Resistance of the Fusarium solani fungus isolated from the roots of Citrus aurantium by Azotobacter chroococcum and poultry fertilizer

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ABSTRACT

This experiment was conducted to evaluate the effect of the bio-agent *Azotobacter chroococcum* and poultry fertilizer with three percentages (10%,15%,20%) against the pathogenic fungus (*Fusarium solani*) under lath house conditions. The results showed that there was a positive effect when using bacteria with fertilizer on the plant and reducing the pathogenicity of the fungus and its effect on the plant, where using *Azotobacter chroococcum* bacteria with poultry fertilizer by 15% gave good results such as the fresh weight, dry weight for the total vegetative and root system, and the plant length for the total vegetative and root system, which amounted to (15.60, 8.90, 9.40, and 5.80 g/plant, 51.00, and 38.06 cm/plant), respectively compared to the control treatment which amounted to (5.60, 2.70, 2.00, and 1.00 gm/plant, 42.60, and 31.00 cm/plant), respectively where the fertilizer encouraged the growth of bacteria as well as inhibited the growth of the fungus and its lack of adaptation in the soil, and the highest percentage of inhibition for the fungus was by treating Azotobacter with the poultry fertilizer (15%) with the pathogenic fungus. The fresh weight and dry weight for the total vegetative and root system, and the plant length for the total vegetative and root system were (10.80, 5.80, 6.20, 3.00 g/plant, 47.10 and 35.10 cm/plant), respectively. The results were compared with the fungus treatment alone, and they were (1.00, 0.60, 0.50, 0.00 g/plant, 9.10 and 5.00 cm plant), respectively. Significant and clear differences appeared when bacteria and fertilizer were used with

Key Words: Resistance fungus, *Fusarium solan* fungus, root infection

1. INTRODUCTION

The original home of citrus is the tropical region that lies between India and China in the northwest, and Australia and Caledonia in the southeast, due to the spread of its wild origins in those regions (2). From an economic point of

view, citrus has an important position in the global agricultural economy and is considered one of the most important fruit trees in the world,

and occupies the first position in global production (13). During the period from 1996 to 2005, citrus production constituted about 20% of the production of fruit at the global level, and the quantity produced globally ranged about 89 million tons for the world of 2004. Citrus is produced in 104 countries and 70% of the production is concentrated in the northern hemisphere, mainly in Brazil, the countries of the Mediterranean basin, the United States, and China, where the total production of these countries constitutes more than two-thirds of the world's production (World Food and Agriculture Organization, 2007). The genus Citrus is located within the Rutaceae family, which includes several groups, including the orange group, including Sweet orange (Citrus sinensis) and Sour orange (Citrus aurantium), the group of mandarin orange, which includes several species including lemon (Citrus limon) and sweet lemon (Citrus limeta) . As for the economic aspect, citrus trees are considered one of the most important fruit trees in the world and have an important place where they occupy the first position in the total global production, which in 1999 amounted to about 98258000 tons (13). The reason for the deterioration is attributed to root rot disease caused by Phytophthora citrophthora (5) as well as *Fusarium solani*, which is almost the dominant fungus among the fungi isolated from citrus roots with high frequency even from apparently infected bio-agent *Azotobacter chroococcum.*

2. MATERIALS AND METHODS

Isolating and diagnosing fungus:

Some samples were taken from citrus roots that showed symptoms of root rot disease from some orchards in Babylon Governorate to laboratory and cut 0.5 cm in size and washed with running water for 15 minutes and superficially sterilized with sodium hypochlorite solution (1%) for 3 minutes, then washed with sterile distilled water for 3 minutes, they were dried with filter paper, transferred by sterile forceps, and 4 pieces were cultured in each Petri dish with a diameter of 8.5 cm containing 15-20 cm^3 of cuilture media (PDA) to which the antibiotic Tetracycline was added at a concentration of 200 mg . The dishes were incubated at 25 ± 2^0 C for 7 days, after which the examination was conducted to check for the presence of the accompanying fungi and purify them by transferring small pieces of the ends of the fungal hyphae and placing them in the center of a Petri dish containing the PDA

culture medium. The dishes were incubated for 7 days (21) and Isolates were then kept in test tubes containing culture media (PDA) to identify the fungi to the level of genus andspecies after the emergence of fungal growths based on the characteristics of the fungal colony, the nature of the mycelium, the spores and the structures that they form and using the approved taxonomic keys (18).

—, _____ the avera ae di.ame ter o f controI co Ion y - ave ra a e di.ame ter o f tre a tment co Iony
The percentage of inhibition =
the average diameter of control colony
X 100

Detection of pathogenic isolates using cabbage seeds

The pathogenicity of isolates of F. solani was tested according to the method of (8). Petri dishes with a diameter of 8.5 cm were prepared that containing 20-15 ml of the culture medium (20 g agar, 1 L distilled water), and the antibiotic was added to it at 200 mg/L. The dishes were inoculated in the center with a disc (0.5 cm) of the fungi cultures grown on PDA culture media at the age of 7 days separately. The dishes were incubated at 25 ± 2 °C for 7 days, after which sterilized local seeds of cabbage were sown with a solution of sodium hypochlorite (1%) in a circular motion near the edge of the dish, at a rate of 25 seeds / dish. The treatments were replicated three times in addition to the control treatment (without fungus) and incubated at a temperature of 25±2°C, and after 7 days the percentage of germination was calculated. Petri dishes containing the culture medium (PDA) were inoculated by placing a piece of 5 mm diameter from the preserved fungus colony and with four replicates for each isolate. The dishes were incubated at a temperature of 25 + 2 ° C for 7 days. A day before the end of the incubation period, the seeds of local millet Panicum miliaceum were taken and washed well to get rid of impurities and dust, then the seeds were soaked for 6 hours, after which the excess water was removed by filtering it with a cloth. The seeds were distributed in 250ml glass beakers at a rate of 50 g / beaker. The beakers were sterilized by autoclave at a temperature of 121 °C and a pressure of 1.5 kg / cm2 for 20 min. After cooling the beakers, they were inoculated with the isolate of the fungus (F. solani) at a rate of 4 tablets of 5 mm in diameter / beaker at the age of 7 days (four replicates for each isolate). The beakers were incubated at a temperature of 25 + 2 °C for two weeks and the flasks were shaken once every 5 days to ensure ventilation and the distribution of the fungus inoculum to

all seeds (11).

Preparing the suspension of bacteria (A.chroococcum)

A quantity of bacterial suspension was prepared and used in the experiments of lath house, where the bacteria were grown on the liquid activated medium N.B by placing 50 ml of this medium in a glass beaker of 100 ml and inoculated with bacteria taken from a one-day-old culture. In order to obtain a larger amount of inoculum for the experiments of lath house, 250 ml conical flasks containing 100 ml of sterile liquid activation medium were used. The inoculated flasks were incubated in an incubator at 37 °C for 48 hours (7).

Determination of the effective concentration of the growth-inhibiting bacterial suspension (A. chroococcum) of the pathogenic fungus (F.solani)

By taking 1 ml of the liquid medium containing bacteria at the age of 1 day, using a sterile pipette, and adding to a test tube containing 9 ml of sterile distilled water. All tubes were inoculated by taking 1 ml from the first tube and adding it to the second tube using a sterile pipette. The process was repeated on the remaining tubes to obtain a series of dilutions 10^{-1}-10^{9}. The dishes containing the PDA culture medium were then inoculated by taking 1 ml/dish of each dilution of the bacterial suspension in the form of circular spots, and placed in its center, a disc with a diameter of 0.5 cm taken from near the edges of the F.solani colony. F.sa isolate grown on PDA medium at the age of 7 days. Four dishes were left for each fungus to compare without bacteria inoculation. 1 ml of sterile distilled water was added to it (3; 4). The dishes were incubated at a temperature of 25 ± 2 °C for 3 days, after which the percentage of inhibition was calculated by calculating the diameter of the colony of the developing fungus in the bacterial treatment and comparing it with the diameter of the colony of the developing fungus in the control treatment. The percentage of fungal growth inhibition was calculated according to the equation of (20).

Evaluating the efficiency of bio-agents (A.chroococcum) in reducing the infection severity of citrus seedlings (with age 103 days) with the pathogenic fungus (F. solani) under lath house conditions.

The experiment was conducted at Al-Musayyib Technical Institute, in which a completely random design (CRD) was adopted. Citrus seeds were planted, and after forty days of planting

the seeds, One seedling was transferred at the age of two months in plastic pots with a diameter of 20 cm, a height of 15 cm, and a capacity of 2.5 kg in a mixture soil, sterilized with oxidizer at a temperature of 121 ° C and a pressure of 1.5 kg / cm^2 for an hour. When the seedlings reached the age of five months, the process of treating was started on them with three replicates per treatment. The experiment included the following treatments:

fungus alone

1. fungus + poultry Fertilizer 10%
2. fungus + poultry Fertilizer 15%
3. fungus + poultry Fertilizer 20%
4. fungus + A. chroococcum
5. fungus + A. chroococcum + Poultry Fertilizer 10%
6. fungus + A. chroococcum+ Poultry Fertilizer 15%
7. fungus + A. chroococcum + Poultry Fertilizer 20%
8. Poultry Fertilizer 10%
9. Poultry Fertilizer 15%
10. Poultry Fertilizer 20%
11. A. chroococcum
12. A. chroococcum + Poultry Fertilizer 10%
13. A. chroococcum + Poultry Fertilizer 15%
14. A. chroococcum + Poultry Fertilizer 20%
15. control treatment

The pathogenic fungus (F. solani) loaded on millet seeds was added at a percentage of 1% (weight/weight) after loading it on local millet seeds. As for A.chroococcum suspension, irrigation water was added with a rate of 100 ml/plant (Larkin, 2004) at a concentration of 5 x 108 CFU/ml, respectively one week before contamination with pathogenic fungi, While the control treatment (bacteria alone) no pathogenic fungi were added to it. As for poultry fertilizer, it was added in three percentages of 10%, 15%, and 20% to the treatments that should be added to it. The results were taken in terms of the percentage of infection severity for the pathogenic fungus (F.sa) and the growth criteria represented by the fresh and dry weight and plant heights for the root system and total vegetative. The infection severity was calculated according to the pathological index consisting of five degrees, which are:

0- A root system of white color that did not show any infection and a well-grown total vegetative with a green color.

1- The rootstock was discolored in a dark brown color by more than 25%-50% with yellowing of a number of the lower leaves to include 25% of them.
2- The root system was discolored in a dark brown color by more than 50%-75% with yellowing of a number of the lower leaves to including 50% of them with the drying of the edges of the leaves.

The root system is discolored in a dark brown color with a percentage of more than 755 with yellowing of a number of the lower leaves,

3- including 75%, with the drying of the edges of the leaves with the fall of the lower leaves.
4- The root system is discolored in a dark brown color with a percentage greater than 76% 100% with yellowing of all leav

The percentage of the infection severity was calculated according to equation of (19).

$$\text{The percentage of the infection severity} = \frac{Number\ of\ plants\ in\ (grade\ 0\ x\ 0) + Number\ of\ plants\ in\ (grade\ 1\ x\ 1) + \ldots + Number\ of\ plants\ in\ (grade\ 4\ x\ 4)}{Total\ number\ of\ plants\ x\ highest\ degree\ of\ infection}$$

3. RESULTS

Isolating and diagnosing fungi:

The results of isolation from the roots of citrus trees showed symptoms of citrus root rot disease represented by discoloration of the roots in brown color and part of them to the presence of the fungus (*Fusarium solani*), whose growth appeared in all samples collected from some citrus orchards in Babylon province with a high presence rate of 90-100% When isolation was carried out from the roots using PDA culture medium, the characteristics of F. solani were also represented in its colonies that were isolated from all samples by the formation of white to milky mycelium. The microscopic examination also revealed the composition of the fungus with three types of microconidia, which are cylindrical to oval in shape, produced from long monophilides bearing an aerial fungal spinule, and macroconidia, which are spindle asymmetric and varying in their dimensions. The third type of spores is Chlamydospores, which produces Single or in pairs in small lateral branches, or in the middle of the mycelium, following the dichotomous key (10; 18).

Detection of pathogenic isolates of F.solani using cabbage seeds.

The results of this test (Table 1) showed that all tested isolates of fungus induced a significant reduction in the percentage of germination of cabbage seeds compared to the control

treatment in which the percentage of seeds germination was 100%. The isolates of the fungus (F. solani) also varied among themselves in reducing the percentage of germination, where the most effective of these isolates in the seeds germination cabbage was F1 isolate, which was isolated from orchards in some areas of Babylon Governorate, where the percentage of germination in its treatment was reduced to 0%. The two isolates (F.b s and F.s c) achieved a significant reduction in their effect on the germination of cabbage seeds, where the percentage of germination in their treatments was 2%, while the percentage of germination of cabbage seeds by the effect of isolate F.s g was 4%.

Table 1: The pathogenicity test of F.solani isolates by using the seeds of cabbage on Water Agar medium.

Location	Symbol of sam	percentage of germina
Al-Sadda (Al-Ealkay	F.s a	00
Al-Sadda (Al-Masayi	F.s b	2
Al-Musayyab (Sector	F.s c	2
Al-Musayyib (Sector	F.s g	4
control		100
LSD		1.406*

*Each number in the table represents four replicates

The difference between isolates belonging to the same species in the extent of their effect on the germination of cabbage seeds may be due to genetic variation due to different areas of sampling or a difference in the number of toxins secreted by these isolates such as Fusarubin and dihydrofusarubin, where isolates with high pathogenic potential are distinguished by their secretion of a greater amount of these metabolites than isolates with weak pathogenicity, and this was confirmed by a number of researchers (30; 8). The reason may be due to the difference in isolates in their ability to secrete enzymes that degrade kinase in the walls of host cells, such as Laccase and Lignin peroxidase, and this is important in causing infection and the spread of fungus toxins and enzymes in those cells (18).

Evaluating the efficiency of A.chroococcum bacteria in inhibiting the growth of the isolate of pathogenic fungus F.solani (F.s a) on PDA medium:

The results of Table (2) indicated that the use of the A. chroococcum bacteria at a concentration of 5 x 810 colony-forming units/ml led to the inhibition of the growth of the pathogenic fungus F. solani isolate to 79.4% on the culture medium compared to the control treatment, where the percentage of inhibition amounted to

0.00%.The effect caused by using these bacteria in inhibiting the growth of pathogenic fungi may be attributed to the ability of these bacteria to produce a number of enzymes that have the ability to degrade the cell walls of pathogenic fungi, including chitinase, laminarinase, and glucanase, and the production of antibiotics such as pyoluteorin, herbicolin, phenazine, as well as Its production is low molecular weight compounds that work to resist pathogenic fungi, including hydrogen cyanide (HCN), where the presence of this compound in high concentrations inhibits the growth of pathogenic fungi (26; 14).

Table 2: Evaluating the efficiency of A.chroococcum bacteria in inhibiting the growth of the isolate of pathogenic fungus F.solani (F.s a).

Treatment	Fungal growth (	The percentage of inhibiti
fungus+ A.chroococ	1.75	79.4
F.solani fungus (F.s	8.5 cm	00
LSD	4.32	45.1

Evaluating the effectiveness of bio-agents (A.chroococcum) and poultry fertilizer in reducing the infection severity of citrus seedlings with the pathogenic fungus F.solani and some growth parameters under lath house conditions:

The results of the study (Table 3) showed that treatment with A.chroococcum bacteria and poultry fertilizer at a rate of 15% achieved the highest values in growth parameters such as fresh weight, dry weight, and plant height for the root system and total vegetative, which were 15.60, 8.90, 9.40, 5.80 g/plant, 51.00 and 38.06 cm/ Plant compared to the control treatment, which amounted to 5.60, 2.70, 2.00, 1.00 g/plant and 42.60 and 31.00 cm/plant, and compared to the treatment of these bacteria with 15% of the fertilizer with pathogenic fungus, which was 10.80, 5.80, 6.20 and 3. 00 g/plant, 47.10 and 35.10 cm/plant, and the fungus alone, where they were 1.00, 0.60, 0.50, 0.00 g/plant, 9.10, and 5.00 cm/plant, respectively. The bacteria with the fertilizer achieved a clearreduction in the infection of the fungus to the plant and The lowest percentage of infection severity was achieved by treating A.chroococcum bacteria, pathogenic fungi, and poultry fertilizer by 15%, where it was 8.29. The reason is due to the different mechanisms that these bacteria possess to influence the pathogen, including the production of enzymes such as chitinase, and protease, antibiotics such

as Bacteriocin and Thuricin, and toxins, including delta-endotoxin, which degrade the cytoplasm of the fungal hyphae (31).

Table 3: Evaluating the efficiency of bio-agents (A.chroococcum) in reducing the infection severity of citrus seedlings (with age 103 days) with the pathogenic fungus (F. solani) under lath house conditions.

Treatments	Infection severity	Fresh Weight (g/plant		Plant heights (cm/pl		dry weight (g/plant)	
		Total vegetative	Root system	Total vegetative	Root system	Total vegetative	Root system
fungus alone	91.33	1.00	0.60	9.10	5.00	0.50	0.00
fungus + poultry Fertilizer 10%	66.66	4.40	2.10	41.00	30.00	1.20	0.00
fungus + poultry Fertilizer 15%	50.00	5.10	2.70	41.30	30.30	2.10	0.20
fungus + poultry Fertilizer 20%	48.33	4.80	2.50	42.70	30.80	2.70	0.00
fungus + A. chroococcum	41.66	6.20	3.00	43.10	31.50	2.80	0.60
fungus + A. chroococcum + Poultry Fertilizer 10%	16.66	9.60	5.10	46.30	34.30	5.40	2.30
fungus + A. chroococcum+ Poultry Fertilizer 15%	8.29	10.80	5.80	47.10	35.10	6.20	3.00
fungus + A. chroococcum + Poultry Fertilizer 20%	16.66	10.20	5.60	46.80	34.70	5.80	2.70
Poultry Fertilizer 10%	00	6.80	3.20	43.60	31.80	3.20	0.80
Poultry Fertilizer 15%	00	7.30	4.00	44.30	32.60	4.00	1.30
Poultry Fertilizer 20%	00	7.10	3.60	43.90	32.20	3.60	1.00
A. chroococcum	00	12.40	6.40	47.80	35.80	7.20	3.70
A. chroococcum + Poultry Fertilizer 10%	00	14.30	8.10	50.10	37.30	8.80	4.90
A. chroococcum + Poultry Fertilizer 15%	00	15.60	8.90	51.00	38.06	9.40	5.80
A. chroococcum + Poultry Fertiliz	00	15.00	8.50	50.80	37.60	9.10	5.20
control treatment	00	5.60	2.70	42.60	31.00	2.00	1.00
L.S.D at probability level of 0.05 .	* 35.40*	3.27*	1.98*	25.66*	17.30*	1.44*	* 0.11*

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