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Research Article

Salicylic acid on mycelial growth and conidial germination of four *Fusarium* pathogens causing head blight

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Abstract: Root and foliar application of exogenous salicylic acid (SA), a critical plant defense hormone, has enhanced adult host resistance to Fusarium head blight (FHB), one of the most devastating fungal wheat diseases. In contrast, information on the direct effects of FHB pathogens on in vitro growth has been hardly available. To elucidate this, the antifungal activity of different concentrations of SA was investigated on four *Fusarium* pathogens with diverse pathogenicity under *in vitro* conditions. SA inhibited mycelial growth and conidial germination of all pathogens in potato dextrose agar medium in a concentration-dependent manner, with the greatest inhibition achieved using the highest SA concentrations. The antifungal activity of SA on mycelial growth was found to be pH-dependent and more efficient in acidic conditions than in alkaline conditions. However, the inhibitory effect of SA on conidia germination is not due to acidification of the medium. Microscopic observations reveal that mycelium from inhibition zones showed a morphological alteration compared to normal mycelium from the untreated control. Our results demonstrate for the first time that, although the inhibitory effect of SA required acidic growth conditions to be observed, SA has a significant and direct impact on *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti* through a reduction in efficiency of germination and growth at higher concentrations. This study has shown that SA could be considered a potent and promising antifungal to FHB infection that is safe for the environment.

Keywords: conidial germination, *Fusarium* species, *in vitro* bio-experiment, mycelial growth, soluble salicylic acid

Introduction

Wheat is a major staple food crop due to its grain yield and unique processing quality. Fusarium head blight (FHB) is one of the most devastating fungal diseases affecting wheat and small-grain cereals worldwide (Buerstmayr *et al.*, 2020). FHB is caused by more than 17 *Fusarium* species of which *F. graminearum* and *F. culmorum* are of greatest relevance and considered the strongest pathogenic pathogens due to their strong viability and wide range of hosts (Sakr, 2022). It directly impacts yield and grain quality, reducing grain weight and changing protein accumulation (Ma *et al.*, 2019). FHB also causes serious health concerns through the contamination of grains by mycotoxins, which are resilient to most transformation processes (Fernando *et al.*, 2021). Economic losses have been estimated to be a total

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of $1.176 billion over 2015 and 2016 in the United States (Dahl and Wilson, 2018). Such losses are expected to increase due to intensified outbreaks promoted by rising temperatures and occasional increases in air humidity expected with climate change (Fernando *et al.,* 2021). Environmental conditions significantly influence the initiation and severity of the disease, where high humidity (> 90%) and moderate temperatures (15 to 30 ºC) favor the fungus and lead to more severe incidences of FHB in the field (Mesterhazy *et al.,* 2020). The combined use of resistant cereal cultivars, chemical control, biological control, and specific management practices (e.g., tillage and crop rotation) can reduce part of the losses due to the disease (Buerstmayr *et al.,* 2020). Knowledge about the most influencing cultivation measures, such as crop rotation, choice of variety and tillage, is important to reduce the risk of Fusarium infection in wheat and was assessed in recent years (Ma *et al.,* 2019). In addition, data about the most susceptible growth stages and favorable climatic conditions, which were shown to affect the infection with FHB pathogens, are necessary to predict the occurrence in a particular field (Buerstmayr *et al.,* 2020). Recently, adding silica to the soil as a fertilizer has been shown to reduce FHB incidence and severity (Sakr and Kurdali, 2023; Sakr and Mohamad, 2023). However, no efficient strategy can fully control FHB epidemics so far, and the enhancement of fungicide-resistance in fungi along with environmental concerns has also brought a great threat to human health and environmental sustainability (Dahl and Wilson, 2018; Fernando *et al.,* 2021). Therefore, recent efforts have focused on developing environmentally safe, long-lasting and effective alternative methods for the management of FHB.

Salicylic acid (SA), a carbon-based secondary metabolite, is a critical plant defense hormone that helps plants defend themselves against various diseases with biotrophic and hemibiotrophic lifestyles (De Vos *et al.,* 2005). SA is a key phytohormone in wheat resistance against FHB (Qi *et al.,* 2012; Zhang *et al.,* 2019). *F. graminearum* infection in wheat heads can significantly increase the accumulation of SA and the expression of SA-related genes, which play an important role in wheat FHB resistance (Makandar *et al.,* 2010). SA triggers systemic acquired resistance and protects wheat plants from further infection after an initial pathogen attack (Sorahinobar *et al.,* 2015). Wheat plants with a deficiency in SA signaling cannot develop SAR and do not show pathogen-related gene activation upon pathogen infection (Makandar *et al.,* 2012). With such background, treating wheat roots and leaves with exogenous application of SA has been shown to affect FHB resistance in wheat positively (Qi *et al.,* 2012; Sorahinobar *et al.,* 2015). Therefore, SA is a valuable chemical target for obtaining wheat germplasms that are immune/highly resistant to FHB (Zhang *et al.,* 2016, 2017). Although SA contributes to wheat/F, *graminearum* interaction remains unclear. It seems likely that SA is a hopeful and valuable target for sustainable control of FHB (Zhang *et al.,* 2019).

Little is known about the direct effect of exogenous salicylic acid on the pathogen, e.g., on colony growth, conidia germination, sporulation, and mycotoxin production of *in vitro* wheat pathogen *F. graminearum*. Qi *et al.* (2012) showed that SA strongly and directly affects fungus by inhibiting conidial germination, mycelial growth and mycotoxin production, indicating that SA has a direct, strong, and toxic effect on *F. graminearum* growth. No studies have determined how exactly SA functions to affect the growth of other FHB species under *in vitro*-environment tests. In contrast, previous studies have highlighted that exogenous application of SA has positive effect against fungal pathogens of great economical and agricultural importance, i.e., *Fusarium oxysporum* f. sp. *niveum*, *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *radicislycopersici*, *F. oxysporum* f. s. *ciceri*, *F. solani*, *F. mangiferae*, *Verticillium dahliae*, *Rhizoctonia solani*, *Colletotrichum coccodes*, *Pythium aphanidermatum*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria solani*, and *Ganoderma* sp. (Saikia *et al.,* 2003; Wu *et al.,* 2008; Abdel-Monaim *et al.,* 2012; Jabnoun-Khiareddine *et al.,* 2015; Kumar and Bains, 2018; Salem, and Shafea, 2020; Saputra *et al.,* 2022).

Developing novel strategies to control FHB in wheat will benefit from studies to clarify the relationship between SA and FHB. Given this evidence and the limited options to control FHB disease, the objective of this study was to assess the possible direct effect of SA on *in vitro F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti* mycelial growth and conidial germination. Here, we tested the hypothesis that an exogenous SA would inhibit FHB fungi, serving as an antifungal element. This would be expected to help understand FHB disease's mechanism and control diverse *Fusarium* pathogens in practice.

Materials and Methods

Pathogen isolates and growth conditions

We used 16 fungal isolates of four *Fusarium* pathogens based on their unique morphological and pathological characteristics (Sakr, 2023) to examine the effect of SA on mycelial growth and conidial germination under *in vitro* conditions. The pathogens were originally isolated from wheat heads showing head blight disease symptoms through the 2015 growing season in Ghab Plain with a FHB history, one of the principal Syrian wheat production areas. Six *F. solani*, five *F. culmorum*, four *F. verticillioides* (synonym *F. moniliforme*) isolates and one *F. equiseti* isolate were monosporic derived cultures of the original wild-type isolates. On 9 cm-Petri dishes with potato dextrose agar (PDA, HiMedia, HiMedia Laboratories) with 13 mg/l kanamycin sulphate added after autoclaving, the isolates were morphologically identified with the aid of the Leslie and Summerell (2006) manual based on microscopic studies of the shape and size of macro- and micro-conidia. They were molecularly distinguished by RAPD markers (Sakr, 2023).

The 16 *Fusarium* isolates were maintained by freezing at-16 °C or in sterile distilled water at 4 °C (Sakr, 2020). Fresh *Fusarium* monosporic-derived cultures were grown separately on PDA medium in an incubator (JSPC, JS Research Inc., year of appliances: 2004) for 7 days at 22 ± 1 ºC in the dark to allow mycelial growth and sporulation. One-wk-old cultures were used to collect macroconidia, and then fungal spores were harvested from the plates with the aid of a sterile glass rod and sterile distilled water (SDW) and then filtered through autoclaved cheesecloth to remove agar and adhering mycelia. The spore concentration was adjusted before use with a Neubauer chamber under an optical microscope.

Effect of salicylic acid on pathogens under *in vitro* conditions

The SA source was an analytically pure SA powder ($C_7H_6O_3$, Rectapur®, composed of a minimum content of 99% SA). The effect of SA on the *in vitro* growth of *Fusarium* pathogens was tested as a previously described method (Qi *et al.*, 2012). The direct effect of SA on mycelia's growth and conidia germination was tested on PDA medium.

Different concentrations of SA were prepared by serial dilution in SDW, then added to the PDA medium. PDA medium was amended with SA (Rectapur®) at 0.0, 0.4, 0.6, 0.8, 1.0 mM for mycelia growth and conidial germination measurements from a 1 M stock solution, respectively. Plates without SA were used as controls. The plates were hermetically closed, sealed with 2 cm ParaFilm strips (Pechiny, Thomas Scientific) to ensure high relative humidity and low air movement and incubated at 22 ± 1 ºC in the dark. The plates without any *Fusarium* colonies were incubated at 22 ± 1 ºC for an extra week to confirm that the conidia could not germinate. The experiments were laid out in a completely randomized design with three replications. The experiment was repeated two times.

Measurement of *Fusarium* mycelia growth

A 5-mm agar plug taken from a 7-d-old PDA culture was inoculated on the center of the plate with/out SA and was incubated for 5 d. The radial growth of mycelia was estimated at two perpendicular points, and the mean was determined by measuring the diameter of the unique colony over five consecutive days. The zone of fungal growth inhibition was assessed. Microscopic examinations of the growth

phenotype of *Fusarium* pathogens under SA treatment as compared with PDA plates without SA were conducted 5 days after incubation periods using a light microscope.

Assessment of *Fusarium* conidial germination

Conidial suspension was diluted to ≤ 400 conidia per mm with SDW. The diluted suspension (0.1 ml) was uniformly spread on the surface of each PDA plate containing SA and incubated for 5 d. The number of colonies was counted daily. Conidia were considered to have germinated if the germ tube length was equal to or greater than the conidial diameter.

Inhibition ratio (%) = (C - E)/C × 100%, where C is the diameter/conidial germination of the control colony, and E is the diameter/ conidial germination of the treatment colonies.

Because SA additions acidify the media (Qi *et al.*, 2012), a series of tests were run to determine if the decrease in pH could contribute to the inhibition of mycelium growth and conidia germination. The effect of different pH (3.6 and 8.0) on the growth of mycelia and germination was also tested on PDA plates, the pH of which was adjusted with 4 M NaOH (Avonchem, SK 116PJ) (the pH of 4 M NaOH was equivalent to 14) before sterilization.

Statistical analyses

Data were evaluated using analyses of variance using DSAASTAT add-in version 2011. Fisher's least significant difference test at P = 0.05 was used to determine if differences were significant for the inhibiting effects of SA on mycelium growth and conidia germination by comparing SA treatments to control.

Results

The inhibitory and direct effect of SA tested at different concentrations was assessed *in vitro* on 16 FHB isolates in two ways: (1) effect on fungal mycelial growth and (2) effect on conidia germination. Regardless of pathogen species, inhibition of *Fusarium* growth and germination of conidia by SA was observed. ANOVA analysis revealed a significant (at $P \leq 0.01$) variation in the average fungal colony diameter and conidia germination of SA treatments relative to SDW controls (Tables 1 and 2). Microscopic observations reveal that mycelium from inhibition zones showed a morphological alteration compared to normal mycelium from the untreated control (Fig. 1).

***In vitro* evaluation of the antifungal activity of SA against *Fusarium* pathogens**

In a concentration-dependent manner, SA inhibited mycelial growth and conidia germination of all Fusarium pathogens in a solid PDA medium (Tables 1 and 2). It is clear that linear growth and germination of conidia of tested fungi decreased significantly with the increase of SA concentrations. The *Fusarium* mycelial growth was not affected at lower concentrations of SA (0.0, 0.4 and 0.6 mM) on a solid PDA medium. Spread of mycelia was significantly decreased ($p < 0.05$) at and above 0.8 mM SA by ~ 60% and completely inhibited by 1 mM SA, when compared to the control with SDW. The conidia germination was not affected ($p < 0.05$) by treatment with SA at concentrations up to 6 mM on PDA plates; however, germination was strongly suppressed at 0.8 mM SA by ~ 80% and stopped by 1 mM SA.

***In vitro* evaluation of the antifungal activity of SA against *Fusarium* pathogens at alkaline condition**

Data shown in Table 3 indicated that the antifungal activity of SA on fungal growth was found to be pH-dependent and more efficient in acidic conditions than in alkaline conditions. A stronger inhibition of mycelial growth was observed in the more acidic condition, where the addition of 1 mM SA caused the pH of the medium to drop below 4.0 (pH 3.6), the threshold where pH contributes to growth inhibition. Interestingly, FHB isolates exhibited normal mycelial growth at basic pH 8.0 with SA at 1 mM as compared to no growth at acidic pH 3.6. However, no effect on the germination of *Fusarium* conidia was observed between pH 3.6 and 8, indicating that the inhibitory effect of SA on conidia germination is not due to acidification of the medium.

Table 1 Effect of different salicylic acid (SA) concentrations on the *in vitro* mycelial growth of *Fusarium* pathogens after 5 days of culture at 22 ± 1 ºC on potato dextrose agar (PDA) medium plates.

Fusarium pathogens	Sterile distilled water	SA concentration (mM)				
		0.0	0.4	0.6	0.8	1.0
F. culmorum	39.1 ± 1.4a	39.6 ± 1.0a	40.4 ± 2.0a	39.6 ± 1.4a	25.9 ± 0.8b (34.6%)*	0.0 ± 0.0c (100%)
F. solani	56.2 ± 0.9a	56.7 ± 0.8a	56.7 ± 0.7a	55.9 ± 0.8a	32.2 ± 1.2b (42.4%)	0.0 ± 0.0c (100%)
F. verticillioides	53.2 ± 1.5a	53.9 ± 1.0a	53.5 ± 1.0a	53.8 ± 1.6a	30.6 ± 1.4b (43.1%)	0.0 ± 0.0c (100%)
F. equiesti	49.2 ± 1.1a	48.4 ± 0.5a	48.4 ± 1.1a	48.6 ± 0.5a	30.0 ± 0.9b (38.2%)	0.0 ± 0.0c (100%)

Data are the mean colony diameters ± standard deviation of three replicates for 5 isolates of *F. culmorum*, 6 isolates of *F. solani*, 4 isolates of *F. verticillioides* and one isolate of *F. equiseti* on PDA added with SA. For fungal pathogens and SA concentrations tested; the mean diameters with the same letter within a line are not significantly different according to the least significant difference (LSD) test (P = 0.05). *: Inhibition rate of mycelial growth (%) of *Fusarium* pathogens obtained by SA.

Table 2 Effect of different salicylic acid (SA) concentrations on the *in vitro* conidia germination of *Fusarium* pathogens after 5 days of culture at 22 ± 1 ºC on potato dextrose agar (PDA) medium plates.

Fusarium pathogens	Sterile distilled water	SA concentrations used (mM)				
		0.0	0.4	0.6	0.8	1.0
F. culmorum	78.2 ± 1.1a	80.5 ± 1.5a	79.6 ± 1.6a	80.9 ± 1.9a	19.6 ± 1.2b (75.8%)*	0.0 ± 0.0c (100%)
F. solani	85.6 ± 1.2a	84.9 ± 0.8a	85.1 ± 1.1a	83.6 ± 1.5a	15.2 ± 0.9b (81.8%)	0.0 ± 0.0c (100%)
F. verticillioides	82.1 ± 2.1a	81.4 ± 1.0a	82.6 ± 0.9a	81.9 ± 1.2a	14.6 ± 1.1b (82.2%)	0.0 ± 0.0c (100%)
F. equiesti	75.5 ± 1.3a	76.1 ± 0.9a	76.8 ± 1.4a	74.9 ± 0.9a	15.8 ± 0.7b (78.9%)	0.0 ± 0.0c (100%)

Data are the mean conidia germination ± standard deviation (%) of three replicates for 5 isolates of *F. culmorum*, 6 isolates of *F. solani*, 4 isolates of *F. verticillioides* and one isolate of *F. equiseti* on PDA added with SA. For fungal pathogens and SA concentrations tested; the mean conidia germinations with the same letter within a line are not significantly different according to the least significant difference (LSD) test (P = 0.05). *: Inhibition rate of conidia germination (%) of *Fusarium* pathogens obtained by SA.

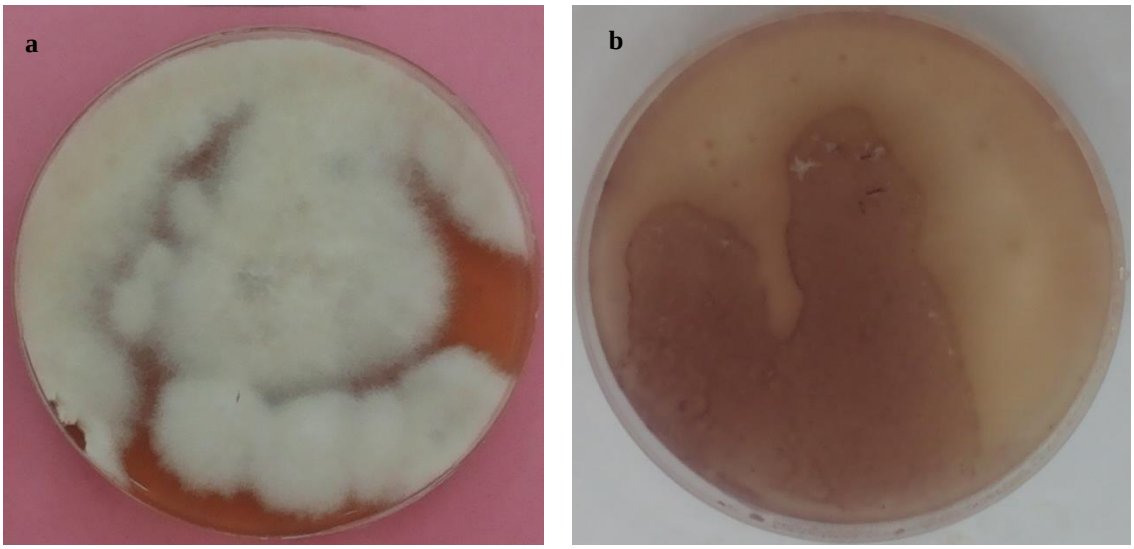

Figure 1 Effect of salicylic acid (SA) on mycelial growth; the microscopic observation of *Fusarium* mycelia showed morphological variations relative to control. Mycelial growth of isolate F2 (*F. culmorum*) on potato dextrose agar medium added with sterile distilled water (a) and 0.8 mM salicylic acid (b) after incubation at 22 ± 1 ºC.

Table 3 Effect of salicylic acid (SA) on mycelial growth and conidia germination of *Fusarium* pathogens after 3 days of culture at 22 ± 1 ºC versus pH of medium. SA concentration of 1.0 mM was added to potato dextrose agar (PDA) medium plates with medium adjusted to pH 3.6 or 8.0.

Fusarium pathogens	Mycelial growth (%)		Conidia germination (%)	
	3.6	8.0	3.6	8.0
F. culmorum	0.0 ± 0.0b	40.2 ± 1.8a	0.0 ± 0.0a	0.0 ± 0.0a
F. solani	0.0 ± 0.0b	56.2 ± 0.9a	0.0 ± 0.0a	0.0 ± 0.0a
F. verticillioides	0.0 ± 0.0b	54.3 ± 1.1a	0.0 ± 0.0a	0.0 ± 0.0a
F. equiesti	0.0 ± 0.0b	49.2 ± 0.6a	0.0 ± 0.0a	0.0 ± 0.0a

Data are the mean colony diameters ± standard deviation of three replicates for 5 isolates of *F. culmorum*, 6 isolates of *F. solani*, 4 isolates of *F. verticillioides* and one isolate of *F. equiseti* on PDA added with SA. For fungal pathogens and SA concentrations tested; the mean diameters/conidia germinations with the same letter within a line are not significantly different according to the least significant difference (LSD) test (P = 0.05).

Discussion

Despite the major economic and health impacts of FHB (Buerstmayr *et al.*, 2020; Sakr, 2022), there is no efficient strategy to manage this disease (Dahl and Wilson, 2018). Therefore, developing effective eco-friendly alternative strategies for controlling it that are safer for human health and the environment is of great importance (Fernando *et al.*, 2021). Since wheat spikes accumulate more SA when infected with *F. graminearum* and wheat defense response to FHB involves SA signaling (Makandar *et al.*, 2010, 2012), it has been widely shown that root application of exogenous SA, one of the key plant defense hormones, is associated with resistance against *F. graminearum* in wheat (Sorahinobar *et al.*, 2015; Zhang *et al.*, 2019). Although the direct effect of SA, rather than SA signaling, has been speculated to play a more important role in wheat resistance against FHB disease (Qi *et al.*, 2019), there is little information on SA- antifungal activity to *F. graminearum* (Qi *et al.*, 2012). However, there is still an important gap about inhibitory and direct effect of SA on other *Fusarium* species implicated in FHB disease complex. To our best knowledge, the results presented here for the first time demonstrate that SA can have a direct effect in a concentration-dependent manner on *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti* mycelial growth and conidia germination under acidic growth conditions. In addition, our data highlighted that the toxic effect of SA was pH-dependent, with toxicity reduced or negligible in neutral to slightly alkaline growth conditions. Moreover, according to the positive effects of SA on FHB, the survey results can be considered a potential approach in managing this disease.

SA is a natural phenolic compound considered a plant hormone growth regulator that may improve disease plant resistance (De Vos *et al.*, 2005). In this research, using SA alone at higher concentrations effectively controlled FHB disease under *in vitro* conditions. Our findings showed that SA caused a potent inhibition of hyphal growth and conidia germination of the four tested *Fusarium* species, causing head blight in wheat. Also, the microscopic observation of *Fusarium* mycelia showed morphological variations. This result confirmed a report that the mycelial growth and germination of conidia of the *F. graminearum* is inhibited by SA addition (Qi *et al.*, 2012). The result also agreed with the study that salicylic acid showed direct fungitoxicity on *Fusarium oxysporum* f. sp. *niveum*, Fusarium wilt (Wu *et al.*, 2008). It is widely known that SA can penetrate the microbial cell and exert its toxic effect. The mechanism of SA effect on inhibiting microorganisms is apparently due to its effect on the cell membrane through the interfering transport of metabolites and maintenance of membrane potential (Salem, and Shafea, 2020). Regarding SA-FHB interaction, SA has a direct, strong, and toxic effect on *Fusarium* pathogens (Qi *et al.*, 2012) partially because SA is capable of destroying the fungal cell membrane by reducing the expression level of *FgLAI12* (a

linoleic acid isomerase gene) and the inner cell wall by inhibiting the expression of *FgCHS8* (a chitin synthase gene), which are required for the response of *F. graminearum* to environmental stresses, including SA (Zhang *et al.,* 2016, 2017). From the results of the current investigation, pathogen-induced salicylic acid also directly inhibits the pathogen's growth. However, for other fungal and bacterial pathogens, i.e. *Pseudomonas syringae* pv syringae causing bacterial cancer (Rasmussen *et al.,* 1991) and *Monilinia fructigena* causing brown rot disease (Lyousfi *et al.,* 2021), SA had little direct activity on the *in vitro* mycelial growth; thus SA probably acts by directly activating host defense mechanisms to defeat these two pathogens (Rasmussen *et al.,* 1991; Lyousfi *et al.,* 2021). It seems that the antibiotic effect of SA is species-dependent.

In the current study, SA inhibited hyphal growth and conidia germination of the *Fusarium* fungi, causing head blight tested in a dose-dependent manner, with the greatest inhibition achieved using the highest SA concentrations. In parallel, the mycelial growth and conidia germination inhibition of *F. graminearum* by SA was concentration-dependent (Qi *et al.,* 2012). A significant negative correlation was detected between SA concentration and *F. oxysporum* f. s. *ciceri* mycelial growth in a Petri-plate assay (Saikia *et al.,* 2003). These authors found no inhibition was observed using SA at 100 µg/ml, but at 2000 µg/ml, the pathogen mycelial growth was stopped entirely. Wu *et al.* (2008) found that the biomass, colony diameter, number of conidium germination, and conidium production of Fusarium oxysporum f. sp. niveum were decreased at 800 mg/L. In the same sense, Abdel- Abdel-Monaim *et al.* (2012) showed that SA tested at 50, 100 and 200 ppm had significantly inhibited radial growth, mycelial dry weight and spore formation of *F. oxysporum* f. sp. *lycopersici* but at different degrees depending on concentrations tested. SA (1-25 mM) inhibited mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *radicislycopersici*, *F. solani*, *Verticillium dahliae*, *Rhizoctonia solani*, *Colletotrichum coccodes*, *Pythium aphanidermatum*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, and *Alternaria solani* in a concentration-dependent manner (Jabnoun-Khiareddine *et al.,* 2015). Salem, and Shafea (2020) observed that SA significantly reduced the linear growth of *Botrytis cinerea*, gray mold rot, *in vitro,* especially in high concentrations (6 g/l and 0.8g/l). Applying 50 and 100 ppm salicylic acid inhibited the growth of *Ganoderma* sp, stem rot and top stem rot disease, by 100% (Saputra *et al.,* 2022). It is well-known that sporulation and conidium germination are important preconditions for pathogenic microorganisms' survival and attacking hosts (Wu *et al.,* 2008). From our results, it is clear that accumulation of SA is unfavorable for *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti* because of suffering from such multiple suppressions, including mycelial growth, biomass, colony growth, conidium's germination and formation. The current results were completely following the accumulation of SA in infected tissues to resist further damage when the host was attacked by FHB pathogen, *F. graminearum* (Makandar *et al.,* 2010, 2012; Sorahinobar *et al.,* 2015; Zhang *et al.,* 2019). We also believe that decreased sporulation and conidial germination of *Fusarium* species by SA would be one of the plant resistance mechanisms against pathogens.

It is widely accepted that variable pH conditions affect the adaptation of many fungi species to grow (Penalva, 2008; Sakr, 2021). In this work, the antifungal activity of SA on mycelial growth of F. culmorum, F. solani, F. verticillioides and F. equiseti was found to be pH dependent and was more efficient in acidic conditions compared to alkaline conditions. This result aligns with those reported on *F. graminearum*; the inhibitory effect of SA required acidic growth conditions to be observed (Qi *et al.,* 2012). Similarly, the SA-mediated mycelial growth inhibition was more pronounced at acidic pH than at basic pH in *Fusarium mangiferae* associated with mango malformation (Kumar and Bains 2018). Amborabe *et al.* (2002) also reported that the antifungal efficiency of SA was higher when the experimental pH was brought to more

acidic values (pH 4). However, basic conditions might allow *F. graminearum* to metabolize SA efficiently through catechol or gentisate pathways (Qi *et al.,* 2012). Thus, *F. graminearum* has an efficient system to deal with SA stress, including the capacity to metabolize, export SA, and strengthen the outer cell wall (Qi *et al.,* 2019). For this, treating wheat spikes with exogenous SA reportedly does not improve wheat FHB resistance (Sorahinobar *et al.,* 2015). However, treating wheat roots and leaves with SA/MeSA (methyl salicylate) can enhance wheat resistance against *F. graminearum*; SA activates antioxidant defense responses and may subsequently induce systemic acquired resistance, which may contribute to the resistance against *F. graminearum* (Sorahinobar *et al.,* 2015).

Conclusion

The present study evaluated the effects of SA with different concentrations and its impact on the rate of conidia germination and growth of mycelium on the wheat pathogens, Fusarium species. To our knowledge, this is the first report on the *in vitro* antifungal activity of SA against *F. culmorum, F. solani, F. verticillioides* and *F. equiseti*. As an antibiotic against plant pathogens, SA may be characterized as having a toxic and direct effect on *Fusarium* species causing head blight under *in vitro* conditions: the inhibition of hyphal growth and conidia germination; suggesting that SA treatment presents a promising and reliable alternative to control the FHB disease preventively. Irrespective of the pathogenic origin of the fungal materials in our study, SA shows an antifungal activity to *Fusarium* infection, suggesting that SA exerts no selective pressure on pathogen populations. Therefore, it can enhance host resistance to infection with any *Fusarium* species, whatever its pathogenic level. Suitable amounts of SA should be used to control the pathogen in field crops via different modes and timings of application. Moreover, this work provides insights into the feasibility of using SA, which is safe in the environment, to enhance the resistant potential against diverse *Fusarium* species. Based on the results, SA can be recommended as an efficient, inexpensive, and safe approach to managing FHB on wheat.

Authors' Contributions

The author's contribution is 100%.

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تأثیر اسید سالیسیلیک بر رشد میسلیومی و جوانه‌زنی کنیدی چهار پاتوژن فوزاریومی عامل بیماری بلایت

نجات ساکر

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چکیده: استفاده از اسید سالیسیلیک (SA) به‌صورت تیمار ریشه‌ای و برگ‌پاشی، مقاومت گیاه میزبان را در برابر بیماری بیماری‌های قارچی بلایت فوزاریومی گندم (FHB)، تقویت می‌نماید. در مقابل، اطلاعات مربوط به تأثیرات مستقیم پاتوژن‌های FHB بر رشد در شرایط کشت آزمایشگاهی به‌ندرت موجود است. برای روشن کردن این موضوع، فعالیت ضدقارچی غلظت‌های مختلف SA روی چهار پاتوژن فوزاریوم با ویژگی‌های بیماری‌زایی متفاوت تحت شرایط *in vitro* مورد بررسی قرار گرفت. SA رشد میسلیومی و جوانه‌زنی کنیدی را در تمامی پاتوژن‌ها در محیط آگار دکستروز سیب‌زمینی به‌صورت وابسته به غلظت مهار کرد و بزرگترین مهار با استفاده از بالاترین غلظت‌های SA حاصل شد. فعالیت ضدقارچی SA نسبت به رشد میسلیومی وابسته به pH بوده و در شرایط اسیدی کارآتر از شرایط قلیایی بود. با این حال، اثر مهاری SA بر جوانه‌زنی کنیدی ناشی از اسیدی شدن محیط نیست. مشاهدات میکروسکوپی نشان می‌دهد که میسلیوم مناطق تیمار شده تغییرات مورفولوژیکی مشخصی نسبت به میسلیوم کنترل نشان دادند. نتایج ما برای اولین بار نشان داد که اگرچه اثر مهاری SA نیازمند وجود شرایط رشدی اسیدی برای مشاهده بود ولی این ماده تأثیر قابل‌توجه و مستقیمی بر گونه‌های قارچی *F. culmorum, F. solani, F. verticillioides* و *F. equiseti* دارد که منجر به کاهش کارایی جوانه‌زنی و رشد در غلظت‌های بالاتر شد. این مطالعه نشان داده است که SA ممکن است یک ضدقارچ قوی و امیدوارکننده برای بیماری قارچی FHB باشد که ایمن برای محیط‌زیست به‌شمار می‌رود.

واژگان کلیدی: جوانه‌زنی کنیدی، گونه‌های فوزاریوم، *in vitro*، رشد میسلیومی، اسید سالیسیلیک