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Evaluating the Efficacy of *Azotobacter chroococcum* Against the Pathogens of Okra Root Rot Disease in Babylon Province

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Abstract: *The field survey results revealed the spread of okra root rot disease in all surveyed areas. The results of isolation and diagnosis showed the presence of 6 fungal isolates associated with the roots of okra plants, differing in appearance in different regions. As Fusarium solani achieved the highest percentage of occurrence, it appeared in all samples with 80%, followed by Macrophomina phaseolina with an appearance of 44%, and Rhizoctonia solani recorded 21%. The results for pathogenicity showed that all tested fungi caused a significant reduction in the germination rate of radish seeds. The results indicated the effect of Fusarium. spp (FA3, FK2, FA1, FR1, FD2, FC1) and M.phaseolina (MB1, ME1) and R.solani (RR1) on germination of okra seeds. In infection of okra plants, the percentage of infection severity reached (88.3, 85)%, compared to the control treatment, which was 0%. The study showed that Azotobacter chroococcum has a high antagonistic capacity against the pathogenic fungi FA3 and ME1, and the inhibition rate varied according to the concentration or dilution used. The lath house results showed that A. chroococcum was controlled by the pathogen F.solani and M.phaseolina fungi. The severity of infection reached (31.66 and 28.33)%, respectively, compared to the treatment of pathogenic fungi alone, which amounted to (77.52 and 83.44%) respectively. The treatment of infection between bacteria and pathogenic fungi in the field experiment also achieved control in the severity of the disease and an increase in growth parameters measured, compared to the treatment of pathogenic fungi alone.*

Keywords: *Okra plant; Azotobacter chroococcum*; *Fusarium solani*; *Macrophomina phaseolina*.

1. INTRODUCTION

Okra plant, *Hibscus esculentus*, belongs to the Malvaceae family. In Iraq, it considered one of the important crops in the agricultural sector due to its high nutritional value. The area cultivated in Babylon province reached 7446 acres and production reached 9689 tons [1].Okra is one of the plants that are affected by many agricultural pests, including fungal diseases, which have increased their spread with protected agriculture, and one of the most important of these diseases, seedlings death disease, root and stem rot, which are common diseases of okra crop in open cultivation and are caused by fungi (*Fusarium* spp., *Macrophomina phasiolian*, *Aspergillus sulphorus*, *Rhizoctonia solani* and *Phytophthora* spp.) [2],[3] There are several methods used to control pathogenic fungi, including chemical control, but the repeated use of them led to the emergence of several problems, including the emergence of the characteristic of resistance as well as its danger to human and animal health as a result of its pollution of the environment [4] And as a result of the increase in the risk of plant diseases on the one hand and the disadvantages of chemical pesticides, on the other hand, the efforts of researchers have turned to the use of the biological

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control method, where many studies indicated the success of biological control in controlling a number of plant pathogens [5],[6] In order for it to be effective, all the elements for its success in the control must be present, so the importance of using more than one biological control agent that works under different environmental conditions and different aspects has emerged to achieve an integrated system for biological control [7]. Therefore, the researchers' efforts were directed to using microorganisms in controlling the pathogens of plants, including root pathogens and among the microorganisms used in this field are types of fungi (*Aspergillus, Trichoderma, Penicillium*) [8] This study aimed to isolate and diagnose pathogens. Okra root rot disease and it's biological control using the bacterium *Azotobacter chroococcum*.these bacteria stimulate plant growth through their ability to produce intibiotics and many growth regulators and increase plant resistance to unfavorable environmental conditions.[9]

2. MATERIALS AND METHODS

2.1.Field survey

Field surveys were conducted in several areas of okra cultivation fields in Babylon province. The survey areas included 8 locations from the province (Table 1). The infected and healthy plants located within the intersection of each location were examined and the number of affected plants was calculated in light of the symptoms appearing on the plants represented by yellowing of leaves, color of stems and wilting, and the Disease incidencewas calculated for each field according to the following equation:

$$\text{Disease incidence (\%)} = \frac{\textit{The number of infected plants}}{\textit{Number of plants tested}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The roots of the infected plants were taken to the laboratory, and after they were placed in polyethylene bags, they were marked and kept in the refrigerator at a degree of 4 ° C. to conduct the isolation of the fungi associated with the roots of the plants from each sample on the next day:

Table1. The temporal and spatial distribution of okra fields from which samples were taken

Sample number	Collection area	The date of taking the sample
1	Jableh sub-district	4/3/2019
2	Al-Mahawil District (Center)	5/3/2019
3	Al-Kifl sub-district	9/3/2019
4	Al-Mahawil District (Al-Aziziyah area)	12/3/2019
5	Al-Nile sub-district (center)	16/3/2019
6	Al-Nile sub-district (Sridiba region)	28/3/2019
7	Al Hamza sub-district	31/3/2019
8	Abu Gharaq sub-district	13/4/2019

2.2.Isolation and diagnosis of okra root associated fungi

The okra plants that had symptoms of infection were brought to the laboratory the next day of the collection, the roots were washed with running water to remove the impurities, they were cut into small pieces and surface sterilized with a solution of sodium hypochlorate at a concentration of (1%) for 3 minutes, after which the roots were washed with sterile distilled water for three times and dried with

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leaves , The pieses was with Petri dishes containing the PDA culture medium added to the Tetracycline antibiotic after sterilization with an autoclave, with four pieces per dish. The dishes were incubated at a temperature of 25 for three days, after which the examination was performed to check for the presence of pathogenic fungi and accompanying and counted, then purified by taking small pieces from them and placed in the center of the plate containing the cultivated media PDA and the races were diagnosed by Dr. Majeed Mutaib Diwan and Dr. Ahmed Kadhim Abd Al Hadi based on the characteristics mentioned by [10], [11] [12], which include the color of the fungal colony, the branching nature of the modern mycelium, and the ability to form stone bodies. The percentage of occurrence of the studied fungi was calculated according to the following equation,

$$\text{Percentage of appearance (\%)} = \frac{\textit{The number of root pieces in which the fungus appeared in the dishes}}{\textit{The total number of pieces used in the sample}} \times 100 \ldots\ldots (2)$$

2.3.Preparing the pathogen inoculum

The fungi were grown on the local millet seeds , *Panicum miliaceum* L., after washing it well to remove the dust and impurities stuck to it, then soaking for 6 hours in water, then leaving on a piece of gauze for half an hour to remove the excess water from it. 0.5 cm diameter of the culture medium containing the pathogen for 15 days, ensuring aeration and distributing the fungal inoculum inside the beaker [13].

2.4. Testing the pathogenicity of Fusarium solani, Rhizoctonia.solani and Macrophomina. Phaseolina isolates using radish seeds

Pathogenicity was tested for isolates of fungi (18) isolates from *Fusarium.solani* and 5 isolates from *Macrophomina phasealina*. and 3 isolates of *Rhizoctonia solani*) on PDA the transplant center 5 days old , both separately. The dishes were incubated at a temperature of 25 for period of three days , after which tye radish seeds were sown topically sterilized with a solution of sodium hypochlorate(1)% and placed in acircular motion near the edge of the plate at a rate of 25 seed per plate . 3 plates were used for each isolate as replicated in addition to to the comparison treatment (without adding a pathogen). the percentage of germination was calculated as in the following equation:

$$\text{percentage of germination (\%)} = \frac{\textit{The number of germinated seeds}}{\textit{The total number of seeds}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

2.5.Preparation of Azotobacter chroococcum bacterial suspension

The bacteria (A.z), which were obtained from the laboratory of postgraduate studies - Al-Mussaib Technical College (isolated from the fields of the wheat and tomato plant farm and previously diagnosed), were grown on the nutrient broth N b) by placing 50 ml of the medium in a 100 ml conical flask and after sterilizing it with an inoculating apparatus. From a modern, one-day-old farm, these bacteria were used using a conveyor, and the flasks were incubated in the vibrating incubator at a temperature of 30 ° C for a period of 3-5 days to obtain a greater quantity of bacterial suspensions for the purpose of using it in the experiments of lath house and field [14].

2.6.Calculate the numerical density of bacteria:

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The Plate Count Technique was followed by the method of counting colonies in the dishes to calculate the total number of bacteria *Azotobacter chroococcum* (Az). Relieve 10^{-1} to 10^{-10} was prepared by transferring 1 ml of the growing bacteria culture to the medium of liquid activation at a 3-day age to a series of sterile distilled water tubes using Sterile pipettes,By transferring 1 ml starting from the first dilution to a petri dish containing the Nutrient Agar medium with the plate moving in a rotating motion, then the dishes were incubated at a temperature of 28 ± 1 ° C for a period of 1-3 days, the number of bacterial cells was calculated as follows: The number of bacteria / ml of the original sample = the number of colonies in the plate x the reciprocal of the sample dilution [15].

2.7.Determination of effective dilution of Azotobacter chroococcum against Fusarium solani and Macrophomina phaseolina

After preparing the dilution of the bacterial suspension, the plates containing the PDA culture media were inoculated and moved in a rotational motion and a disc was taken from the edge of the fungal colony of the pathogen *Fusarium solani* and *Macrophomina.phaseolina* grown on the PDA culture medium at 7 days old each separately, at an average of 3 plates per dilution, leaving 3 plates of fungus pathogen for comparison and left for three days in the incubator, after which the percentage of inhibition was calculated according to the following equation [16]:

$$\text{Percentage of inhibition (\%)} = \frac{\text{fungus growth in control treatment} - \text{fungus growth in treatment}}{\text{fungus growth in control treatment}} \times 100 \quad \text{…….(4)}$$

2.8.Evaluating the effect Azotobacter chroococcum In the severity of infection with pathogenic fungi and some growth parameters of okra under lath house conditions.

The experiment was conducted in the wooden canopy of the College of Technology / Al-Mussaib on 1/10/2020 (*Azotobacter chroococcum*) was added to the soil according to Singh et al.2008 method, where 10 ml of bacterial suspension was added to each bag at a concentration of 106 × 26 (colony formation unit). Three days before adding the pathogenic fungus, Beltanol was added within the aforementioned treatment at a concentration of 1 ml / liter after one day of adding the pathogen, which was added at 1% (weight / weight) and left the control treatment without any addition [17 and 18]. As for the experiment treatments, they are as follows:

1-Fusarium solani (F.s3)

2.Macrophomina phaseolina (M.ph8)

3-Bacteria Azotobacter chroococcum (A.z)

4- Bacteria (A.z) + fungus (F.s)

5-Bacteria (A.z) + Fungus (M.ph)

6-Beltanol + Fungus (F.s)

7 - Beltanol + fungus (M.ph

8- control treatment (Control)

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Then the percentage of infection severity and some growth parameters of okra were calculated.

2.9.Evaluating the effect of the Azotobacter chroococcum In the severity of infection with pathogenic fungi and some growth parameters of okra under field conditions

The field experiment was conducted in the spring season 15/3 of 2020 in one of the fields of the Al-Mahaweli, using The Randomized Complete Block Design (RCBD), the bacterial suspension was added at a rate of 30 ml / bottle when culture [19], and the chemical pesticide Beltanol was added at a concentration of 1 ml / liter of water In the amount of 15 ml / pit according to the recommendations of the producing company for the required treatment. As for the control treatment, only okra seeds were used. The pathogenic fungus vaccine was added to the slit, each according to its treatment, loaded on the seeds of local millet at an average of a 250 ml beaker containing 50 g of fungal inoculum for each breed [20]. Results were taken after 100 days of cultivated, the percentage of infection severity and some growth parameters of okra were calculated.

3. RESULTS AND DISCUSSION

3.1. Field survey

Table (1) shows the spread of okra root rot disease in all fields covered by the survey, where the percentage of infection ranged between (22-44)%. The highest percentage of infection was recorded in the fields of Jabla sub-district, which amounted to 44%, and the lowest percentage of infection was recorded in Nile sub-district, which amounted to 22%.

Table 2. Percentage of infection with okra root rot disease in some fields of Babylon province.

No	Location	The percentage of infection (%)
1	Jabla sub-district	44
2	Al-Mahawil District (Center)	33
3	Al-Kifl sub-district	37
4	Al-Mahawil District (Al-Azzawiya region)	41
5	Nile sub-district	22
6	Nile Sub-district (Sridiba Region)	25
7	Al-Hamza sub-district	33
8	Abi Gharaq sub-district	37

3.2. Isolation and diagnosis of fungi associated with the roots of infected okra plants

Several genera of fungi were isolated and diagnosed in the roots of infected okra, *Fusarium solani*, isolated from all surveyed areas, where the percentage of its appearing amounted to (80%), followed by the *Macrophomina phaseolina* fungus which was isolated from 5 regions, and the percentage of its appearing amounted to (44%), and the *Rhizoctonia solani* fungus found in three regions, and the percentage of its appearing amounted to (21%) as shown in Table (3) [21]. The results of the diagnosis showed the presence of many fungi associated with the roots of okra plants, such as *Aspergillus* spp., *Penicillium* spp. And *Alternaria* spp.

3.3. Testing the pathogenicity of isolates of F.solani, M. phaseolina, and R.solani fungi, using radish seeds

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Table (4) shows that the pathogenic fungi isolates led to a reduction in the percentage of germination, where it ranged between 0-52% compared to the control treatment in which the percentage of germination amounted to 100% and The fungal isolates (F_{A3} F_{E1}, F_{K1}, F_{R1}, M_{B1}, M_{E1} F_{C1}, F_{D2}) have excelled in reducing the percentage of germination over the rest of the isolates, which amounted to (0%).

Table 3. The percentage of the presence of fungi associated with the roots of okra plants infected in different locations of Babylon province.

Name of fungi	The presence of fungi in locations								The highest percentage of infection
	Jabla sub-district	Al-Mahawil District	Al-Kifl sub-district	Al-Mahawil District (Al-Azzawiya region)	Nile sub-district	Nile Sub-district (Sridiba Region)	Al-Hamza sub-district	Abi Gharaq sub-district	
Fusarium sp	75	33	50	50	56	42	80	25	80
Macrophomina phaseolina	44	25	-	-	25	14	-	25	44
Rhizoctonia solani	19	8	-	-	-	21	-	12	21
Aspergillus spp	-	-	-	18	14		-	-	18
Penicillium spp	-	16	7	-	-	14	-	18	18
Alternaria spp	6	-	50	6	-	-	-	-	50

- It means that the fungus isolate did not appear at the above location

Table 4. Testing the pathogenicity for the isolates of pathogenic fungi, F.solani and M. phaseolina and R.solani fungi using radish seeds on PDA agriculture media.

isolate	**The percentage of germination**	**isolate**	**The percentage of germination**	**isolates**	**The percentage of germination**
F_{A1}	8	M_{A1}	12	R_{A1}	52
F_{A2}	20	-		-	
F_{A3}	0	-		-	
F_{A4}	12	-		-	
F_{B1}	30	M_{B1}	0	R_{B1}	40
F_{B2}	12	-		-	
F_{C1}	0	-		-	
F_{D1}	20	-		-	
F_{D2}	0	-		-	
F_{E1}	0	M_{E1}	0	-	
F_{E2}	20	-		-	
F_{k1}	6	-		-	
F_{k2}	0	-		-	
F_{k3}	36	-		-	
F_{k4}	40	-		-	
F_{H1}	12	M_{H1}	8	-	
F_{R1}	0	-		R_{R1}	6
F_{R2}	16	-		-	
L.S.D					**2.14**

* Each number in the table represents an average of three replicates
** The following symbols represent (A = Jabla sub-district, B = Al-Mahawil District (Center), C = Al-Kifl sub-district, D = Al-Mahawil District (Al-Azzawiya region), E = Nile sub-district, K = Nile Sub-district (Sridiba Region), H =Al-Hamza sub-district, R = Abi Gharaq sub-district)

*3.4. The severity of okra plant infection with the pathogens of okra root rot disease,*by *Fusarium solani, Macrophomina phaseolina, and Rhizoctonia solani.*

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Table (5) shows that the severity of infection for the two isolates (FA3 and ME1) amounted to (88.3, 85)%, respectively, followed by the other isolates (FK2, FA1, FR1, FD2, FC1, MB1, RR1) which amounted to (76.66, 74, 65, 62.5, 60, 58.23, 56.5%), respectively. The reason for the difference in isolates is attributed to their ability to secrete enzymes that degrade pectin, cellulose and lignin, which can play a role in penetrating plant roots [22]. These results confirmed the results of the experiment with the pathogenicity of these isolates on radish seeds in the previous experiment.

Table 5. The percentage of The severity of okra plant infection with the pathogens of okra root rot disease, *Fusarium solani, Macrophomina phaseolina*, and *Rhizoctonia solani*.

Symbol of Isolate (%)	The percentage of the severity of infection
F_{A1}	74
F_{A3}	88.3
F_{C1}	60
F_{D2}	62.5
F_{E1}	71.6
F_{k2}	76.66
F_{R1}	65
M_{B1}	58.23
M_{E1}	85
R_{R1}	56.6
Control	0

Each number represents an average of three replicates

3.5. Testing the antagonistic capability of Azotobacter chroococcum against pathogenic Fusarium solani and Macrophomina phaseolina fungi on the PDA

Table (6) shows that the effect of *A. chroococcum* on the pathogenic fungi *F.solani* and *M.phaseolina* amounted to (86, 78.66)%, respectively, compared to the fungi treatments without adding bacteria, which amounted to (0.00%). The effect of using these bacteria in inhibiting the growth of the pathogen may be attributed to their ability to compete with the fungal pathogen in utilizing the nutrient media well, which limits the growth of the fungal pathogen [19].

Table 6. Test of the antagonistic capability for the bacterial control factor against isolates of pathogenic fungi (F.s and M.ph) and the percentage of inhibition on the PDA agriculture media.

Bacteria	Pathogenic fungi	The diameter of Colony (cm)	%inhibition
A.chroococcum	*F.solani*	1.25	86
	M.phaseolina	1.92	78.66
	Control	9	0.00

3.6. Evaluating the effect of the Azotobacter chroococcum on the severity of infection with the pathogenic fungi Fusarium.solani and Macrophomina phaseolina and some growth parameters of okra plants under lath house conditions.

Table (7) shows that the interaction treatment between *A. chroococcum, F.solani* and *M.phaseolina* bacteria caused a significant reduction in the percentage of the severity of infection, which amounted to

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(28.33, 31.66)%, respectively compared to the treatment of pathogenic fungi alone, which amounted to (77.52 and 83.44)% respectively. The interaction treatment between bacteria in the presence of pathogenic fungi also achieved a significant increase in growth parameters such as height and fresh weight of the total vegetative and root system, which amounted to (29.31, 12.33) cm and (11, 28.10 cm), respectively, and (19.62, 10.92, 6.66, 3.13 g), and (19.08, 8.76, 6.6, 3.33 g), respectively, compared to the treatment of pathogenic fungi alone, which amounted to (12.2, 4.96 cm) and (83.44, 10.86 cm), respectively and (9.09, 3.55, 3.85, 1.46 g) and (7.84, 2.38, 3.52, 1.09 g), respectively. The reason for this is that the *A.chroococcum* bacteria produce anti-fungal metabolites, including Siderophores that are used to control fungal diseases[23].thes bacteria A.chrooccocum produced and secretes various growth regulators such as Auxin. Cytokinin. Gibberellin. Which positively affects plant growth by increasing the root system and its ability to absorb nutrient and increase the fixation of atmospheric nitrogen. All of help the plant grow significantly and increase its productivity[24].

Table 7. Evaluating the effect of the biological control factor *Azotobacter chroococcum* and Beltanol on the severity of infection with the pathogenic fungi .

Treatment	%infection	Plant length cm		Fresh weight gm		Dry weight gm	
		vegetative	root	vegetative	root	vegetative	root
A.z	0	31.06	16.33	21.86	13.11	8.64	3.75
F.s	77.52	12.2	4.96	9.09	3.55	3.85	1.46
M.ph	83.44	10.86	3.66	7.84	2.38	3.52	1.09
A.z+F.s	28.33	29.31	12.33	19.62	10.92	6.66	3.13
A.z+M.ph	31.66	28.10	11.00	19.08	8.76	6.6	2.33
Beltanol+F.s	23.33	30.72	14.00	19.55	12.55	6.64	3.07
Beltanol+M.ph	25	29.64	13.33	17.61	11.93	5.16	2.66
Control	0	27.92	12.33	18.77	10.4	6.44	3.38

3.7. Evaluating the effect of the biological control factor Azotobacter chroococcum and Beltanol on the severity of infection with the pathogenic fungi .

Table (8) shows that the interaction treatment between *A.chroococcum*, *F.solani* and *M.phaseolina* had a significant reduction in the percentage severity of infection, which amounted to (30, 30)%, respectively, compared to the treatment of pathogenic fungi alone, which amounted to (78.66, 83)% respectively. The treatment of the interaction between bacteria in the presence of pathogenic fungi also achieved a significant increase in growth parameters such as height and fresh weight of the total vigitative and root system, which amounted to (64.99, 23.66 cm) and (66.66, 25.44 cm), respectively and (142.06, 36.5, 64.5, 19.82 g) and (164.2, 34, 77.45, 19.3 g), respectively, compared to the treatment of pathogenic fungi alone, which amounted to (62.33, 21.88) cm and (65.55, 22.66 cm), respectively, (104.54, 25.25, 47, 12.81 g) and (114.11, 31.5, 51.3, 14.66 g), respectively. *A. chroococcum* bacteria produce and secrete some growth regulators such as Auxin, Cytokinin, Gibberellins, acid Indole 3−acetic, which positively affect plant growth by increasing 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, the speed of plant growth significantly, and increase its productivity [25], [26].

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Table 8. Evaluating the effect of the biological control factor Azotobacter chroococcum and Beltanol on the severity of infection with the pathogenic fungi .

Treatment	The percentage of infection	Plant length		Fresh weight		Dry weight	
		vegetative	root	vegetative	root	vegetative	root
A.z	0	76.33	29.88	207.96	46	105.35	30.67
F.s	78.66	62.33	21.88	104.54	25.25	47	12.81
M.ph	83	65.55	22.66	114.11	31.5	51.3	14.66
A.z+F.s	30	64.99	23.66	142.06	36.5	64.5	19.82
A.z+M.ph	30	66.66	25.44	164.2	34	77.45	19.30
Beltanol+F.s	25.83	73.55	29.77	231.06	46	116	34.00
Beltanol+M.ph	30.83	68.99	26.66	169.4	39.5	138.5	25.66
Control	0.0	72.77	28.32	169.86	33.5	98.4	21.16

4. CONCLUSIONS

It was found that there are mony fungi that cause okra root rot disease (*Fusarium solani* , *Macrophomina phaseolina*) that have a high pathogenic ability against okra plant andThe bacterium *Azotobacter chroococcum* has a high antagonism against that fungi pathogenic to okra root rot disease. These Azotobacter chroococcum possesses many enzymes that inhibit the action of pathogenic fungi.

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