

Effect of Salicylic Acid on Induction of Resistance Against Green Mold in Orange Fruits

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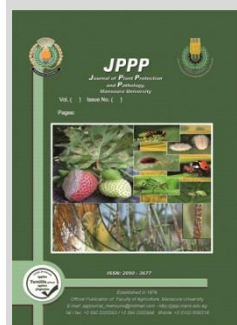
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ABSTRACT

Postharvest diseases significantly reduce the quantity and quality of the fruit crop. Its causes the loss of more than half of the agricultural production of fruits and vegetables. Therefore, this study aimed to find safe and environmentally friendly alternative fungicides to induce resistance against postharvest disease by inducing the natural resistance of fruits and vegetables by increasing the antioxidant enzymes that minimize the disease and enhance the quality of fruits and prolong the storage period. The following fungus is the most important pathogen of orange fruits after harvest (*Penicillium digitatum*). This study showed that the salicylic acid (SA), at 14 mM, inhibited the growth of mycelium with a percentage of 100 compared to the untreated control, while the percentage of inhibition of spore germination was 90.8%, compared to the untreated control. Moreover, SA inhibited the development of treated orange fruit rots by 39.06% and enhanced the enzyme peroxidase (POD), Polyphenoloxidase (PPO), and total phenols contents. We recommend the use of salicylic acid to reduce the severity of infection caused by *P. digitatum* that affects orange fruit.

Keywords: Postharvest diseases, Salicylic acid, Orange, Green mold, Induced resistance

INTRODUCTION

Postharvest diseases are of great importance that should not be overlooked, as they cause the loss of more than half of the agricultural production of fruits and vegetables. The following fungi are the most important pathogens of fruits and vegetables after harvest *Aspergillus flavus*, *A. niger*, *A. parasiticus*, *Alternaria alternata*, *Botrytis cinerea*, *Penicillium digitatum*, *Sclerotinia sclerotiorum*, *Fusarium sambucinum* (Adss *et al.* 2017; Attia *et al.* 2019; Soliman *et al.* 2019; Elsherbiny *et al.* 2021). The most important pathogenic fungi such as *Penicillium*, *Sclerotinia*, *Fusarium*, *Alternaria*, and *Botrytis*, especially in conditions of high temperature and high humidity shorten the storage period and cause global economic losses (Chen *et al.* 2014). The fungus *P. digitatum* infects mostly mature fruits of the Rutaceae family and has a small host population (Costa *et al.* 2019). *P. digitatum* causes this infection and penetrates the fruits through wounds on the peel during harvesting, handling, and storage (Costa *et al.* 2019). At 25°C, the pathogen has a life cycle of between 3 to 5 days of disease course and a wide range of spore production one to two billion (Zhu *et al.* 2019).

Plants can detect the presence of pathogens and can respond and defend themselves after infection, a downstream defense pathway involving a various range of components and signals transferred systemically out of plant tissues is activated (Fu and Dong, 2013; Soliman *et al.* 2021; El-Sharkawy *et al.* 2022). Various natural and synthetic compounds were discovered to mimic the effect of pathogens and provoke plant defense responses. These

components, known as "elicitors or inducers" can be applied exogenously during various plant growth stages, and their actions in enhancing plant defense can last for some time after enforcement, conferring plants with resistance against a broad range of plant pathogens (Walters *et al.* 2013; Soliman *et al.* 2021).

Salicylic acid is one of the first compounds to be discovered to activate systemic resistance in plants (Métraux *et al.* 1990). It is a naturally occurring organic compound that plays an important role in SAR signaling pathways (Durrant and Dong, 2004; Soliman *et al.* 2021; Rashad *et al.* 2021). Therefore, this study aimed to find safe and effective alternatives to the use of fungicides, which cause risks to human health, environmental pollution, and biodiversity imbalances, as well as the formation of fungal strains that are resistant to fungicides.

MATERIALS AND METHODS

1. Pathogen and chemical elicitor

A virulent isolate of *P. digitatum* was obtained from the Department of Plant Pathology, Faculty of Agriculture, Mansoura University. Salicylic acid was obtained from Sigma Chemical Co. Egypt.

2. Antifungal activity

For mycelial growth, SA was tested in a variety of concentrations, including 8, 10, 12, and 14 mM. For each concentration, an Erlenmeyer flask containing 250 ml PDA medium was prepared. Just before pouring SA into Petri plates, they were mixed with the melted PDA. The plates were inoculated with 7-day-old of *P. digitatum*

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cultured 5 mm mycelial discs. As a control, plain PDA plates inoculated with *P. digitatum* mycelia discs were used. Four plates were used as replicates for each treatment and incubated at 25 °C. The diameter of the fungal growth in the different treatments was then measured, and the inhibition in growth was calculated as:

$$\text{Inhibition \%} = \frac{a - b}{a} \times 100$$

Where

a is the growth diameter in the control and b is the growth diameter in treatment.

For spore germination, the conidial suspension of *P. digitatum* (10⁶ conidia mL⁻¹) was mixed with potato dextrose broth (PDB) in glass tubes and SA to achieve the final concentrations of 0, 8, 10, 12, and 14 mM. After 15 h of incubation, spores were microscopically examined for germination rate. Spore germination inhibition was calculated using the formula (Elsherbiny et al. 2017):

$$\text{Inhibition (\%)} = [(\text{conidia germinated in control} - \text{conidia germinated in treatment}) / \text{conidia germinated in control}] \times 100$$

3. Orange fruit assay

The following experiment was conducted with the fruits of orange obtained from Sherbeen city, Dakahlia, Egypt. The fruits were thoroughly rinsed under running water. After that, the orange fruits were submerged in a solution of 2 percent NaOCl for 2 min. The fruits were washed in sterile distilled water before being air-dried. Then, at the vegetative tip, a wound was formed on the outer surface of the orange fruits (3 mm depth to 3 mm breadth). The following quantities of SA were injected into 25 µL of SA 6, 8, 10, and 12 mM. As a control, distilled water was used. After 3 hours, inject the fruits with 20 µl of *P. digitatum* spore suspension at 1 × 10⁶ spore mL then store the fruits in plastic cartons at 25°C, and 95-100% humidity for seven d. Each treatment had three replicates, each with ten fruits, and the experiment was repeated two times. The disease incidence and severity were calculated as:

$$\text{Disease incidence (percentage)} = [(\text{number of rotten wounds} / \text{total wounds})] \times 100.$$

$$\text{Disease severity percentage} = [(\text{treatment lesion diameter} / \text{control lesion diameter})] \times 100.$$

4. Biochemical analysis

Inoculation was done at the greatest dosage utilized in the growth of fruit mold tests, where wounds were formed on the surface of the fruits, and samples were taken from the inoculated fruits with chemical elicitors and pure water as a control (3 mm deep and 3 mm wide and inoculated after washing with running water and sterilization). At a temperature of 24°C, the cells were incubated in plastic boxes. On the first, second, third, and fourth days, samples were obtained from the entire wounded area as well as the surrounding area. For each treatment, three replicates were employed, and the process was performed twice. According to (Maria et al. 1981; Maxwell and Bateman (1967), POD and PPO enzymes were extracted and assayed. Total phenol content was also determined using the Folin-Ciocalteu method (Malick and Singh 1980).

5. Statistical analysis

The statistical analysis system CoStat (CoHort Software, USA) version 6.4 was used to analyse all data. Duncan's multiple range test was used to determine whether there were significant treatment differences at *P* ≤ 0.05.

RESULTS AND DISCUSSION

1. Antifungal activity

All concentrations of SA showed a significant decrease in the mycelial growth of *P. digitatum* (Table 1). The inhibition of mycelial growth was 84.76%, 95.77, and 100 for concentrations 10, 12, and 14 respectively.

Table 1. Effect of salicylic acid on *P. digitatum* growth

Treatment	Concentration (mM)	Mycelial growth inhibition (%)
SA	8	82.87 d
	10	84.76 c
	12	95.77 b
	14	100 a
Control	0	0.0 e

Means with the same letter are insignificantly different (Duncan multiple range test at *P* ≤ 0.05).

For germination inhibition, Table 2 showed that SA showed a significant decrease in the spore germination of *P. digitatum* compared to the untreated control. The percentage of inhibition of the spore germination% of SA ranges ranged from 88.51, 94.25, 94.25, and 90.8 for concentrations 8, 10, 12, and 14 mM respectively, while the lowest concentration of spore growth inhibition was at 8 mM, but the highest inhibition of spore germination% was the concentration 12 and 14 mM. These findings are consistent with those of Panahirad et al. (2012), who found that 5 mM of SA reduced the growth of *Rhizopus stolonifera* in vitro. The increasing demand for safe and effective alternatives to chemical fungicides has led to an increased interest in the field of induced resistance, in which organic and synthetic chemicals can be used to induce resistance against plant pathogens and thus control or reduce the effects of plant diseases (El-Sharkawy et al. 2022).

Table 2. Effect of SA on spore germination inhibition of *P. digitatum*.

Treatment	Concentration (mM)	Spore germination inhibition (%)
SA	8	88.51 c
	10	94.25 a
	12	94.25 a
	14	90.80 b
Control	0	0.0 d

Means with the same letter are insignificantly different (Duncan multiple range test at *P* ≤ 0.05).

2. Disease parameters

The application of SA at a concentration of 12 mM decreased disease incidence and severity by 25 and 39.06%, respectively (Table 3). These results are in harmony with Adss et al. (2017) who found that tomato fruits with SA elicitor enhanced the resistance to *A. solani* and reduced the rotten area. Also, Atia et al. 2019 found that *in vivo*, SA minimized the disease severity of

tuber rots caused by *F. solani* or *S. sclerotiorum* compared with control.

Table 3. Efficacy of SA at different concentrations for controlling orange green mold.

concentration (mM)	Disease incidence %	Disease severity %	Efficacy %
0	100 a	100 a	0.0
6	89.28 b	90.78 b	9.22
8	89.28 b	61.80 c	38.2
10	85.71 c	61.66 c	38.34
12	75 d	60.94 d	39.06

Means with the same letter are insignificantly different (Duncan multiple range test at $P \leq 0.05$).

3. Enzymes activities and total phenols

The results showed that the POD activity of orange fruits treated with SA at 12 mM was higher on day 4 compared to the untreated control on the first day of storage at 24 °C. Activity levels during 4 days of storage on the second and then first days compared to control as in (Table 4), while the activity of total phenols was highest on the third and fourth day, where SA significantly enhanced the activity of total phenols compared to control untreated.

These results are in harmony with findings by (Adss *et al.* 2017), who found that treatment of tomato fruits by elicitors after harvesting increased the activity of the PAL, PPO, and POD enzymes. The mechanisms of defense include earlier physical and chemical barriers that inhibit the pathogen (Hilal *et al.* 2016; Shafie *et al.* 2016; Yousef *et al.* 2016; Farouk *et al.* 2017; El-Sharkawy *et al.* 2018; El-Sharkawy *et al.* 2022).

Table 4. Effect on defense-related enzyme activity and phenolic contents.

Day	PODc (U)	PODt (U)	PPOc (U)	PPOt (U)	Total phenols c	Total phenols t
1	0.14	0.94	5.01	5.78	51.41	35.25
2	0.79	0.66	4.98	5.85	49.27	56.48
3	0.80	0.70	5.22	5.95	54.26	67.84
4	1.24	1.83	7.69	7.38	67.57	83.33

PODc (POD control), PODt (POD treatment), PPOc (PPO control), PPOt (PPO treatment), Total phenols c (control) and Total phenols t (treatment). One unit of POD, and PPO activity was defined as the change in absorbance at 460, and 398 nm, respectively per mg of protein per minute.

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تأثير حمض الساليسيليك على استحثاث المقاومة ضد العفن الأخضر في ثمار البرتقال

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تقلل أمراض ما بعد الحصاد بشكل كبير من كمية ونوعية ثمار الفاكهة، حيث تتسبب في خسارة أكثر من نصف الإنتاج الزراعي من الفاكهة والخضروات. لذلك هدفت هذه الدراسة إلى إيجاد وسائل بديلة وصديقة للبيئة للحث على مقاومة أمراض ما بعد الحصاد من خلال استحثاث المقاومة الطبيعية للفاكهة والخضروات عن طريق زيادة إنزيمات مضادات الأكسدة التي تقلل المرض وتعزز جودة الفاكهة وتطيل فترة التخزين. أظهرت هذه الدراسة أن حمض الساليسيليك (SA)، عند 14 ملي مولار، كان الأعلى في تثبيط نمو *Penicillium digitatum* بنسبة 100%، بينما كانت النسبة المئوية لتثبيط إنبات الجراثيم 90.8%. علاوة على ذلك، أعاق SA نمو المسبب المرضي على ثمار البرتقال المعالجة بنسبة 39.06% وعزز زيادة إنزيم بيروكسيداز (POD) وبوليفينولوكسيداز (PPO) ومحتوى الفينولات الكلي.