Dijlah Journal of Agricultural Sciences
Journal homage: https://djas.uowasit.edu.iq/index.php/djas/en

Research article

Evaluating effect of Salicylic acid and Proline on inducing resistance in Tomato(*Solanum lycopersicon* L.) plants against Eearly blight caused by the *Alternaria alternate* fungus

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ARTICLE INFO

Keywords:
Salicylic acid
Proline
Eearly blight
Alternaria
Tomato
inducing resistance

Article history:
Received: 17 May 2026
Accepted: 2 Jun 2026
Online: 30 Jun 2026

ABSTRACT

A factorial experiment was conducted in the greenhouse during the 2024-2025 year to study the effect of spraying tomato plants with six concentrations (0.0, 0.5, 1.0, 1.5, 2, and 2.5 ml.L^{-1}) of Salicylic acid and Proline on their resistance to infection by the fungus *Alternaria alternate* which caused early blight disease . The results showed decrease in the average number of flowers, fruits, and fruit weight with increasing concentrations of Salicylic acid and Proline. Conversely, the results showed an increase in defensive enzymes in the tomato plant, as recorded the activity of the phenylalanine ammonia-lyase (PAL) enzyme in plants treated with salicylic acid, with activity increasing with increasing concentration. The highest value was recorded at 2.5 ml. L^{-1} (3.978 g. L^{-1}), while the lowest value was 2.953 g. L^{-1} at a concentration of 0.5 ml. L^{-1}. Conversely, enzyme activity decreased with increasing proline concentration, reaching a peak of 3.286 at 0.5 ml. L^{-1} and a minimum of 0.879 at 2.5 ml. L^{-1}. In contrast, the levels of PO, total protein, and lignin increased with increasing salicylic acid and proline concentrations, reaching peaks of 0.365, 1.839, and 31.13, respectively, at 2.5 ml. L^{-1}, and minimums of 0.212, 1.489, and 16.40, respectively, at 0.5 mL/L. Salicylic acid coefficients were highest at 2.5 mL/L, at 0.290, 1.818, and 20.66, respectively, and lowest at 0.5 ml. L^{-1}, at 0.153, 1.484, and 13.66, respectively. Regarding the salicylic acid content in the plant, its coefficients decreased with increasing concentration, reaching a high of 41.18 at 2.5 ml. L^{-1}and a low of 16.47 at 0.5 ml. L^{-1}. In contrast, the proline content in the plant increased with increasing concentration, reaching a high of 119.06 at 2.5 ml. L^{-1}and a low of 93.50 at 0.5 ml. L^{-1}. By exploiting the plants' innate resistance to pathogens, the use of resistance inducers may reduce the need for environmentally toxic chemicals in disease management. Therefore, we propose using proline and salicylic acid as unconventional and environmentally friendly alternatives for plant protection and promoting sustainable agriculture.

1. Introduction

Solanum lycopersicum L. is one of the plants of the Solanaceae family and is an economically important plant (Naika et al., 2005). It is considered one of the most widespread and popular vegetables worldwide due to its nutritional value and variety of types (Mohit et al., 2019). It is one of the crops that

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yield high financial returns for many farmers worldwide. Its production can be marketed fresh or processed, as tomatoes are a rich source of carbohydrates, proteins, fatty acids, minerals, vitamins, amino acids, and phenolic compounds (Goka et al., 2021). Tomato are an annual staple crop grown in more than 170 countries worldwide, including Iraq, where they have spread to both covered and open fields in arid and temperate regions (Ntonifor et al., 2013). The area planted with tomatoes in 2018 amounted to about 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 Bureau of Statistics, and the Food and Agriculture Organization of the United Nations, it totaled 744 million tons in Iraq (Aziz and Hussein, 2023).

Tomato plant is affected by many diseases, including early blight (Hassine et al., 2014), caused by the fungus *Alternaria alternate*, which is among the most common diseases of agricultural crops (Chaerani and Voorrips, 2006). Symptoms begin on the lower leaves as a blight, then spread to the upper leaves. As the disease develops, the leaves turn yellow, wilt, and fall. The infection season occurs in the spring, when the weather is humid, after planting seedlings (Rouma et al., 2021). The disease affects the fruit during ripening (Rotem, 1994). At this stage, the infection is sunken, with skin-like spots ranging from dark brown to black and concentric rings. The infection may cover the entire fruit, and the infected area at this stage is loaded with mature fungal spores (Rahman, 2013).

Chemical pesticides cause significant harm to the environment and human health, prompting researchers to develop alternative, non-chemical methods to combat plant diseases. Studies have recently turned to the technology of stimulating natural defense systems in plants by generating synthetic or chemical defense mechanisms using resistance-inducing materials (Li et al., 2020). This resistance can be stimulated by using natural and synthetic chemicals that are environmentally friendly and effective against diseases, known as resistance inducers, which activate induced resistance in the plant (Ramezani et al. 2018). Induced resistance is the appropriate reaction plants mount against biotic or abiotic stressors, especially attacking pests and pathogens, as part of the plant response (Walters et al., 2007). Therefore, the aim of the current study was to evaluate the effect of spraying tomato plants with different concentrations of salicylic acid and proline on stimulating plant resistance against the pathogenic fungus *Alternaria alternate*, the causative agent of early blight.

2. Materials and Methods

2.1 Isolation of the pathogenic fungus *Alternaria alternate*

Tomato samples exhibiting disease symptoms were collected from the local market in Baqubah, Diyala, Iraq, between February 2024 and February 2025. The samples were transferred to the Mycology Laboratory of the Department of Biology, College of Education for Pure Sciences, University of Diyala. Next, the samples were washed thoroughly with running water and dried on filter paper. The fruits were then superficially sterilized with a 1% sodium hypochlorite solution for 2 minutes, after which they were washed 3 times with sterile water, then dried on sterile filter paper. Next, the infected parts of the fruit were removed and cut into 1 cm long pieces, then transferred to 9 cm diameter plastic dishes containing the Sabouraud Dextrose Agar (SDA) culture medium, which was sterilized in a steam sterilizer (Autoclave) at a temperature of 121 C° and a pressure of 15 psi for 15 minutes. Next, the dishes were incubated at 25±2 h until fungal growth appeared (Mahmoud et al., 2020). *Alternaria alternate* was diagnosed based on the morphological and microscopic characteristics described by Barnett and Hunter (1986).

2.2 Growing tomato seedlings

The field experiment was carried out on tomato seedlings *Solanum lycopersicum* L. Super Rogina variety, whose seeds were previously grown in cork dishes with an area of 1 m×1 m, and after 40 days of age, the tomato seedlings were transferred to the plastic house in the nursery department of the

Agricultural Research Department in Baqubah - Diyala Governorate – Iraq, during the agricultural season 2024-2025, where In this experiment, a mixture of sandy mixed soil and peat moss was used (3:1) The soil and peat moss were previously sterilized according to the method mentioned in the reference (Zidane, 2007) using the heat of the sun. Then I packed the soil into 3 kg pots and added Dab fertilizer 2 days before transferring the 40-day-old seedlings to the pots, one seedling per pot.

2.3 Creating artificial infection

To test the effect of inducers on 40-day-old tomato seedlings using resistance inducers salicylic acid and proline, artificial infection by the pathogenic fungus *Alternaria alternate* was induced by preparing a suspension of pathogenic fungus spores. 10 ml of sterile distilled water was added to a plate containing a seven-day-old fungal colony. Then the germs were collected. After filtering the sample through two layers of gauze, the concentration of spores in the resulting suspension was adjusted to 1×10^{-5} spores.mL using a cell counter chip.Treatment was carried out by artificial infection with the pathogenic fungus *Alternaria alternate*, using six concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5) ml in 1 liter of a salicylic acid and proline solution, and spraying 2 days after artificial infection. The spraying process for both treatments was repeated three times after planting, at 10-day intervals. As for the control treatments, they were sprayed only with distilled water. For the pathogenic fungus, treatment was carried out by spraying the suspension onto the entire plant at a rate of 10 ml per plant. After the last spray, the following measurements were made: Calculate the number of flowers and fruits, the weight of the fruit.

2.4 Measuring resistance induction mechanisms in tomato plants

2.4.1 Enzyme activity of phenylalanine ammonia lyase (PAL)

by followed The method described in (Dickerson et al. 1984) throw take 1 gram of plant sample and weighed (vegetative total) and the sample was placed in a ceramic mortar inside an ice container and 3 ml of cooled sodium borate medium was added to it (pH 7, 0.1 M), then the mixture was placed in a 2 ml Eppendorf tube and then placed in a Centrifuge microcentrifuge at a speed of 16,000 rpm One minute for 15 minutes, then the precipitate, which represents the enzyme extract, was transferred to a new 2 ml tube. Enzyme activity was measured by monitoring the conversion of L-phenylalanine to cinnamic acid. 0.4 ml of the enzyme extract was taken and placed in a 10 ml glass tube to which 0.5 ml of borate buffer was added (pH 8.8), 0.1 M The reaction began with the addition of 0.5 ml of phenylalanine-L, after which the mixture was placed in an incubator at a temperature of 30 m5 for 30 minutes, after which the rate of conversion from phenylalanine-L to acid cinnamic-Trans was measured using a spectrophotometer at a wavelength of 290 nm. As for the Blank standard tube, all previous reaction materials were added, except for the enzyme extract, to which the same volume of Distilled water was added. The enzyme's enzymatic activity was calculated using the equation described previously (1985, Nezih).

Enzymatic activity (unit of absorption/g wet weight) = Device reading / Model weight / extraction volume x volume taken for reading

2.4.2 Enzyme activity of Peroxidase (PO)

It followed the method of Hammerschmidt (et al. 1982). One gram of the plant sample kept in the refrigerator was taken and crushed in a ceramic mortar with 2 mL of phosphate buffer. The mixture was placed in a tube, then centrifuged at 16,000 rpm for 15 minutes. The slurry, representing the enzyme extract, was transferred to a new tube. Take 0.5 ml of the enzyme extract and put it in a 10 ml glass tube. Add 1.5 ml of 1% O2H2 hydrogen peroxide. The change in optical density (OD) was measured directly with a spectrophotometer at 420 nm. The measurement was taken every 30 seconds for 3 minutes, and the enzyme activity rate was calculated using the equation described in the previous paragraph (1-2-1).

2.4.3 Measurement of total protein concentration

I followed the method of (Whitaker et al. 1987) where 0.5 g of plant sample was weighed and placed in a ceramic mortar, then the plant sample was crushed with 10 ml of phosphate buffer (0.01 M, PH7.5), after which the mixture was filtered using Whatman No. 1 filter paper and 1 ml of the filtrate was taken and placed in a 2 ml tube, after which ammonium sulfate was added to it at a concentration of 51.6% At a rate of 0.8 ml per tube, then shake the tube well, then keep the tubes for 2 hours at a temperature of 4°C, then place the tubes in the cooled centrifuge at a speed of 9000 rpm for 30 minutes at a temperature of 4:5°C, then pour the slurry and add 1 ml of cooled acetone to the precipitate to remove plant dyes. Immediately after that, place the tubes in the cooled centrifuge at 9000 rpm for 30 minutes. The process was repeated twice until the dye disappeared. Then, 0.4 ml of phosphate buffer was added to the precipitate. The protein concentration was measured using a UV-Vis Microplate Spectrophotometer using the following equation:

$$\text{Protein concentration} = \text{OD280} - \text{OD260} / 2.51$$

OD: represents optical density

2.4.4 Measuring the content of defense hormones

The examination took place at the Scientific Research Authority, Environmental and Water Research Center. High-performance liquid chromatography (HPLC) was used. The device was of German origin. Salicylic acid estimation (extraction of salicylic acid from the plant) According to the method described by Zhi et al. (2015), tomato leaves were dried at room temperature. The samples were weighed and cooled. Methanol and phosphoric acid were added to the tube, which was then centrifuged at 5000 rpm for 10 min. After that, the organic layer was taken and ethyl acetate was added to it, then a primary secondary amine (PSA) was added to it and placed on the Vortexes mixer for two minutes, then returned to the centrifuge at a speed of 8000 rpm for 5 min Then the supernatant was collected and evaporated until dry, then methanol was added and injected with a device (HPLC). proline estimation (extraction of proline from the plant) According to the method by Rasmus Dahl-Lassen (2018), proline extraction began by placing the sample in a volumetric vial and adding hydrochloric acid. The mixture was incubated at 55°C for 3 hours. After incubation, the sample was dried using a rotary evaporator. Sodium citrate was added, and the mixture was filtered through a 0.45 um plastic filter. The filtrate was then injected into the HPLC device for analysis.

2.4.5 Measuring the total content of lignin

To detect the formation of lignin in the vegetative system of tomato plants, they used the method (Lin and Kao, 2001), where 3 grams of the vegetative system were crushed in a ceramic mortar with 10 ml of ethanol at a concentration of 95%, then the mixture was placed in 20 ml glass tubes, which in turn were placed in a centrifuge at a speed of 10,000 rpm for 5 minutes Then pour the broth and wash the precipitate three times with 95% ethanol and twice with a mixture of ethanol and hexane (2:1), volume / volume (then leave the precipitate to dry under a dry air stream, then wash the dry precipitate once by adding 2 ml of bromide in acid acetic acid (3:1), volume / volume (, 1 ml of bromide acetyl and acid acetic mixture (3:1), volume / volume (was added to the precipitate and kept at 70 m5 for 30 minutes. After leaving the mixture to cool below room temperature, 0.9 ml of NaOH (M2) and 0.1 ml of hydroxylamine hydrochloride (M7.5) were added. The volume was supplemented to 10 ml by adding acid acetic acid

Then the glass tubes were placed in the centrifuge at a speed of 1000 rpm for 5 minutes and the slurry was taken and the amount of lignin was measured by a spectrophotometer at a wavelength of 280 nm and the standard curve was prepared using Alkali lignin and the lignin content is expressed in mg .g^{-1} wet weight.

2.5 Statistical analysis

A field experiment was conducted using a complete randomised block design (CRBD). The results were statistically analyzed using SPSS software. The differences between the averages were compared using Duncan's multiple range test at a probability level of 0.05 and a P value.

3. Results and Discussion

3.1. Average number of flowers, fruits, fruit weight and yield

The results of Table 1 showed significant differences in the effect of spraying Salicylic acid and Proline on the average number of flowers of tomato plants. Salicylic acid spray treatments showed a decrease in the average number of flowers with increasing concentration, with the highest value in the number of flowers at concentration being (0.5) ml.L^{-1} amounted to (9.50) flowers.Plant^{-1}, while the lowest value in the number of flowers was at a concentration of 2.5 mL.Liter^{-1} reached (6.25) flowers. Plant^{-1}. As for the effect of Proline spraying on the average number of flowers, the results showed a decrease in the average number of flowers with increasing concentration, with the highest concentration being 1 ml.L^{-1} The highest value in the number of flowers was 9.50 flowers.Plant^{-1}, while the concentration was recorded as (2.5) ml.Liter^{-1} The lowest value in the number of flowers was 6.50 flowers.Plant^{-1}, and by comparison, treatment at a concentration of 0 ml.L^{-1} gave a lower value of (7.75) flowers. Silicic acid is a growth and defense regulator that, at low concentrations, stimulates flower formation by activating physiological processes associated with flowering and signaling hormones, such as auxins and gibberellins. It has also helped improve photosynthesis, leading to increased nutrient uptake and stronger bud and flower growth. High concentrations have transformed a stimulant into a stressor that affects hormone action and disrupts their balance in the plant, leading to slowed growth and the fall of buds and flowers. The same thing happened in proline. Low and medium concentrations improved the number of flowers due to their role in maintaining the water balance inside the cells and supporting photosynthesis processes and protecting enzymes, while high concentrations led to a decrease in the number of flowers due to an imbalance in the osmotic balance of the cells and a decrease in the efficiency of photosynthesis processes that affected the number of flowers. This study agrees with (Altaf et al., 2022) and (Khan et al., 2020)

Table 1. Effect of Salicylic acid and proline treatment on the number of flowers of tomato plants growing under biostress caused by the pathogenic fungus *Alternaria alternate*

Concentrations	Flowers number Proline	Salicylic acid
0	7.75 b	7.75 b
0.5	9.50 a	8.25 ab
1	8.50 ab	9.50 a
1.5	7.75 ab	9.00 a
2	7.00 bc	8.25 ab
2.5	6.25 c	6.50 c
Control	9.75 a	9.75 a

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

The results of Table 2 show significant differences in the effects of Salicylic acid and Proline spraying on the average number of fruits, with Salicylic acid spraying showing lower values at higher concentrations, with the highest recorded at 0.5 ml.L^{-1} has the highest value, with an average of 5.50 fruits.Plant^{-1}, while the lowest value was at a concentration of 2.5 ml.L^{-1} amounted to (3.50) fruits.Plant^{-1}, while the effect of spraying proline recorded the highest value, with an average of 5.50 fruits.Plant^{-1} at a concentration of 1 ml.L^{-1}, while the lowest value with the average number of fruits was at a concentration of 2.5 ml.L^{-1} amounted to(3.75) fruits.Plant^{-1}, while the comparison treatment recorded lower values than the high concentration values at the salicylic acid and proline treatments, reaching 4.75 fruits.Plant-1 at concentration (0) ml.L^{-1}.

Table 2. Effect of Salicylic acid and proline treatment on the average number of fruits of tomato plants growing under biostress caused by the pathogenic fungus *Alternaria alternate*

Concentrations	Fruits numbeer Salicylic acid	Proline
0	4.75bc	4.75bc
0.5	4.75bc	5.50a
1	5.50a	4.75b
1.5	5.25ab	4.25c
2	4.25cd	3.75cd
2.5	3.75d	3.50d
Control	6.25a	6.25a

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

The results of Table 3 also showed significant differences in the effects of spraying Salicylic acid and Proline on the average fruit weight. Salicylic acid spray treatments recorded a decrease in the average fruit weight with an increase in concentration, with the concentration recording (0.5) ml.L^{-1} had the highest average fruit weight value of (41.95) g, while the lowest concentration value was (2.5) ml.L^{-1} amounted to (36.44) g, while the effective proline coefficients for the average fruit weight were at a concentration of (1.5) ml.L^{-1} amounted to (47.46)g, while the lowest value amounted to (36.99)g at a concentration of (2.5)g.L^{-1}. By comparison with the comparison treatment (without spraying) at a concentration of 0 ml.Liter-1 recorded the highest value (48.75 g). Salicylic acid and proline helped the plant withstand the pathogenic stress caused by the fungus under study, leading to decreased photosynthetic efficiency and impaired nutrient transport. Therefore, plant resources were diverted from growth to defense. These processes consumed the plant's food resources and energy that were allocated for growth, which resulted in a decrease in the outcome As for low concentrations, they improved growth and resistance characteristics due to stimulating defense proteins and activating enzymes, and facilitated the transfer of water and nutrients in plant tissues, and this helped reduce the effect of stress, thus equalizing growth and defense processes, thus giving good values in the result. This is consistent with other studies (Kaya et al., 2023; Yang et al., 2023; Altaf et al., 2022).

Table 3. Effect of Salicylic acid and proline treatment on the average fruit weight of tomato plants growing under biostress caused by the pathogenic fungus *Alternaria alternate*

Concentrations	Fruits weight Salicylic acid	Proline
0	48.75 b	48.75 b
0.5	41.09 b	41.95 a
1	47.16 a	39.46 b
1.5	47.46 a	38.77 bc
2	38.99 c	38.11 bc
2.5	36.99 d	36.44 c
Control	57.31a	57.31a

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

The results in Table (4) showed significant differences in the effect of treatment materials on the productivity of the tomato plant product, as Salicylic acid spray treatments recorded a decrease in the product production with an increase in concentration, as the concentration recorded (0.5)ml.L^{-1} had the highest production value at (167.81)kg, while the lowest concentration value was (2.5)ml.L^{-1} amounted to (145.77) kg, while for proline coefficients, the most effective value in yield was at a concentration of (1.5) ml.L^{-1} amounted to (189.85) kg, while the lowest value was at concentration (2.5) ml.L^{-1} amounted to (147.98) kg, and compared to the comparison treatment (without spraying), which recorded a higher value in yield, amounting to (195.02) kg than all treatments at a concentration of (0) ml.L^{-1}. When a plant is infected with a fungus, it is stimulated, its defenses are activated, and the production of defensive compounds increases, so there is a redistribution of nutrients that is reflected in vegetative growth, so there is a decrease in vegetative growth, and the plant's energy is directed towards the fruits, and there is an increase in the weight and number of fruits. This is what happened in the comparison treatment and what is shown in Tables 2 and 3, where there was an increase in the number and quantity of production. As for the coefficients of silicic acid and proline at low concentrations, production increased as the plant's growth and defense processes were balanced, and decreased at high concentrations. The reason may be the additional physiological stress on the plant, which led to defense processes being directed towards increased oxidative stress, the accumulation of free radicals, and hormonal imbalance. This process negatively affected growth at these concentrations, resulting in reduced growth and production. These results are consistent with those of Altaf et al. (2023) and Sabry et al. (2023).

Table 4. Effect of Salicylic acid and proline treatment on the experimental unit yield of tomato growing under biostress caused by the pathogenic fungus *Alternaria alternate*

Concentrations	Fruit yield per Experimental Unit (kg) Salicylic acid	Proline
0	195.02 a	195.02 a
0.5	167.81 a	164.36 b
1	157.86 ab	188.66 a
1.5	155.11 b	189.85 a
2	152.44 b	155.96 c
2.5	145.77 c	147.98 d
Control	229.26 a	229.26 a

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

3.2 Mechanisms for inducing resistance in tomato plants

3.2.1 Phenylalanine ammonia lyase (PAL) content

The results of Table 5 showed the effect of spraying tomato plants with the induced substances Salicylic acid and Proline on the enzymatic activity of Phenylalanine ammonia lyase (PAL). The results of Table (5) showed clear significant differences in all coefficients after the third spray of the induced substances under study, and the Salicylic acid coefficients recorded an increase in the enzyme value with an increase in concentration, as the concentration was (2.5) ml.L^{-1} recorded the highest average enzyme activity reading of (3.978) and was higher than the comparison treatment (without spraying) when the concentration was (0) ml.L^{-1}, which amounted to (3.965), while the lowest reading was at concentration (0.5) ml.L^{-1} amounted to (2.953), while the results of proline spray treatments recorded a decrease in the enzyme value with increasing concentration, as the highest average enzyme activity at concentration was (0.5) ml.L^{-1} amounted to (3.286), while the lowest value with average enzyme activity was at a concentration of (2.5) ml.L^{-1} amounted to (0.879). By comparison with the comparison treatment (without spraying) at a concentration of (0) ml.L^{-1}, the highest was at (3.965).

Table 5. Effect of Salicylic acid and proline treatment on the average reading of Phenylalanin ammonia lyase (PAL) content after the third spray of tomato plants growing under biostress caused by the pathogenic fungus *Alternaria alternate*

Concentrations	Enzyme content Phenylalanin ammonia layase (PAL) μ/g Salicylic acid	Proline
0	3.965 a	3.965 a
0.5	2.953cd	3.286 bc
1	3.216 bc	2.181 de
1.5	3.531 b	1.980 e
2	3.765 ab	1.218 f
2.5	3.978 a	0.879 g
Control	3.146 bc	3.146 bc

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

3.2.2 Peroxidase (PO) enzyme content

The results of Table 6 showed significant differences in the effect of spraying the tomato plant with the induced substances Salicylic acid and Proline on the activity of the enzyme Peroxidase (PO). The results showed an increase in the enzyme value in all treatments after the third spray of the induced materials under study. In the Salicylic acid spray treatments, there was an increase in the enzyme value at all concentrations, as the concentration was recorded at (2.5) ml.L^{-1} is the highest reading with an average enzyme activity of (0.365), and higher in value than the comparison treatment of (0.239) at a concentration of (0) ml.L^{-1}, and the lowest value of enzyme activity was at a concentration of (0.5) ml.L^{-1} reached (0.212), and as for proline spray coefficients, the average measurement of enzyme activity increased with increasing concentration, as the highest value of enzyme activity at concentration reached (2.5) ml.L^{-1} reached (0.290) and was higher in value than the comparison treatment, which reached (0.239) when the concentration was (0) ml.L^{-1}, and the lowest value was at concentration (0.5) ml.Liter^{-1}, which amounted to (0.153).

Table 6: Effect of Salicylic acid and proline treatment on the average reading of Peroxidase (PO) enzyme content after the third spray of tomato plants growing under biostress caused by the pathogenic fungus *Alternaria alternate*

Concentrations	Enzyme content Peroxidase (PO)µ/g Salicylic acid	Proline
0	0.239 cd	0.239 cd
0.5	0.212 d	0.153 d
1	0.270 bc	0.178 cd
1.5	0.303 ab	0.243 cd
2	0.349 a	0.279 bc
2.5	0.365 a	0.290 abc
Control	0.075 e	0.075 e

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

3.2.3 Total protein content

The results of Table 7 showed significant differences in the effect of spraying tomato plants with the induced substances, Salicylic acid and Proline, on total protein content. The table results showed an increase in total protein content in all treatments after the third spray. In Salicylic acid spray treatments, there was an increase in the total protein value with increasing concentration, and the highest value was at a concentration of 2.5) ml.L^{-1}, which amounted to 1.839, and the lowest value amounted to 1.489 at a concentration of 0.5) ml.L^{-1}. As for the proline spray coefficients, the protein values were also high with increasing concentration, as the concentration recorded (2.5) ml.L^{-1} The highest value in measurement was (1.818) and the lowest value was (1.484) when the concentration was (0.5) ml.L^{-1}, and the comparison treatment (without spraying) recorded a higher value than all treatments in total protein content at a concentration of 0 ml.Liter^{-1}, which amounted to (2.877).

Table 7: Effect of Salicylic acid and proline treatment on the average reading of total protein content after the third spray of tomato plants growing under biostress caused by the pathogenic fungus *Alternaria alternate*

Concentrations	Total protein µ/g Salicylic acid	Proline
0	2.877a	2.877a
0.5	1.489 f	1.484 f
1	1.514 f	1.597 e
1.5	1.663 d	1.699 cd
2	1.711 cd	1.791 bc
2.5	1.839 b	1.818 b
Control	1.318 g	1.318 g

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

3.2.4: Lignin content

The results of Table 8 showed significant differences in the effect of spraying tomato plants with the induced substances, Salicylic acid and Proline, on alkene content. The table results showed a clear increase in both color values across all transactions after the third spray. Salicylic acid spray coefficients recorded high values with increasing concentration, and the highest value was with the lignin content at a concentration of 2.5 ml.L^{-1}, which amounted to 31.13, and the lowest value was at a concentration of 0.5 ml.Liter-1 amounted to (16.40), while the comparison treatment (without spraying) recorded low values of the alkene at concentrations of (0) ml.Liter^{-1} reached (16.40). As for the effect of proline spraying, the concentrations under study recorded an increase in the value of the alkene, and the highest value was at the concentration (2.5) ml.L^{-1} amounted to (20.66), while the lowest value was at concentration (0.5) ml.L^{-1} amounted to (13.66), and the comparison treatment (without spraying) recorded a low value compared to the value of the higher concentration and a high value compared to the value of the lower concentrations, which amounted to (16.40) at a concentration of (0) ml.L^{-1}.

Table (8): Effect of Salicylic acid and proline treatment on the average lignin content after the third spray of tomato plants growing under biostress caused by pathogenic fungus *Alternaria alternate*

Concentrations	Lignin content µ/g Salicylic acid	Proline
0	16.40 g	16.40 g
0.5	16.40 g	13.66 i
1	20.60 d	14.83 h
1.5	23.40 c	18.33 f
2	27.20 b	19.23 e
2.5	31.13 a	20.66 d
Control	12.50 j	12.50 j

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

The above results demonstrated the positive effects of externally applied salicylic acid and proline on the plant, including their effect on fungal infection, thereby reducing it. Spraying with salicylic acid stimulated plant biodefense mechanisms by activating secondary metabolic pathways represented by an increase in the activity of the enzyme (PAL), which in turn worked to activate and stimulate Defense mechanisms that enhanced resistance by producing phenolic compounds It also increased the enzyme (PO), which worked to strengthen cell walls, oxidize phenolic compounds, and its ability to get rid of free radicals (ROS), and stimulate defense proteins and resistance enzymes by raising the content of lignin and total protein As for proline, its role was preventive, limited to reducing stress on cells, regulating osmotic balance, reducing oxidative stress, and reducing the activity of secondary defense pathways. Consequently, PAL activity decreased and contributed to improve plant tolerance by increasing PO activity and lignin and total protein content. This study is consistent with studies by Khan et al. (2020), Silva et al. (2017), and Hayat et al. (2008).

3.2.4 Salicylic acid and proline content in plants

The results of Tables 9 showed the effect of spraying tomato plants with the induced substances Salicylic Acid and Proline on the rate of measuring their content. The results of Table 9 showed clear significant differences in transactions after the third workshop. Salicylic acid coefficients recorded lower values of acid content with increasing concentration, with the concentration recording (2.5) ml.L^{-1} The highest value measured (SA) was (41.18), while the lowest value was (16.47) at a concentration of (0.5) ml.L^{-1} The comparison treatment (without spraying) recorded a low acid content value of (12.20) at a concentration of (0) ml.L^{-1}. In plants treated with salicylic acid, the percentage of acid increases as it is a growth regulator and defensive signal, so it is naturally active and activates the pathways (SAR) and enhances defenses when the plant is exposed to stress. Its level rises inside the tissues when sprayed externally, which leads to accumulation inside the cells, and it decreases relatively after the plant's defenses stabilize The reason for the decrease in the number of untreated infected plants is the plant's dependence on the amount produced naturally in it, and this amount is limited in the plant as a defensive state when exposed to stress. (Kaya et al., 2023; Chao et al., 2025).

Also Table 10 showed that there were significant differences in measuring the proline content in the proline spray coefficients, as the results showed a clear increase in the coefficients after the third spray, and the coefficients recorded high values in the proline content with an increase in concentration, the concentration recorded (2.5) ml.L^{-1} had the highest value of (119.06), while the lowest value was at concentration (0.5) ml.L^{-1} amounted to (93.50), and compared to the comparison treatment (without spraying), which recorded a low value at a concentration of 0 ml.L^{-1}, reaching (90.28). Proline is an amino acid found naturally inside the plant that protects the plant from external stress. It stabilizes cell membranes, reduces mycotoxins, and acts as an antioxidant. External spraying increases its absorption and accumulation in tissues, thereby improving resistance and protecting cells. Therefore, its percentage inside the plant increases with increasing spray concentration The decrease in comparative treatment is its consumption by the plant to resist pathological stress, since fungal infection affects physiological processes such as photosynthesis and the manufacture of proteins and amino acids, thus causing its value to decrease in the plant, and these results are consistent with other studies (Mudasir et al., 2016; Sofia et al., 2023).

Table 9: Effect of treatment with Salicylic acid and proline on the average Salicylic acid and proline content after the third spray of tomato plants growing under biostress caused by pathogenic fungus *Alternaria alternate*

Concentrations	Salicylic acid	Proline
0	12.20 g	90.28 f
0.5	16.47 f	93.50 e
1	20.70 e	97.11 d
1.5	27.28 d	103.61 c
2	33.41 c	111.49 b
2.5	41.18 b	119.06 a
Control	55.22 a	33.51 g

Note: Similar characters indicate no statistically significant differences between the averages, at a probability level of 0.05.

4. Conclusion

The results of this study showed the role of Salicylic acid and Proline in stimulating systemic resistance in tomato plants susceptible to early blight, as the growth characteristics represented by the weight and number of fruits were improved, especially at optimal concentrations Systemic resistance was also stimulated when Salicylic acid and proline were sprayed externally on the plant, where defense mechanisms were enhanced instead of growth as a natural regulatory response, especially at high concentrations, by raising the level of hormones, enzymes, proteins, and lignin and increasing the content of Salicylic acid and proline as a defense response to reduce the spread of the pathogenic fungus under study within Plant tissues The results of this study are promising in combating plant diseases without resorting to the use of chemical pesticides with harmful environmental effects, which supports the goals of sustainable development and thus preserving the environment.

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