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The Influence of Resistance Chemical Inducers Against Anthracnose on Cucumber and Pepper Plants

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ABSTRACT

This study aimed to investigate the effects of boric acid (BA), ascorbic acid (ASA), and salicylic acid (SA) as chemical inducers on the linear growth and sporulation of *Colletotrichum orbiculare* and *Colletotrichum acutatum* using cucumber and pepper plants. Scanning electron microscopy (SEM) analysis of SA-treated samples at concentrations of 5mM and 2.5mM against *C. orbiculare* and *C. acutatum*, respectively, revealed noticeable deformations, shrinkage, and collapse. Under greenhouse conditions, the efficacy of SA on disease severity index (DSI%) was evaluated, with the Super zad cucumber hybrid F1 cultivar displaying a disease severity of 19.7%, followed by the Rania cucumber hybrid F1 cultivar at 27.57%. In pepper plants, SA treatment demonstrated reduced DSI of anthracnose in both the hot pepper ES 752 F1 and pepper super Ammar F1 cultivars compared to the control treatment. Additionally, SA treatment significantly elevated the levels of non-enzymatic substances, such as free phenolic compounds, total flavonoid content, and total protein, as well as the activities of antioxidative enzymes (peroxidase and polyphenol-oxidase) in both cucumber and pepper cultivars when compared to the controls.
Keywords: Anthracnose; Chemical inducers; *Colletotrichum orbiculare*; *Colletotrichum acutatum*; Cucumber; Pepper.

INTRODUCTION

Plants have intrinsic immune systems that can protect them from numerous pathogen attacks. Both biotic and abiotic inducers can trigger these defense systems (Floryszak-Wieczorek *et al.*, 2016). Therefore, substantial efforts have been made to develop plant defense systems to control plant diseases and simultaneously trigger resistance inducers. In the Systemic Acquired Resistance (SAR) state, different plant tissues can act as signal conductors in response to various strange stimulators resulting in a high level of resistance against many biotic or abiotic inducers (Fu and Dong, 2013). During various stages of plant growth, several kinds of natural or synthetic compounds known as resistance inducers and elicitors are applied exogenously to improve plant resistance (Walters *et al.*, 2013; Gao *et al.*, 2014). Additionally, using environmentally benign compounds like these elicitors is thought to be a successful tactic for boosting plant defense against plant diseases (Briache *et al.*, 2020).

Numerous chemicals, both natural and synthetic, have been discovered to be effective plant systemic resistance inducers, including oxalic acid, sodium saccharin dihydrate, glycerol-3-phosphate, and acibenzolar-S-methyl (ASM) Also, salicylic acid (SA), jasmonic acid (JA), Ascorbic acid(ASA), Boric acid (BA) and ethylene (Arora, 2013; El-Sharkawy *et al.*,2016; Li *et al.*, 2016, Svoboda *et al.*, 2021). Significant modifications to the metabolic functions within plant cells occur in conjunction with the activation of resistance responses in plants (Apel and Hirt 2004). Also, The SAR in plants has been studied at the molecular and biochemical levels, and it has been found that different chemicals' activities, whether enzymatic or nonenzymatic, may fluctuate dramatically during the induced resistance process (Prusky and Romanazzi, 2023). Anthracnose, caused by diverse species of the genus *Colletotrichum*, which is a destructive disease in a number of crops including fruit, vegetables, trees and shrubs due to its incidence in pre-harvest and post-harvest stages. It occurs worldwide and causes significant economic losses, particularly in tropical and subtropical regions (Baroncelli *et al.*, 2016; Chakraborty *et al.*, 2019; Guevara-Suarez *et al.*, 2022). It can affect all parts of plants at any stage of growth, and symptoms include dark, sunken circular or oval-shaped lesions on stems, leaves and petioles. Also, fruit lesions become sunken, round, wet and black in colour ,water-soaked cotyledons become limited and pale in colour with chlorotic (yellow) or necrotic (brown) lesions then dry up eventually and die (Yousuf *et al.*, 2018; Tamilmalar *et al.*, 2022). Conidial masses are formed when the conditions are favorable.

Chilli anthracnose is mainly caused by *Colletotrichum truncatum, C. gloeosporioides, C. coccodes* , *C. capsici* and *C. acutatum* (Sim.) which, frequently mentioned globally and linked to anthracnose in bell peppers and chilli peppers (Montri *et al.*, 2009; Damm *et al.*, 2012). *Colletotrichum orbiculare* (Berk. and Mont.) can attack Cucurbitaceae and members of host species worldwide (Chen and Dai, 2012; Keinath, 2015). It is commonly found in cucumber under both greenhouse and field condition resulting in premature plant death due to the reduction in the photosynthetic surface area leading to yield losses (Chen and Dai, 2012).

Controlling diseases caused by *Colletotrichum* spp. poses a challenge due to their high plasticity and genetic variability, which enables the development of new strains resistant to fungicides (Hahn, 2014). To mitigate the adverse effects of fungicides on humans and the

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environment, chemical elicitors have been explored as an alternative approach for managing diseases caused by *Colletotrichum* spp. across various plant hosts (Arora, 2013; Li *et al.*, 2016; da Silva *et al.*, 2018). Therefore, the objectives of this study were: a, to assess the impact of boric acid, ascorbic acid, and salicylic acid on the linear growth and sporulation of *C. orbiculare* and *C. acutatum* in cucumber and pepper plants under in vitro conditions; b, to evaluate the activity of oxidative enzymes and total phenolic compounds to elucidate the potential correlation between their activity and the induced defense mechanisms; c. to analyze micrographs obtained through scanning electron microscopy (SEM) to examine the effects of salicylic acid on *C. orbiculare* and *C. acutatum*; d, to investigate the effectiveness of salicylic acid in controlling anthracnose in cucumber and pepper plants under glasshouse condition.

MATERIALS AND METHODS

Isolation and Identification

Colletotrichum spp. was isolated from cucumber and chili pepper plants exhibiting characteristic anthracnose symptoms. Lesions measuring 5 mm in diameter were sliced, and the plant tissues were subjected to surface sterilization. This involved treating them with 1% NaOCl for 1-2 minutes, followed by 70% ethyl alcohol for 30 seconds. The tissues were then rinsed twice with sterilized distilled water, dried using sterilized filter paper, and cultured on potato dextrose agar (PDA) medium supplemented with streptomycin. The plates were incubated at 25±1 °C for 7 days, as described by Dutta *et al.* (2019). The isolates were identified based on morphological and cultural characteristics, in accordance with Sutton (1992). Verification of the identification was performed at the Assiut Mycological Centre, Faculty of Science, Assiut University (AUMC). *Colletotrichum acutatum* was identified from the pepper plant, while *Colletotrichum orbiculare* was identified from the cucumber plant. The cultures were stored at 4 °C until further use.

Effect of resistance inducing chemicals (RICs)

Effect of RICs on the linear growth of C. orbiculare and *C. acutatum*

In this study investigation the inhibitory effects of resistance-inducing chemicals (ascorbic acid, salicylic acid, and boric acid) on the linear growth of *C. orbiculare* and *C. acutatum* was carried out. Selected concentrations of these chemicals (2.5, 5, 10, and 20 mM) were individually added to sterilized potato dextrose agar (PDA) medium before solidification, with a volume of 10 mL per plate. The plates were then inoculated at their centers with equal-sized discs (5 mm in diameter) obtained from the periphery of 7-day-old cultures of *C. orbiculare* or *C. acutatum*. Each treatment and control consisted of five plates containing either *C. orbiculare* or *C. acutatum*. Incubation of the cultures was carried out at a constant at 25±1°C (Dutta *et al.*, 2019). To determine the rate of growth reduction, measurements were taken when the mycelial mats in the control treatment covered the surface of the medium, following the calculation method described by Siripornvisal *et al.* (2009).

Effect of RICs on Sporulation of C. orbiculare and C. acutatum

The sporulation of *C. orbiculare* and *C. acutatum* on an amended growth medium were assessed. Mycelial growth was allowed to reach the edge of the control plate. Firstly, A sterile, clean glass rod was used to rub the surface after each plate received 5 ml of sterile, distilled water. A total of 50 ml of sterilized water was used to filter the suspensions from five plates (Ketabchi and Shahrtash, 2011). To quantify the final conidia concentration of spores, a hemocytometer under a compound microscope (40X) was used by adding 10 µl suspension pipetted into each side. Also, here each value indicates the mean and standard error. The number of spores per ml produced by each replicate was recorded after 7 days of incubation (five replicates /treatment).

Scanning electron microscopy (SEM)

SEM analysis was performed on the treatments that exhibited the most promising results *in vitro* in which potato dextrose agar (PDA) plates were prepared by adding salicylic acid as the chemical inducer at concentrations of 5 mM for *C. orbiculare* and 2.5 mM for *C. acutatum*. The PDA medium was sterilized, and the salicylic acid was incorporated separately into the medium before it solidified, with a volume of 10 ml per plate. Next, mycelial discs measuring 5 mm in diameter were obtained from 7 day-old cultures of either *C. orbiculare* or *C. acutatum* and placed at the center of each Petri plate. These plates were then incubated at 25±1°C until the mycelial growth reached the edges of the control plates without any chemical treatment.

For SEM sample preparation, mycelial discs with a diameter of 8 mm from both the treatments and the control were directly placed into glass vials containing 3 ml of a solution consisting of 2.5% buffered glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium phosphate buffer at pH 7.4. The samples were left overnight at 4°C. Subsequently, the samples underwent a series of washing steps. They were washed three times for 15 minutes each in 0.1 M sodium phosphate buffer supplemented with 0.1 M sucrose. Following this, the samples were post-fixed in a 2% sodium phosphate buffered osmium tetroxide solution at pH 7.4 for duration of 90 mins.

To prepare the samples for SEM analysis, a series of steps were followed. Initially, the samples underwent three 15-minute washes in 0.1 M sodium phosphate buffer at pH 7.4. Subsequently, the samples were subjected to two 15-minute dehydration treatments in 50% ethanol. Overnight contrast was then achieved by immersing the samples at 4°C in a solution consisting of 70% acetone, 0.5% uranyl acetate, and 1% phosphotungstic acid. Further dehydration was carried out by exposing the samples to ethanol with increasing concentrations (ranging from 80% to 96% v/v). Each concentration step involved two 15-minute treatments. Following this, three 20-minute treatments were performed using 100% ethanol for additional dehydration. The samples were then dried using the critical point method. To enhance conductivity, the samples were coated with gold-palladium membranes. Finally, the prepared samples were examined using a Jeol

JSM-6510 L.V SEM operated at 30 kV at the EM Unit, Mansoura University, Egypt (Karnovsky, 1965). This meticulous sample preparation process ensured optimal conditions for SEM analysis, allowing for accurate visualization and examination of the samples' surface characteristics and morphology.

Effect of SA on cucumber and pepper anthracnose under glasshouse conditions

Conidial suspensions were obtained from the surface of a 7-day-old of *C. acutatum* and *C. orbiculare* in Petri dishes by adding 30 mL of sterile distilled water to a PDA medium. The conidia were removed using a soft bristled brush, and were filtered through two layers of sterile cheesecloth. Conidia were counted with a haemocytometer and the concentration was adjusted to $1x10^6$ spores/ml. The one-month-old seedlings of pepper plants, specifically Pepper super Ammar F1 cv. and Hot pepper ES 752 F1 cv., along with cucumber plants, specifically Rania cucumber hybrid F1 cv. and Super zad cucumber hybrid F1 cv., were used in the study and placed in a glasshouse. After 24 hr plants were sprayed with concentrations of 5mM for cucumber and 2.5 mM for pepper plants or sterile ddH_2O (4 ml per plant, 10–15 psi) and kept for 2 days in glasshouse under >90% relative humidity, 16 hrs photoperiod at 25˚C. Inoculation with the conidial suspension was conducted 72 hrs post SA or sterilized distilled water (ddH_2O) treatment by spraying the entire pepper and cucumber plants with 3 ml conidial suspension per plant ($1x10^6$ spores/ml in 0.02% Tween 20). Sterilized ddH_2O containing 0.02% Tween 20 was used as negative control. The inoculated plants were covered with transparent polyethylene bags for 24 hrs to maintain high humidity. Disease severity was evaluated at 7 days post-inoculation using the scale proposed by Dutta *et al.* (2019). However, disease severity index (DSI%) was calculated according to Chiang *et al.*(2017). Three separate trials were used for the experiment, each with three plants in each cultivar and treatment.

Sample collections

After 14 days from the application of SA, leaves from each treatment were randomly collected and tagged to determine non-enzymatic chemicals and the activity of antioxidant enzymes.

Determination of non- enzymatic compounds

Free phenolic compounds

Studying the effect of chemical inducer (SA) on the phenolic contents of cucumber and pepper plants using two varieties of pepper (Pepper super Ammar F1 cv. and Hot pepper ES 752 F1 cv.) and cucumber plant (Rania cucumber hybrid F1 cv. and Super zad cucumber hybrid F1 cv.). In brief, samples of 5 g of the tested cultivars were taken from SA treatment and control. Fresh leaves were extracted by ethanol followed the method of Singh, (2023). The extracted solution was then filtered and mixed with 1 ml of ethanolic extract with 1 ml of 2 N Folin-Ciocalteu reagent, 1 ml of Na_2CO_3 solution consentration14%, was mixed at 7 ml distilled water, then heated in a water bath at 70°C. The contents of free phenolic compounds were estimated by Folin-Ciocalteu method (Horwitz, 1975) at 650 nm using the correction factor 0.0042 from catechol standard curve.

Total flavonoid content

By using an aluminum chloride colorimetric approach, as described by Chang *et al.* (2002), the total flavonoid concentrations were calculated. In a nutshell, 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water were combined with 0.5 ml of extract and left to stand at room temperature for 30 min. At 415 nm, the absorbance was calculated using a spectrophotometer. Based on fresh weight, the results were recorded as mg equivalents of quercetin per 100 g^{-1} (mg/100 g^{-1} FW).

Total protein

Leaf extract was made by mixing 0.2 g of fresh leaves with 1 ml of 0.1 M phosphate buffer (pH 7) and homogenizing the mixture. Centrifuging the suspension at 10,000 rpm for 15 minutes after filtering it was done (Urbanek *et al.*, 1991). According to Bradford (1976), the soluble protein concentration of the supernatant leaf extract was calculated as mg/g FW using a Bovine serum albumin reference curve and a correction factor of 0.00233.

Determination of antioxidant enzyme activity

All measured enzyme extracts were prepared according to Urbanek *et al.* (1991).

Peroxidase (POD) activity

POD activity was determined following the method described by Allam and Hollis (1972). Briefly, 0.1 mL of enzyme extract was mixed with 4 ml of guaiacol solution, which consisted of 3 ml of 0.1 M potassium phosphate buffer (pH 6.5), 0.5 ml of 2% guaiacol, and 0.3% H_2O_2. The POD activity was then measured using a spectrophotometer, and the change in absorbance at 425 nm per gram fresh weight per 5 minutes was used to express the enzymatic activity.

Polyphenol-oxidase (PPO) activity

PPO activity was measured according to Matta and Dimond (1963). The mixture reaction contained from 0.1 ml enzyme extract, 1 ml of 0.2 M potassium phosphate buffer (pH 7) and 1 ml of 10^{-3} M catechol, and completed to 6 ml with distilled water. This mixture was incubated at 30 °C for 30 min. PPO activity was measured using a spectrophotometer every 0.5 minutes at 430 nm/g FW/5 min.

Statistical analysis

A completely randomized design (CRD) was used to evaluate the collected data. The collected data were analyzed using a One-way ANOVA test with CoStat Software version 6.311. Significance of the results was determined by evaluating the F values. In cases where the F values were found to be significant, post-hoc analysis was conducted using the least significant difference (LSD) method at a significance level of 0.05. This approach allowed for the comparison and separation of means between different groups or treatments.

RESULTS

Effect of RICs on the linear growth of *C. orbiculare* and *C. acutatum*

The data presented in Figures (1) and (2) demonstrate that the tested Resistance-Inducing Chemicals (RICs)

significantly suppressed the mycelial growth of *C. orbic-ulare* and *C. acutatum*. Furthermore, the inhibitory effect of these RICs increased as the concentration of RICs increased. Specifically, SA (Salicylic Acid) exhibited complete inhibition of mycelial growth in *C. orbiculare* at the higher concentrations of 10 and 20 mM. Similarly, SA completely inhibited mycelial growth in *C. acutatum* at the three higher concentrations of 5, 10, and 20 mM, resulting in the greatest reduction in growth percentage. Ascorbic acid followed closely behind SA in terms of growth reduction efficacy. In comparison, boric acid exhibited the lowest efficacy in reducing mycelial growth among the tested RICs.

Effect of RICs on spore production of *C. orbiculare* and *C. acutatum*

As shown in Table 1, the control treatment showed the highest number of *C. orbiculare* and *C. acutatum* spores, at 169.9 and 194.7×10^5 spores/ml, respectively. Moreover, the RICs had a considerable impact on the spore production of *C. orbiculare* and *C. acutatum*, besides, their production decreased as concentrations of RICs increased. SA was the most effective RICs in reducing spore production at all tested concentrations. Regarding the *C. orbiculare* treatment of SA, 62.4 and 22.7×10^5 spores / ml were recorded for 2.5 and 5 mM, respectively, whereas concentrations of 10 and 20 mM inhibited spore generation.

Boric acid had the lowest effect on the number of spores production at 131.8, 124.2, 89.6 and 61.4×10^5 spores /ml for the respective conc-entrations. Ascorbic acid came second in its negative impact on spore production, but it still differed signi-ficantly with control treatment (Table 1). As for *C. acutatum*, SA treatment was the most effective treatment at concentrations of 5 mM and over totally prevented *C. acutatum* spore development. Ascorbic acid and boric acid were the next most effective treatments.

Scanning electron microscopy

SEM analysis was performed to examine the effects of Salicylic Acid (SA) on *C. orbiculare* and *C. acutatum*. The results revealed that at a concentration of 5 mM, SA exhibited significant effects on *C. orbiculare* compared to the control treatment, as observed in Figure (3A and B). The micrographs clearly showed distorted, lysed, and swollen fungal hyphae in the treated samples. Similarly, when applied at a concentration of 2.5 mM, SA had a noticeable impact on *C. acutatum* compared to the unt-reated hyphae (control), as represented in Figure (4A and B). In the control treatment for both fungi, the mycelia appeared healthy with no deformation. However, in the treated fungi, the mycelia appeared thinner, deformed, and ultimately collapsed compared to the control treatment. These findings provide visual evidence of the significant effects of SA on the morphology and integrity of both pathogens.

Effect of SA on DSI of anthracnose in cucumber and pepper under glasshouse conditions

In comparison to the control treatment, SA treatment considerably decreased the DSI of anthracnose in cucumber and pepper plants grown in glasshouse, for two different cultivars of each studied crop (Table 2).

Results revealed that SA reduced the anthracnose severity in cucumber cultivar (Super zad cucumber hybrid F1) showed 19.70 % followed by cultivar (Rania cucumber hybrid F1) at 27.57 %. In pepper plants, SA treatment under glasshouse conditions reduced anthracnose severity in cultivar (Hot pepper ES 752 F1) and cultivar (Pepper super Ammar F1) at 22.25 and 35.70 % compared with control treatment (*C. acutatum*). Significant differences existed among RICs and control treatment (Table 2).

Effect of SA treatment on phenol content in cucumber and pepper plants

SA treatment elevated the phenolic compounds contents over those of controls in cucumber and pepper plants under glasshouse conditions (Table 3). In cucumber cultivars, spraying leaves with SA at 5 mM elevated the phenolic compounds over those of controls (with or without *C. orbiculare* spores inoculation). The highest total phenols were observed in (Super zad cucumber hybrid F1 cv.) at 322.84 mg/100 g fresh weight (FW), whereas the lowest content was recorded for (Rania cucumber hybrid F1 cv.) at 315.49 mg/100g FW. There were significant differences among the tested SA and controls treatments (Table 3). Furthermore, compared to that of controls (with or without *C. acutatum* spores inoculation), spraying pepper leaves with SA at 2.5 mM, cultivar Hot pepper ES 752 F1 showed the highest total phenols at (772.56 mg/100 g FW), whereas pepper super Ammar F1 cv. recorded (537.49 mg/100g FW).

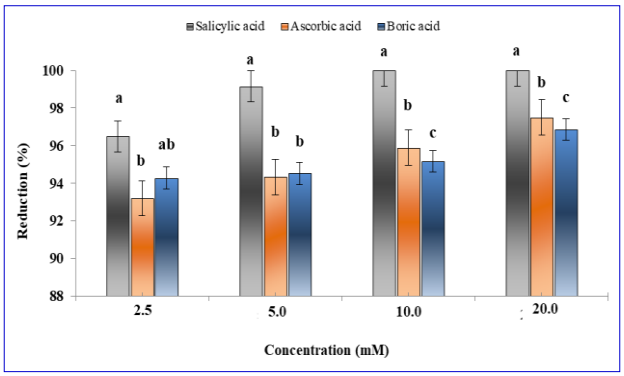

Figure (1): Effect of resistance-inducing chemicals, salicylic acid, ascorbic acid and boric acid, on growth reduction percentage of *C. orbiculare in vitro*. Bars represented by different letters are significant difference (LSD, $p \leq 0.05$).

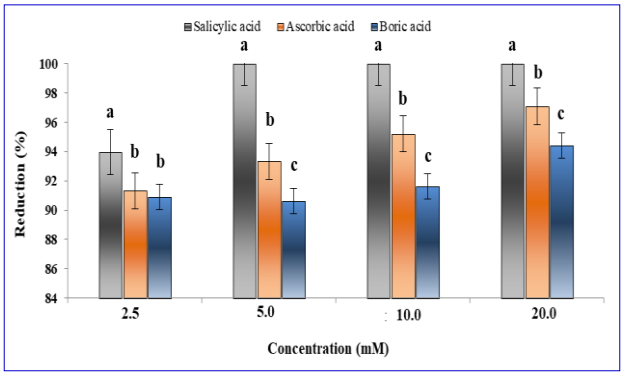

Figure (2): Effects of resistance-inducing chemicals, salicylic acid, ascorbic acid and boric acid, on growth reduction percentage of *C. acutatum in vitro*. Bars with different letters indicate significant difference (LSD, $p \leq 0.05$).

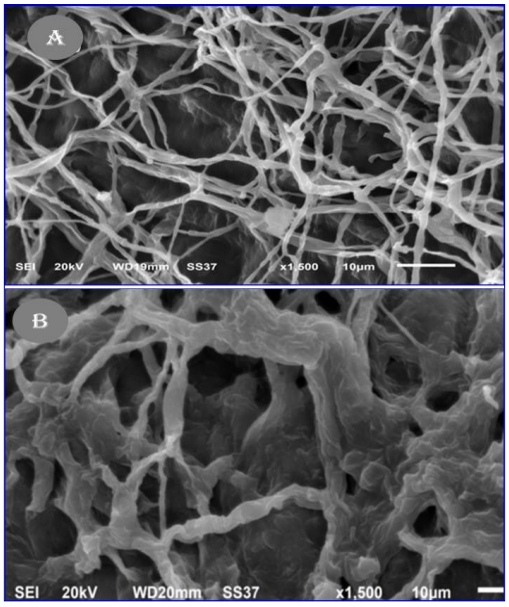

Figure (3): SEM microphotographs of hyphae of *C. orbiculare*. (A), Untreated (control) fungal hyphae grown on PDA. (B), Treated hyphae with 5mM of SA.

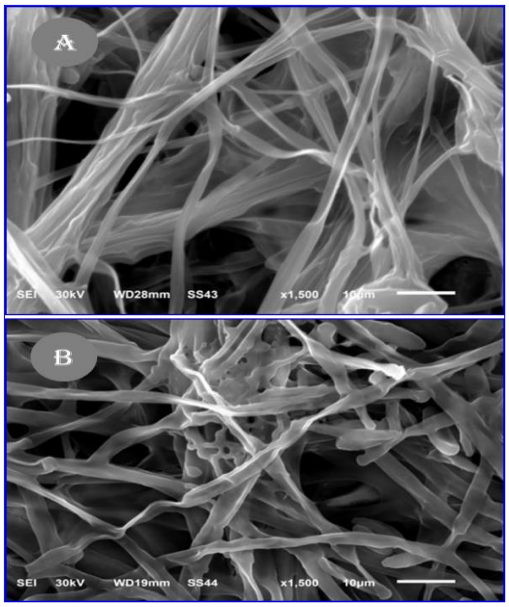

Figure (4): SEM microphotographs of hyphae of *C. acutatum*. (A), untrea-reated (control) fungal hyphae grown on PDA. (B), Treated hyphae with 2.5mM of SA.

Effect of SA on the total flavonoid content in cucumber and pepper plants

The non-enzymatic compounds (total flavonoid contents) in cucumber and pepper plants were significantly affected by the application of SA treatment as compared with the controls under glasshouse conditions (Table 4).

In cucumber, spraying leaves with SA at 5ppm elevated the flavonoid content over those of controls. The highest total flavonoid content was observed in (Super zad cucumber hybrid F1 cv.) at 89.93 mg/100 g FW), whereas the (Super zad cucumber hybrid F1 cv) recorded 79.99 mg/100g FW as compared to that of control (1) (79.79b and 72.98 mg/100g FW) and control (2) with *C. orbiculare* inoculation (81.44 and 76.83 mg/100g FW). Pepper cultivar (Hot pepper ES 752 F1 cv) showed the highest total flavonoid content at (223.1 mg/100 g FW), whereas (Pepper super Ammar F1 cv.) recorded 167.29 mg/100g FW as compared to controls (Table 4).

Effect of SA treatment on protein content of cucumber and pepper plants

Data in (Table 5) showed that the applied SA treatments considerably influenced the non-enzymatic compounds (total protein) and increased the protein content in both cultivars of cucumber and pepper compared to the controls. Hot pepper ES 752 F1 reported 24.26 mg/g FW, whilst Rania cucumber hybrid F1 recorded 19.94mg/g FW.

Effect of SA on POD and PPO activities in cucumber and pepper plants

SA treatment increased POD and PPO in cucumber and pepper cultivars compared to control treatments (Tables 6 and 7). The cucumber cv (Super zad cucumber hybrid F1 and Rania cucumber hybrid F1) showed the highest POD activity at 7 and 6.3 unit/g fresh weight/5 min., respectively.

Similarly, PPO activity was measured with SA treatment at 4 and 3.9 unit/g fresh weight/5min, respectively in (Cucumber cv Rania cucumber hybrid F1) followed by (Super zad cucumber hybrid F1) as compared to control. Also, Hot pepper ES 752 F1 showed the highest POD and PPO activity at 5.44 and 2.24 unit/g FW/5 min followed by cv pepper super Ammar F1 at 3.80 and1.01 unit/g fresh weight/5min, respectively.

Table (1): Effects of RICs on spore production of *C. orbiculare* and *C. acutatum* .

Treatment	Spore production (Spores×10^5 ml^{-1})							
	C. orbiculare				*C. acutatum*			
	Concentration used (mM)							
	2.5	5.0	10.0	20.0	2.5	5.0	10.0	20.0
Control	169.9±5.29^a	169.9±5.29^a	169.9±5.29^a	169.9±5.29^a	194.7±2.15^a	194.7±2.15^a	194.7±2.15^a	194.7±2.15^a
Salicylic acid	62.4±1.2^c	22.7±0.7^c	0.0±0.0^c	0.0±0.0^d	74.2±6.59^d	0.0±0.0^d	0.0±0.0^d	0.0±0.0^d
Ascorbic acid	156.5±10.54^a	121.1±4.92^b	74.9±8.42^b	34.1±4.1^c	107.6±2.94^c	87.2±1.44^c	72.9±8.2^c	30.8±1.12^c
Boric acid	131.8±2.64^b	124.2±1.39^b	89.6±7.21^b	61.4±2.32^b	148.9±11.96^b	141.5±3.9^b	102.1±9.67^b	91.2±2.03^b
LSD 0.05	19.8	12.04	20.02	11.54	23.04	7.6	20.9	5.16

Data are represented in means±SE. Means followed by different letters, per column, are significantly different (LSD, $p \leq 0.05$).

Table (2): Effect of salicylic acid (SA) treatment on disease severity index of *C. orbiculare* and *C. acutatum* on cucumber and pepper plants.

Treatment	Anthracnose Disease Severity (%)			
	C. orbiculare		*C. acutatum*	
	Tested Plant cultivar			
	Cucumber		Pepper	
	Rania hybrid F1	Super zad hybrid F1	Super Ammar F1	Hot pepper ES 752 F1
Control	70.14±0.6[a]	66.74±1.41[a]	59.45±1.33[a]	64.72±2.2[a]
SA	27.57±2.31[b]	19.70±0.11[b]	35.70±2.69[b]	22.25±0.38[b]
LSD	6.62	3.92	8.32	6.19

Data are represented in means±SE. Means followed by different letters, per column, are significantly different (LSD, $p \leq 0.05$).

Table (3): Effect of salicylic acid (SA) treatment on phenol content in cucumber and pepper plants.

Treatment	Phenol content (mg/100 g FW)			
	Tested Plant cultivar			
	Cucumber		Pepper	
	Rania hybrid F1	Super zad hybrid F1	Super Ammar F1	Hot pepper ES 752 F1
Control (1)	226.29±0.44[c]	274.29±0.56[c]	473.5±0.28[c]	540±1.04[c]
Control (2)	229.33±0.52[b]	283.34±0.39[b]	479.95±0.12[b]	615.70±0.52[b]
SA	315.49±0.92[a]	322.84±0.78[a]	537.49±0.34[a]	772.56±0.15[a]
LSD	2.29	2.08	0.9	2.34

Means in the same column followed by different letters are significantly different (LSD, $p \leq 0.05$). Control (1), treated with ddH_2O containing 0.02% Tween 20; Control (2), inoculated with *C. orbiculare* for Cucumber and with *C. acutatum* for Pepper.

Table (4): Effect of salicylic acid (SA) treatment on the total flavonoid content in cucumber and pepper plants.

Treatment	Total flavonoid content (mg/100 g FW)			
	Tested Plant cultivar			
	Cucumber		Pepper	
	Rania hybrid F1	Super zad hybrid F1	Super Ammar F1	Hot pepper ES 752 F1
Control (1)	72.98±1.38[b]	79.79±0.4[b]	118.32±1.12[c]	159.38±0.61[c]
Control (2)	76.83±0.58[a]	81.44±0.58[b]	141.73±0.58[b]	185.87±0.57[b]
SA	79.99±0.78[a]	89.93±1.21[a]	167.29±1.24[a]	223.15±0.18[a]
LSD	3.38	2.8	3.54	1.71

Means in the same column followed by different letters are significantly different (LSD, $p \leq 0.05$). Control (1), treated with ddH_2O containing 0.02% Tween 20; Control (2) inoculated with *C. orbiculare* for Cucumber and with *C. acutatum* for Pepper plants.

Table (5): Effect of salicylic acid (SA) on measured protein content of cucumber and pepper plants.

Treatment	Protein Content (mg/g FW)			
	Tested Plant cultivar			
	Cucumber		Pepper	
	Rania hybrid F1	Super zad hybrid F1	Super Ammar F1	Hot pepper ES 752 F1
Control (1)	16.32±0.17b	16.30±0.66b	18.49±0.21b	22.24±0.43b
Control (2)	18.39±0.21a	16.28±0.07b	20.04±0.15b	23.69±0.34a
SA	19.94±0.96a	18.01±0.13a	22.11±1.10a	24.26±0.40a
LSD	1.98	1.3	2.25	1.35

Means in the same column followed by different letters are not significantly different (LSD, $p \leq 0.05$). Control (1), treated with ddH_2O containing 0.02% Tween 20; Control (2) inoculated with *C. orbiculare* for Cucumber and with *C. acutatum* for Pepper.

Table (6): Effect of salicylic acid on measured Peroxidase activity (POD) in cucumber and pepper plants.

Treatment	Peroxidase activity (unit/g fresh weight/ 5min)			
	Tested Plant cultivar			
	Cucumber		Pepper	
	Rania hybrid F1	Super zad hybrid F1	Super Ammar F1	Hot pepper ES 752 F1
Control (1)	1.11±0.52[b]	2.49±0.40[b]	2.08±0.04[b]	3.60±0.58[b]
Control (2)	2.07±0.09[b]	2.69±0.32[b]	2.97±0.07[ab]	4.61±0.35[ab]
SA	6.34±0.51[a]	7.00±1.25[a]	3.80±0.45[a]	5.44±0.21[a]
LSD	1.47	2.71	0.91	1.42

Means in the same column followed with different letters are significantly different (LSD, p±0.05). Control (1) treated with ddH_2O containing 0.02% Tween 20. Control (2) inoculated with *C. orbiculare* for Cucumber and with *C. acutatum* for Pepper.

Table (7): Effect of salicylic acid on measured Polyphenol-oxidase activity (PPO) in cucumber and pepper plants.

Treatment	Polyphenol-oxidase activity(unit/g fresh weight/ 5min)			
	Tested Plant cultivar			
	Cucumber		Pepper	
	Rania hybrid F1	Super zad hybrid F1	Super Ammar F1	Hot pepper ES 752 F1
Control (1)	1.09±0.06[b]	2.16±0.51[b]	0.52±0.18[b]	1.58±0.51[a]
Control (2)	3.65±0.72[a]	3.25±0.21[ab]	0.86±0.13[ab]	1.57±0.13[a]
SA	4.00±0.52[a]	3.99±0.55[a]	1.01±0.04[a]	2.24±0.32a
LSD	1.78	1.56	0.45	1.24

Means in the same column followed with different letters are significantly different (LSD, $p \leq 0.05$. Control (1), treated with ddH_2O containing 0.02% Tween 20; Control (2), inoculated with *C. orbicularе* for Cucumber and with *C. acutatum* for Pepper cv.

Statistical analyses showed that POD and PPO activity differed significantly in plants treated with SA as compared with those recorded in control with or without *C. orbiculare* or *C. acutatum* inoculation (Tables 6 and 7).

DISCUSSION

The tested RICs reduced the linear growth of *C. orbiculare* and *C. acutatum* and caused disease reductions under glasshouse conditions. The most efficient RIC was SA, followed by ascorbic acid, and the least efficient was boric acid. Similarly, all tomato pathogens, including *Colleto-trichum coccode*s, had their mycelial growth suppressed by SA (1-25 mM) in a conc-entration-dependent manner, according to Jabnoun-Khiareddine *et al.* (2015) findings. Also, an *in vitro* experiment Grellet-Bournonville *et al.* (2012) showed that SA at concentration 50 mM demonstrated an inhibitory impact, 5 mM SA had no effect on the growth of the virulent isolate M11 of *C. fragariae*.

SA at a minimum concentration of 2.5 mM has been shown to have similar effects on a variety of pathogens, including *R. stolonifer, F. oxysporum, R. solani, S. rolfsii, M. phaseolinae, Pythium sp*., and *Phytophthora* (Panahirad *et al.,* 2012, El-Mohamedy *et al.,* 2013,). On the other hand, (Jendoubi *et al.*, 2015) found that SA promoted the mycelial growth of *Alternaria solani* and *F. oxysporum* f. sp. *radices-lycopersici* at lower concentrations. Similarly, Kumar and Bains (2018) found that the applied SA promoted mycelial growth at low concentrations (0.5 mM). However, concentrations greater than 0.5 mM inhibited growth of two isolates of *F. mangiferae* in the laboratory.

In this study the studied RICs reduced the spore production of *C. orbiculare* and *C. acutatum*. As compared to the control treatment, SA was the strongest (RICs) that reduced spore production and inhibited it in a concentration-dependent way. Similarly, SA directly inhibited the germination of *C. gloeosporioides conidia* (*Zhang et al.,* 2016). This finding was in accordance with other studies on other pathogens (Abdel-Monaim *et al.,* 2012). In their study, they showed that SA had significantly inhibited spore formation of *F. oxysporum* f. sp. *lycopersici* at different degrees depending on concentrations. Additionally, SA prevented the germination of *P. expansum* conidia at 2.5 mM and resulting in pathogen protein leakage into the medium, detectable lipid degradation, and intracellular disorganization (da Rocha Neto *et al.*, 2016).

SA treatments showed great impact on the mycelia of *C. orbiculare* and *C. acutatum* including distorted, lysis, swelling, thinning or deformed fungal hyphae. The control treatment showed turgid mycelia. Similar findings have been documented under SEM with SA against *C. gloeosporioides* including deformed hyphae, nodule development, thinned cell walls, and decreased

hyphae diameter (Ramos-Guerreo *et al.,* 2018).

In glasshouse experiment and when SA was applied as foliar sprays on cucumber and pepper, disease severity index in pepper cv. Hot pepper ES 752 F1was lower than those in Pepper super Ammar F1. Similarly from the glasshouse experiment and field trial, Oanh *et al.* (2006) found that the application of SA as foliar sprays induced chemical resistance in chilli against anthracnose. Disease incidence was lower in chilli cv. Man Dum than in Mae Ping. It is possible that the Man Dum variety contained constitutive genes involved in resistant response. (Glazebrook, 2005) stated that plant-pathogen interactions exist on a spectrum ranging from complete resistance to extreme susceptibility. SAR was effective against anthracnose caused by *Colletotrichum lagenarium* in cucumber, and SA treatment induced resistance to this pathogen (Rasmussen *et al.*,1991).

Cucumber and pepper cultivars treated with SA contained substantially more flavonoids and phenolic compounds overall than control cultivars with or without *C. orbiculare* or *C. acutatum* inoculation. Briache *et al.* (2020) confirmed that SA had a comparable impact and that the susceptible genotype Lobab had less phenolic compound accumulation with *Orobanche crenata* infestation than the resistant genotype Giza 843. Phenolic substances improved the mechanical strength of the host cell wall while reducing parasite invasion. Furthermore, some phenolic compounds were toxic to pathogens, and their accumulation at the infestation site was linked to pathogen development inhibition. The phytochemical compounds were increased in cell suspensions in response to a biochemical stress caused by an increase in SA (El-Gaied *et al.*, 2013). The application of SA increased phenoloic and total flavonoids in chili pepper fruits (Sanchez- Chavez *et al.*, 2011).

The non-enzymatic compounds (total protein) were significantly affected by the application of SA and it elevated the protein content in both cultivars of cucumber and pepper as compared to controls. Similarly, Gunaeni *et al.* (2021) recorded that the absorbance value of protein levels in healthy chili pepper fruits was lower than that found in fruit samples infected with *Colletotrichum acutatum* Sukabumi isolate. Protein content looked different between varieties and higher than sensitive controls, which indicates that the increase in protein activity is a form of functional resistance of genes from chili varieties (Gunaeni *et al.*, 2021). By controlling the expression of pathogenesis-related (PR) genes, SA is necessary for the formation of the SAR (Shine *et al.*, 2019). The transcriptional activation of PR genes and systemic resistance activation in plants are regulated by an endogenous buildup of SA, which is produced locally and systemically (Durrant and Dong, 2004).

Under glasshouse, SA treatment raised POD and PPO activity in cucumber and pepper cultivars in comp-arison to control treatment. Many plant enzymes, including oxidative enzymes such as PPO, PO and other enzymes are involved in defense reactions against plant pathogens (Avdiushko *et al.*, 1993). These enzymes were found to be more abundant in cucumber plants treated with abiotic and biotic inducers (Avdiushko *et al.*, 1993). In greenhouse experiments the produced resistance abilities with plant inducers differed between plant inducers in chilli varieties, and that all plant inducers could cause disease resistance to anthracnose in cv. Man Dum (Oanh *et al.*, 2006). The high activity of antioxidant enzymes has been showing in many tolerant/resistant cultivars under various stresses indicating the importance of enzymes in imparting tolerance to biotic and abiotic stresses (Reddy *et al.*, 2004; Pérez-de-Luque *et al.*, 2006). POX is found in plant species and performs a wide range of functions, including detoxification of H_2O_2 and the production of reactive oxygen species. These toxic intermediates cause an oxidative burst that leads to the biosynthesis of lignin, which forms the structural barrier against pathogen invasion (Passardi *et al.*, 2004, Kösesakal and Ünal, 2009). Also, SA and IAA plant treatment increased PAL and PPO activities in resistant and susceptible genotype, which had identified high PAL, POX, and PPO activities in non-infested and infested conditions (Briache *et al.*, 2020).

CONCLUSION

Based on the previous study, it can be concluded that resistance chemical-inducers such as boric acid, ascorbic acid, and Salicylic Acid have demons-trated significant potential in controlling anthracnose disease in cucumber and pepper plants. SA, in part-icular, has shown the ability to induce systemic resistance in both cucumber and pepper plants against *C. orbiculare* and *C. acutatum*. Furthermore, the application of SA through spray treatment led to a notable increase in non-enzymatic compounds and antioxidative enzyme activities in cucumber and pepper cultivars, exceeding the effects of control treatments. However, it is important to note that adjustments in doses and treatment methods may be necessary to optimize the desired outcomes. The induction of Systemic Acquired Resistance (SAR) could serve as an additional tool to enhance genetic resistance against anthracnose. This control strategy presents a promising approach for managing anthracnose in cucumber and pepper plants, while also minimizing the environmental impact associated with chemical fungicides.

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تأثير مستحثات المقاومة الكيميائية على مرض الأنثراكنوز فى نباتات الخيار والفلفل

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الملخص العربـــــي

استخدمت هذه الدراسة نباتات الخيار والفلفل لدراسة آثار كلا من حمض البوريك (BA)، الأسكوربيك (ASA)، وحمض الساليسيليك (SA) كمحفزات كيميائية على تثبيط النمو الخيطي وتكون الجراثيم لكلا من فطر *Colletotrichum orbculare* و *Colletotrichum acutatum*. وأظهرت الصور المجهرية، بواسطة المجهر الإلكتروني الماسح (SEM)، لـ SA ضد *C. orbculare* عند تركيز 5 مم و*C. acutatum* عند 2.5 مم تشوه وانكماش وانهيار فى هيفات الفطر المسبب للمرض. كما أظهرت النتائج ان تأثير SA في مكافحة الإصابة بمرض الأنثراكنوز في الخيار والفلفل تحت ظروف الصوبة الزجاجية أن صنف الخيار (سوبر زاد هجين F1) سجل أقل نسبة لشدة الاصابة بالمرض 19.7%.، يليه الصنف (خيار رانيا هجين F1) بنسبة 27.57 % في نباتات الفلفل، أدت المعالجة بـ SA إلى خفض نسبة الإصابة بالأنثراكنوز في الصنف (الفلفل الحار ES 752 F1) والصنف (Pepper super Ammar F1) مقارنة بمعاملات بالكنترول. كما أدت أيضا المعاملة بحمض SA إلى زيادة كبيرة في مستويات المواد غير الأنزيمية (المركبات الفينولية الحرة، محتوى الفلافونويد الكلي، والبروتين الكلي) وكذلك أنشطة الإنزيم المضاد للأكسدة (peroxidase و polyphenol-oxidase) في أصناف الخيار والفلفل المختبرة.