Evaluation of the efficiency of the Azotobactar chroococcum and Penicillium cyclopium and some plant extracts in controlling pepper root rot disease under laboratory and lath house conditions

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Abstract

The study aimed to survey pepper root rot disease in some fields of Babylon province, isolate and diagnose the fungi that cause the disease, and evaluate the efficiency of some biological control agents against pathogens in the laboratory and under lath house conditions. The results showed the presence of the disease in all areas covered by the survey, with infection rates ranging between 20-60%. The fungi, Fusarium solani and Macrophamina phaseolina, was causing agent of disease. The results showed that Penicillium cyclopium and Azotobacter chrocooccum bacteria had a high antagonistic ability against the pathogenic fungi F.solani and M.phaseolina. The results of the antagonism of the aqueous extracts of plants used in the study (Cariza, Schanginia, wild mustard, and Cinnamon) in the concentration (5,10,15%) against the pathogenic fungi. The results of the wooden canopy experiment showed that the interaction treatment of A. chrocooccum and P. cyclopium and the extract of the cinnamon reduction the disease incidence and severity of infection with the pathogenic fungi F.solani and M.phaseolina, where the disease incidence and severity reached 23.33-21.67% and 33.33 % respectively, compared to treatment with pathogenic fungi alone The disease incidence 100% and severity 81.67-83.33%. All treatments achieved a significant increase in the average of height, wet and dry weights and the percentage of seed germination in the woody canopy. The interaction treatment with A.chrocooccum bacteria and the P.cyclopium and the extract of cinnamon enhancement growth parameters compared to the control treatment.

Key Words: Pepper, Root rot, Cinnamon, Biological control, Pathogenic fungi

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Introduction

The pepper plant Capsicum annum L. belongs to the Solanaceae family and is considered one of the important vegetable plants in many countries of the world, and in economic terms it is the third crop after tomato and potato (Tewari, 2001 and Ebert, 2020).Pepper is cultivated in Iraq by the method of protected cultivation at the beginning of autumn and open cultivation at the beginning of spring. The cultivated area of pepper in Iraq for the year 2019 was about 13052 dunums, and the total production reached 23,112 tons (Central Statistics Organization, 2019).The pepper plant is infected with many pathogens, which leads to great economic losses (Manisha, 2018 and Yin, 2020), and one of the fungi that causes root rot diseases of the pepper crop is Phytophthora spp. and Rhizoctonia solani as well as the fungus Fusarium spp. and Macrophomina phuseolina.This fungus infects the pepper plant during its different growth stages as well as infecting seeds and seedlings in the soil before and after emergence (Musa et al., 2020 and Matloob and Al-Baldawy, 2020).

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Many workers in the field of plant diseases were interested in thinking about finding other means of control and avoiding chemical pesticides and the use of biological control agents through the use of non-pathogenic microorganisms for plants to discourage pathogenic organisms
in the cultivated soil without affecting the rest of the groups of microorganisms (Scott et al. , 2020 and Wang and Ji, 2021), Among those microorganisms used in biological control are Plant Growth Promoting Rhizobacteria (PGPR) such as Azotobacter spp. and Azospirillum and Bacillus spp.Also, Pseudomonas spp. and Rhizobium, as well as some fungi such as Penicillium, Aspergillus and spp. Trichoderma, and the use of powders and plant extracts for parts of some plants and their addition to the soil has attracted the attention of many researchers and achieved effectiveness in reducing the pathogen vaccine and reducing the severity of the disease to a number of pathogens found in fungal soils, such as Fusarium solani, Rhizoctonia solani, Phytophthora cinnumoni, as well as Fusarium oxysporium and Fusarium moniliforme (Pang et al., 2021), The decomposition of plant parts added to the soil releases compounds that inhibit plant pathogens and increase the effectiveness of microorganisms that have the inhibitory ability of these pathogens, which increases the control process well with the biological control factor (Elshahawy et al., 2021).Based on the foregoing and the importance of pepper root rot disease and the losses caused by and to try to combat it biologically, this study came to aim at: Isolating and diagnosing the fungi that cause pepper root rot disease in Babylon province and assessing the efficiency of the bacteria Azotobactar chroococcum Penicillium cyclopium and some plant extracts in controlling pepper root rot disease under lath house laboratory conditions

3- Materials and methods:

1-3 Field Survey:

A field survey was conducted for eleven locations of pepper cultivation fields for the period from 5/10 to 27/9/2020 (Table, 1) The infected and healthy plants were randomly tested and located within the intersection of the diameters for each site and the number of infected plants was calculated based on the symptoms appearing on the plants and the percentage of infection was extracted for each field using the following equation:

percentage of infection%$=\frac{The\ number\ of\ infected\ plants}{The\ total\ number\ of\ plants\ tested}\times 100$

Infected plants were taken to the laboratory after being placed in (polyethylene) bags. The samples were marked and stored in the refrigerator at a temperature of 4°C for isolation from each site on the day following collection.

Table (1) temporal and spatial distribution of pepper fields and cultivation locations covered by the field survey.

Cultivated area (Dunam)	Sample collection date 2020	Location	Sampel number
½	5/10	Muwailiha	1
1	5/15	Alhuswa	2
1	5/30	Alwatfia	3
½	7/11	Al-Azzawiyah	4
5	7/18	Al Muhaweel	5
1	7/29	Albadea	6
1	8/18	Jelawiyah	7
1	9/1	Abu Al-Jassem	8
1	9/11	Aljafjafa	9
¼	9/15	Hilla/Sardib	10
1	9/27	Hilla/Tahmaziyah	11

2-3 Isolation and diagnosis:

The process of isolation from each sample of the affected pepper plants was carried out on the day following the scanning process. The roots of the affected plants were washed with running water for one hour to remove the soil from the soil and the roots were cut into small pieces of 0.5 cm length.It was superficially sterilized by immersing it in sodium hypochlorite solution (1% free chlorine) for two minutes, then washed with sterile distilled water for 2-3 minutes, then the free water was removed from it with sterile filter paper and then the pieces were transferred by sterile forceps to Petri dishes with a diameter of 9 cm containing the culture medium with more dextrose. Potato Dixtrose Agar. Tetracycline antibiotic was added at a concentration of 250 mg / liter after sterilization of the medium with an autoclave device at a temperature of 121 ° C and a pressure of 1 atmosphere for 20 minutes. 4 pieces were used for each plate. The dishes were left in the incubator at a temperature of 1 ± 25 ° C for 3 days. The different fungi were purified and tested under the micro-

powers of the compound microscope, and the genera and species were identified according to the approved taxonomic keys (Parmeter and Whitney, 1970 and Ellis, 1971, Booth, 1971 and 1977, and Summerell and Lesile (2006). The appearance rate of the studied fungi was calculated according to the following equation:

The percentage of the presence of fungi=

$$\frac{\textit{The number of root pieces in the dishes in which the fungus appeared}}{\textit{The total number of cut the roots used for each sample}} \times 100$$

3-5 Pathological ability test:

Detection of pathogenic isolates of M. phaseolina and F. solani using radish seeds The pathogenicity of 11 isolates of Fusarium solani was tested and 6 isolates of the fungus Macrophomina phaseo (Table 2) according to the method of Bolkan and Butler (1974), the results were taken after 7 days by calculating the percentage of germination according to the following equation:

germination percentage=$\frac{\textit{The number of germinated seeds}}{\textit{The number of seeds sown}} \times 10$

Table (2) Isolates of Fusarium solani and Macrophomina. phaseolina, whose pathogenicity was tested using radish seeds.

Isolates symbol	Isolates symbol	Location	No
Mp_1	Fs_1	Muwailiha	1
Mp_2	Fs_2	Alhuswa	2
Mp_3	Fs_3	Alwatfia	3
Mp_4	Fs_4	Al-Azzawiyah	4
Mp_5	Fs_5	Al Muhaweel	5
Mp_6	Fs_6	Albadea	6
_	Fs_7	Jelawiyah	7
_	Fs_8	Abu Al-Jassem	8
_	Fs_9	Aljafjafa	9
_	Fs_{10}	Hilla/Sardib	10
_	Fs_{11}	Hilla/Tahmaziyah	11

Fs: means F.solani , Mp :M.phaseolina

6-3 Effect of isolates of the pathogenic fungi Fusarium solani and Macrophomina phaseolina on pepper plants

This test was conducted in a greenhouse and this test was conducted according to a Completely Randomized Design (CRD) The fungal inoculum was prepared for the isolates of the two fungi F.solani, isolate No. Fs7 and M.phaseolina, isolate No. Mp5, which achieved the highest percentage of inhibition in the germination of radish seeds in previous experiments. The soil was sterilized with an autoclave and after 24 hours it was sterilized again and left for 7 days with stirring before use to expel toxic gases. Distributed in a cork, then it was contaminated with the fungus F.solani in isolate Fs7 and M.phaseolina in Mp5 isolate. The fungus pollen loaded on the local millet seeds was added. Each treatment was repeated 3 times and 3 replicates were left with the addition of sterile millet seeds only as a control .Cork was planted with Quantum pepper seeds 7 days after adding the fungal inoculum to the soil with one seed for each eye. The severity of the disease of pepper root rot caused by the pathogenic fungi F.solani and M.phaseolina was calculated after 35 days of planting the seeds using the following pathological evidence:

0 - A healthy vegetative system, the root system is white in color and healthy root hairs. 1- More than 0-25 % of the root is colored light brown and a specific number of root branches. 2- More than 25-50 % of the root is discolored in dark brown and a large number of root branches with drying of the lower leaves. 3- More than 75-50% of the root is dark brown with the lower leaves falling off. 4- Discoloration of more than 75% of the root in a dark color or the death of the plant.

The severity of the injury was calculated according to the 1923 (Mickenny) equation.

the infection severity=

$$\frac{\textit{Number of pla of category}(0*0)+\ldots\ldots+\textit{Number of plants of category}(}{\textit{The group of plants tested} * \textit{highest degree of infection}} 1000$$

7-3 Test the antagonistic ability of Azotobacter chrocooccum against the pathogenic fungi F.solani and M.phaseolina on culture media.

3-7-2 Determination of the effective concentration of Azotobacter chrocooccum, which inhibits the growth of the pathogenic fungi F.solani and M.phaseolina. A series of A.chrocooccum suspension dilutions were prepared by taking 1 ml of Nutrent Broth liquid media in which the bacteria grew by means of a medical syringe and adding to a test tube containing 9 ml sterile distilled water to transfer 1 ml of the 101-dilution to the second tube and transferring 1 ml from the second tube. to the third tube and thus the process was repeated on

the rest of the tubes to obtain a series of dilutions 106-.....101- After that, the plates containing the PDA culture medium were inoculated by taking 1 ml / dish of each dilution of the bacterial suspension and moving the dish in a capillary motion to distribute the bacterial inoculum and it was taken A disc from the edge of the fungal colony with a diameter of 0.5 cm.F.solani isolate Fs7 and M.phaseolina isolate Mp5 grown on PDA medium at 7 days old were taken separately and at an average of 3 dishes for each dilution. 3 dishes were left for each fungus for comparison without inoculation with bacteria added to it 1 ml sterile distilled water.The dishes were incubated at a temperature of 1 ± 25 ° C for 3 days, after which the amount of inhibition was calculated by calculating the diameter of the colony of growing fungus in the treatment of bacteria and comparing it with the diameter of the colony of developing fungus in the control treatment, and the percentage of inhibition was calculated according to the following equation (Clark 1965)).

2-1-8-3 Effect of some plant extracts on inhibiting isolates of fungi causing root rot disease of pepper plants on PDA culture media.

The method of Seema et al. (2011) was used to prepare the aqueous extracts. The aqueous extract of the selected plants was mixed with the dissolved PDA nutrient medium after being sterilized and cooled to 45°C. Concentrations (15, 10, 5%) of the extract were taken. The dishes were inoculated in the center with a 0.5 cm diameter disc of a fungal colony growing on the medium of PDA in the center of the dish containing one of the previously mentioned concentrations. The dishes were incubated at a temperature of 1 ± 25 °C.After the diameter of the fungal culture for the comparison treatment (without extract) reached the edge of the plate, the results were taken to calculate the average of two perpendicular diameters from the growth of each colony, as well as to calculate the percentage of inhibition as in the following equation:

$$\text{fungal growth to inhibit\%} = 1 - \left[\frac{\textit{Fungal growth in bacteria treatment}}{\textit{Fungal growth in control treatment}} \times 100\%\right.$$

plant extracts:

Four plants were selected to study their effect against the pathogenic fungi F.solani and M.phaseolina, which included Cariza, Schanginia, wild mustard, and Cinnamon (Table 3).

Table (3) Plants used in the research.

collaction area / Babylon	used part	The family	scientific name	local name
Al-Mussaib	Leaves	Lauraceae-Laurels	Cinnamomum Cassia	Cinnamon
Al-Mussaib	Seeds	Brassicaceae	Sinapis Arvensis	wild mustard
Gardens of Al-Mussaib Technical College	Leaves	Apocynaceae	Carissa Macrocarpa	Cariza
Project-Al-Mussaib	Leaves	Moraceae	Schangini tribracteata	Schanginia

2-1-8-3 Effect of some plant extracts on inhibiting isolates of fungi causing root rot disease of pepper plants on PDA culture media.

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9-3 Evaluation of the efficiency of the aqueous extract of the study plant and the biological agent in the percentage of the severity of infection with the fungi F.solani and M.phaseolina and some growth parameters of pepper samples under the conditions of the lathhouse bed.

This test was conducted in a plastic house on 12/1/2020 using cork cultivation dishes. In each eye, sterilized sandy soil was placed in the reflux. Pepper seeds are sown unbroken Quantum cultivar were planted with two seeds for each eye in the cork, which were added according to the following Treatments:

1- Fs alone.
2- Fs+ A.chrocooccum bacteria.
3- Fs+ fungus P. cyclopium.
4- Fs+ Cinnamon extract.
5- Fs+ Beltanol irragation pesticide.
6- Fs + A. chrocooccum + P. cyclopium.
7- Fs + A. chrocooccum + Cinnamon extract.
8- Fs+ P.cyclopium+ Cinnamon extract.
9- Fs + A. chrocooccum + P. cyclopium fungus + Cinnamon extract.
10 -Mp alone.
11-Mp + A.chrocooccum bacteria.
12 - Mp + fungus P.cyclopium.
13 - Mp + Cinnamon extract.
14- Mp + Beltanol Pesticide irrigation.
15 - Mp + A. chrocooccum bacteria + P. cyclopium fungus.
16 - Mp + A. chrocooccum bacteria + Cinnamon extract.
17 - Mp + P.cyclopium mushroom + Cinnamon extract.
18-Mp + A. chrocooccum + P. cyclopium + Cinnamon extract.
19- A. chrocooccum alone.
20- The fungus P.cyclopium alone.
21- The Cinnamon extract alone.
22- A. chrocooccum + P. cyclopium.
23- A. chrocooccum bacteria + Cinnamon extract .
24- P.cyclopium mushroom + Cinnamon extract .
25- A. chrocooccum bacteria + P. cyclopium + Cinnamon extract.
26- An unpolluted control.

The experiment was conducted using a completely randomized design (CRD) with 3 replications for each treatment. The vaccine of pathogenic fungi F.solani and M.phaseolani was added on local millet seeds (the fungal vaccine was prepared according to the Dewan method 1989) to all treatments that require the addition of pathogenic fungi vaccine at a average of 1% Weight / weight, as for the biological control agent P.cyclopium.It was added at a average of 1% w/w taken from a 7-day-age culture 3 days before adding the pathogenic fungi vaccine. The bacterial suspension was added 3 days before planting at a average of 5 ml/refined. As for the treatment of the aqueous Cinnamon extract , it was added at a concentration of 5 ml/eye directly after adding the pathogenic fungi vaccine. As for the control treatment, only sterilized millet seeds were added to it. The results were calculated after a month and a half from conducting the experiment, and the rate and severity of infection was estimated according to the pathological evidence mentioned in paragraph 3-5, and the fresh and dry weight and length of the vegetative and root groups of pepper plants were calculated.

4- Results and discussion.

1-4 field survey.

The results of the survey conducted in the fields of pepper plants showed the presence of pepper root rot disease in all areas covered by the survey, with percentages of infection ranging between 20-60% (Table 4). Al-Mahaweel field, the infection rate has reached 50%.The reason for the spread of the disease in this percentage may be due to the repeated cultivation of the pepper crop or to the cultivation of other crops belonging to the Solanaceae family in the same fields. Mougy et al., 2011). The results showed that the lowest rate of infection was in the Al-Haswa area, which amounted to 20%. This may be due to the fact that it is the first time that the pepper crop is planted. 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. It is known that the environmental factors of moisture and heat are Significant effect in increasing the fungus pollen as well as increasing the pathogenicity of fungi.

Table (4) Percentage of infection with root rot of pepper plants in some fields of Babylon province.

Percentage of infection%	location	No.
40	Muwailiha	1
20	Alhuswa	2
50	Alwatfia	3
60	Al-Azzawiyah	4
50	Al Muhaweel	5
60	Albadea	6
40	Jelawiyah	7
30	Abu Al-Jassem	8
40	Aljafjafa	9
40	Hilla/Sardib	10
30	Hilla/Tahmaziyah	11

2-4 Isolation and identification of fungi accompanying the roots of infected pepper plants.

Several types of fungi were isolated and identified from the roots of pepper plants infected with root rot disease, Table (5). The most frequent pathogenic fungi was Fusarium solani, which was isolated from all areas covered by the survey. The highest percentage of its appearance was 94%, followed by Macrophomina phaseolina. Which was isolated from 6)) regions, and the highest percentage of
appearance was 93%.These results are consistent with what Emmanual et al. (2010) stated that the fungi F.solani and M.phaseolina are among the most important pathogens of root rot of many plants, and that the characteristics of each of these pathogenic fungi were that the colony of F.solani was characterized by cream color with the formation of Macroconidia, which form after 4-7 days from the beginning of growth on simple carriers, but then form on short, many-branched conidia that form what is known as the conidia (Sporodochium)
As for the microconidia, they are formed on long lateral branches that are often at the beginning elongated side bulges known as (phialids) and they are oval in shape and in large numbers, and Chlamydospores are unicellular, with a rough wall and divided by two divisions, which It may be terminal or between the cells of the hyphae, and sometimes it may form in chains or within the semicircular spore cells, thus maintaining the survival of the fungus F. solani for long periods. This was confirmed by Summerell and Lesile (2006),and the colony of M. phaseolina was characterized by its rapid growth and its transparent color, which later turns to black, and this transformation is central and includes the colony, and growths with a high fluffy appearance appear above the colony. Upon microscopic examination, black sclerotia stone bodies appeared, which gave the colony a dark color (Muyolo et al., 1993).The results of the diagnosis showed the presence of many fungi accompanying the roots of pepper with less frequency, such as Rhizoctonia solani, Trichoderma spp. and Penicillium spp. Mucar sp, Alternaria alternata, Aspergillus niger, Chatemium

Table (5) the percentage of the appearance of fungi in the roots of pepper plants

	Hilla/Sardib	Aljafjafa	Abu Al-Jassem	Jelawiyah	Albadea	Al Muhaweel	Al-Azzawiyah	Alwatfi
	43	53	40	86	94	12	76	1
	—	—	—	—	20	62	61	1
	—	—	—	—	—	—	—	-
	44	—	—	—	33	31	96	6
	—	—	—	17	—	22	8	3
	53	—	10	—	—	—	—	-
	—	41	15	—	—	—	—	-
	—	—	—	—	—	—	—	-
	—	—	—	—	—	—	—	-

Each number represents an average of three replicates.

3-4 Test of pathological ability

1-3-4 Testing the pathogenicity of isolates of pathogenic fungi using radish seeds on PDA culture medium.

The results in Table (6) showed that all tested isolates of fungus led to a significant reduction in the percentage of germination compared to the control treatment, in which the percentage of seed germination was 96.67%.The isolate of the fungus F.solani Fs7) outperformed the isolate of Jilawia) in reducing the percentage of germination than the rest of the isolates, where the average of the percentage of germination in it reached 23.33%, followed by isolate Fs2, where the percentage of germination in it was 30%, while the percentage of germination of the rest of the isolates ranged between -40 60%.The results in Table (6) also indicated that all tested isolates of M. phaseolina caused a significant reduction in the germination of radish seeds compared to the control treatment in which the germination rate reached 96.67%. The isolates varied among themselves in reducing the germination rate. The first place in decreasing the percentage of germination, as it reached 23.33%, followed by isolate Mp2 isolate the gallstone, in which the percentage of seed germination reached 36.67%, while the percentages of germination in the rest of the isolates ranged between 43.33% - 73.33%.The reason for the variation of isolates in their effect on the percentage of germination of radish seeds may be due to the genetic difference between isolates of fungus collected from different regions or it may be due to the difference in isolates in their ability to secrete enzymes that degrade pectin and cellulose in the early stages of infection and these enzymes play a role in penetrating the host, including Pectinase, Phosphatase, Cellulase, Methylesterase pectin, lyase pectin Which has a significant impact on the pathogenicity of the fungus (Wrather et al., 1997), and from the results of this test, the two isolates that reduce the germination of radish seeds, namely Fs7 and Mp5, were selected for subsequent tests.

Table (6) Detection of pathogenic isolates of Fusarium solani and Macrophomina phaseolina using radish seeds on PDA medium.

germination %	**isolates**
50.00	Fs_1
30.00	Fs_2
56.67	Fs_3
60.00	Fs_4
53.33	Fs_5
43.33	Fs_6
23.33	Fs_7
53.33	Fs_8
40.00	Fs_9
60.00	Fs_{10}
50.00	Fs_{11}
73.33	Mp_1
36.67	Mp_2
43.67	Mp_3
66.67	Mp_4
23.33	Mp_5
60.00	Mp_6
96.67	control
5.4	L.S.D)0.05(

Each number represents an average of three replicates, Fs = Fusarium solani, Mp = Macrophomina phaseolina.

4-4 Test the antagonistic ability of A.chroococcum against the pathogenic fungi Fusarium solani and Macrophomina phaseolina on PDA culture medium.

The test results in Table (7) showed the ability of A.chroococcum bacteria at a concentration of 610×75 to inhibit the growth of pathogenic fungi F.solani and M.phaseolina isolate Fs7 and Mp5 on PDA culture media, and A.chroococcum bacteria showed the highest effect on the growth of pathogens F.solani and M. .phaseolina if the percentage of inhibition reached 87.04%, respectively, compared with the treatment of pathogenic fungi alone, if it reached 0.00%. The results showed that there is a direct proportion to the percentage of inhibition with an increase in the concentration of bacteria, as it caused a significant reduction in the growth of pathogenic fungi, and whenever the concentration of A.chroococcum bacteria was greater, the percentage of inhibition was higher compared to the treatment of the two fungi alone, which amounted to 0.00%.A. chroococcum bacteria have a high ability to inhibit the growth of pathogenic fungi and produce metabolites, organic compounds, indole acetic acid, and a number of enzymes that have the ability to degrade the cell walls of pathogenic fungi. These

enzymes include Glucanase, Chitinase, Iaminarinase and the production of a number of antibiotics such as Phenazin, Pyoluteorin herbicolin, and A. chroococcum bacteria have the ability to produce compounds with low molecular weights that work to resist pathogenic fungi, including hydrogen cyanide (HCN), as the presence of this compound in high concentrations inhibits the growth of pathogenic fungi.These results are in agreement with Kaiser's (2021) finding of the efficacy of Azotobacter.chroococcum against pathogenic fungi solani Fusarium and Macrophpmina phaseolina that cause okra root rot, as the percentage of inhibition in PDA culture media was 78.66 - 86.00%.

Table (7): Testing the antagonistic ability of A.chroococcum against the pathogenic fungi Fusarium solani and Macrophamina phaseolina on PDA culture medium.

% inhibit	fungus growth average(cm)	Dilution	Treatments
0.00	9	$^{0}10$	Fs_7 + A
87.04	1	10^{-1}	
74.44	2	$^{2-}10$	
62.41	3	$^{3-}10$	
52.70	4	$^{4-}10$	
42.59	5	$^{5-}10$	
34.25	6	$^{6-}10$	
24.44	7	$^{7-}10$	
23.30	7	$^{8-}10$	
16.67	8	$^{9-}10$	
14.74	8	$^{10-}10$	
0.0	9	$^{0}10$	A+Mp_5
87.04	1	$^{1-}10$	
74.44	2	$^{2-}10$	
62.41	3	$^{3-}10$	
52.70	4	$^{4-}10$	
44.07	5	$^{5-}10$	
36.67	6	$^{6-}10$	
34.45	6	$^{7-}10$	
27.41	7	$^{8-}10$	
23.30	7	$^{9-}10$	
16.67	8	$^{10-}10$	
4.221	0.3321		)0.05 (L.S.D

Each number represents an average of three replicates, Fs = Fusarium solani, Mp = Macrophomina phaseolina.

5-4 Test the antagonistic ability of Penicillium cyclopium against the pathogenic fungi Fusarium.solani and Macrophmina.phaseolina on PDA culture medium.

The results in (Table 8) showed that P.cyclopium has a high antagonistic ability against pathogenic fungi F.solani and M.phaseolina on PDA culture media. The fungus P.cyclopium inhibited isolates of pathogenic fungi Fs7 and Mp5, with an inhibition rate of 88.33%, 74.44% for each of the isolates. Fs7 and Mp5, respectively, after seven days of double culture compared to the treatment of pathogenic fungi alone, which had a percentage of inhibition of 0.00%.The reason for this is due to the ability of the fungus P.cyclopium to produce antibiotics, including Penicillic acid, Vioxanthin, Viomellein, Xanthomegnin, which have a role in inhibiting the growth of pathogenic fungi on PDA culture media, in addition to secondary compounds, namely dehydrocyclopeptin, cyclopenin, cyclopenol, puberulinesurine, , cyclopeptin, verrucofortine, 3-methoxyviridicatin which have a great ability to inhibit the growth of many plant pathogenic fungi (El-Hawary et al., 2019).

Table (8) Testing the antagonistic ability of Penicillium cyclopium against the pathogenic fungi Fusarium solani and Macrophomina phaseolina on PDA culture medium.

% inhibit	fungus growth average(cm)	Treatments
0.00	9	fungus Fs alone
0.00	9	fungus Mp alone
88.333	1.200	fungus P.cy + fungus Fs
74.443	2.300	fungus .cy+fungus Mp
14.88	1.39	L.S.D at (0.05)

Each number represents an average of three replicates, Fs = Fusarium solani, Mp = Macrophomina phaseolina, Pc = Peincillium cyclopium.

1-6-4 Testing of the effect of aqueous extracts of Schanginia , Carissa and wild mustard plants on the growth of the pathogenic fungi Fusarium solani and Macrophomina phaseolina.

The results in Table (9) showed that the aqueous extracts of Carissa, Schanginia , and wild mustard, with concentrations of 15.10.5%, had effective inhibition against the pathogenic fungi F.solani and M.phaseolina. For Schanginia 26.59 and 34.07%, for Cinnamon 16.67 and 24.52% and for wild mustard 25.37 and 25.04%, respectively, compared to the control treatment, which had a growth rate of 9 cm and an inhibition rate of 0.00%.As for the concentration of 10%, the fungus Carissa reached 26.22 and 24.44%, the fungus 26.3 and 30.37%, Cinnamon 22.78 and 61.85%, and the wild mustard 27.04-36.22% against the pathogenic fungi F.solani and M.phaseolina, respectively, compared to the control treatment, which was the growth rate It has 9 cm and the inhibition rate is 0.00%.As for the concentration of 15%, the extracts showed a significantly excelled on the rest of the extracts in the percentage of growth inhibition of the pathogenic fungi F.solani and M.phaseolina, as the percentage of carisa was 53.89 and 52.89%, and tadpoles were 25.59 and 54.82%, and the Cinnamon were 84.81 and 75.11% and wild mustard was 23.71 and 72.7%, respectively, compared to the control treatment, which had a growth rate of 9 cm and an inhibition rate of 0.00%.The test proved that the extract of the Cinnamon at a concentration of 15% was excelled on the rest of the extracts in inhibiting the two pathogenic fungi on the PDA food medium. The physiological action of plant aqueous extracts in the effect may be due to the nature of their content of active substances that have the ability to inhibit the growth of the two fungi, as noted by Wen-Bao et al. (2000).They interpreted that the inhibitory effect of these extracts may be due to their effect in preventing spore germination, their effect in changing the permeability of cell walls, or their effect in preventingthe fungal hyphae in its early stages, which leads to the inhibition of the growth of these fungi. As noted by EL-Mehalawy (2006) that the effective inhibitory compounds found in the plant extracts anti- tagonistic microorganisms reduce the total carbohydrate and protein content, and they also work to increase the activity of the enzymes Saccnic dehydroyenase, Malik dehydrogenase, Fumaras, at the same time it works on Reducing the activity of catalase enzyme in both R.solani and F.oxysporium, which leads to increased toxicity and then reduced growth average. Thobunluepop et al. (2007) confirmed that some plants contain effective compounds capable of inhibiting the growth of microorganisms and that these compounds have chemical compositions and efficacy different from the traditional fungicides used to control the growth and survival of these organisms.

Table (9): Effect of aqueous extracts of Cariza, Schanginia, and wild mustard and Cinnamon plants on the growth of the pathogenic fungi Fusarium solani and Macrophomina phaseolina on PDA culture media.

inhibit %	fungus growth average(cm)	councentration	Extracts	
0.00	9.00	0	Cariza	Fs
25.18	6.73	5		
26.22	6.63	10		
53.89	4.35	15		
0.00	9.00	0	Schanginia	
26.59	6.60	5		
26.3	6.80	10		
26.59	6.63	15		
0.00	9.00	0	wild mustard	
25.37	6.73	5		
27.04	6.56	10		
23.71	6.86	15		

0.00	9.00	0	Cinnamon	
16.67	6.73	5		
22.78	6.93	10		
84.81	1.36	15		
0.00	9.00	0	Cariza	
33.63	5.97	5		Mp
24.44	6.80	10		
52.89	4.24	15		
0.00	9.00	0	Schanginia	
34.07	5.93	5		
30.37	6.26	10		
54.82	4.06	15		
0.00	9.00	0	wild mustard	
25.04	5.71	5		
36.22	6.40	10		
72.7	2.46	15		
0.00	9.00	0	Cinnamon	
24.52	6.93	5		
61.85	3.43	10		
75.11	2.24	15		
8.34	1.67			L.S.D (0.05)

Each number represents an average of three replicates, Fs = Fusarium solani, Mp = Macrophomina phaseolina.

The test proved that the extract of the Cinnamon at a concentration of 15% was excelled on the rest of the extracts in inhibiting the two pathogenic fungi on the PDA food medium. The physiological action of plant aqueous extracts in the effect may be due to the nature of their content of active substances that have the ability to inhibit the growth of the two fungi, as noted by Wen-Bao et al. (2000).They interpreted that the inhibitory effect of these extracts may be due to their effect in preventing spore germination, their effect in changing the permeability of cell walls, or their effect in preventing the fungal hyphae in its early stages, which leads to the inhibition of the growth of these fungi.As noted by EL-Mehalawy (2006) that the effective inhibitory compounds found in the plant extracts anti- tagonistic microorganisms reduce the total carbohydrate and protein content, and they also work to increase the activity of the enzymes Saccnic dehydroyenase, Malik dehydrogenase, Fumaras, at the same time it works on Reducing the activity of catalase enzyme in both R.solani and F.oxysporium, which leads to increased toxicity and then reduced growth rates. Thobunluepop et al. (2007) confirmed that some plants contain effective compounds capable of inhibiting the growth of microorganisms and that these compounds have chemical compositions and efficacy different from the traditional fungicides used to control the growth and survival of these organisms.

7-4 Evaluation of the efficiency of the aqueous extract of the Cinnamon plant, the biological factors and the pesticide Beltanol on the percentage of germination of pepper seeds, the percentage and severity of infection with the pathogenic fungi Fusarium solani and Macrophomina phaseolina and some growth parameters of pepper plants under lath house conditions.

The results of the experiment in Table (10) showed that the addition of the pathogenic fungi F solani and M phaseolina led to a reduction in seed germination, where the lowest percentage of seed germination was when the pathogenic fungi were added alone, where the germination rate was 25.00%.The treatment of interactions between A. chrocooccum and P cyclopium and the extract of the study with the pathogenic fungi F solani and M phaseolina achieved a significant increase in seed germination, reaching 91.67% compared to the treatment of pathogenic fungi alone. And all the biological combined treatments of bacteria, fungi, and the extract of students with the pathogenic fungi achieved a significant increase in the

percentage of seed germination compared to the control treatment with the two pathogenic fungi alone.As for the biological treatments added without the pathogenic fungi, the percentage of seed germination was 100%, and this confirms that the interaction between the biological control factors is more effective in raising the standards of plant growth.The results of the experiment in Table (10)show that all treatments that included the biological control Az.chrocooccum bacteria, P.cyclopium fungus, aqueous extract of Cinnamon extract and Beltanol pesticide had a significant effect at the level of 5% in reducing the effect of pathogenic fungi, especially when the treatments were combined together, where the percentage reached for infection and severity of infection in the treatment of pathogenic fungi F.solani and M.phaseolina. Alone, as the infection rate was 100%, and the severity of the infection reached 81.67 and 83.33%, respectively. The results agree with what was indicated by Babay-Ahari et al. (2009) and Almammory and Matloob (2019) that these two fungi are causes of pepper root rot disease and are also considered as one of the most important pathogens on many plant families. A. chrocooccum bacteria induce systemic resistance in plants, which in turn inhibits pathogenicity-related proteins at the sites of infection, including the enzyme Chitinase, Amylase, and Peroxidase (Asma et al., 2012).This was confirmed by Kaviyarasan et al. (2020) also that A. chrocooccum bacteria works to produce and secrete some growth regulators and growth stimulants such as Auxin, Indole3-acetic acid, Gibberellins, Cytokinin, which positively affect the growth and activity of the root system and its ability to absorb nutrients and increase atmospheric nitrogen fixation. All this helps in the germination of seeds and the speed of plant growth significantly.The biological control fungus P.cyclopium also achieved a significant reduction in the rate and severity of infection with pathogenic fungi F.solani and M.phaseolina. These results show that the fungus has a high ability to protect pepper seedlings from pathogenic fungi.The reason for this is that the fungus produces many important and

toxic compounds and antibiotics, which are Penicillic acid, Xanthomeynin, Viomellein, Vioxanthin, in addition to secondary compounds with unknown toxicity, and these compounds are dehydrocyclopeptin, 3-methoxyviridicatin, viridicatol, cyclopenin cyclopenol. Which has a high ability to inhibit the pathogenic fungus and reduce the severity of the infection of pathogenic fungi to pepper seedlings, and that the fungus Penicillium spp. has a significant effect on the plant growth indicators of Refai (2015).The treatment of the aqueous Cinnamon extract of the study plant at the concentration of 15% achieved a significant reduction in the rate of infection with the pathogenic fungi F.solani and M.phaseolina 58.33% and 58.33%, respectively, and the severity of infection reached 43.33% and 36.67%, respectively, and this is due to the efficiency of the Cinnamon extract in Inhibition of pathogenic fungi, as Cinnamon extract have shown that upon chemical diagnosis, Cinnamon contain 14 types of volatile oils with antifungal activity, terpenes, ketones, hydrocarbons and other compounds.Also, the Cinnamon extract possesses a fungi-inhibiting activity represented by granulation of the cytoplasm, rupture of the cytoplasmic membrane, and inhibition of the synthesis of intracellular and extracellular enzymes of pathogenic fungi (Cowan, 1999 and Takizawa, 2001).The two treatments of A.chrococcum integration with P.cyclopium and the aqueous Cinnamon extract with the presence of the pathogenic fungi F.solani and M.phaseolina achieved excelled and high effectiveness in reducing the infection rate 33.33-33.33% and the infection severity reached 23.33-21.67% and this decrease indicates on the existence of agreement between Az.chrocooccum bacteria and the biological control fungus P.cyclopium and the aqueous extract of the two Cinnamon together and that their action was synergistic.

Table (10) Evaluation of the efficiency of the aqueous extract of the Cinnamon, the biological factors and the pesticide Beltanol in the percentage of germination, infestation and the severity of infection with the pathogenic fungi F.solani and M.phaseolina under the conditions of lath house.

%infection average	infection severity%	germination%	Treatments
100.00	81.67	25.00	Fs
66.67	61.67	41.67	A+Fs
66.67	53.33	50.00	Pc+Fs
58.33	43.33	58.33	Fs+Extract

50.00	36.67	66.67	Pc+A+Fs
41.67	33.33	75.00	A+Fs+Extract
33.33	23.33	83.33	Pc+Fs+Extract
16.67	15.00	91.67	Pc+A+Fs+Extract
33.33	28.33	58.33	Fs+Besticide+Beltanol
100.00	83.33	25.00	Mp
75.00	53.33	33.33	A+Mp
66.67	43.33	41.67	Pc+Mp
58.33	36.67	50.00	Mp+extract
50.00	31.67	58.33	Pc+A+Mp
41.67	28.33	66.67	A+Mp+Extract
33.33	21.67	83.33	Pc+Mp+Extract
16.67	15.00	91.67	Pc+A+Mp+Extract
8.333	28.33	50.00	Mp+Beltanol
0.00	0.00	100.00	A
0.00	0.00	100.00	PC
0.00	0.00	100.00	extract
0.00	0.00	100.00	PC+A
0.00	0.00	100.00	A+Extract
0.00	0.00	100.00	PC+Extract
0.00	0.00	100.00	Pc+A+Extract
0.00	0.00	100.00	control
10.35	4.35	6.72	L.S.D at (0.05)

Each number represents an average of three replicates, Fs = Fusarium solani, Mp = Macrophomina phaseolina.

The results in Table (11) also showed that all treatments led to a significant increase in the average plant height in soil contaminated with the pathogenic fungi F.solani and M.phaseolina, and the highest plant height was in the treatment of integration of bacteria A.chrocooccum and bio-fungus P.cyclopium and the aqueous extract for the two Cinnamon, the vegetative length reached 20.59 and 21.5 cm, respectively, and an increase in the fresh weight, which reached 44.89 g and 45.4 g, respectively, and the dry weight was 0.144 g and 0.172 g, respectively.Compared to the treatment of the pathogenic fungi F.solani and M.phaseolina alone, which caused a clear effect in reducing plant height and fresh weight, which were 4.5 cm, 4.233 cm, 4.14 g and 4.207 g, respectively. The average dry weight of pepper plants was 0.05 g and 0.057, respectively .The treatment of bacteria and fungi individually added to the contaminated soil achieved a significant increase in the growth parameters of the studied plants under the conditions of the lathhouse. The results showed that there was a significant increase in the treatment of pepper plant with the biological factors alone and the integration treatment that included the bio-fungi, the bio-bacteria and the extract of the Cinnamon , as it excelled on all the treatments with the control treatment without any addition, the plant length reached 18.17 cm, the fresh weight was 25.04 g, and the dry weight was 0.134 g.These results are in agreement with what was found by Xu et al. (2010) and Xu and Jeger (2013) that A.chrocooccum is a highly effective biological control agent against pathogenic fungi where it carries siderophores and has an important role in the production of antibiotics that inhibit the growth of many pathogenic fungi. These include Fusarium spp. Fusarium spp. These bacteria also secrete growth stimulants such as Gibberllins that increase plant growth. P.cyclopium is a highly effective biological control agent against pathogenic fungi

It produces many important toxic compounds and antibiotics, including Xanthomegnin, penicillic acid, as well as secondary compounds with unknown toxicity, such as cyclopenin, viridicatol, where this fungus has a great effect on plant growth in a positive methods

Table (11) Evaluation of the efficiency of the aqueous extract of the Cinnamon plant, the chemical pesticide Beltanol, and the biological factors against the pathogenic fungi Fusarium solani and Macrophomina phaseolina and their effect on plant height, fresh and dry weight of pepper plants under lathhouse conditions.

dry weight(g)	Fresh weight(g)	plant height(cm)	Treatments
0.05	4.14	4.5	Fs
0.136	12.64	8.333	A+Fs
0.138	16.18	9.5	Pc+Fs
0.142	19.33	12.38	Fs+Extract
0.152	23.28	14.17	Pc+A+Fs
0.158	26.46	16.5	A+Fs+Extract
0.168	32.73	17.07	Pc+Fs+Extract
0.173	44.89	20.59	Pc+A+Fs+Extract
0.144	23.43	16.5	Fs+Beltanol
0.057	4.207	4.233	Mp
0.134	13.25	11.59	A+Mp
0.138	16.14	14.35	Pc+Mp
0.142	18.63	16.08	Mp+extract
0.147	22.09	18.35	A+Mp+Pc
0.153	27.52	19.5	A+Mp+Extract
0.162	32.28	22.42	Pc+Mp+Extract
0.172	45.4	21.5	Pc+A+Mp+Extract
0.142	22.93	17.5	Mp+Beltanol
0.163	47.24	20.75	A
0.168	49.62	22.5	PC
0.169	49.91	23.5	extract
0.172	53.31	23.83	PC+A
0.173	55.45	25.5	A+Extract
0.174	56.44	26.5	PC+Extract
1.183	60.05	28.92	Pc+A+Extract
0.134	25.04	18.17	control
0.028	2.434	1.800	L.S.D

Each number represents an average of three replicates, Fs = Fusarium solani, Mp = Macrophomina phaseolina.

1-5 conclusions

The spread of pepper root rot disease in all areas covered by the field survey. It was found that the two pathogenic fungi Fusarium solani and Macrophomina phaseolina from the main causes of pepper root rot disease have a high pathogenicity against pepper plant. Azotobacter.chrocooccum, Penicillium.cyclopium and Aldera aqueous extract possess high antagonistic efficiency against pathogenic fungi F.solani and M.phaseolina in vitro on PDA culture medium. Efficiency of biological control agents A. chrocooccum, P. cyclopium and schistosomiasis aqueous extract (alone or in combination with each other) in reducing the rate and severity of infection with the pathogenic fungi F.solani and M.phaseolina that cause pepper root rot disease and increasing the growth parameters of pepper plants and their fruits under conditions lath house and field.

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