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ORIGINAL ARTICLE

THE CONTROL OF SOME FUNGI THAT CAUSE TOMATO ROOT ROT DISEASE BY EM1 AND NORMAL AND NANO-CHITOSAN

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Abstract: This study aimed to evaluate EM11 microorganism preparations and of normal and Nano chitosan to control the causes of tomato root rot disease. The results of the field survey that was conducted in some fields of Karbala, Baghdad and Babylon governorates showed the spread of root rot disease in the areas covered by the field survey, with an infection rate ranging between 62-100% and an infection severity of 35-57%. Several types of fungi were isolated and identified from the roots of tomato plants infected with root rot disease. The most frequent pathogenic fungi were *Fusariumsolani*, followed by *Rhizoctonia solani* and *Macrophomina phaseolina*. The results showed that all tested fungi isolates were pathogenic and led to any significant reduction in the percentage of germination, compared to the comparison treatment. The results showed the ability of the EM1 microorganism preparation to inhibit the growth of pathogenic fungi *F.solani* Fs1, *R.solani* Rs5 and *M. phaseolina* Mp8 on PDA culture medium. The EM1 showed the highest effect at 5, 10 and 15% concentrations on pathogenic fungi growth, as the percentage reached .The percentage of inhibition is 100%. The results of the study showed that the inhibitory activity of chitosan in the growth of the fungi *F. solani, R. solani and M. phaseolina* on PDA culture media increased with increasing concentration.

***Key words*:** Tomato, Fungi, Root rot disease, Effective microorganism.

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1. Introduction

The tomato crop is affected by a group of pathogens that have different pathogenic mechanisms that reduce the yield of tomato and affect the quality of the product and many fungi, bacterial species, viruses and some types of Mycoplasma are included under the pathogens and among these important diseases are *Fusarium* wilt, leaf rot, root knot, early blight on tomatoes and others fungi and the most famous of them are *Fusarium solani, Rhizoctonia solsni, Microphomina phasiolina, Fusarium oxysporium* and *Pythium* spp. [Campos *et al*. (2022)]. Excessive use of chemical pesticides in combating diseases led to many environmental problems and toxic effects for humans and non-target organisms, which motivated researchers to develop alternative inputs to combat plant diseases, including the use of biological control [Aboutorabi *et al*. (2018)], biological control agents act as alternatives. For pesticides, these organisms are used as biocides and fertilizers in sustaining and enhancing crop production and protection and are usually biological strains isolated from the soil. Induced resistance was also used that works on stimulating plants and pushing them to resist disease, whether through the use of biological or non-biotic product. Its production promised a good alternative to the control of plant pathogens, and the application of Nanotechnology in agriculture showed promising results in terms of development and use of products such as fertilizers and pesticides that significantly enhanced the induction of resistance in plants [Paramo *et al*. (2020)]. The biologically safe techniques based on effective microorganisms (EM1 Effective Micro-Organisms)

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were used to maintain soil fertility, many of which are highly resistant to toxic substances and have positive effects on plant growth and plant protection from toxic substances under different stress conditions [Ivanova and Ryabchikova (2022)]. In recent years, the use of nanomaterials to improve soil properties, increase plant growth and improve its production has expanded, and it has been promised as a good alternative to control plant pathogens. The application of nanotechnology in agriculture has shown promising results with regard to the development and use of products such as fertilizers and pesticides, which have significantly enhanced the stimulation of resistance in plants [Paramo *et al*. (2020), Shihab and Abood (2019)] and many recent studies have reported the use of chitosan, which is a component of the cell walls of some organisms and for the protective skin of various crustaceans such as crabs, shrimp and insects [Abdul-Karim (2021)]. Because of the importance of the disease of seedling death and tomato root rot and to search for methods of resistance in line with modern global trends in reducing the use of chemicals in agriculture, this study aimed to isolate some pathogenic fungi from tomato plants infected with root rot disease, diagnose them, test their pathogenicity and try to combat the causes of root rot disease. Tomato using some normal and nano chemical inducing agents and EM-microorganism preparation under laboratory conditions.

2. Materials and Methods

2.1 Field survey

A field survey was conducted for the season 2021-2022 in three governorates of Baghdad, Babylon and Karbala for the period 20/6/2021 to 5/9/2021. The percentage of infection was calculated and the percentage of infection severity for the root system was calculated according to the pathological index consisting of 6 degrees, as follows:

0 =healthy plant, 1 = More than 0-25% of the roots are affected, 2 = More than 25%-25% of the roots are infected 3=More than 75%-50% of the roots are infected, 4= more than 75% of the roots are infected, 5 = plant death in a dark color or the death of the plant. The percentage of severity was calculated according to the Mckinney equation (1923) as follows:

$$\textbf{Severity}\ (\%) = \frac{\text{Plants in 1 degree} \times 1 + \ldots \text{ Plants in 5 degree} \times 5}{\text{all plants} \times 5} \times 100\%$$

2.2 Isolation and identification of fungi associated the infected roots of tomato plants

The affected roots were washed with running water to remove dust for 5 minutes, and they were cut into small pieces with a length of 0.5 cm, and superficially sterilized by dipping them with a solution of Sodium hypochlorite (1% free chlorine) for one minute. Small pieces from the ends of the fungal hyphae were placed in the center of a petri dish containing PDA medium. The dishes were incubated for 4 days in the incubator and examined under the compound microscope. The fungi were diagnosed for the level of sex and species after the appearance of the fungal growths and depending on the characteristics of the colony. The appearance of fungi was calculated as following:

$$\text{Repeat the fungus in the sample} = \frac{\text{The number of fungus appeared in dishes}}{\text{Total number of pieces used in the sample)}} \times 100$$

2.3 Detection of pathogenic isolates of *Rhizoctonia solani, Fusarium solani,* and *Macrophomina phaseolina* using cabbage seeds on Potato Dextrose Agar (PDA) medium

Seven isolates of *M. phaseolina*, five isolates of *F. solani* and 10 isolates of *R. solani* were tested. According to the method of [Bolkan and Butler (1974)], the cabbage seeds were planted in a circular motion near the edge of the plate, at a rate of 10 seeds per plate. The dishes were incubated in the incubator at a temperature of 25±1°C. After seven days, the results were taken by calculating the percentage of germination.

2.4 Evaluation of the antagonistic ability of effective microorganisms EM1 to inhibit the growth of *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* on PDA medium.

The inert basic EM1 solution, were activated by mixing it with molasses, chlorine-free warm water at a ratio of 5 ml of inert EM1 solution, 5 ml of molasses, and 95 ml of chlorine-free water. A warm place away from sunlight for 20 days at a temperature between 35-40°C. During this period the package was opened more than once to leak gas and form a layer of precipitate at the bottom of the package. Add 1, 3, 5, 10 and 15% ml of activated EM1 solution to a beaker. 100 ml glass was completed and the volume was completed to 100 ml of PDA culture medium, each concentration individually, then the mixture was poured into Petri dishes. The dishes was inoculated by isolates of *F. solani* (Fs1) and *R. solani* (Rs5) and *M.*

phaseolina (Mp8) at 5 days age in the center of the dish with 3 replicates, the plates were placed in the incubator at a temperature of 25+1°C for 7 days and the colony diameter and the percentage of inhibition were measured based on the comparison treatment to evaluate the efficiency of the EM1 preparation according to the following equation:

$$\% \text{ Inhibition} = \frac{R-r}{R} \times 100\%$$

Where, r is the radius of the fungal colony against the bioagents and R is the radius of the fungal colony without the bioagents.

2.5 Evaluation of the efficiency of normal and Nano-chitosan in inhibiting the growth of *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* on PDA medium

Ordinary and Nano-chitosan was used in this experiment, which is a white powder extracted from shrimp shells. Chitosan was obtained from Dr. Ahmed Adnan / Al-Furat Al-Awsat Technical University / Plant Production Departments) while Nano-chitosan was produced by the American research company, Inc. Nanomaterials. Dissolve 20 g of normal and Nano chitosan separately in 50 ml of acetic acid and complete the volume to 1000 ml of distilled water, for the purpose of preparing the concentrations 1, 3, 5, 10 and 15% for each of the regular chitosan and Nano chitosan, each concentration was taken separately and placed in A glass flask with a capacity of 100 ml and the volume was completed to 100 ml of the medium of Potato Dextrose Agar (PDA) with a capillary motion to homogenize the mixture and then it was poured into Petri dishes with a diameter of 9 cm and then inoculated with a 0.5 cm diameter disc of pathogenic isolates of *F. solani* (Fs1) and *R. solani* (Rs5) and *M. phaseolina* (Mp8) at 5 days of age with three replicates for each concentration as well as three replicates left without adding any concentration (medium only) as a control treatment and incubated at a temperature of 25 + 1°C for 7 days. The results were as mentioned in paragraph (2-4).

3. Results and Discussion

3.1 Field survey of tomato root rot

The results of Table 1 showed the field survey that was conducted in some fields of Karbala, Baghdad and Babylon governorates, the spread of root rot disease in the areas covered by the field survey, with an infection rate ranging between 62-100% and an infection severity of 35-57% which reached 80, 85, 85, 96 and 100%, respectively. The reason for the high infection rate in these fields may be attributed to the repeated cultivation of the tomato crop in them or the cultivation of other crops belonging to the Solanaceae family in the same fields, which leads to the accumulation of fungal pollen for pathogens, especially sclerotia, which remain in the soil for a long time, and appropriate environmental conditions, especially temperatures suitable for the growth of pathogenic fungi, hoeing and weeding processes, lead to the making of wounds in the roots, which facilitates the process of fungal invasion and infection. While the highest severity of infection was in the fields of Tarmiyah, which amounted to 57%, followed by Al-Azzawiya area with 54%, and then Al-Haidariya area with 57%. While the infection severity was less in the Bada'a and Al-Mahaweel region, reaching 35%, and the reason for this is that the field was newly planted with the tomato crop, and the severity of the infection varied depending on the operations of crop service, soil, regular irrigation and control methods, as well as the difference in environmental factors such as temperature and humidity, which have a significant impact on Increase in fungal infection.

3.2 Isolation and identification of fungi accompanying the roots of infected tomato plants

Several types of fungi were isolated and diagnosed from the roots of tomato plants infected with root rot disease (Table 2) and the most frequent pathogenic fungus was *Fusarium solani*, which was isolated from all areas covered by the survey, its appearance rate

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

Region	Disease induce (%)	Severity (%)
Babel / Al-Azzawiya	96	54
Babylon / Tahiriya	62	42
Babylon / heresy	85	35
Babylon / Mahaweel	77	35
Baghdad / Tarmiyah	85	47
Baghdad / Yusufiyah	66	43
Baghdad / Latifiya	80	42
Karbala / Haidaria	100	57
Karbala / Khan Al-Nass	71	40
Karbala / Husseiniya	70	44

Table 2: Percentage of the appearance of fungi in the roots of tomato plants infected with root rot.

Fungus	Appearance rate (%)	High ratio of appearance
Fusarium solani	96	54
Rhizoctonia solani	62	42
Macrophomina phaseolina	85	35
Pencillium spp.	77	35
Aspergillus nigeria	85	47
Chaetomium globosum	66	43
Acremonium spp.	80	42
Lichtheimia ramosa	100	57

was 55.9% and the highest percentage of its appearance was 91%, followed by the fungus *Rhizoctonia solani*, which was isolated from seven regions, at a rate of 61.8%, and the highest rate of its appearance was 95%, followed by the fungus *Macrophomina phaseolina*, which was also isolated from seven regions, where the highest percentage of its appearance was 92%, with a rate of 58.5%. These results show that the fungi *F.solani*, *R.solani* and *M. phaseolina* are among the most important pathogens associated with root rot that infect many field crops.

These results are consistent with many studies that confirmed the association of these fungi with root rot diseases in many crops [Emmanual *et al*. (2010)]. The diagnostic results showed the presence of many Fungi associated the roots of tomatoes and less frequent, such as *Pencillium* spp, *Aspergillus niger*, *Chaetomium globosum* and *Acremonium* spp. and *Lichtheimia ramose* with a higher percentage of 81, 73, 44, 13 and 16%, respectively. Studies agree with what was found by Alsudani (2020) and Muslim *et al.* (2021) that these fungi *F. solani, R. solani* and *M. phaseolina* infect tomato and cause root rot and seedling fall diseases.

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

The results of **Table 3** showed that all tested fungi isolates led to any significant reduction in the percentage of germination, compared to the comparison treatment in which the percentage of seed germination was 100%. The rest of the isolates, as the percentage of germination in them was 13%, followed by isolates of Fs3, Fs6 and Fs8, where the percentage of germination reached 26.67%, while the percentage of germination in the rest of the isolates ranged between 36.67-66.67%, as the results of the Table 3 indicated that all isolates. The tested fungus *R.solani* caused a significant reduction in the germination of the seeds of cabbage compared to the comparison treatment, and the isolates varied among themselves in reducing the germination rate. Rs8 Isolation of Karbala / Al-Haidariya governorate, in which the percentage of seed germination reached 16.67%, while the percentage of germination in the rest of the isolates ranged between 23.33-63.33%, and the results in the Table 3 showed that all isolates of *M. phaseolina* showed a significant reduction in the percentage of germination of cabbage seeds compared to the comparison treatment, which reached the percentage of germination 100%, and it was more superior among isolates of Mp8 isolate belonging to Karbala / Al-Haidariya governorate, the percentage of

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

Region	Isolate symbol	No. germinated seeds	Germination (%)
Babel / Al-Azzawiya	control	10	100
Babylon / Tahiriya	Fs1	1.33	13.33
Babylon / heresy	Fs2	3.67	36.67
Babylon / Al-Mahaweel	Fs3	2.67	26.67
Baghdad / Tarmiyah	Fs4	5	50
Baghdad / Yusufiya	Fs5	3.33	33.33
Baghdad / Latifiya	Fs6	2.67	26.67
Karbala / Haidari	Fs7	4.33	43.33
Karbala / Khan Al-Nass	Fs8	2.67	26.67
Karbala / Hussainiya	Fs9	6.67	66.67
Babylon / heresy	Fs10	4.33	43.33
Baghdad / Tarmiyah	Rh3	2.33	23.33
Baghdad / Yusufiya	Rh5	0	0
Karbala / Haidari	Rh6	4.67	46.67
Karbala / Hussainiya	Rh8	1.67	16.67
Babel / Al-Azzawiya	Rh10	6.33	63.33
Babylon / Tahiriya	Mp1	5	50
Babylon / heresy	Mp2	4.67	46.67
Babel Al / Mahaweel	Mp3	6	60
Baghdad / Tarmiyah	Mp4	3.33	33.33
Karbala / Haidari	Mp5	3	30
Karbala / Hussainiya	Mp8	0.33	3.33
LSD (0.05)	-	0.815	8.16

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

germination in it was 3.33%, followed by isolate Mp9 isolate Karbala Al-Hussainiya governorate and the percentage of germination was 23.33%, while the percentage for the rest of the isolates ranged between 30.00-60.00%.

The reason for the variation of isolates in their effect on the percentage of germination of cabbage seeds is due to genetic differences between isolates of fungi collected from several regions in different governorates, or the difference of these isolates in their ability to produce toxins or enzymes degrading pectin and cellulose in the early stages of infection and these enzymes Phosphatase, pectinase, cellulase, pectin methylesterase, pectin lyase, which have an effective role in the pathogenesis of fungi, have a significant role in penetrating the plant host and causing infection. This was confirmed by Issa *et al*. (2019). In this test, the isolates with the most reduced germination of cabbage seeds, namely Fs1, Rs5 and Mp8, were selected for subsequent tests.

3.4 Evaluation of the antagonistic ability of effective microorganisms (EM1) in inhibiting the growth of the fungi *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* on PDA medium.

The test results are shown in **Table 4**. The ability of the EM1 microorganism preparation to inhibit the growth of pathogenic fungi *F.solani* Fs1, *R.solani* Rs5 and *M. phaseolina* Mp8 on PDA culture media. The microorganism EM1 showed the highest effect at 5, 10 and 15% concentrations on the growth of pathogenic fungi, as the percentage of inhibition reached 100% compared with the control treatment of fungi alone, where the percentage of inhibition was 0.00%. While the concentration of 1 and 3 ml showed a decrease in the growth level of the colony diameter of the fungus Fs1, which reached 2.55 and 1.33 cm, respectively, and the percentage of inhibition was 71.29 and 81.18%, respectively. As for the isolate of the fungus *R.solani* Rs5, the diameter of the colony growth of the fungus at concentrations 1 and 3% was 2.67 and 1.25 respectively. It was clear that the higher the concentration, the higher the percentage of inhibition, reaching 70.37 and 86.11%, respectively, and the isolate of *M. phaseolina* Mp8. The percentage of inhibition at concentration 1 and 3 ml was 83.33 and 87.96%, respectively, and the diameter of the mushroom colony growth was 1.50 and 1.08. straight.. The results showed

Table 4: Effect of EM1 on the growth of the pathogenic fungi *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* on PDA medium.

Treatment	Concentration	Colony diameter.cm	Inhibition (%)
Control	0	9	0
EM1 + Fs1	1	2.58	71.29
	3	1.33	85.18
	5	0	100
	10	0	100
	15	0	100
Control	0	9	0
EM1 + Rs5	1	2.67	70.37
	3	1.25	86.11
	5	0	100
	10	0	100
	15	0	100
Control	0	9	0
EM1 + Mp8	1	1.5	83.33
	3	1.08	87.96
	5	0	100
	10	0	100
	15	0	100
LSD (0.05)	-	0.095	1.047

Each number represents an average of three replicates
Fs1 = *Fusarium solani*, Rs5 = *Rhizoctoniasolani*,
Mp8 = *Macrophomina phaseolina*, the number near the symbol represents the isolate number

that there is a direct proportion to the percentage of inhibition with an increase in the concentration of the biological preparation EM1 if it causes a significant reduction in the growth of pathogenic fungi, and the greater the concentration of the preparation, the higher the percentage of inhibition compared to the treatment of fungi alone, which amounted to 0.00%.

The inhibition of the pathogenic fungus is successful due to the by-products produced by microorganisms such as the antibiotics produced by the bacteria present in the EM1 antibiotic during fermentation, which directly affects the growth of many pathogens as well as the presence of yeasts and fungi.

3.5 Evaluation of the efficiency of different concentrations of normal and Nano-chitosan in inhibiting the growth of the fungi *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* on PDA culture medium

The results in Table 5 showed that the addition of

normal chitosan to the culture media at a concentration of 1, 3, 5, 10 and 15% led to the inhibition of the growth of the fungi *R. solani, F. solani* and *M.phaseolina*, on the PDA culture medium, if the percentage of inhibition of the Fs1 isolate for *F. solani* 65.73, 78.70, 100.00, 100.00, and 100.00%, respectively, and the addition of Nano-chitosan to the culture media at the same concentrations led to the inhibition of the growth of *F. solani* on the PDA culture media with inhibition rates of 77.77, 87.03, 100.00, 100.00, and 100.00% on the respectively, compared to the comparison treatment, where the percentage of inhibition was 0%. As for the fungus *R.solani*, the results showed that all the normal chitosan concentrations had an effective effect in reducing the growth of pathogenic fungi and increasing the percentage of growth inhibition of the fungus isolate Rs5 compared to the comparison treatment, which amounted to 0% and it led to an increase in the percentage of fungal inhibition, reaching 68.51, 82.40, 100.00, 100.00, and 100.00%, respectively, and when adding Nano-chitosan, the percentage of inhibition increased at the same concentrations used in regular chitosan, as the percentage of inhibition reached 75.00, 84.26, 100.00, and 100.00 100.00%.

The percentage of growth inhibition of the fungus M. phaseolina when adding the same concentrations of normal chitosan to the culture media reached 65.73 and 85.18%, while the concentrations of 5, 10 and 15% reached 0%, and the percentage of growth inhibition of phaseolina increased. M when adding Nano-chitosan at concentration 1 and 3% reached 69.44 and 88.88%, respectively, and reached 0% at concentrations 5, 10 and 15%.

The results of the study showed that the inhibitory activity of chitosan in the growth of the fungi *F. solani*, *R. solani* and *M. phaseolina* on PDA culture media increased with increasing concentration. The best inhibitory activity was found at 5% concentration compared to the comparison treatment, which did not have inhibition. The superiority of the concentration of 5% on all concentrations and the comparison treatment is explained by the fact that the concentrations of the active substances were high, so the inhibitory activity was the best, and this may be due to the effect of chitosan on the DNA of pathogenic fungi, which led to the suspension of the activity of some enzymes and proteins necessary for the growth of fungi. Emmanual *et al.* (2010) indicated the mechanism of chitosan against the growth of pathogens lies in increasing the permeability of the cell membrane as a result of the interaction of the positively charged chitosan with the membrane of the negatively charged fungi and inhibits the synthesis of essential enzymes and proteins due to the change in DNA.

Table 5: Different concentrations of normal and Nano chitosan elicit growth inhibition of *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* on PDA culture medium.

Treatment	Concentration	Colony diameter.cm	Inhibition (%)
Control	0	9.00	0.00
chitosan + Fs1	1	3.08	65.73
	3	1.92	78.70
	5	0.00	100.00
	10	0.00	100.00
	15	0.00	100.00
control	0	9.00	0.00
chitosan + Rs5	1	2.83	68.51
	3	1.58	82.40
	5	0.00	100.00
	10	0.00	100.00
	15	0.00	100.00
control	0	9.00	0.00
chitosan + Mp8	1	3.08	65.73
	3	1.33	85.18
	5	0.00	100.00
	10	0.00	100.00
	15	0.00	100.00
Control	0	9.00	0.00
Nano-chitosan + Fs1	1	2.00	77.77
	3	1.17	87.03
	5	0.00	100.00
	10	0.00	100.00
	15	0.00	100.00
control	0	9.00	0.00
Nano-chitosan + Rs5	1	2.25	75.00
	3	1.33	84.26
	5	0.00	100.00
	10	0.00	100.00
	15	0.00	100.00
control	0	9.00	0.00
Nano-chitosan + Mp8	1	2.75	69.44
	3	1.00	88.88
	5	0.00	100.00
	10	0.00	100.00
	15	0.00	100.00
L.S.D 0.05		0.182	1.898

The results of this study also showed that the Nano-chitosan had the highest inhibition rate. This is due to the conversion of ordinary chitosan to Nano-chitosan. The physical, chemical and biological properties of ordinary chitosan change. The Nano-chitosan has the unique properties of Nanoparticles such as small size, high surface area, surface charge and its ability to break down biological barriers, accumulate in the target region and penetrate the cell membrane**.**

4. Conclusion

We conclude from the current study the inhibitory activity of chitosan in the growth of the fungi *F. solani, R. solani* and *M. phaseolina* on PDA culture media increased with increasing concentration,the microorganism preparation had high antagonistic ability against fungi caused root rot disease.

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