Biological control of *Azotobacter chroococcum* on *Fusarium solani* in tomato plant

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Abstract. This experiment aimed to study the effect of *Azotobacter chroococcum* in reducing infection of the tomato plant by *Fusarium solani* under the greenhouse condition. The results show that *Fusarium solani* isolates proved high pathogenicity and chosen isolate (Fs4) for being given a lower percentage of germination of cabbage seeds and the highest disease severity to tomato seedling. Also, the results were indicated that T6 (Biofertilizer at concentrations (25 ml/2Kg soil) with *F. solani* (F.s4) was the past this treatment reduced significantly the disease severity (73.10%) which also recorded a positive effect on the growth parameter of tomato plant compare to T1 (pathogen only).

Keywords. Biocontrol, Tomato, Root rot, *Azotobacter*, *Fusarium*.

1. Introduction

*Lycopersicon esculentum* is one of the important vegetable crops in most agricultural areas in Iraq and used for food and industrial purpose [1]. Tomato plants are infected by several fungal pathogens such as Fusarium spp., which cause several diseases as wilt and root rots and finally reduced crop yield [2, 3]. Several strategies are used to control and reduce the risk of diseases; especially fungicides [4, 5]. However, the application of fungicides would affect the environment and human health negatively but is not always satisfactory, as well as the emergence of resistant strains of fungi towards these pesticides [6] (Cooper and Dobson,2007). Therefore, studies have tended to find alternatives methods to control plant diseases by using biological control agents [7] (Lee, et al., 2008). The success of some biological resistance factors has been a major role in pushing some researchers to find other organisms such as bacteria and fungi that can be hosted as successful biological resistance agents in showing the best performance to combat pathogens and to enhance plant growth. *Azotobacter chroococcum* is one of the organisms that were used in the biocontrol and also plays a large role against many pathogens, as it works directly or indirectly as a biological resistance agent for a visit to plant growth and the prevention of harmful effects of pathogens as well as play another important role, such as producing inhibitory antibiotics that stop fungi growth, including Agrocin 84, Agrocin434, 2,4-diacetyl phorogluinol, Herbicolin, Phenazin, Phenazin, Oomycin, Pyoluteorin, as these antibiotics act as inhibitors of pathogenic fungi, including *Fusarium* [8, 9]. In our research, the biofertilizers and
biological control with Azotobacter chroococcum will be studied in terms of controlling root rot disease which is usually caused by the fungus (Fusarium solani). Also, its effect on the growth parameter of the tomato plant and the resistance of the Fusarium solani under greenhouse condition.

2. Materials and Methods

2.1. Isolation and diagnosis of Fusarium from tomato plants

2.1.1. Sample collection

Samples were collected from the roots of tomato plants grown in greenhouses from some farms in Baghdad city, which showed symptoms of infection. The samples were placed in polyethylene bags, transferred to the laboratory, and then kept in the refrigerator until the isolation process was performed.

2.1.2. Isolation from infected tomato plants

The roots of tomato plants were rinsed under tap water for 30 minutes to remove the suspended soil, chopped the roots into small pieces, and disinfected with sodium hypochlorite solution (1%) for 3 minutes and rinsed with sterile distilled water for 2-3 minutes and then dried using sterile filter papers. The pieces were placed on Potato Dextrose Agar (PDA) plates. The plates were then incubated at (25±2 °C)/ 5 days. Microscope examinations were then carried out of the growing fungi colonies. The colonies of the Fusarium appeared which were transferred to fresh PDA medium.

2.1.3. Diagnosis of Fusarium

Fusarium solani has been diagnosed based on certified classification keys [10, 11].

2.2. Pathogenicity test

Four isolates of F. solani were taken from roots of tomato plants and tested based on [12] process. PDA disk of 0.5 cm/7 days old Fungus colonies were put in the center of water agar medium petri dishes. Other petri dishes used as a control treatment, and containing the same medium but not inoculated with fungus. All petri dishes (inoculated and non-inoculated) were incubated at 25±2°C for 72 hours. After that, the local cabbage seeds were sterilized using 1% solution sodium hypochlorite for 2 min and then washed with distilled water 3 times and then twenty five seeds were cultured circularly edge of inoculated and non-inoculated petri dishes. This culture was done twice so end up with 3 treatments for each. Then they were incubated at 25 ± 2°C. Seven days later, the percentage of seed germination calculated using:

\[
\text{The Percentage of germination} = \frac{\text{The number of seeds germinated}}{\text{The total number of seeds}} \times 100
\]

2.3. The pathogenicity of Fusarium solani on tomato plants under greenhouse conditions

This experiment was conducted in greenhouse of Agriculture College / Baghdad University in 20/12/2019 and it was done in three randomly replicate. This experiment was included in fungal treatment and control treatment without fungal inoculum. The plastic pots were filled with sterile soil, tomato seedlings were transplanted in each pot at 35 days after sowing. Previously, the fungal inoculum was prepared according to the [13], the inoculum was added after 3 days from seedling transplant to pots. The percentage of disease severity was calculated after 30 days based on [14] equation by the following.
Disease severity(%) \[= \frac{\sum \text{scale} \times \text{number of plants infected}}{\text{Highest scale} \times \text{total number of plants}} \times 100 \]

0 refers to healthy plants

1 = 1- less than 25% of the plant root are slightly brown color.

2 = 25- less than 50% of plant roots are a dark brown color.

3 = 50- less than 75% of plant roots are a dark brown color.

4 = 75- less than 100% of plant roots are a dark brown color.

2.4 Isolate and diagnose of Azotobacter chroococcum

Soil samples were collected from rhizospheric soil from the rhizosphere of the different crops (tomato, wheat, cotton). About 10 g of dry soil sample was put in 90 ml of D.W in a conical flask. Basic dilution was prepared to 10\(^{-6}\). Bacterial isolation was performed based on [15] manner. Azotobacter chroococcum were identified phenotypically based on their appearance under the microscope and biochemical test. Later on, Azotobacter chroococcum strain (A.ch1) was used in a greenhouse experiment due to its high ability against the pathogenic fungal.

2.5. Antagonistic effect of A. chroococcum against F. solani (F.s4)

For studying the antagonistic effect of A. chroococcum bacteria against F. solani (F.s4), PDF plates were inoculated with two days old bacterial colony by streaking with needle on opposite sides of periphery plates. Each plates then were inoculated in the center with F. solani (F.s4) disk as control and incubated at (28)\(^\circ\)C. Antagonistic effect of pathogen fungal growth on control petri dishes surface was calculated by [16].

\[
\text{Inhibition percentage} \% = \frac{A_1 - A_2}{A_1} \times 100
\]

Where the A\(_1\) refers to the fungus growth area on the control surface.

A\(_2\) refers to the fungus area in the dual culture.

2.6. The effect of A. chroococcum on rot disease under greenhouse condition

The experiment was carried at greenhouse during 2019 following a completely randomized design. There were 3 replication for each treatment. Antagonistic bacteria isolates was cultured on Nutrient agar plates (28\(^\circ\)C/45 hours). Then, bacterial suspension of (108CFU/ml) was prepared by using distil water and O.D of 0.1(600nm) [spectrophotometer model 6405UV VIS].The plastic pots (2 Kg capacity) were filled with a mixture of sterile soil and peat-moss 2:1. After that seedlings were transplanted to pots (30 days after sowing). The experiment was included in the following treatments (Table 1).
Table 1. Treatments of experiment.

| Symbol treatment | Treatment                                                                 |
|------------------|---------------------------------------------------------------------------|
| T1               | F. solani (F.s4) only (pathogen only)                                      |
| T2               | Biofertilizer at concentrations (5ml/2Kg soil) with F. solani (F.s4).     |
| T3               | Biofertilizer at concentrations (10 ml/2Kg soil) with F. solani (F.s4).   |
| T4               | Biofertilizer at concentrations (15ml/2Kg soil) with F. solani (F.s4).    |
| T5               | Biofertilizer at concentrations (20ml/2Kg soil) with F. solani (F.s4).    |
| T6               | Biofertilizer at concentrations (25 ml/2Kg soil) with F. solani (F.s4).   |
| T7               | Chemical fertilizer only (Beltanol 1ml/L) with F. solani (F.s4).          |

The some pots were inoculated with the A. chroococcum (A.ch1) at concentrations (5, 10,15,20,25 ml/2Kg soil). It was applied 7 days before fungal inoculation millet seeds which contain on F. solani (F.s4) isolate. Some pots were treated with a chemical fungicide. The fungicide was applied one day before fungi inoculation. The plants were harvested and calculated the following:

- Disease severity.
- Height and weight of shoot (dry and fresh).
- Number of leaf and flower.
- Weight of one fruit per plant.
- Number of fruit per plant.
- Yield fruit.

2.7. Data analysis

ANOVA variance analyzer with the aid of GENSTAT computer software package were used to analyze the data and the means different between treatments were compared by using 0.05 probability level.

3. Results and Discussion

3.1. Isolation and diagnosis of Fusarium spp. from tomato plants

The colonies of 4 Fusarium solani isolates showed creamy pigmentation. The thin microconidia were oval to ellipsoid shaped. The macroconidia were subcylindrical, usually predominantly with three septate. The chlamydospores were single, in pairs, chains or clusters, arranging terminal or intercalary.

3.2. Pathogenicity tests

Table (2) showed that all isolates Fusarium solani were caused a significant reduction in the percentage of seed germination compared to control treatment. The results revealed that isolate Fusarium solani (F.s4) was more virulent than other isolates. These results may be attributed to that Fusarium solani are secreted many toxins such as (Fusarubin, Javanicin, Polypeptide, Anhydrofusarubin, and Protenoneons) that is important in pathogenicity [17].
Table 2. Pathogenicity tests of *Fusarium solani* (F.s4) on cabbage seeds.

| Isolates         | Percentage of germination |
|------------------|---------------------------|
| *F. solani* (F.s1) | 11                        |
| *F. solani* (F.s2) | 9                         |
| *F. solani* (F.s3) | 14                        |
| *F. solani* (F.s4) | 7                         |
| Control          | 95                        |
| LSD=0.05         | 1.97                      |

3.3. The pathogenicity of *Fusarium solani* on tomato plants under greenhouse conditions.

Disease severity was recorded after 30 days of transplantation. Disease severity of root tomato was found within the range of 48.10-73.10%. The isolate *Fusarium solani* (F.s4) has maximum disease severity was further used in the experiment. As well as, three isolates (F.s1, F.s2, F.s3) were the least aggressive disease severity compared with isolate (F.s4) Table (3). [18] found that isolates have different abilities to the secretion of analyzing enzymes that play role in penetrating the plant roots.

Table 3. The pathogenicity of *Fusarium solani* on tomato plants under greenhouse conditions.

| Isolates         | Disease severity% |
|------------------|-------------------|
| *F. solani* (F.s1) | 52                |
| *F. solani* (F.s2) | 55.10             |
| *F. solani* (F.s3) | 48.10             |
| *F. solani* (F.s4) | 73.10             |
| control          | 0                 |
| LSD=0.05         | 1.85              |

3.4. Isolate and digamous of the *Azotobacter chroococcum* and inoculum prepare.

Tables (4, 5, 6) shows the morphological, microscopic characteristics, and physiological test of bacterial isolates of rhizosphere of tomato, wheat, cotton crops. Colonies of bacteria exhibit viscous, brown-color, on pairs resampling *Azotobacter chroococcum* in its characteristics. Bacteria were gram-negative with rounded ends. These results were similar to as described by Bergey’s manual of Determinative Bacteriology [19]. Table (7) reports the biochemical patterns of bacteria tested in this study. Three *A. chroococcum* isolates were positive in each test. Also, these bacterial shows cannot able to grow on Burk’s medium and the same was used inoculation.

Table 4. Colonies morphology characteristics of *Azotobacter chroococcum*.

| Morphological characters | A. chroococcum (A.ch1) | A. chroococcum (A.ch2) | A. chroococcum (A.ch3) |
|--------------------------|------------------------|------------------------|------------------------|
| Plant type               | tomato                 | wheat                  | cotton                 |
| Growth of colonies       | +++                    | ++                     | +                      |
| Consistency color         | Very viscous           | viscous                | viscous                |
| Surface                  | Light brown            | Dark brown             | Dark brown             |

Table 5. Microscopic characteristics of *Azotobacter chroococcum* (G⁺).

| Microscopic characters | A. chroococcum (A.ch1) | A. chroococcum (A.ch2) | A. chroococcum (A.ch3) |
|------------------------|------------------------|------------------------|------------------------|
| Aggregation            | pairs                  | pairs                  | pairs                  |
| Cell shape             | Rod                    | Rod                    | Rod                    |
| Cyst formation         | +                      | +                      | +                      |
Table 6. Physiological test of *Azotobacter chroococcum*.

| Physiological test | *A. chroococcum* (A.ch1) | *A. chroococcum* (A.ch2) | *A. chroococcum* (A.ch3) |
|--------------------|--------------------------|--------------------------|--------------------------|
| Growth at NaCl (1%) | +                        | +                        | +                        |
| Growth at temperature 37°C | +                  | +                        | +                        |
| Motility           | +                        | +                        | +                        |
| N₂ fixed (mg.l⁻¹)  | +                        | +                        | +                        |

Table 7. Biochemical test of *Azotobacter chroococcum*.

| Biochemical test | *Azotobacter chroococcum* (A.ch1) | *Azotobacter chroococcum* (A.ch2) | *Azotobacter chroococcum* (A.ch3) |
|------------------|----------------------------------|----------------------------------|----------------------------------|
| Burk’s medium    |                                  |                                  |                                  |
| Starch           | +                                | +                                |                                  |
| Manitol          | +                                | +                                | +                                |
| Rhaminose        | -                                | -                                | -                                |
| Sucrose          | +                                | +                                | -                                |
| Fructose         | +                                | +                                | +                                |
| Glucose          | +                                | +                                | +                                |
| Nitrate reduction| +                                | +                                | +                                |
| Catalase test    | +                                | +                                | +                                |
| Gelatine hydrolysis | +                        | +                                | +                                |
| Utilize citrate  | +                                | +                                | +                                |

3.5. Antagonistic effect of *A. chroococcum* against *F. solani* (F.s4).

Table (8) showed higher reduction in mycelia linear growth of the *F. solani* (F.s4) isolate by *A. chroococcum* (A.ch1).

Table 8. Antagonistic effect of *A. chroococcum* against *F. solani* (F.s4) isolate.

| Bacterial isolates | Radial growth (cm) | Inhibition percentage (%) |
|--------------------|--------------------|---------------------------|
| *A. chroococcum* (A.ch1) | 2.45               | 69.37                     |
| *A. chroococcum* (A.ch2) | 2.63               | 67.13                     |
| *A. chroococcum* (A.ch3) | 2.89               | 63.87                     |
| control             | 8.00               | 0.0                       |

3.6. Evaluation of *A. chroococcum* against root rot disease some growth parameter of tomato plants in greenhouse environmental condition

Table 8 reported *A. chroococcum* and Beltanol treatments with pathogens were led to the lowest disease severity compared with pathogen treatment only. Also, *A. chroococcum* (25 ml/2Kg soil) with pathogen treatment was recorded highest disease severity (0.0%) compared with other treatments treated with the pathogen. Plant height records of growth parameters have been noticed in tomato inoculation with *A. chroococcum* (25ml/ 2Kg soil) with *F. solani* (F.s4). All treatment inoculation with *A. chroococcum* gave higher records of growth parameters in combination with control. Inoculation of *A. chroococcum* can increase plant growth through the ability of bacteria to synthesis IAA, fix nitrogen, provide P nutrients, and other elements [20]. The production of plant growth regulates by bacteria in the rhizosphere around roots was increased by the fixation of nitrogen [21]. The weight of one fruit recorded increase due to the application of *A. chroococcum* in combination with T1 (treated with pathogen only) (Table 9). The maximum weight of one fruit was recorded from the untreated plants. The fungicide application significantly increased the weight of one fruit. These results are in
agreement with [22] who found that inoculation with *A. chroococcum*, Alipoferum+ NPK resulted in earlier flowering, increasing fruits, and increased yields. *A. chroococcum* has many benefits like N2 fixing, antibacterial and antifungal production growth regulators, and siderophores. It is reported that the application biofertilizer such as *Azospirillum*, *Azobacter* and *Bacillus* sp. significantly increased tomato fruits and total yield/seed compared with control [23].

Table 9. Evaluation of *A. chroococcum* against root rot disease some growth parameter of tomato plants under greenhouse condition.

| Treatments | Disease severity | Height plant (cm) | fresh weight of shoot | dry weight of shoot | No. of leaves | No. of flowers |
|------------|------------------|-------------------|-----------------------|--------------------|---------------|---------------|
| T1         | 73.00            | 30.33             | 12.67                 | 3.33               | 16.33         | 8.33          |
| T2         | 21.67            | 37.67             | 20.33                 | 5.67               | 25.33         | 12.33         |
| T3         | 11.00            | 38.67             | 24.67                 | 6.33               | 28.00         | 13.00         |
| T4         | 12.00            | 43.33             | 27.33                 | 6.67               | 30.33         | 13.00         |
| T5         | 5.67             | 44.67             | 30.33                 | 7.33               | 35.67         | 13.33         |
| T6         | 0.00             | 50.33             | 32.00                 | 7.67               | 40.00         | 15.67         |
| T7         | 6.67             | 47.33             | 25.33                 | 5.67               | 29.33         | 12.00         |
| LSD        | 1.26             | 1.50              | 1.22                  | 1.03               | 1.57          | 1.06          |

Table 10. Evaluation of *A. chroococcum* against root rot disease some growth parameter of tomato plants under greenhouse condition.

| Treatments | Weight of one fruit per plant | Number of fruit per plant | Yield fruit  |
|------------|-------------------------------|---------------------------|--------------|
| T1         | 7.33                          | 3.67                      | 0.2733       |
| T2         | 18.33                         | 5.33                      | 0.3233       |
| T3         | 31.33                         | 6.67                      | 0.5467       |
| T4         | 46.67                         | 9.33                      | 0.7200       |
| T5         | 56.00                         | 10.67                     | 0.9267       |
| T6         | 65.00                         | 12.33                     | 1.0400       |
| T7         | 46.67                         | 10.67                     | 0.9200       |
| LSD        | 1.700                         | 0.868                     | 0.04580      |

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