 minutes to remove the excess water. About 50 g of seeds were placed in a 250 ml glass flask and were sterilized for half an hour in an autoclave. After 24 h, these were sterilized again and were left to cool. The glass flasks were then inoculated by placing 5 discs (0.5 cm in diameter) of the PDA medium containing *R. solani* and *F. solani* fungi, each separately. Then, the flasks were incubated at a temperature of 25 ± 1°C for 14 days, stirring every two days for ventilation and distributing the fungal inoculum to all millet seeds.

Effect of F. solani and R. solani fungal isolates on pepper seed germination

This experiment was conducted at the Al-Mussaib Technical College of the Al-Furat Al-Awsat Technical University. Local millet seeds inoculated with the fungal isolates of *F. solani* (2-Fs, 4-Fs, and -6 Fs) and *R. solani* (1-Rs, Rs-3, and Rs-5) were added to the sterilized soil mix, and were distributed in 1.0 kg plastic pots at a rate of 1.0% (weight/weight). Ten pepper seeds were planted in each pot, superficially sterilized with 1.0% sodium hypochlorite solution. Each treatment was repeated 3 times, and 3 replicates were left without adding the pathogenic fungus as a comparison. The pot was watered. The percentage of germination was calculated after the seeds of the comparison treatment were fully germinated.

Testing antagonistic ability of EM-1 against R. solani and F. solani

The inactive EM-1 solution was activated mainly by mixing it with molasses and warm sterile chlorine-free water at a ratio of 5 ml of inactive EM-1 solution, 5 ml of molasses and 90 ml of sterile chlorine-free water, and was placed in a closed plastic container, protected from light, in a warm place at a temperature of 35–40 °C (Kazem, 2022). During this period, the package was opened several times to discharge gas, and a layer of sediment was formed at the bottom of the package, as indicated by Di Turo and Hurtak (1998). Then, 5, 10, and 15% of the activated EM-1 solution was added to 100 ml glass flasks, the volume was completed to make 100 ml of PDA medium. Each concentration was separated. Then the mixture was poured into a Petri plate, and after the medium solidified, it was inoculated with 0.5 cm diameter disc of *R. solani* and *F. solani* isolates with 3 replicates for each isolate, compared to the control treatment without any EM-1 added. The Petri plates were placed in an incubator at 25 ± 1 °C for 7 days, after which the colony diameter and the percentage of fungal growth inhibition were measured according to the following equation:

$$\text{Growith inhibition }(\%) = \left(\frac{R - r}{R}\right) \times 100$$

Where, r = the radius (mm) of the fungal colony against the bio-agents, R = the radius (mm) of the fungal colony without the bioagents.

Evaluation of antagonistic ability of Trichoderma species against F. solani and R. solani

The antagonistic ability of four species of *Trichoderma* fungus (*T. viride.*, *T. reesei*, *T. harzianum*, and *T. koningiopsis* obtained from the University of Karbala, College of Agriculture) was testedagainst pathogenic fungi *F. solani* and *R. solani*. This test was based on the double culture method. The sterile PDA medium was prepared in an autoclave at a temperature of 121°C and a pressure of 15 psi, poured into sterile glass Petri plates (diameter of 9 cm), and divided into two equal parts. The first part of the plate was inoculated with each pathogenic fungus separately, where a 7-day-old 0.5 cm diameter disk was taken from the fungus culture, while the other part of the dish was inoculated with a 7-day-old 0.5 cm diameter disk from *Trichoderma* spp. culture. The experiment was carried out with three replicates for each treatment. Petri plates were placed in an incubator at a temperature of 25 ± 1°C for one week, and the antagonistic potential was estimated according to the scale of Bell *et al*. (1982). A biological control agent is considered antagonistically effective when it showed an antagonism score of 2 or less with the pathogenic fungal isolate under study. The colony diameter and the percentage of inhibition were also measured according to the equation used in the previous paragraph.

Results and Discussion

Field survey of pepper root rot disease

The results of the field survey, as shown in Table 1, showed the spread of pepper root rot disease in all areas covered during the field survey in Babylon Governorate, Iraq with the percentages ranging between 33–100 %. The highest percentage of infection was recorded for the Imam, Al-Sabbaghiya, Al-Nil and Sinjar districts, which reached 100%, followed by Al-Haidari, Al-Mahawil and Al-Himyari districts, which reached 87, 80 and 70% disease incidence, respectively. The lowest percentage of infection was found in the Muwailha and Al-Azzawiya districts, which reached up to 33 and 40%, respectively. The reason is due to the repeated cultivation of pepper crops annually in these fields or the cultivation of crops belonging to the Solanaceae family, which lead to the accumulation of pathogen inoculum and its long-term survival in the soil (Vadakattu and Patterson, 2005; EL-Mougy, 2011) as found in Brazil (Felix *et al*., 2012). The other reason may be due to the widespread use of synthetic fungicides, which led to the development of resistance in many pathogens, in addition to the weeding and hoeing process, which causes scratching of plant wounds, thus facilitating the invasion of pathogenic fungi (Cheng *et al*., 2020; Naqvi *et al*., 2025). The highest infection severity was found in the Sinjar region, which reached 72%, and the lowest infection severity was recorded for the Al-Azzawiya region, which reached 20%. This might be due to the cultivation of pepper crop for the first time in the field, which reduces humidity, which is an important factor in the occurrence of the disease. These results are consistent with those of Shanwer (2021) and Alanzi (2024) regarding the spread of pepper root rot disease in the Babylon Governorate, Iraq.

Table 1: *Incidence and severity of root rot disease in pepper (Capsicum annuum) crops recorded during 2020-2024 in Babylon Governorate, Iraq.*

Sr. No.	Sampling site	Diseases incidence (%)	Disease severity (%)
1	Imam	100	50
2	Al-Sabbaghiya	100	68
3	Al-Mahawil	80	24
4	Al-Dulaimi	100	56
5	Al-Himyari	70	63
6	Al-Nil	55	42
7	Muwailiha	33	26
8	Sinjar	100	72
9	Al-sayahi	57	28
10	Al-Haidari	87	47
11	Al-Azzawiya	40	20

Isolation and diagnosis of fungi associated with infected pepper roots

The results presented in Table 2 regarding the isolation and diagnosis of several types of fungi associated with the roots of pepper plants infected with root rot disease showed the symptoms of disease represented by rotting of the entire root or part of it and by its brown color with wilting and yellowing of the plant leaves. The most frequently occurring pathogenic fungi were *F. solani* with 56.12–100 % incidence rate, followed by *R. solani*, also isolated from 8 areas with an incidence rate of 45.5% and the highest incidence rate was 80% (Figure 1), followed by *M. phaseolina*, isolated from 5 areas with an incidence rate of 25.2%. These results are in agreement with what Al-Ghanimi (2023) found: that the fungi *F. solani and R. solani* are the most important causes of pepper root rot. These results are also in line with many previous studies showing the presence of these pathogenic fungi and their spread on various crops (Al-Mammory, 2019; Al-Baldawy *et al*., 2021; Al-Janabi, 2022; Satar, 2022). The study results also showed the appearance of many other fungi accompanying the roots of pepper plants at lower rates of recurrence, such as the fungus *Trichoderma* spp., *Aspergillus niger, Penicillium* spp., *Mucor* spp., *Chaetomium globosum*, and *Alternaria alternate*.

Table 2: *Percent incidence of fungal diversity associated with the roots of pepper plants infected with root rot disease.*

Highest presence rate (%)	Rate (%)	Sinjar	Al-Sayahi	Al-Haidari	Muwailiha	Al-Nil	Al-Dulaimi	Al-Azzawiya	Al-Himyari	Al-Mahawil	Al-Sabbaghiya	Imam	Names of fungi
100	56.12	-	-	-	50	38	50	100	68	58	40	45	Fusarium solani
80	45.5	36	43	40	25	48	56	36	80	-	-	-	Rhizoctonia solani
32	25.2	-	-	-	-	23	22	25	24	32	-	-	Macrophomina phaseolina
14	14.00	-	-	-	-	-	-	14	-	-	-	-	*Trichoderm*a
38	24.2	38	17	-	-	-	22	8	-	-	25	36	*Aspergillus nige*r
33	21.1	-	16	-	-	-	33	15	26	14	-	23	*Penicillium* spp.
25	2. 18	14	16	-	-	-	-	12	25	24	-	-	*Mucor* spp.
14	14.00	-	-	-	14	-	-	-	-	14	-	-	*Chaetomium globosu*m
53	31.5	-	-	-	-	53	-	-	-	-	-	10	*Alternaria alternat*a

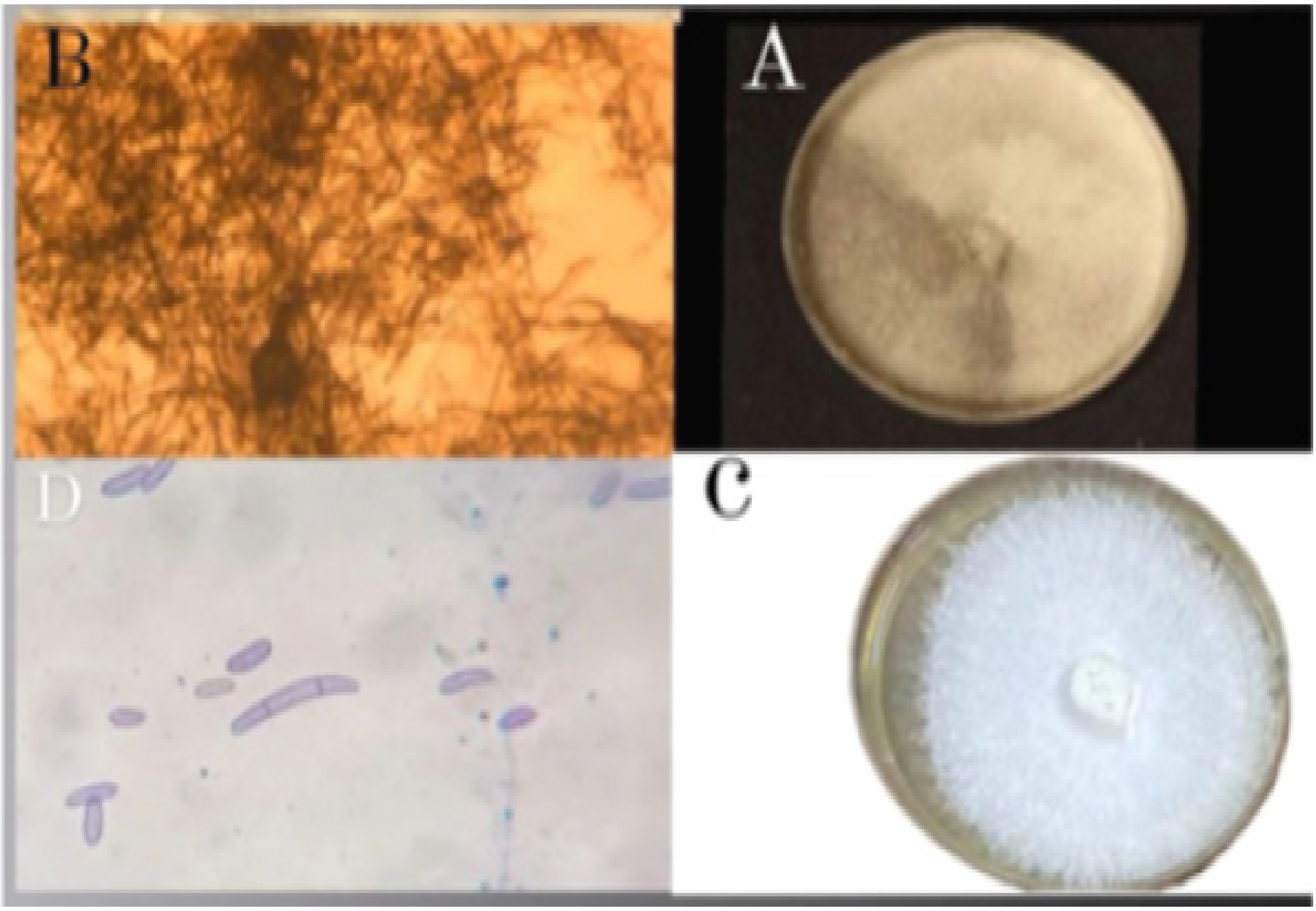

Figure 1: *Morphological and microscopic diagnostic characteristics of some pathogenic fungi causing pepper root rot disease. Photos from 7-day-old fungal culture. A = the fungus Rhizoctonia solani culture on PDA medium, B = sclerotia of R. solani, C = the fungus Fusarium solani culture on PDA medium, D = the large and small conidia of F. solani.*

The effect of fungal isolates against cabbage seed germination

All the tested *R. solani* and *F. solani* isolates exhibited a significant reduction in the seed germination percentage, compared to the comparison treatment in which the seed germination percentage reached 100%, as the *R. solani* isolate-1 (Al-Hamri isolate) outperformed the rest of the isolates in reducing the germination percentage, as the germination percentage rate reached 0.00, followed by *R. solani*-3 (Al-Dulaimi

Table 3: *Pathogenicity tests of pepper root rot-associated fungal isolates on the cabbage seeds.*

Sr. No.	Sample Sites	Pathogenic fungal isolates	Number of seeds germinated	Seed Germination (%)
1	-	control	10.00	100.00
2	Al-Himyari	Rs1	0.00	00.00
3	Al-Azzawiya	Rs2	4.33	43.33
4	Al-Nil	Rs3	1.00	10.00
5	Al-Nil	Rs4	3.00	30.00
6	Muwailiha	Rs5	1.33	13.33
7	Al-Haidari	Rs6	4.00	40.00
8	Al-sayahi	Rs7	3.00	30.00
9	Sinjar	Rs8	2.66	26.67
10	Imam	Fs1	2.66	26.66
11	Sabbaghiya	Fs2	0.00	0.00
12	Al-Mahawil	Fs3	3.33	33.33
13	Al-Himyari	Fs4	1.00	10.00
14	Al-Azzawiya	Fs5	4.33	43.33
15	Al-Dulaimi	Fs6	1.33	13.33
16	Al-Nil	Fs7	5.00	50.00
17	Muwailiha	Fs8	2.66	26.67
18	Al-Mahawil	Mp1	4.33	43.33
19	Al-Himyari	Mp2	3.33	33.33
20	Al-Azzawiya	Mp3	2.33	23.33
21	Al-Dulaimi	Mp4	4.33	43.33
LSD (0.05)		-	1.2811	12.811

Each number in the table represents the average of three replicates. Fs = Fusarium solani, Rs = Rhizoctonia solani, Mp = Macrophomina phaseolina.

isolate) that had a germination percentage of 10.00, while the germination percentage of the remaining isolates ranged between 13.33–43.33% (Figure 2). The results also indicated that all tested *F. solani* isolates caused a significant reduction in the germination percentage of cabbage seeds as compared to the control treatment, in which the germination of cabbage seeds reached 100%. The isolates varied among themselves in reducing the germination percentage, as the isolate *F. solani*-2 (Al-Sabaghiya isolate) excelled, as the seed germination percentage reached 0.00, followed by F. solani-4 (Al-Hamri isolate), as the germination percentage reached 10.00, and the germination percentage of the remaining isolates ranged between (13.33–50.00%).

These results are consistent with the findings of Matloob *et al.* (2019) that these pathogenic fungi are among the main causes of root rot in many crops (El-Kazzaz et al., 2022). For the root rot disease of the tested plants, it caused a significant reduction in the germination rate of cabbage seeds in PDA culture medium as compared to the control treatment. This is attributed to the secretion of secondary toxic metabolites by fungi that led to the death of embryos, in addition to the production of many decomposing enzymes responsible for seed rot, and thus preventing them from germinating (Coleman *et al.*, 2016; Ben Salem *et al.*, 2019). From the results of this test, the isolates that caused a maximum reduction of cabbage seed germination were selected, namely isolate-1 *R. solani*, *R. solani*-3, *F. solani*-2, and *F. solani*-4, to conduct the subsequent experiments. Moreover, the isolates of *M. phaseolina* did not cause a significant reduction in the germination of cabbage seeds.

The pathogenicity test of F. solani and R. solani against the germination of pepper seeds

The results in Table 4 indicated that all the tested isolates of the fungi *F. solani* and *R. solani* caused a significant reduction in the percentage of germination of pepper seeds, as the isolate *R. solani*-1 achieved

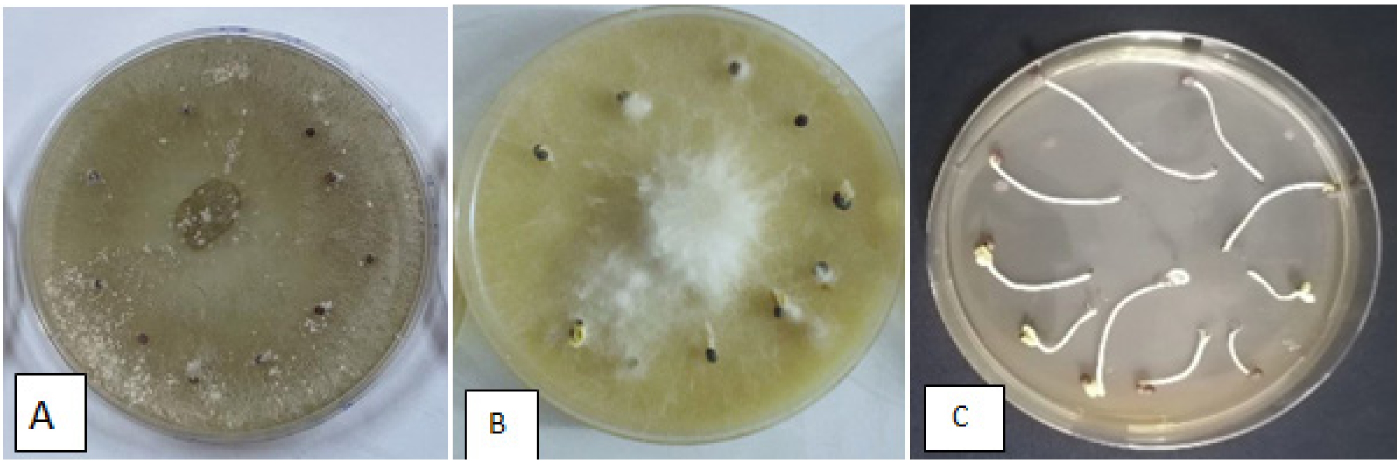

Figure 2: *The pathogenicity or inhibition potential of pepper root rot-associated fungal isolates on the cabbage seeds. A = germination inhibition by the fungus Rhizoctonia solani, B = germination inhibition by the fungus Fusarium solani, C =control.*

the highest percentage of reduction in the percentage of germination, which reached 0.00%, and the percentage of germination for the rest of the isolates ranged (16.7-46.7) with a significant difference with the comparison treatment without adding the pathogenic fungus, in which the percentage of germination was 100%. Moreover, fungus *F. solani*-2 caused a significant reduction in the percentage of seed germination, which reached 0.00%, and the remaining isolates ranged between 23.3–26.7%.

Table 4: *The effect of pepper root rot-associated pathogenic fungal isolates on the germination of pepper seeds.*

Sr. No.	Isolate	No. of seeds germinated	Seed germination (%)
1	Control	10	100
2	*F. solani*-2	0.00	0.00
3	*F. solani*-4	2.67	26.7
4	*F. solani*-6	2.33	23.3
5	*R. solani*-1	0.00	0.00
6	R. solani-3	4.67	46.7
7	*R. solani* -5	1.67	16.7
LSD (0.05)		1.011	10.11

Effect of Effective Microorganisms (EM-1™) on growth of R. solani and F. solani

The results of this bioassay showed the effectiveness of the EM-1 microorganisms in inhibiting the growth of pathogenic fungi *R. solani* and *F. solani* on PDA medium. The highest effect of the EM-1 microorganism in inhibiting pathogenic fungi was shown at concentrations of 10 and 15%, where the inhibition rate reached 100% compared to the comparison treatment of pathogenic fungi only, where the inhibition rate reached 0.00%, while the concentration of 5% exhibited a significant reduction of 94.07 % and the average diameter of the *F. solani* fungus colony reached 0.53, while the *R. solani* fungus isolate had a colony growth diameter of 0.63 mm at a concentration of 5% and the percentage of inhibition reached 92.96% The results showed that the percentage of inhibition increased when the concentration of the EM-1 microorganisms increased as compared to the comparison treatment (Figure 3). These results are consistent with Almumin (2024) demonstrating that the EM-1 microorganisms' preparation has a significant role in inhibiting the fungus *R. solani*, as the percentage of inhibition reached 100% at concentrations of 10 and 15%.

Table 5: *Inhibition potential of the effective microorganisms (EM-1) on the growth of pathogenic fungi Rhizoctonia solani and R. solani on PDA medium.*

Treatments	Concentration (%)	Colony diameter (cm)	Growth inhibition (%)
Control	-	9.00	0.00
EM-1+Fs-2	5	0.53	94.07
	10	0.00	100.00
	15	0.00	100.00
Control	-	9.00	0.00
EM-1+Rs-1	5	0.63	92.96
	10	0.00	100.00
	15	0.00	100.00
LSD (0.05)		0.04997	0.5546

Each number in the table represents the average of three replicates. Rs = Rhizoctonia solani, Fs = Fusarium solani.

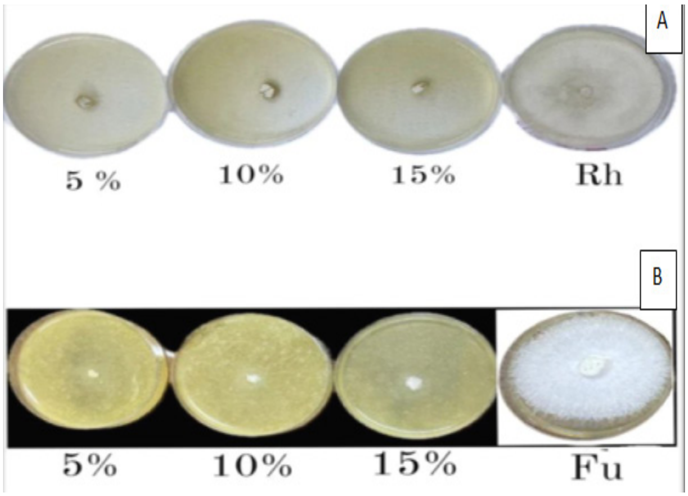

Figure 3: *Effect of the effective microorganisms (EM-1) on the growth of pathogenic fungi assessed on PDA media. A = Rhizoctonia solani, B = Fusarium solani.*

Table 6: *Inhibition potential of the Trichoderma fungi on the growth of pathogenic fungi Rhizoctonia solani and R. solani on PDA medium.*

Treatment	Colony diameter.cm	Inhibition (%)
Control	9.0	0.00
T. reesei + F. solani-2	0.76	91.33
T. reesei + R. solani-1	0.63	92.66
T. viride + F. solani-2	0.16	97.33
T. viride + R. solani-1	0.13	97.66
T. koningiopsis + F. solani-2	0.56	93.33
T. koningiopsis +R. solani-1	0.73	91.66
T. harzianum + F. solani-2	0.23	96.66
T. harzianum + R. solani-1	0.26	96.33
LSD (0.05)	0.1065	1.059

Each number in the table represents the average of three replicates.

The antagonistic ability of Trichoderma fungal strains against F. solani and R. solani

The fungal species of the *Trichoderma* genus exhibited a high antagonistic ability against the pathogenic fungi *R. solani* and *R. solani* under laboratory conditions (Table 6). The highest antagonistic ability was showed by *T. viride*. The treatments *T. viride* + *F. solani*-2 and *T. viride* + *R. solani*-1 had the highest antagonistic capacity against both *F. solani* and *R. solani*. The percentage of inhibition reached 97.33 and 97.66%, respectively, and the diameter of the fungal colony reached 0.16 and 0.13%, respectively. According to the Bell *et al.* (1982) scale, the isolates of the fungus *T. viride* were the most effective against the fungi *F. solani* and *R. solani*. These results are also consistent with what Al-Jubouri (2024) indicated regarding the role of *T. viride* in inhibiting *Fusarium solani* on bean plants. Then, the rest of the *Trichoderma* species varied in their antagonistic ability. The reason for this is attributed to the fact that the fungus *Trichoderma* can inhibit the growth of plant pathogenic fungi using various mechanisms such as mycoparasitism, production of antifungal substances, and competition for food and space (Verma *et al.*, 2007; Kaewchai, 2009; Yao *et al.*, 2023).

These results also corroborate the findings of many studies showing the inhibitory effect of *T. viride* on many phytopathogenic plants (Rao *et al.*, 2022). The fungus *Trichoderma* spp. produces many secondary metabolites, including antibiotics such as trichodermine, gliotoxin, and virindin, which have proven antifungal activity against *Trichoderma* to inhibit many pathogens that spread through the soil (Kareem and Al-Araji, 2017; Inovejas nd Divina, 2018; Mukherjee *et al.*, 2023). Modrzewska *et al.* (2022) also demonstrated the efficiency of *Trichoderma* spp. fungi in inhibiting the growth of pathogenic fungi, including *Fusarium* spp.

The isolate *T. harzianum* + *F. solani*-2 exhibited a 96.66% inhibition and a diameter of 0.23 mm of the fungal colony, followed by the isolate *T. harzianum* + *R. solani*-1 with a 96.33% inhibition and 0.26 mm colony diameter (Figure 4). These results corroborate that the fungus *Trichoderma* spp. has anti-pathogenic potential (Inovejas nd Divina, 2018; Tyśkiewicz *et al.*, 2022).

Conclusions and Recommendations

Based on the overall study results, it is concluded that pepper root rot disease is one of the most prevailing plant diseases in Babylon Governorate, Iraq. *Fusarium solani* and *Rhizoctonia solani* are the major pathogenic fungi causing this disease. The effectiveness microorganisms EM-1™ preparation had a considerable inhibition potential against pathogenic fungi as compared to the control treatment. Moreover, it is concluded that the application of different types of biological agents, such as *Trichoderma* fungal species, including *T. viride*, *T. reesei*, *T. harzianum*, and *T. koningiopsis*, had a high antagonistic ability against both pathogenic fungi *F. solani* and *R. solani*. Future perspectives of this study include the field demonstration of the effectiveness of these biological agents in controlling

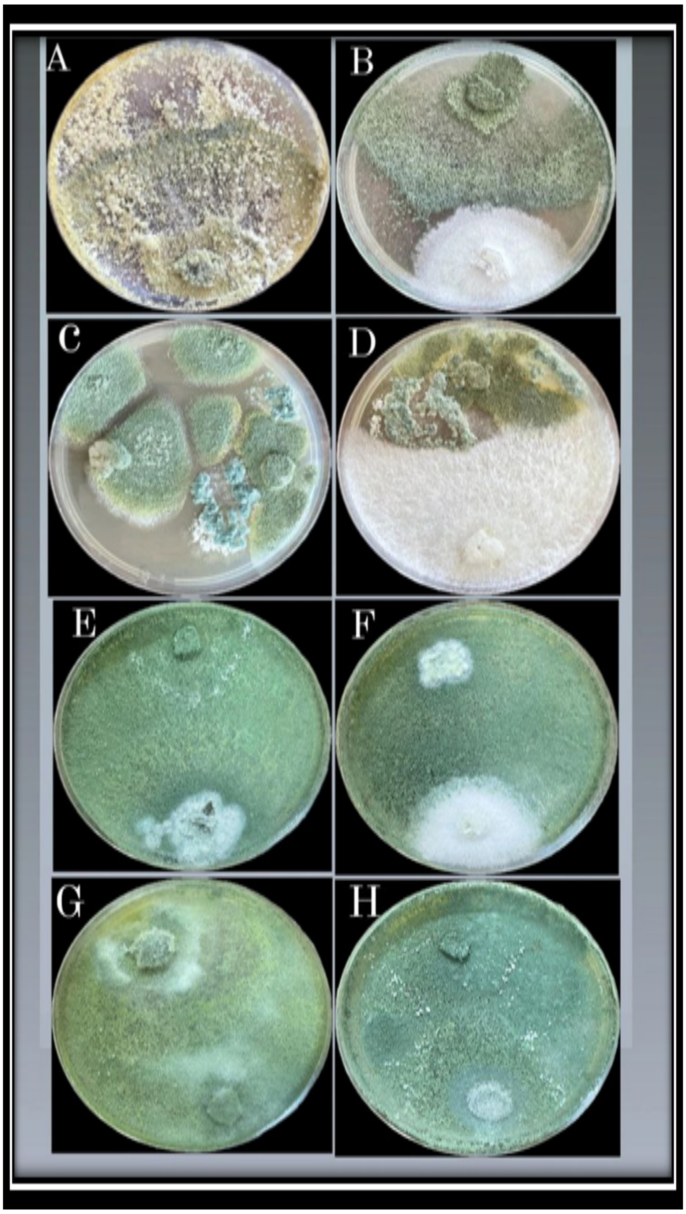

Figure 4: *The antagonistic ability of Trichoderma fungal species against the growth of pepper root rot-associated fungal isolates on PDA media. A = T. koningiopsis + R. slonai-1, B = T. koningiopsis + F. solani-2, C = T. reesei + R. solani-1, D = T. reesei + F. solani-2, E = T. harzianum + R. solani-1, F = T. harzianum + F. solani-2, G = T. viride + R. solani-1, H = T. viride + F. solani-2.*

plant pathogens and their role in finding safe natural biological alternatives and reducing the use of harmful chemical pesticides in the agricultural field.

Novelty Statement

Effective microorganisms and Trichoderma can improve plants' ability to prevent and control diseases. With the focus of Trichoderma as a biocontrol fungus as the future basis of plant immunity against Pathogenic Fungi (*Rhizoctonia solani* and *Fusarium solani*).

Author's Contribution

Baidaa R. Jasim: Writing and editing.
Ahed A.H. Matloob: Data curation, writing – original draft preparation, writing and editing.
Both authors read and agreed to the published version of the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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