Evaluation of Salicylic Acid and Proline Treatment on Tomato Plant Growth under Biotic Stress by Alternaria Alternata Fungus

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Abstract: An experiment was conducted in 2024-2025 to examine the effects of treatment with different concentrations of salicylic acid SA and proline on pathogenic fungus *Alternaria alternata* on tomato plants. Seven fungal isolates of A. alternata fungi were obtained from tomato fruits showing symptoms of late blight infection, the pathogenicity of these isolates showed that two isolates (Ala2) and (Ala4) gave the highest percentage of pathogenicity, as the seedling death rate, and Ala4 isolate was chosen for the laboratory experiment and the greenhouse experiment. The results of the tested experiment also showed the inhibitory effect of SA on the growth of fungal mycelium on SDA medium, as the inhibition rate was 100% at all concentrations, As for proline, the concentration 0.5 recorded highest inhibition rate of 91.27%, Then the effect of spraying with five concentrations of resistance inducing agents (SA and proline) was tested, which are (0.5, 1, 1.5, 2, 2.5) ml. L-1 were sprayed on 40 day old tomato seedlings after treatment with the pathogenic fungus ,The results showed that superiority of spraying with SA in reducing the infection rate with early blight disease, the infection rate with the disease decreased with increasing the concentrations of spraying with salicylic acid. Accordingly, the lowest infection rate was in the spraying treatment with 2.5 ml. L-1 and was 1.96%. The results also show that spraying with proline had different results than spraying with SA, as the infection rate did not decrease with increasing concentration, and increasing the concentration of the treatment did not affect the pathogen. The results show that the treatment with SA and proline did not affect the average number of leaves or the leaf area of the plant compared to the control treatment without the pathogenic fungus, but it outperformed the control treatment with the pathogenic fungus.

1 INTRODUCTION

Tomato (Solanum lycopersicon L.) is one of the most important and popular vegetables in the world. It is a strategic crop due to its high nutritional value [1]. It is also an economically important plant, as it is the second most important crop after potatoes [2]. Tomato is an annual staple crop, produced in more than 170 countries worldwide, including Iraq, where tomato cultivation has spread in covered and open fields in arid and temperate regions [3]. The cultivated area in 2018 amounted to 18,186 hectares, with a production capacity of 266,294 tons. As for tomato production in 2020, according to statistics from the Ministry of Planning, the Central Statistical Organization (CSO), and the Food and Agriculture Organization of the United Nations, Iraq’s tomato production amounted to 744 million tons [4]. Tomato crop is exposed to many diseases, which are the main limiting factor in its production, causing significant losses in the crop. Infection is often very rapid when the environmental conditions are suitable, especially in contaminated soil. Tomato productivity is affected by many fungal and non-fungal infections worldwide, as these diseases can spread from one plant to another in the field [5]. One of the most widespread fungal pathogens is the fungus *Alternaria alternata*, which is responsible for late blight of tomato plants [6]. A. alternata is a common pathogenic fungus of tomato plants. This fungus invades the fruits due to the high nutritional content. The high moisture content of the fruit and the availability of a thin outer skin make tomato fruits susceptible to infection and colonization through infected or weakened tissues, causing fruit rot disease [7]. Ripe fruits are more susceptible to infection than fruits in the early stages of development [8] with infection occurring either in the field or during harvest or storage [9]. The fungus infects plant parts such as leaves, stems, fruits, tubers, and roots.

The disease manifests as necrotic disease causing black spots on the surface of ripe fruits, causing fruit spoilage [10]. Recent studies have shown that the residual effects of chemical pesticides on plants exceed the permissible limit. This, in turn, poses a significant risk to consumers and the environment. Therefore, studies and research have focused on finding alternative and safer methods for combating plant diseases. Among these methods is the use of plant resistance-inducing substances, which work to control the pathogen [11]. Plants have developed a defense system to survive, where plants resort to several methods and mechanisms to defend themselves against pathogens, such as physical defenses such as thick cell walls, which are the main site and target of infection by pathogens, or through the formation of a waxy surface on leaves or the presence of thorns [12].The other type of defense is chemical defenses, which are represented by the production of chemical compounds that kill or inhibit the pathogen, such as the production of phytoalexins [13], or through systemic acquired resistance (SAR) that occurs after infection with a non-fatal disease, which leads to the stimulation of defense mechanisms in all parts of the plant, which gives the plant the ability to resist subsequent diseases. Therefore, the current study aimed to study the effect of treatment with different concentrations of salicylic acid and proline on the pathogenicity of the fungus *Alternaria alternata* that caused late blight on tomato plants in the laboratory and on the symptoms of the disease on the tomato plant and the growth of the tomato plant under biotic stress by the pathogenic fungus.

2 MATERIALS AND METHODS

2.1 Isolation of the Pathogenic Fungus Alternaria Alternata

Several samples of tomato fruits showing symptoms of the disease as shown in Figure 1 were collected from the local market of Baqubah, the capital of Diyala Governorate, during February 2024-2025. The samples were transferred to the Mycology Laboratory in the Department of Life Sciences, College of Education for Pure Sciences, University of Diyala. Afterwards, the samples were washed well with running water and dried on blotting paper. The fruits were then superficially sterilized with 1% sodium hypochlorite solution for 2 minutes, after which the fruits were washed with sterile water three times, then the samples were dried using sterile filter papers. After that, the infected parts of the fruit were removed and cut into 1 cm long pieces and transferred to 9 cm diameter plastic dishes containing the Sabouraud Dextrose Agar (SDA) culture medium, sterilized by an autoclave at 121°C and a pressure of 15 psi for 15 minutes. After that, the dishes were placed in an incubator at 25 ± 2°C until the appearance of fungal growths [14]. The fungus *Alternaria alternata* was diagnosed according to the morphological and microscopic characteristics of the fungus mentioned in [15]. To prepare a suspension of pathogenic fungal spores, ten ml of sterile distilled water was added to a plate containing a seven-day-old fungal colony. The harvested spores were then filtered through two layers of cheesecloth, and the spore concentration in the resulting suspension was adjusted to 1×105 spores.ml-1 by using a hemocytometer slide.

Figure 1: Some samples of Tomato fruits collected from local markets in Baqubah city show symptoms of early blight.

2.2 Pathogenicity Test

To test the pathogenicity Tomato seeds Solanum lycopersicum L. variety Superrogena were used for this. These seeds were obtained from the agricultural offices in Diyala Governorate and their variety was confirmed by testing them in the laboratories of the Ministry of Science and Technology, Baghdad. The seeds were sterilized in sodium hypochlorite solution 1% for 10 minutes, then washed three times with distilled water, and dried on paper towels, then placed in suspension of pathogenic fungal spores for 10 minutes. Then the seeds soaked in the pathogenic fungus were transferred to plastic penny dishes containing filter papers to maintain the moisture of the growth medium. The dishes were left in a dark place and were watered when the filter paper dried. As for the control treatment, seeds not soaked in the pathogenic fungus were used. After 15 days of planting, the Percentage of seedling death were calculated as in the following equations [16]:

Germination rate (%) = (N. of germinated seeds / Total N. of seeds) × 100.

Death rates (%) = (N. of dead seeds / Total N. of tested seeds) × 100.

2.3 Effect of Salicylic Acid and Proline on Alternaria Alternatalinear Growth

According to the poisoned food technique [17], the effect of salicylic acid and proline on the most virulent fungal isolates was evaluated in a pathogenicity experiment for the growth of A. alternata (Ala4), where 6 concentrations of each were tested, namely (0.0, 0.5, 1.0, 1.5, 2, 2.5) ml. L-1. The pathogenicity test was performed using the culture intoxication technique, and sterile SDA medium was used for this purpose. Plates were inoculated with a 5 mm diameter disc taken from a freshly grown fungal colony, and each plate was inoculated with one disc. Each treatment was repeated 3 times, after which the plates were incubated in an incubator at 25 ± 1 °C for 10 days. Then, the readings were taken by measuring the diameters of the fungal cultures (mm) and the inhibition percentage was calculated according to the equation [18] as follows: Percent inhibition%= (Radial growth in control- Radial growth of treatment / Radial growth in control) × 100.

2.4 Greenhouse Experiment

2.4.1 Preparing Tomato Seedlings

Tomato seeds variety Super Rogina were used, which were planted in plastic cork plates after filling them with peat moss sterilized by an autoclave at a temperature of 121°C and a pressure of 15 pounds for 20 minutes. The seeds then planting in it and watered as needed. The seedlings were used after they had grown and reached the age of 40 days in the greenhouse experiment.

2.4.2 Experiment Implementation

A factorial experiment was conducted in the greenhouse of the Nursery Department of the Agricultural Research Department in Baqubah - Diyala Governorate - Iraq during the agricultural season 2023-2024 on tomato plant Solanum lycopersicum L. Super Rogina variety, which was previously grown in special dishes. The seedlings were transferred at the age of 40 days to plastic pots filled with sandy mixture soil mixed with peat moss at a ratio of 3:1 and sterilized by solar pasteurization according to the method of [19]. The pots were planted at a rate of one plant per pot. The experiment was carried out with the aim of studying the effect of spraying with salicylic acid and proline on the resistance of tomato plants to infection with the pathogenic fungus *Alternaria alternata*.

The treatment was carried out by artificial infection with the pathogenic fungus and at 6 concentrations (0.0, 0.5, 1.0, 1.5, 2, 2.5) ml. L-1 of salicylic acid and proline spraying after two days of artificial infection with the pathogenic fungus. Spraying was repeated for both methods three times after planting at equal intervals. As for the control treatment, it was sprayed with distilled water only. As for the pathogenic fungus, the treatment was carried out by spraying the pathogenic fungus suspension on the entire plant at a rate of 10 ml per plant. After 45 days of planting in plastic pots, the following measurements were taken:

- Disease incidence was computed according to [20]: Disease incidence % = (N. of Diseased Plants /Total N. of Plant Inspected) ×100;
- Average of leaves number ;
- Leaf area calculation (cm2): was calculated for tomato leaves at all concentrations using Image J [21].

2.5 Statistical Analysis

A factorial experiment was conducted using a Randomized Complete Block Design (R.C.B.D.). The results were statistically analyzed using SPSS. The differences between the means were compared using Duncan's multiple range test at a probability level of 0.05 and P value.

3 RESULTS AND DISCUSSION

3.1 Isolation of the Fungus Causing Early Blight Disease

The results of isolating the fungus causing early blight on tomato fruits showed that the causative agent was one, namely the fungus *Alternaria alternata*, as 7 pure isolates were obtained, namely Ala1, Ala2, Ala3, Ala4, Ala5, Ala6, and Ala7. Figure 2 shows a colony of the fungus *Alternaria alternata* on SDA medium, Colonies were olive-black to black or gray, resembling tanned to tangled leather, and the fungus grew quickly on SDA media. Under a microscope, simple, occasionally branched, short, or oblong sporangia were used to create branching spore chains of multicellular spores simultaneously. The spores had smooth or warty walls, were dull brown in color, spherical or spheroidal, occasionally oval or ellipsoidal, and frequently had a short, conical or cylindrical beak, and this agreement with [22].

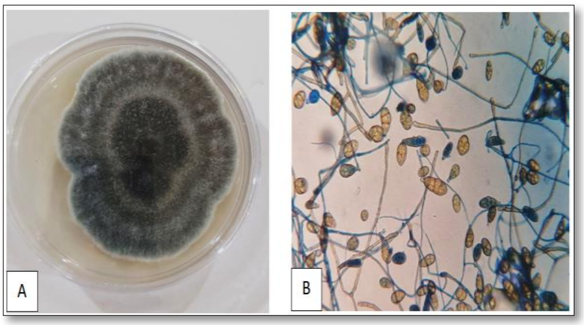

Figure 2: Colony of *Alternaria alternata* fungus on SDA medium a), *Alternaria alternata* fungus under a light microscope at ×40 magnification b).

3.2 Pathogenicity Test

The results in Table 1 show the pathogenicity test results for 7 fungal isolates of *Alternaria alternata* on tomato seeds after 15 days of germination. All isolates were pathogenic to varying degrees. The two isolates Ala6 and Ala7 recorded the highest percentage of seedling death, which was 100%, followed by the two isolates Ala3 and Ala5, with a seedling death percentage of 97%. As for the isolate Ala1, it recorded a percentage of 90%, while the two isolates Ala4 and Ala2 recorded the lowest percentage of seedling death, which was 66% and 50%, respectively, compared to the control treatment, in which the percentage of seed germination was 100% and the percentage of seedling death was 0%.

Table 1: Testing the effect of different fungal isolates of Alternaria alternata on the germination rate and seedling death of tomato after 15 days of growth in plastic dishes.

Fungi isolates	Germination rate, % after 15 day	Seedling death rate, % after 15 day
Ala1	1.00 d	90 a
Ala2	5.00 b	50 c
Ala3	0.33 d	97 a
Ala4	3.33 c	66 b
Ala5	0.33 d	97 a
Ala6	0.00 d	100 a
Ala7	0.00 d	100 a
Control	100 a	0 d

Note: Similar letters indicate that there are no significant differences between the means, at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

The decrease in germination rate and the increase in seedling death rate resulting from seed infection by pathogenic fungi are attributed to the ability of the fungus to produce decomposing enzymes as well as to secrete mycotoxins, all of which can reduce seed germination rate or cause seedling death after germination [23], [24]. Also the difference between fungal isolates in their pathogenicity can also be attributed to the difference in environmental conditions between one region and another or the difference in the genetic composition of the fungus, which allows its development, spread, and high adaptability [25].

3.3 Effect of Salicylic Acid and Proline on Alternaria Alternata Linear Growth

Table 2 shows and Figure 3 that treatment with different concentrations of salicylic acid led to inhibition of the growth of the fungus *Alternaria alternata*, where the average diameter in all treatments was 0.00 mm compared to the control treatment, in which the diameter of the fungal colony was 75.0 mm, with an inhibition rate of fungal growth of 100%. The treatment with proline shows that the average colony diameter decreased with an increase in the concentration of proline, and the treatment with a concentration of 2.5 ml. L-1 achieved the lowest rate of diameter growth, which was 19.12 mm, with an inhibition rate of 74.51%, followed by treatments (2, 1.5, 1, 0.5) ml.L-1 in which the average diameters were (28.00, 46.22, 59.56, 68.45) mm, respectively, with inhibition rates of (62.67, 38.37, 20.59, 8.73)%, respectively. It is known that salicylic acid plays an important role in stimulating plant defenses against pathogens and also has the ability to directly affect pathogens, especially fungi, by inhibiting fungal growth. This is what the results of the current study confirmed and is consistent with the results of previous studies, including [26], [27].

3.4 Greenhouse Experiment

The results in Table 3 show the effect of spraying with salicylic acid and proline on the infection rate of tomato plants with early blight disease on leaves. The table shows the superiority of spraying with salicylic acid in reducing the infection rate with early blight disease. As we note from the results in the table, the infection rate with the disease decreased with increasing the concentrations of spraying with salicylic acid. Accordingly, the lowest infection rate was in the spraying treatment with 2.5 ml.L-1 and was

1.96%. As for the spraying treatment with a concentration of 0.5 ml.L-1 it recorded the highest infection rate with the disease and was 6.66%, while the control treatment infected with the pathogenic fungus and without spraying recorded an infection rate of 44.11%. The results of the table also show that spraying with proline had different results than spraying with silicic acid, as the infection rate did not decrease with increasing concentration, and increasing the concentration of the treatment did not affect the pathogen. We find that the 0.5 ml.L-1 concentration treatment achieved the lowest infection rate of the disease, which was 16.66%, while the 2.5 ml.L-1 treatment recorded a rate of 23.43%.

The results in Table 4 show the effect of spraying with salicylic acid and proline on the average number of leaves of tomato plants growing under biological stress with the pathogenic fungus *Alternaria alternata*. The spraying treatments with salicylic acid show that the average number of leaves decreased compared to the control treatment without pathogen, which was 15.5 leaves, and the concentration treatment of 0 ml.L-1 (pathogen without spraying), which was 13.6 leaves. The results show that the average number of leaves decreased with increasing salicylic acid spray concentration, which was (12, 10.2, 10.8, 11, 10.2) leaves, respectively, for concentrations (0.5, 1, 1.5, 2, 2.5) ml.L-1, respectively. As for the proline spray treatments, the results show that the number of leaves increased with increasing concentrations, which were (0.5, 1, 1.5, 2) ml.L-1, respectively, and the average number of leaves was (13.2, 13.4, 15.5, 15.6) leaves, respectively, while the 2.5 ml.L-1 concentration treatment recorded a decrease in the average number of leaves to become 12.8 leaves.

Table 5 and Figure 4 shows the effect of spraying with salicylic acid and proline on the average leaf area of tomato leaves under biological stress by the pathogenic fungus *Alternaria alternata*. The results show the varying effect of spraying with different concentrations of salicylic acid on the leaf area, as the largest leaf area was in the 0.5 ml/L concentration treatment, which was 10.85 cm, and the smallest leaf area was in the 1.5 ml/L concentration treatment, which was 7.66 cm. As for the proline spraying treatment, the results show that the 0.5 ml/L concentration treatment achieved the highest average leaf area, which was 14.43 cm, while the 0 ml/L concentration treatment, which was without spraying, recorded the lowest average leaf area, which was 8.03 cm, while the leaf area in healthy plants in the control treatment was 11.25 cm.

The above results confirm the positive role of salicylic acid and proline in reducing the incidence of tomato plants with early blight disease caused by the pathogenic fungus *Alternaria alternata*, through their direct effect on the growth of the fungus, as shown in the results in Table 2 and Figure 3. The decrease in the incidence of the disease is also explained by the ability of both salicylic acid and proline to induce resistance in the plant by increasing the concentration of secondary metabolites or active compounds, in addition to increasing the concentration of antioxidant enzymes and proteins associated with pathogenesis. Consequently, the symptoms of the disease decreased in the treated plants [28] , [29] , [30] , [31], [32]

Table 2: The effect of treatment with salicylic acid and proline in inhibiting the growth of pathogenic fungi Alternaria alternata.

Concentrations	Salicylic acid		Proline	
	Radial growth (mm)	Percent of inhibition (%)	Radial growth (mm)	Percent of inhibition (%)
0	75.00	-------	75.00	-------
0.5	0.0	100	68.45	8.73
1	0.0	100	59.56	20.59
1.5	0.0	100	46.22	38.37
2	0.0	100	28.00	62.67
2.5	0.0	100	19.12	74.51

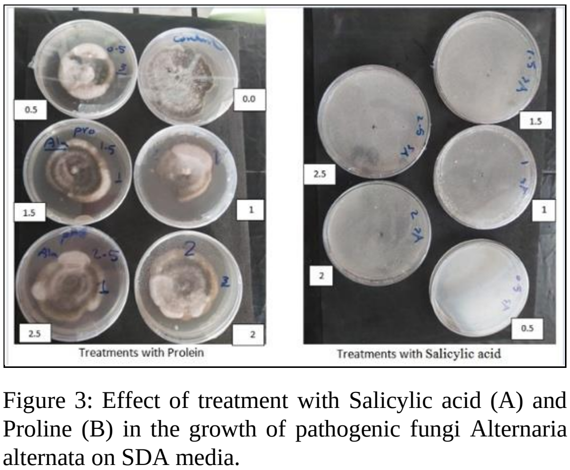

Figure 3: Effect of treatment with Salicylic acid (A) and Proline (B) in the growth of pathogenic fungi Alternaria alternata on SDA media.

Table 3: Effect of treatment with Salicylic acid and Proline on the incidence rate (%) of early blight disease on tomato plants caused by the fungus Alternaria alternata.

Concentrations	Incidence rate %	
	Salicylic acid	Proline
0	44.11 a	44.11 a
0.5	6.66 c	16.66 b
1	3.92 c	20.89 b
1.5	3.70 c	19.35 b
2	1.81 c	17.94 b
2.5	1.96 c	23.43 b

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

Table 4 : Effect of treatment with salicylic acid and proline on the number of leaves of tomato plants growing under biotic stress by the pathogenic fungus *Alternaria alternata* .

Concentrations	Leaves number	
	Salicylic acid	Proline
0	13.6 b	13.6 b
0.5	12 cd	13.2 bc
1	10.2 d	13.4 b
1.5	10.8 cd	15.5 a
2	11 cd	15.6 a
2.5	10.2 d	12.8
Control	15.5 a	

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

Table 5: Effect of treatment with salicylic acid and proline on leaf area (cm2) of tomato plants growing under biotic stress by the pathogenic fungus *Alternaria alternata* .

Concentrations	leaf area (cm^2)	
	Salicylic acid	Proline
0	8.03 e	8.03 e
0.5	10.85 bc	14.43 a
1	8.07 e	11.75 b
1.5	7.66 e	10.76 bc
2	9.76 cd	10.06 bcd
2.5	8.84 de	11.10 bc
Control	11.25 bc	

Note: Similar letters indicate that there are no significant differences between the means, at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

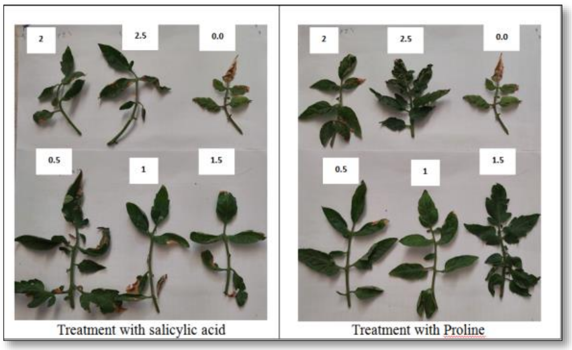

Figure 4: The effect of treatment with salicylic acid and proline on disease symptoms in tomato plants infected with the fungus *Alternaria alternata*.

4 CONCLUSIONS

The results of the current study show that the isolates of the pathogenic fungus causing early blight on tomato plants were highly virulent, as the seven isolated strains had a strong effect in inhibiting the growth of tomato seedlings. The results of the induction factors used in the study, including salicylic acid and proline, also indicated that they had an inhibitory effect on the growth of the pathogenic fungus in the laboratory and also led to the inhibition of the disease infection on tomato plants grown in greenhouse conditions. The results showed that the effect of salicylic acid on disease inhibition increased with increasing concentration. Therefore, the results of this study are considered promising results for combating plant diseases without resorting to the use of chemical pesticides that have harmful environmental and biological effects, which supports the goals of sustainable development and environmental conservation.

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