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Research Article

Effect of Salicylic Acid on Mycelial Growth and Conidial Germination of *Fusarium oxysporum*

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ABSTRACT

Fusarium wilt is a significant disease of chilli (*Capsicum annuum* L.) caused by *Fusarium oxysporum* f. sp. *capsici*. Pakistan is known as a major chilli producer; however the chilli industry is suffering from lower yields affected by biotic and abiotic stresses including *F. oxysporum*. The objective of the study was to evaluate the role of exogenous application of salicylic acid on mycelial growth and conidial germination of the pathogen in controlled lab conditions. Using various concentrations of SA (0-1.5 mM), a clear concentration major effect was observed. At lower concentrations (0.05-0.5 mM) the fungal growth was stimulated, while higher concentrations (0.5–1.5 mM) significantly inhibited fungal growth, being the highest level of inhibition at 1.5 mM where mycelial growth was reduced to 77.8% compared with controls. Moreover, the rate of conidial germination also decreased with increasing levels of SA, with a reduction of 47 % at 1.5 mM indicating its potential as an antifungal agent. The results of this study shows the dual role of SA in regulating plant-pathogen interactions and demonstrate the potential of SA for managing Fusarium wilt in chilli crops, opening avenues for its use as a sustainable agricultural practice to improve the resistance of crops to fungal diseases.

Keywords: Conidial Germination, *Fusarium* Species, Mycelial Growth, Salicylic Acid, Resistance Inducers.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is of significant economic importance all over the world with Pakistan being the sixth largest chilli producing country (Pakistan China Joint Chamber of Commerce and Industry, PCJCCI). In 2024, the total cultivated area under chilli production in Pakistan was about 150,000 acres, and the total production of chilli was approximately 143,000 tonnes. However, in spite of a worldwide reputation and importance, chilli production in Pakistan has shown a decreasing tendency, that is due to a wide variety of biotic and abiotic stresses (Delai et al., 2024). FOC attacks the root tissues, quickly colonizing the xylem vessels (El-Kazzaz et al., 2008), inhibiting water and nutrient movement, and generating characteristic symptoms of upward and inward curling, yellowing and finally plant withering. This emerging threat to chilli production urgently requires research and management practices to minimize its effects and maintain Pakistan's role as a major chilli producer in the world.

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Fusarium wilt is one of such diseases, caused by the soil-borne pathogen. *F. oxysporum* f. sp. *capsici*, is among the most destructive diseases, causing significant yield reductions. Depending on climatic, soil, nutritional conditions and number of pathogens in the soil, this disease occurs at different growth stages, from seedling to maturity.

In order to manage Fusarium wilt, various approaches have been used including crop rotation, cultivation of resistant varieties, biological control, soil solarization and chemicals (Abo-Elyousr et al., 2009). Some of these have severe impacts on the environment while others are costly to employ. Conventional fungicides have limited efficacy and raise concerns over environmental safety and resistance development. Recent studies have identified salicylic acid (SA), a key phytohormone involved in plant defense signaling, as a potential biocontrol agent capable of enhancing plant resistance against pathogens. Natural plant hormone salicylic acid holds a key role in plant-microbial interaction. Exogenous applications of salicylic acid also have demonstrated a wide-range of effectiveness against fungal pathogens in both in-vivo and in-vitro conditions in previous studies (Wu et al., 2008a; Mandal et al., 2009; Qi et al., 2012).

According to Ali et al. (2024), SA restricts fungi from growing and reproducing by changing the levels of reactive oxygen species and activating systemic acquired resistance in host plants. Nevertheless, the direct antifungal properties of SA, especially regarding the mycelial growth and spore germination of *F. oxysporum*, are inadequately investigated and poorly recorded. It is very important to understand these pathways in order to use SA in long-term disease management plans. Consequently, it is imperative to assess the impact of exogenously administered salicylic acid on the in vitro development of *F. oxysporum* to a certain of its potential as an environmentally being antifungal drug (Wu et al., 2008b).

The aim of this study was to evaluate the in vitro efficacy of salicylic acid against *F. oxysporum* on mycelia growth and conidial germination.

MATERIALS AND METHODS

Salicylic acid preparation

The SA stock solution (100 mM) was prepared by dissolving 13.81 g of salicylic acid in a minimal volume of ethanol and then to make final volume upto 1 L by adding autoclaved distilled water. This stock solution was used to obtain different concentrations of salicylic acid (0, 0.05, 0.15, 0.5, 1.0, and 1.5 mM) through serial dilutions. Distilled water alone was used as the control.

Isolation and identification of *F. oxysporum*

Infected samples of Chilli showing typical Fusarium wilt disease symptoms were collected from different regions of Bahawalpur for the isolation of causal pathogens. Samples with wilting symptoms were collected and placed in a cool box for storage. Isolation of pathogens was carried out from the infected samples. The infected samples were cut aseptically into small pieces of about 4–6 mm. These pieces were washed with distilled water to remove debris from the surface and sterilized by dipping in 1% sodium hypochlorite (NaOCl) solution for 30 seconds than three times washed with sterilized distilled water to remove residual NaOCl and air dried under aseptic conditions. Under sterile conditions, the samples were carefully shifted to PDA plates using sterilized forceps. The plates were kept in an incubator maintained at 28 ± 2°C and were checked each day for the formation of fungal colonies. In order to isolate and purify the fungal pathogen, sub culturing was done on freshly prepared PDA media. The diagnosis of *F. oxysporum* f. sp. c*apsici* were performed by light microscope, and the morphological characterization based on critical features such as colony size, pigmentation (white to pale pink), and the presence of diagnostic structures including micro conidia and conidiophores.

Effects of salicylic acid on mycelial growth

The *In-vitro* effect of salicylic acid (SA) on mycelial growth of *F. oxysporum* was observed on PDA supplemented with different concentrations of SA. Salicylic acid was added to PDA to final concentrations of 0.0 mM (control), 0.05 mM, 0.15 mM, 0.5 mM, 1.0 mM, and 1.5 mM. Under aseptic conditions, the prepared medium was poured into sterile 90 mm diameter Petri dishes. 5 mm agar plug taken from the growing margin of a 3–5 day old pure *F. oxysporum* culture, was placed in the center of each plate. Experimental controls consisted of plates prepared with sterile water instead of SA. Inoculated plates were incubated at 27 ± 1°C, in complete darkness to allow for optimal fungal growth. Radial mycelial growth (diameter) was measured for six days and expressed in millimeters (mm). Three replicates for each treatment were used in each experiment. The experiment was performed twice to ensure the accuracy of the results (Kumar, and, Bains, 2018).

Effects of salicylic acid on conidial germination

The influence of salicylic acid (SA) on the germination of conidia of *F. oxysporum* was evaluated on Potato Dextrose

Agar (PDA) medium. A 5 mm agar plug taken from 7 day old PDA culture was distributed aseptically to Potato Dextrose Broth (PDB) to prepare conidial suspensions. All the liquid cultures were kept at 27 ± 1°C with continuous agitation at 120 rpm for 7 days. After incubation, the fungal broth was filtered through four layers of sterile cheesecloth to obtain conidial suspension. Conidia density was measured by counting with a hemocytometer and adjusted to 1×10^6 conidia/mL in sterile distilled water. To assess the effect of salicylic acid on conidial germination, 100 conidia were aseptically inoculated onto the surface of PDA plates supplemented with various concentrations of SA. Plates were kept at 27 ± 1°C in the dark for 60 hours. Used a microscope to count the amount of germinated conidia to find out how fast they germinated. Observed germination by seeing if the conidia had produced germ tubes that were at least as long as the diameter of the conidium (Kumar, and, Bains, 2018).

Pathogenicity test

Chilli seedlings were cultivated in plastic pots containing 200 grams (w/w) of sterilized peat soil. Evaluations were conducted 30 days after sowing. To prepare the fungal inoculum, a fungal isolate was grown in 250 mL of potato dextrose broth (PDB). For inoculation, 5×5 mm agar segments taken from an actively growing fungal culture were introduced into the broth. This culture was incubated for three days at 120 rpm using a shaker to promote conidia production. The resulting suspension was then applied to the soil at a concentration of 1×10^6 colony-forming units per gram (cfu/g). Sterile distilled water was used in control pots as a negative control.

Ten chilli plants were inoculated with the prepared fungal suspension by adding it directly to the soil. The entire experiment was conducted twice to ensure clarity. Disease incidence was determined by counting the number of infected plants out of the total number of plant. The disease severity was recorded 40 days post-incubation following the 0–5 scale vascular browning scale with some modification (Fatima et al., 2023); where 0 = no vascular browning; 1 = 1–20% vascular browning; 2 = 21–40% vascular browning; 3 = 41–60% vascular browning; 4 = more than 61-80% vascular browning; 5= 81-100 plant death.

***In-vivo* assessment of salicylic acid**

Surface sterilization of seeds of susceptible variety of Chilli was done by sodium hypochlorite 1% solution followed by soaking in different concentration (0.0, 0.05, 0.15, 0.5, 1.0 and 1.5 mM) of salicylic acid ($C_7H_6O_3$) for 24 hours while sterilized distilled water was used for control. Fifteen days after sowing, seedlings were uprooted, washed under a running tap water to remove clay particle from the roots and dipped in *F. oxysporum* spore suspension (10^6 spore/mL) for 4 hours before transplanting in pots contained sterilized soil and transferred to the greenhouse. Disease incidence was calculated using the following formula with some modification (Maalik et al., 2024); Disease incidence (%) = Number of plants observed/Total number of plants observed × 100.

Experimental Structure (Treatments, SA concentrations):

T0: Control
T1: 0.05 mM
T2: 0.15 mM
T3: 0.5 mM
T4: 1.0 mM
T5: 1.5 mM

Parameters:

Plant height (cm)
Fresh biomass (g)
Dry biomass (g)
Disease incidence (%)
Disease severity index (0–5)
Wilting percentage (%)

Statistical Analysis

The experiments were conducted in a completely randomized design (CRD). The data was subject to ANOVA and the treatment means were compared by using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p \leq 0.05$. The statistical package used for the analysis of data was Statistix (Ver. 10).

RESULTS AND DISCUSSION

Metabolite colour

Pigments of specific colour are shown by *F. oxysporum* on the PDA culture. From the top, the colony was fluffy,

white with a pinkish appearance, while from the bottom view it was pink. In addition, it formed macro conidia and micro conidia on PDA medium (Figure 1).

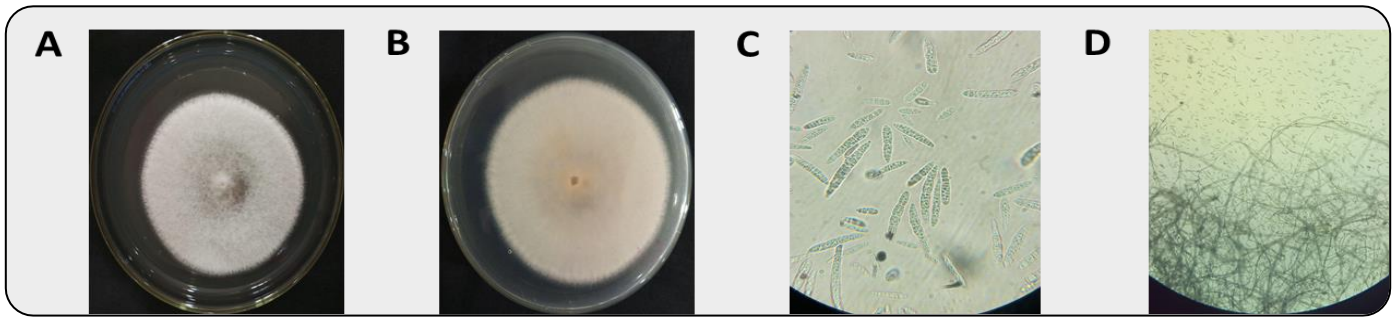

Figure1. Pigments of specific color are shown by *F. oxysporum* on the PDA culture and it formed macro conidia and micro conidia on PDA medium. (A) upper view of petri plate (B) lower view of petri plate (C) microscopic spores, and (D) mycelium with spores.

Effects of salicylic acid on mycelial growth of *F. oxysporum*

The mycelial growth of *F. oxysporum* on PDA medium was significantly affected by salicylic acid (SA) concentrations and showed a concentration different response. At lower concentrations of salicylic acid (0.05 - 0.5 mM), fungal growth was enhanced as compared to control (6.06 cm). The fungal diameter was 7.50 cm at 0.05 mM SA, whereas further increase in growth was recorded at 0.15 mM SA (8.65 cm). At 0.15 mM SA, promotion of growth was maximal with a 42.7% increase compared with the control. In the presence of 0.5 mM SA, the fungal diameter is 6.475 cm and at 1.0 mM SA significantly reduced it to 5.39 cm. Growth inhibition reached its highest value at 1.50 mM SA with the fungal diameter reduced to 4.98 cm which corresponds to 17.8% inhibition relative to the control. SA was confirmed to be a growth stimulant at concentrations between 0.05 - 0.5 mM (which possibly promotes cell proliferation) and at a higher concentration, above 0.5 mM, it may act as an antifungal agent with the best inhibition at 1.50 mM SA (Figure 2A).

Effects of salicylic acid on conidia germination of *F. oxysporum*

Salicylic acid (SA) is an important signaling molecule in plant-microbe interactions and it plays an essential role in plant defensive response. It is endogenously produced in plants, and its level rise significantly under biotic and abiotic stress conditions. Differences in concentration of SA contribute to plant defense by modifying the physiology of the host or by directly inhibiting pathogen development and infection. Previous studies have shown SA plays a key role in plant defense by modifying host physiology or directly inhibiting pathogen development and infection. This study examined the in vitro inhibitory effects of SA on the mycelial growth and conidial germination of *F. oxysporum*. The results showed a concentration dependent response, conidial germination gradually decreased with increasing SA concentrations, up to a minimum value of 82 % in presence of 1.5 mM SA. The significantly reduced germination of conidia in SA suggests that the antifungal effect of SA is dose-dependent, reinforcing its significant potential in the control of *Fusarium* infections. These findings suggest that use of exogenous SA can significantly inhibit infection and colonization of the host tissues by *F. oxysporum*, and may be a potential strategy for controlling Fusarium wilt in chilli crops (Figure 2B).

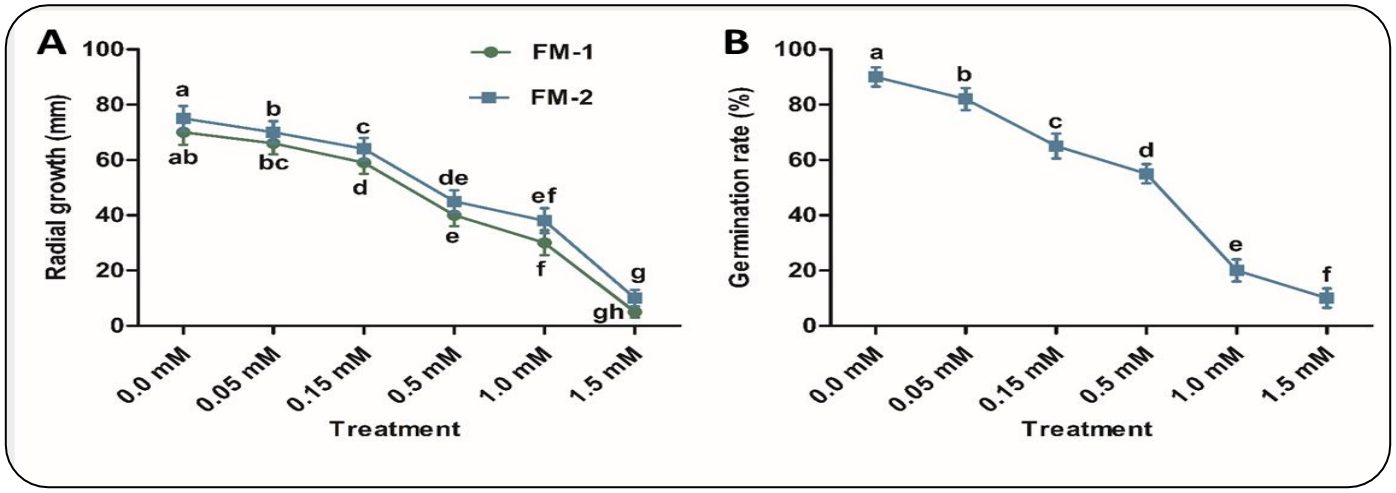

Figure 2. (A) Radial growth (mm), and (B) germination rate (%)

The data was assessed using the One-way ANOVA Tukey's HSD test, using a significance level of P ≤ 0.05. The presence of distinct lowercase letters signifies significant differences between the treatments.

Plant Biomass

Plant Height (cm) at 35 dpi

The plant height data shows a varying trend in response to different treatment concentrations. In the untreated control group, the average height was 8 cm. When treated with 0.05 mM and 0.15 mM, the average heights dropped to 7.2 cm and 6.8 cm respectively, indicating a slight reduction in growth at lower doses. A mild recovery was seen at 0.5 mM with a height of 7 cm. Interestingly, the plants showed improved growth at higher concentrations, reaching 9.2 cm at 1.0 mM and 11 cm at 1.5 mM. This pattern suggests that the treatment may have an inhibitory effect at low doses and a stimulatory effect at higher levels. Such dose-dependent responses are not uncommon in plant systems and could be linked to the compound influencing hormonal or metabolic activity depending on concentration levels, as discussed in prior plant physiology studies (Calabrese & Baldwin, 2002), as shown in (Figure 3A).

Fresh plant weight (g) at 35 dpi

The recorded data reveals that the average fresh weight of plants varied significantly with different treatment concentrations. In the control group, the biomass was 12 g. A decrease was observed at lower concentrations, with 10.8 g at 0.05 mM and 10 g at 0.15 mM, indicating a possible stress or inhibitory effect at minimal doses. A slight improvement occurred at 0.5 mM with a weight of 10.5 g, but a significant increase was noted at higher concentrations 13.8 g at 1.0 mM and reaching 16 g at 1.5 mM. These findings suggest a concentration-dependent response where low doses may restrict biomass accumulation, while higher doses enhance it. This trend is an example of a classic hormetic effect, in which a chemical may first stop physiological activity at lower levels but then encourage growth and production at greater levels. Such growth stimulation at elevated treatment levels may result from improved food absorption or the activation of metabolic pathways. (Calabrese & Baldwin, 2002), as shown in (Figure 3B).

Dry plant weight (g) at 35 dpi

The data clearly reveals that the average dry weight changes depending on the treatment level. The dry weight in the control group, which didn't get any treatment, was 3.2 g. At the lower concentrations of 0.05 mM and 0.15 mM, biomass reduced to 2.8 g and 2.5 g, respectively. This could mean that these dosages had a suppressive effect. The dry plant weight improved a little to 2.7 g at 0.5 mM. But there were big changes at higher concentrations: 3.6 g at 1.0 mM and 4.2 g at 1.5 mM. This upward trend at higher dosages indicates a beneficial effect of the treatment on biomass accumulation, either due to improved metabolic efficiency or the activation of physiological processes. The general trend supports the idea of hormesis, which says that a substance has harmful effects at low doses and helpful effects at larger doses (Calabrese & Mattson, 2009). These findings underscore the significance of dose adjustment in investigations of plant growth, as shown in (Figure 3C).

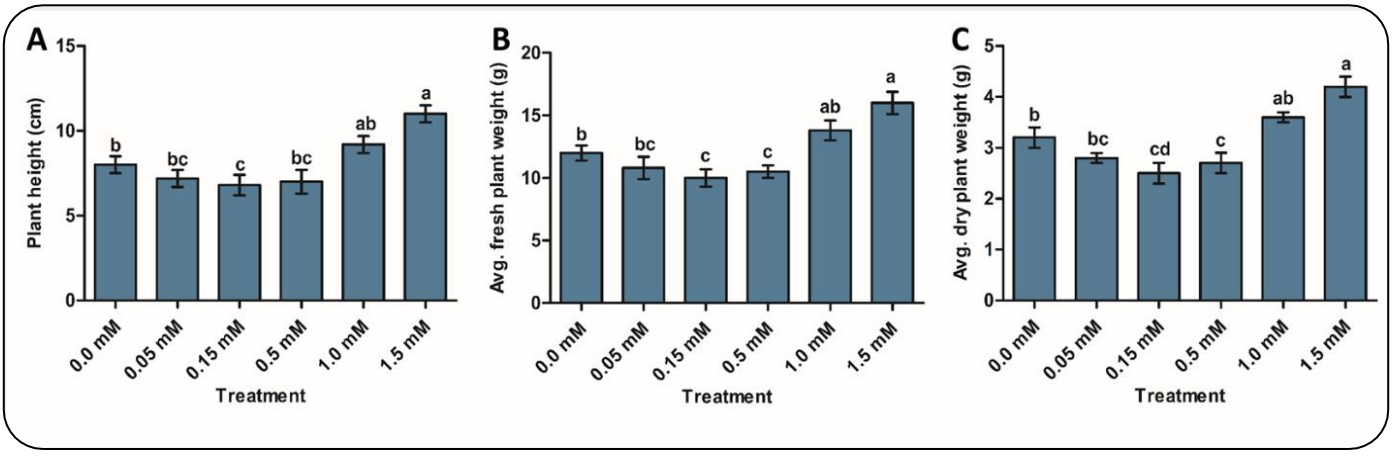

Figure 3. (A) Plant height (B) Avg. fresh plant weight, and (C) Avg. dry plant weight

The data was assessed using the One-way ANOVA Tukey's HSD test, using a significance level of P ≤ 0.05. The presence of distinct lowercase letters signifies significant differences between the treatments.

Disease incidence, disease severity index, and wilting percentage

Disease incidence (%) at 35 dpi

At lower concentrations, disease incidence was increased, reaching 85% at 0.05 mM and 90% at 0.15 mM. This

suggests that little doses may have made plant more likely to get disease or had no protective effect. At 0.5 mM, the incidence dropped a little to 88%, which suggests that the disease incidence was lower. But at greater concentrations, there was a big drop: 60% at 1.0 mM and 35% at 1.5 mM. This indicates that elevated treatment levels exerted a significant protective or inhibitory influence on disease progression. The results show that there is a point beyond which the therapy works, which could be because it activates the plant's defensive systems or stops the growth of pathogens.

This type of dose-dependent shift in disease incidence aligns with previous findings where optimal concentrations are critical for achieving control efficacy (Calabrese & Mattson, 2009), as shown in (Figure 4A).

Disease severity index (0–5) at 35 dp

The Disease Severity Index (DSI) changed with different treatment concentrations, revealing a clear pattern based on dose. The DSI was measured at 4 in the untreated control group. When given smaller dosages, 0.05 mM and 0.15 mM, the DSI went up to 4.3 and 4.7, respectively. The DSI reduced to 4.5 at 0.5 mM, which was a small improvement.

However, a marked reduction was observed at higher concentrations, with the DSI decreasing to 2.5 at 1.0 mM and further to 1.2 at 1.5 mM. These findings indicate that higher doses significantly reduced disease severity, likely by enhancing the plant's defense mechanisms or suppressing pathogen development. The results highlight the importance of using an effective concentration, as sub-optimal doses may not only be ineffective but may also increase vulnerability to disease. Similar dose-dependent responses have been reported in earlier studies on plant-pathogen interactions and stress management (Calabrese & Blain, 2005), as shown in (Figure 4B).

Wilting percentage (%) at 35 dpi

The percentage of wilting observed across treatments shows a clear pattern related to the concentration applied. In the control group, wilting was 75%, indicating a high level of stress or disease impact. At lower concentrations, wilting increased 80% at 0.05 mM and 85% at 0.15 mM suggesting that minimal levels of treatment were not only ineffective but may have worsened plant stress. A slight improvement was noted at 0.5 mM, with wilting reducing to 82%. However, a significant decrease was observed with higher concentrations: 50% at 1.0 mM and 25% at 1.5 mM. These results suggest that higher treatment doses substantially reduced wilting, possibly by strengthening the plant's defense mechanisms or reducing pathogen activity. The pattern indicates a threshold effect, where the treatment becomes effective only at sufficient concentration levels. This kind of dose-dependent response is consistent with findings in plant protection studies, where higher doses of a compound often lead to better physiological resilience (Calabrese & Mattson, 2009), as shown in (Figure 4C).

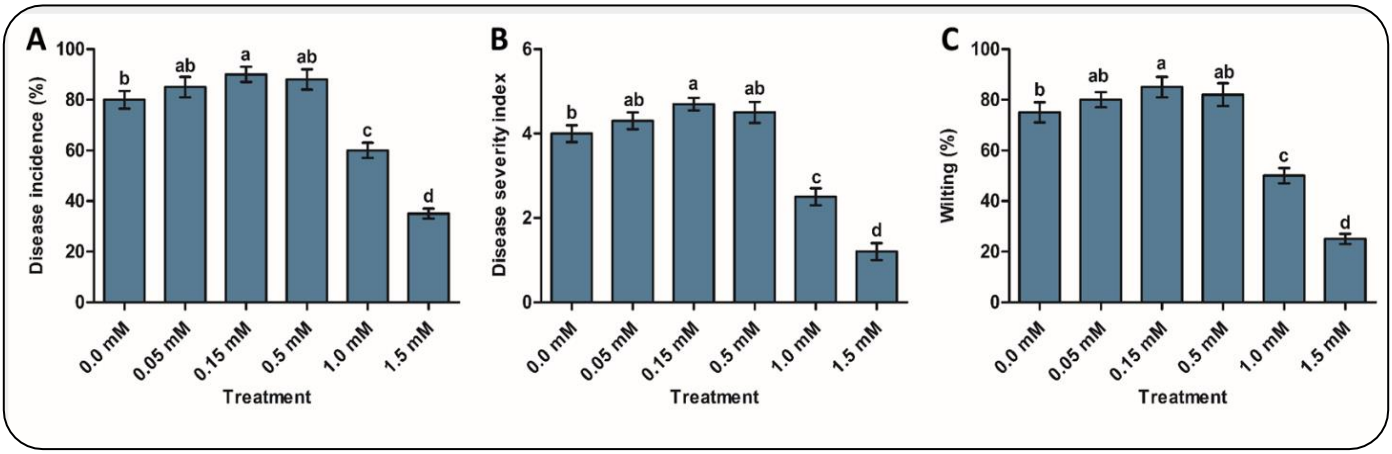

Figure 4. (A) Disease incidence, (B) Disease severity index, and (C) wilting.

The data was assessed using the One-way ANOVA Tukey's HSD test, using a significance level of $P \leq 0.05$. The presence of distinct lowercase letters signifies significant differences between the treatments.

CONCLUSION

The results of this study clearly showed that the exogenous application of the tested compound at varying concentrations had a significant impact on plant health and disease resistance. Lower concentrations (0.05 mM–0.5 mM) showed minimal to no improvement, while in some cases, worsened physiological parameters such as height, biomass, and disease symptoms. However, higher concentrations, particularly 1.0 mM and 1.5 mM, consistently enhanced plant growth parameters including height, fresh and dry biomass, while also reducing disease incidence, severity index (DSI), and wilting percentage. These findings suggest that optimal concentration plays a crucial role in

achieving positive physiological and protective outcomes in plants under stress. Hence, the application of 1.5 mM was the most effective in promoting growth and improving resistance, indicating its potential utility in managing Fusarium related stress in chilli crops under controlled conditions. The dual role of SA in regulating plant-pathogen interactions and demonstrate the potential of SA for managing Fusarium wilt in chilli crops.

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AUTHOR CONTRIBUTIONS

All authors contributed equally.

COMPETING OF INTEREST

The authors declare no competing interests.

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