Efficiency of *Trichoderma viride* and chitosan in control of pathogenic fungi *caused* cowpea root rot diseases in Babylon province

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Abstract:

the study aimed to isolating some pathogenic fungi from cowpea plants infected with root rot, and controlling the causes using some plant resistance-inducing agents and the *Trichoderma* viride. The results showed the presence of cowpea root rot disease in all areas included in the survey in Babylon Governorate, with varying rates of disease incidence ranging between 40-100% and infection severity ranging from 18-75%. The highest infection severity was in samples from the areas of Al-Hashimiya, Al-Qasim, Al-Badaa, and Taliah, respectively, with infection rates of 100% in most of these areas. The *F. solani* was the most abundant fungus as it appeared in most samples with varying frequency rates 55.9%, followed by the fungus *Macrophomina phaseolina* with an appearance rate of 45.55%, respectively. The results of the experiment showed that all treatments, which included the biological fungus *T. viride* and chitosan, had a significant effect in reducing the growth of pathogenic fungi, especially when the treatments were combined together, as measured by the percentage of infection and the severity of infection in the treatment of the pathogenic fungi *F. solani* and *M. phaseolina*. On its own, the infection rate reached 100.00%, while the infection severity reached 77.3 and 74.7%, respectively. The treatment using the biological fungus *T. viride* also achieved a significant reduction in the infection rate with the pathogenic fungi *F. solani and M. phaseolina*, which reached 46.7 and 40.0%, while the severity of infection reached 28.0 and 24.0%, respectively. The results showed that all treatments used to combat the causes of the root rot disease of cowpea led to a significant increase in the average plant height, wet and dry weight in soil contaminated with the pathogenic fungi *F. solani* and *M. phaseolina*.

Keywords: Cowpea, *Trichoderma viride*., Chitosan, Fungi, Root rot diseases.

1-Introduction:

The cowpea plant (*Vigna unguiculata* L. Walp.), Leguminaceae, is one of the most important plants of this family, as it is grown worldwide for its high nutritional value, as its fruits contain many carbohydrates, proteins, and Lipids. Cowpeas are grown in order to obtain their dry seeds and green pods, which are used cooked as food for humans. Or it is used as green fertilizer to improve soil characteristics and increase its fertility [26]. The area cultivated with the cowpea crop in Iraq for the year 2021 was estimated at 17596 dunams. The total production of this area in Iraq reached 26606 tons (Iraqi Central Statistics, 2021). The cowpea crop is infected with a group of pathogens, the most important of which are types of fungi, bacteria, viruses, and some types of mycoplasma. Root rot and seedling damping off are among the diseases that most affect plants and are caused by infection with soil-inhabiting fungi [8].
Several methods are used to control these pathogens, including the use of chemical pesticides, but the extensive use of these materials has led to the emergence of many risks to human and animal health and the environment, in addition to their high economic cost and the emergence of resistance by pathogens towards them, which has prompted the efforts of researchers in the field of plant protection to search for less risky and safer methods, the most prominent of which is the use of microorganisms as vital agents to resist plant pathogens, reduce their pollen, and

increase the quantity and quality of the crop [13], [35]. One of the most important biological agent is the biological fungus *Trichoderma* spp. The species belonging to the genus *Trichoderma* are among those antibiotics that have given encouraging results in the field. This fungus possesses various antagonistic properties against pathogens and is important in improving plant growth and production, as well as the ability to induce resistance [18]; [44]; [1]; [20]. Many recent studies have reported that the use of Chitosan, which is one of the components of the cell walls of some organisms in various crustaceans, such as crabs, shrimp, and insects, in inducing resistance in plants against pathogens, including cowpea, cucumber, eggplant, grapes, and sugar cane [37]; [31]; [30]. Due to the lack of studies related to the causes of cowpea root rot diseases in Iraq and to try to find new and safe ways to combat them, the study aimed to isolating some pathogenic fungi from cowpea plants infected with root rot, resisting the causes using some plant resistance-inducing agents and the biological fungus *Trichoderma* spp.

2-Methods and materi als:

2-1 Field survey:

The field survey of the 2022-2023 season was carried out in some cowpea fields in the province of Babylon. From 25/03/2023 to 1/7/2023, samples were collected from a plant by randomly. then put the samples in polyethylene bags. The percentage of the diseases incidence was calculated by the equation:

% Disease incidence = (Number of infected seedlings\Total number of total seedlings) × 100.

The percentage of severity according to Mckinney (1923) was calculated as follows: The percentage of infection severity for the root system was calculated according to the 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, 5=plant death. Disease Severity (%) = ((Plants in 1 degree ×1+… Plants in 5 degree ×5)/ all plants ×5) ×100%.

2-2 Isolation and diagnosis of fungi accompanying the roots of cowpea plants.

The isolation process was carried out from samples of cowpea plants that showed symptoms of root rot disease, represented by wilting, yellowing of the leaves, and general weakness in growth, with the presence of brown rot on the main and branch roots on the next day of the field survey. Pieces of cowpea roots that were sterilized with sodium hypochlorite1% were transferred using sterile forceps to Petri dishes, 4 pieces in each dish (dish diameter 9 cm) containing the potato dextrose Agar (PDA) medium with addition of the antibiotic Tetracycline at a rate of 250 mg/L. The dishes were incubated at 25+1°C for three days. The diagnosis of fungi was carried out based on the taxonomic keys [40]; [19]; [32]; [6] and[7]; [12]; [10]; [39]. The occurrence rate of the fungi that appeared was calculated according to the following equation:

The percentage of the presence of fungi= N/n ×100

N = (Number of root pieces in dishes in which pathogenic fungi appear , n= The total number of root pieces used for each sample.

2-3 Efficiency of the biological fungus *Trichoderma viride* and chitosan in infection of the cowpea plant.

The experiment was carried out in the canopy of the Plant Production Department on 14/7/2023. Using plastic pots with a capacity of 2 kg/pot, sterilized sandy soil (in the autoclave at a temperature of121°C and a pressure of one atmosphere). The sterilization was

repeated the next day, then it was left for seven days before use and distributed in plastic pots at a rate of 2 kg/pot. planted with local cowpea seeds at a rate of 5 seeds per pot. The experiment was carried out using a Complete Random Design (C.R.D) and with three replicates for each treatment. The pathogenic fungi inoculum was added on local millet seeds to all treatments that required the addition of the pathogenic fungi inoculum at a rate of 1%. The biological control agent *T. viride*, a supplement was added to the wheat bran at a rate of 1% three days before adding the pathogenic fungal inoculum, and as for the Chitosan Ch, the plants were sprayed, Until wetness at a concentration of 5%, while the chemical pesticide Beltanol was added at a concentration of 1%, one day after adding the pathogenic fungi as for the Control treatment, only sterilized millet seeds were added to it. The treatments included the following:
1-*Fusarium solani*, 2- *F. solani* + *Trichoderma viride*, 3- *F. solani* + chitosan, 4- *F. solani* +Beltanol, 5- *F. solani* + *Trichoderma viride* + chitosan, 6- Control (without addition) , 7- *Trichoderma viride*, 8- chitosan, 9- *Trichoderma viride* + chitosan, 10- *Macrophomi phaseolina*, 11- *M. phaseolina* + *Trichoderma viride*, 12- *M. phaseolina* + chitosan, 13- *M. phaseolina* + *Trichoderma viride* + chitosan, 14- *M. phaseolina* + Beltanol.

3-Results and discussion

3-1 Field survey of cowpea root rot disease

The results (Table 1) showed the presence of cowpea root rot disease in all areas included in the survey in Babylon Governorate, with varying rates of disease incidence ranging between 40-100% and infection severity ranging from 18-75%. The highest infection severity was in samples from the areas of Al-Hashimiya, Al-Qasim, Al-Badaa, and Taliah, respectively, with infection rates of 100% in most of these areas. The reason for the high incidence of infection in these areas may be due to the fact that they are areas specialized in the cultivation of cowpeas, as this crop is grown there continuously annually, which has led to the accumulation of inoculum from pathogenic fungi, especially sclerotia, which remain in the soil for a long period of time, perhaps up to five years [27]; [43]; [40]. The results also showed that the lowest incidence and severity of infection appeared in samples from the Al-Rashidiyah area, and this may be a result of the field being planted with the crop for the first time.

Table (1) Areas included in the field survey and date of sampling in Babylon Governorate

No.	Region	Date of survey	Field area / dunums	Disease incidence (%)	Severity (%)
1	Al-Qasim	25/3/2023	2	100	73
2	Tabo	6/5/2023	1	80	58
3	Taliah	26/3/2023	3	100	60
4	Al Mashroa	27/4/2023	3	90	56
5	Al Azawia	18/5/2023	5	100	56
6	Al-Qasim- Al fayadia	10/6/2023	3	100	52
7	Al-Rashidiyah	15/6/2023	1	40	18.0
8	Al-Badaa	29/6/2023	2	100	62.8
9	Al-Hashimiya	1/7/2023	4	100	75

3-2 Isolation and diagnosis of fungi accompanying the infected roots of cowpea plants

Through microscopic examination of fungal growths formed from planting infected plant cuttings on PDA culture medium, it was revealed the presence of 13 species of fungi accompanying the roots of the cowpea plant (Table 2). The *F. solani* was the most abundant fungus as it appeared in most samples with varying frequency rates 55.9%, followed by the fungus *Macrophomina phaseolina* with an appearance rate of 45.55%, respectively, while the fungus *F. sulphureum* appeared in samples from the Al-Qasim, Taliah, and Al-Qasim areas. AlRashidiyah, at a rate of 7.6%. This is the first recording of the fungus *F. sulphureum* on the cowpea plant in Iraq. It was noted that the dominance of some species was observed in samples of some areas despite their repeated appearance in samples of other areas, as the fungus *F. solani* recorded a percentage of 92% in the samples of the Al-Qasim area, and the fungus *M. phaseolina* in the Bidaa area reached 0.0%. The results are consistent with what was obtained by [34] regarding the emergence and spread of soil fungi pathogenic and caused of Beans root rot diseases. The examination results showed the presence of many fungi accompanying the roots of the cowpea plant, such as *Alternaria alternata, Aspergillus niger,* and *Stemphylium* sp. The presence of these types of fungi may be attributed to the growth and penetration of their mycelium into the cells of decomposing tissues that were previously infected with the fungi causing this condition, which provided them with protection from the action of the surface disinfectant, or they may include species with parasitic ability on plants [29].

Table 2. Fungi accompanying the roots of infected cowpea plants, their locations, and their frequency in samples

Fungus	No. sample*	Appearance rate (%)**	Highest ratio of appearance
Fusarium solani	1‹4‹5	55.9	92
Alternaria alternata (Fres.) Keissler	3‹8‹ 4‹5	15.5	32.9
Pencillium spp.	5‹1‹8	52.6	81
Aspergillus Nigeria	3‹5‹6‹1	29.6	18
Mucor spp.	1‹4	12.33	14
Fusarium oxysporum.	6‹7	1.27	1.3
Fusarium solani (Mart.) Sacc.	1-3‹5-9	37.4	65.0
F. sulphureum schlecht.	1‹3‹7	7.6	15.4
Macrophomina phaseolina (Tassi) Goid.	1-3‹5‹7‹9	45.55	91
Rhizoctonia solani Kuhn	1-4‹7‹9	28.21	32.14
Trichoderma harzianum	3‹1	5.7	5.7
Stemphylium sp.	1‹5-7	4.3	7.9
Torula sp.	6	6.6	6.6

*No. sample, numbers represent sample collection areas (Table 1).

%** repeat the fungus in the sample = (The number of fungus appeared in dishes\Total number of pieces used in the sample)x 100.

The fungus *T. harzianum* was also isolated from a sample from the AlQasim area, with an occurrence rate of 5.8%. The presence of the fungus may have come as a result of its ability to parasitize the mycelium of pathogenic fungi inside the plant tissues, which made them immune to the action of the surface sterilizer [16]. Another group of fungi was isolated with less frequency. *Fusarium oxysporum, F. semitectum, Pencillium* spp., and *Torula* sp. . According to the priority of their isolation, the isolates were given numbers next to the fungus symbol to distinguish them from other isolates.

3-3 Efficiency of *Trichoderma viride* and chitosan in infection of the cowpea plant under woody canopy conditions.

The results of the experiment (Table3) showed that all treatments, which included the biological fungus *T. viride* and chitosan, had a significant effect in reducing the growth of pathogenic fungi, especially when the treatments were combined together, as measured by the percentage of infection and the severity of infection in the treatment of the pathogenic fungi *F. solani* and *M. phaseolina*. On its own, the infection rate reached 100.00%, while the infection severity reached 77.3 and 74.7%, respectively. The treatment using the biological fungus *T. viride* also achieved a significant reduction in the infection rate with the pathogenic fungi *F. solani and M. phaseolina*, which reached 46.7 and 40.0%, while the severity of infection reached 28.0 and 24.0%, respectively, compared to treating the pathogenic fungi alone. These results show that the *T. viride* has a high ability to protect the seeds of the cowpea plant from infection with the pathogenic fungi, and the reason for this is due to Because it possesses mechanisms of direct parasitism on pathogenic fungi through its production of enzymes that degrade the cell walls of the pathogen, its production of antibiotics, and competition with it, in addition to the secretion of enzymes that stimulate systemic resistance in plants and enzymes that encourage plant growth, and this is what [17] found, which was found among living organisms. Microflora species of fungi spp. *Trichoderma* is one of the most useful microorganisms as it can be exploited as fungal biological agents all over the world in order to manage soil-borne diseases, which works to enhance plant growth and stimulate systemic resistance in the plant to enhance its level of resistance to diseases and contains secondary metabolites that promote plant growth [42]. These results are consistent with what was found by [3], which contains many microorganisms that work to nourish the plant and induce systemic resistance in the plant, which in turn works to inhibit proteins associated with diseases at the sites of infection, including the enzymes Peroxidase, Amylase, Chitinase, . These results are also consistent with what was found by[33] and [25] where they found that the fungus spp. Trichoderma has the ability to inhibit a wide range of soil-borne plant pathogenic fungi such as *Sclerotium rolfsii, Sclerotinia sclerotiorum, Rhizoctonia solani, Macrophomina phaseolina*, in addition to *Fusarium* species. Resistance to the fungus spp. The use of chitosan also achieved spraying on the foliage, as the rate and severity of infection with the pathogenic fungi *F. solani* and *M. phaseolina* when using chitosan reached 53.3 - 46.7%, and the severity of infection ranged from 38.7 - 22.7%, and the results are consistent with what was shown by experiments carried out by [38]. When spraying the plant (the shoot) with different concentrations of chitosan and at different spraying times, it led to a significant reduction in tomato root rot disease. It confirmed the presence of a systemic effect of the chitosan that is transmitted from the shoot To the root system to cause structural and chemical defense changes that lead to the reduction of the disease. The systemic effect of the chitosan compound may be due to stimulating plants to produce low-molecular-weight proteins that have the ability to have a systemic effect, called pathogenicity-related proteins, such as chitinase, glucanase, peroxidase, phenylalanine ammoniale, and total phenol content, which have been proven to be induced by chitosan [11]; [5]; [15].

The results of Table (4) also showed that all treatments used to combat the causes of the root rot disease of cowpea, which included the biological fungus *T. viride* and chitosan, led to a significant increase in the average plant height in soil contaminated with the pathogenic fungi *F. solani* and *M. phaseolina*. The integrated treatment between the *T. viride* and chitosan, there was a significant superiority against all pathogens. The highest length in the integration treatment was 29.33 and 37.33 cm. As for the biological fungus *T. viride*, the plant length was 38.33 and 39.67, and an increase in The wet weight was 948 and 875 g, and the dry weight was 315.0 and 373.0g. As for the treatment of the biological fungus *T. viride* and chitosan, the length of the shoot of the plant reached 52.67 cm, and an increase in the fresh weight was 1.185 g, and the dry weight also reached 414.0 g. Compared to treating the pathogenic fungi *F. solani and M. phaseolina* alone, which caused a clear effect in reducing the plant height and fresh weight, reaching 14.00 and 11.67 cm, 515 and 611 g. The average dry weight of the cowpea plant reached 140.0 and 149.7 g. Therefore, the use of biological resistance agents provided good protection for the crops from infection by pathogens and increased growth, as was proven. [37] that the higher the concentration of chitosan, the greater the effectiveness of the peroxidase enzyme in cucumber plants. A group of researchers concluded that the use of chemical stimuli leads to an increase in the peroxidase enzyme [36] and [2]. The effect of the biological fungus *T. viride* is due to its ability to combat a wide range of pathogenic fungi through several resistance mechanisms. Antagonism negatively affects plant pathogens, such as its high ability to compete for nutrients, as well as its production of enzymes that degrade the fungal cell wall, which facilitates their penetration, such as the Cellulase [21] and[23] and[4]. In addition to possessing a parasitism mechanism, in a study conducted by[14] on the effect of the resistant fungus *T. viride* on the pathogen *Ceratocystis paradoxa* in the laboratory, it was found that the resistant fungus moves towards the pathogenic fungus and wraps itself around the mycelium, penetrating it and hindering its growth. Chitosan is a natural control agent that acts as a biostimulant for plant growth and protection. It also stimulates many responsive genes, proteins, and secondary metabolic compounds in plants [9].

Table (3) Efficiency of the biological fungus *Trichoderma viride* and chitosan in reducing the rate and severity of infection with the pathogenic fungi *Fusarium solani* and *Macrophomina phaseolina* under woody canopy conditions.

Transaction	Incidence rate%	Severity of injury%
Fu-6	100.0	77.3
Fu-6+Beoll	16.7	16.0
Fu-6+Th	46.7	28.0
Fu-6+Ch	53.3	38.7
Fu-6+Th+Ch	15.0	13.3
Mac-5	100.0	74.7
Mac-5+Beoll	36.7	28.0
Mac-5+Th	40.0	24.0
Mac-5+Ch	46.7	22.7
Mac-5+Th+Ch	16.0	0.0
Th	0.0	0.0
Ch	0.0	0.0
Th+Ch	0.0	0.0
Control	0.0	0.0
L.s.D = 0.05	5.69	2.26

Each number represents the average of three replicates: 6 Fu = *Fusarium solani*, Mac5 = *Macrophomina phaseolin*, Ch = chitosan, *Trichoderma viride*.

Table (4): Efficacy of the probiotic fungus *Trichoderma viride* and chitosan against the pathogenic fungi *Fusarium solani* and *Macrophomina phaseolina* and their effect on plant height and fresh and dry weight of cowpea plants under woody canopy conditions.

Treatments	Plant length (cm)	wet weight (g)	Dry weight (g)
Fu6	14.00	515	140.0
Fu6+Beoll	34.00	830	208.3
Fu6+Th	38.33	948	215.0
Fu6+ch	29.33	810	115.0
Fu6+Th +ch	39.76	985	230.7
Mac5	11.67	611	159.7
Mac5+Beoll	31.00	710	182.3
Mac5+Th	39.67	875	373.0
Mac5+Ch	37.33	739	235.0
Mac5+Th+Ch	41.00	912	335.0
Th	53.00	1.162	415
Ch	44.33	685	175.0
Th+Ch	52.67	1.185	314.0
Control	42.33	664	150.5
L.s.D=0.05	5.251	4.36	5.31

Each digit represents an average of 6FU = 6FU = *Fusarium Solani*, Mac5= *Macrophomina Phaseolin*, Ch= Kitusan, *Trichoderma Viride*.

Conclusions:

The cowpea root rot disease was spread in all areas in Babylon Governorate, The *Fusarium solani* and *Macrophomina phaseolina* were the most abundant fungi as it appeared in most samples. The biological fungus *Trichoderma viride* and chitosan, had a significant effect in reducing the growth of pathogenic fungi, especially when the treatments were combined together and increase in the growth of cowpea plants.

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