

Effect of salicylic and ascorbic acids on growth of two pathogenic fungi of pepper plants

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Abstract

This study was conducted to investigate the effectiveness of two types of acids, namely salicylic acid and ascorbic acid, against two fungi that are pathogenic to pepper plants, namely *Fusarium oxysporum* and *Botrytis cinerea*, under laboratory and greenhouse conditions. The results showed that the effect of salicylic acid increased with increasing acid concentration, as inhibition of the growth of the pathogenic fungus *F. oxysporum* increased, and when the acid concentration increased to 200 mg per liter, the fungal growth was completely inhibited. While the concentration of 300 mg/L of ascorbic acid completely inhibited the growth of the pathogenic fungus. It was also found that the dry weight of the pathogenic fungus *F. oxysporum* could reduce to 62 mg (or inhibition rate 53.73%) from 134 mg for spraying zero mg per liter salicylic acid with increasing the concentration of salicylic acid up to 50mg per liter. Raising acid concentration to 150 mg l (-1) resulted in dry fungal weight reduction to 14 mg (89.55% inhibition rate), whereas 200 mg l (-1) effectively inhibited growth. Both the selected compounds ascorbic acid had fully inhibited the dry weight of the pathogenic fungus in control conditions at a concentration of 300 mg/L. Tests showed that 100 mg/L of the acid concentration inhibited fungal growth to 42 mm (inhibition rate of 54.99%), inhibition increased to 86.43% at a concentration of 150 mg/L, and growth rate was limited to 12.66 mm; 200 mg per liter of salicylic acid completely suppressed fungal growth. The results also revealed that a 200 mg l (-1) concentration of the acid was enough to completely suppress fungal growth, with relative inhibition being 100%. Concerning ascorbic acid, the results showed that this effect was enhanced with increasing of concentration of acid, and resulted in decreasing the dry weight of *Botrytis cinerea* biomass. Growth was completely inhibited at 400 mg/L, as observed from the results. The results showed that the various treatments affected differently pepper plant growth parameters such as shoot and root length per plant, dry weight of plants and the percentage infected plants. The data indicated a clear impact of the tested pathogen *Fusarium oxysporum* in suppressing most plant growth and pathogenicity traits for the various treatments. It was obvious that the influence of salicylic acid and ascorbic acid to enhance the plant growth parameters and reduce percentage of *Fusarium* wilt diseases in comparison to various treatments.

Keywords: Salicylic acids, Ascorbic acids, *Fusarium oxysporum*, *Botrytis cinerea*, Pepper plants

Introduction

Pepper, is considered as an important cash crop all over the world and especially in Iraq. This crop is vulnerable to many pests including pathogenic fungi at its different stages of growth. Peppers are a family of plants from the nightshade. EN they are commonly used nutrients. They are rich in vitamins C and A, and contain antioxidants that promote body health (Shim et al., 2023).

Pepper is an annual, herbaceous, culinary and medicinal plant of the specie, *Capsicum frutescens* valued in countries other than Iraq in view of its requirement for climatic regimes-hot humid and well-drained soil 2023. Fungal diseases are significant to pepper plants, the most common ones being *Fusarium oxysporum* and *Botrytis cinerea* which contribute significantly to the deterioration of health and productivity in this crop (Abdel-Aziz et al, 2023). To control these fungal diseases, the application of toxic and cancer causing chemical

pesticides to humans and environment needs to be minimized. Consequently, the scientists and experts in plant diseases have focused on employing a safe, economic method. One of the ways to control these diseases is field application of organic acids like salicylic, ascorbic acid.

Salicylic acid is a natural substance which, because of its antioxidant properties, plays an important role in plant stress perception paramount. This acid also enhances the immunity of plant to fungal infection (Decsi et al., 2025). Some reports have suggested that this acid might be potential to serve as a fungicide for some plant diseases, besides conferring the resistance of plants to fungal pathogens. Linxuan et al. (2022) reported that this acid can effectively suppress the mycelium growth and spore production, and inhibit the growth of *F. oxysporum*. While Mufti et al. (2023) explained the possibility of using salicylic acid to control fusarium wilt disease in chickpea plants. While El-Sharkawy and El-Sharawy (2024)

concluded that treating pepper plants with salicylic acid significantly increased phenolic compounds, total flavonoid content, and total protein, in addition to activating peroxidase and polyphenol oxidase enzymes, compared to plants not treated with the acid. Muhammad et al. (2022) confirmed that salicylic acid showed less disease severity in pepper plants infected with *Fusarium* wilt compared to benzoic, citric and ascorbic acids. Abou-Zeid et al. (2016) found that treating pepper plants with salicylic and ascorbic acids significantly reduced the infection of pepper plants with the The management of *Fusarium* wilt, the soil-borne fungus that afflicts bananas, spices, ginger and other crops, cuts into yield. But at the same time it enhances plants' growth traits. It is much more than normal-- evidently because (Boubakri 2018, Fatima et al., 2025). As recently written by ND Amiri, ASPexpress 13.1 ameliorated a great many indicators of aluminum stress for rice plantlets. Additionally, it was evident that the application of citric acid decreases P solubilization by either of these *Aspergillus* species (Zhang and Pettifor, 2021, Abbas et al., 2024). Therefore, this study was designed to evaluate the effectiveness of salicylic and ascorbic acids in inhibiting and controlling the growth of the two pathogenic fungi, *Fusarium oxysporum* and *Botrytis cinerea*, which cause *Fusarium* wilt and gray mold diseases of pepper plants under laboratory and greenhouse conditions.

Materials and Methods

Pathogenic fungi

This study used a single isolate of the two pathogenic fungi, *Fusarium oxysporum* and *Botrytis cinerea*, isolated from infected pepper plants. *Fusarium oxysporum* was isolated from the rhizosphere of a pepper plant showing symptoms of wilt, while *Botrytis cinerea* was isolated from a pepper fruit infected with the pathogenic fungus.

Preparing concentrations of salicylic and ascorbic acids:

Sterile distilled water was used to prepare several concentrations of the two acids (salicylic acid and ascorbic acid). Concentrations of 50, 100, 150, and 200 mg per liter were prepared, while concentrations of 100, 200, 300, and 400 mg per liter of ascorbic acid

were prepared.

Culture media used:

The culture medium used was potato extract dextrose agar media to test the effectiveness of specific concentrations of both acids on the radial growth of the two pathogenic fungi under study under laboratory conditions. The culture medium used was potato extract dextrose broth media to test the effectiveness of specific concentrations of both fungi on inhibiting the average dry weight of the biomass of the two pathogenic fungi under study.

Study of the effect of several concentrations of salicylic and ascorbic acids on the linear growth of two fungi that are pathogenic to pepper plants on PDA culture media.

For every acid, five different set-ups were employed each was subjected from 0-200 mg/L agar concentration, thus allowing these that appeared on either side of 150 mg/L in order to be examined as optimum concentrations--i.e: 50 mg/L, 100mg/L and 200mg/L. To determine the minimal effective concentration of salicylic acid on suppressing fungal growth, we examined its effect at different levels; ascorbic acid was tested along the same lines with concentrations that ranged from 0-400mg/L mg/L but different from salicylic acid.

* This was followed by sterilization, then cooling to 40°C before pouring into the dishes. After it had set (ie. solidified) on-its-own whereupon completely solid cultures could be manipulated therewithout danger. Then, the center of each Petri dish was inoculated with a small piece of the pathogenic fungus culture grown on the medium at 7 days of age, and then incubated at 25°C. The linear growth rate of the pathogenic fungus was recorded continuously until the growth in the control treatment (acid concentration zero) was reached.

The percentage of growth inhibition was also calculated using the following equation:

Growth inhibition (%) = {(growth in treatment - growth in control)/ growth in control} × 100

Study of the effect of several concentrations of salicylic and ascorbic acids on the linear growth of two fungi that are pathogenic to pepper plants on PDB culture media.

Five concentrations of each acid were used to study the effect of these two acids on the growth of the two pathogenic fungi, *Fusarium oxysporum* and *Botrytis cinerea*, on the liquid biosafety medium (PDB). Five 1-liter glass flasks containing 500 ml of the autoclaved liquid biosafety medium were inoculated with one 1 cm diameter disc of a pure culture of one of the two pathogenic fungi (1×10^6 cell/ml), then the plates were incubated at 25°C for 10 days, and then the fungal biomass was extracted using pure sterile filters using a vacuum device. After that, the biomass was dried at 80°C for 24 hours, and the dry weight of the different concentrations was taken and compared with the control treatment (0 mg per liter), and then the percentage of growth inhibition was calculated.

Study of the effect of salicylic acid and ascorbic acid in stimulating the growth of pepper plants against *Fusarium* rot disease caused by the pathogenic fungus *Fusarium oxysporum* under greenhouse conditions:

We used 10 kg plastic pots with sterile mixed soil then planted the pots with local pepper seeds at a rate of 20 seeds per pot. Then the pots were distributed inside the greenhouse at $25 \pm 5^\circ\text{C}$. The pots were treated according to the following treatments:

- 1- Positive control treatment (without the addition of F.o.)
- 2- Negative control treatment (with the presence of F.o.)
- 3- Soil treatment with salicylic acid only
- 4- Soil treatment with salicylic acid + F. o
- 5- Soil treatment with ascorbic acid only
- 6- Soil treatment with ascorbic acid + F. o
- 7- Soil treatment with salicylic acid + ascorbic acid only
- 8- Soil treatment with salicylic acid + ascorbic acid + F. o

Table 1. Effect of multi concentrations of salicylic acid and ascorbic acid on radial growth rate of *Fusarium oxysporum* on potato dextrose agar medium

Salicylic acid			Ascorbic acid		
Conc. mg/L	RG(mm)	% Inhibition	Conc. mg/L	RG (mm)	% Inhibition
0	84.0	-	0	85.67	-
50	75.33	10.32	100	38.33	55.25
100	59.67	28.96	200	6.0	92.99
150	16.67	80.15	300	0	100.0
200	0.0	100.0	400	0	100.0
LSD = $P \leq 0.05$	3.98	-	LSD = $P \leq 0.05$	4.12	-

RG=Radial growth of pathogen, Conc.=Concentration of acid.

Infection Rate=(Total number of plants / number of infected plants) $\times 100$

To calculate the number of infected plants per treatment: Count the number of plants showing wilt symptoms. Total Plant Count: Count the total number of plants in the treatment under study.

Statistical analysis

Statistical analysis of the data was conducted using the GeneStat statistical program VER 22.0 where averages and the least significant difference between treatments were found at a 5% significance level.

Results and Discussions

Effect of multi concentrations of salicylic acid and ascorbic acid on radial growth rate of *Fusarium oxysporum* on potato dextrose agar medium:

The results of Table 1 showed that the effect of salicylic acid increased with increasing acid concentration, as inhibition of the growth of the pathogenic fungus *F. oxysporum* increased, and when the acid concentration increased to 200 mg per liter, the fungal growth was completely inhibited. While the concentration of 300 mg/L of ascorbic acid completely inhibited the growth of the pathogenic fungus. In conclusion, both acids showed an inhibitory effect on the growth of the pathogenic fungus *F. oxysporum* with increasing concentration, but salicylic acid had a stronger effect at higher concentrations. The potential inhibitory effect on the growth of both acids demonstrates the importance of concentration in determining the effectiveness of the acid in inhibiting fungal growth (Rasouli et al, 2019, El-Shazly et al, 2017).

The results of Table 2 showed that increasing the

concentration of salicylic acid to 50 mg per liter led to

a decrease in the dry weight of the pathogenic fungus *F. oxysporum* to 62 mg with an inhibition rate of 53.73% compared to 134 mg for the zero mg per liter treatment. By increasing the acid concentration to 150 mg per liter, the dry weight of the fungus decreased to 14 mg, with an inhibition rate of 89.55%, while growth was completely inhibited at a

concentration of 200 mg per liter. In the case of ascorbic acid, a concentration of 300 mg/L completely inhibited the dry weight of the pathogenic fungus. In conclusion, both acids significantly affected the dry weight of the pathogenic fungus *F. oxysporum*, but salicylic acid was more effective than ascorbic acid (Guo et al., 2021).

Table 2. Effect of multi concentrations of salicylic acid and ascorbic acid on biomass dry weight of *Fusarium oxysporum* on potato dextrose broth medium

Salicylic acid			Ascorbic acid		
Conc. mg/L	D. W.(mg)	% Inhibition	Conc. mg/L	D. W. (mg)	% Inhibition
0	134.0	0.0	0	104.0	0.0
50	62.0	53.73	100	54.33	47.75
100	14.0	89.55	200	32.67	68.58
150	12.3	91.04	300	8.0	92.30
200	0	100.0	400	0.0	100.0
LSD =P≤0.05	12.16	-	LSD=P≤0.05	4.27	-

DW= Dry weight of pathogen biomass.

The results of Table 3 showed that the effect of acid on the growth of the pathogenic fungus *Botrytis cinerea* increased with increasing its concentration. It was found that a concentration of 50 mg per liter of acid led to a reduction in fungal growth to 83.33 mm with an inhibition rate of 10.71% compared to 93.33% in the control treatment. While the concentration of 100 mg per liter of the acid reduced the fungal growth to 42 mm with an inhibition rate of 54.99%, the inhibition rate increased to 86.43% at the concentration of 150 mg per liter with a growth rate of 12.66 mm, while the concentration of 200 mg per liter of salicylic acid completely inhibited the

fungal growth. The results also showed that the concentration of 100 mg per liter of ascorbic acid reduced the growth of *Botrytis cinerea* to 53.66 mm and an inhibition rate of 43.71% compared to 95.33 mm for the control treatment. The concentration of 200 mg per liter of acid also reduced the growth of the fungus to 24.33 mm and an inhibition rate of 74.47%, while the concentration of 400 mg per liter of acid completely inhibited the growth of the fungus. The inhibitory effects of these two acids are due to the toxic effects of high concentrations, which lead to inhibition of the growth of the two fungi (Noor and Little, 2022, Viviana et al, 2021, Vinod and Sabah, 2018).

Table 3. Effect of multi concentrations of salicylic acid and ascorbic acid on radial growth rate of *Botrytis cineria* on potato dextrose agar medium

Salicylic acid			Ascorbic acid		
Conc. Mg/L	RG(mm)	% Inhibition	Conc. Mg/L	RG(mm)	% Inhibition
0	93.33	0.0	0	95.33	0.0
50	83.33	10.71	100	53.66	43.71
100	42.0	54.99	200	24.33	74.47
150	12.66	86.43	300	6.67	93.01
200	0.0	100.0	400	0.0	100.0
LSD=P≤0.05	4.81	-	LSD=P≤0.05	3.18	-

RG=Radial growth of pathogen, Conc. =Concentration of acid.

The results of Table 4 showed that the concentration of 50 mg per liter of salicylic acid led to the inhibition of the dry weight of the biomass of *Botrytis cinerea* to 83.33 mg, with an inhibition rate of 49.89%,

compared to 166.3 mg for the control treatment, while the concentration of 200 mg per liter of the acid was sufficient to completely inhibit the growth of the fungus, reaching 100%.

As for ascorbic acid, the results indicated that the effect increased with increasing acid concentration and led to a reduction in the dry weight of the biomass of *Botrytis cinerea* fungus. The results showed that a concentration of 100 mg per liter of acid led to a reduction in the dry weight of the fungus

to 81 mg, with an inhibition rate of 51.59%, while a concentration of 300 mg per liter recorded a significant reduction in the dry weight of 6 mg, with an inhibition rate of 96.41%, and a concentration of 400 mg per liter completely inhibited growth.

Table 4. Effect of multi concentrations of salicylic acid and ascorbic acid on biomass dry weight of *Botrytis cineria* on potato dextrose broth medium

Salicylic acid			Ascorbic acid		
Conc. Mg/L	DW(mg)	Inhibition %	Conc. %	DW(mg)	Inhibition %
0	166.3	0.0	0	167.33	0.0
50	83.33	49.89	100	81.0	51.59
100	37.3	77.57	200	45.67	72.70
150	17.7	89.35	300	6.0	96.41
200	0.0	100.0	400	0.0	100.0
LSD=P≤0.05	8.74	-	LSD=P≤0.05	6.33	-

DW= Dry weight of pathogen biomass

The results of Table 5 showed that the different treatments varied in their effect on the studied pepper plant growth parameters, which included the length of the shoot and root system per plant, the dry weight of the plant, and the percentage of disease. It was observed that the impact of the tested pathogen, *Fusarium oxysporum*, in decreasing most plant growth and pathogenicity characters was evident for each treated and untreated characteristics. The impact of salicylic and ascorbic acid is also evident in the enhancement of plant growth parameters, and reduction in *Fusarium* wilt per cent for various treatments. Alternative to salicylic acid, relations of effect among compounds with effects comparable to thoseresponse of leaf metabolism was observed for these effects. the results revealed that the SA + AA treatment in the absence of pathogenic fungus was significantly higher than other treatments. The dimensions of the vegetative mass, the root mass and the dry weight were found to be 41 x 40, 24 x 63 and 60 x 50 compared with respective values of 30 x 23,16 x M and 31x07 for positive control while negative control gave dimensions of u20. ote that sensitivity between two DFT calculations does not depend on scaling factor s.In this paper we have found A which gives optimal agreement . Results also showed that the application of SA+AA in combination with pathogenic fungus suppressed the increase of disease percentage estimated from 81.43% in negative control to be 4.17%. In summary, the results have shown that it is possible to apply these two acids for controlling *Fusarium* wilt affecting pepper plants.

It has been found that, along with its significant role in the plant's response to abiotic stress,, and its effective therapeutic role against several pathogens,, salicylic acid acts as an internal signalling 2_SC between intra-plant defence responses,,, systemic immunity induction within a plant, against diverse pathogens, besides its pivotal function in the control of physiological and metabolic processes along the plant life (Mimouni et al, 2016, Vicente and Plasencia, 2011). With regard to ascorbic acid, it has been demonstrated that this organic acid is involved in a wide variety of plant metabolic and signaling processes. This acid also contributes to buffering oxidative stress in the plant by detoxifying reactive oxygen species. This acid also plays a crucial role in plant health, growth and development (Hossain et al, 2017, Naz et al, 2016).

Table 5. Study of effect of salicylic acid and ascor acids in stimulating the growth of pepper plants against *Fusarium* rot disease caused by the pathogenic fungus *Fusarium Oxysporum* under greenhouse conditions

Treatment	Shoot length Cm	Root length Cm	Dry weight of plant	Disease incidence %
Positive control	30.23	16.03	31.07	0.0
Negative control	17.20	8.23	20.07	81.43
SA only	35.77	19.57	45.57	0.0
SA + F. o	30.33	16.27	41.03	6.07
AA only	33.07	20.03	34.83	0.0
AA + F. o	31.43	18.87	31.27	9.18
SA+AA only	41.40	24.63	60.50	0.0
SA+AA+ F. o	34.47	23.0	55.33	4.17
LSD=P≤0.05	2.01	1.54	1.96	2.17

SA=Salicylic acid, AA=Ascorbic acid , F.o=*Fusarium oxysporum*

Infection rate=(Total number of plants/Number of infected plants)×100

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