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# Antibiotic effect of exogenously applied salicylic acid on *in vitro* soilborne pathogen, *Fusarium oxysporum* f.sp.*niveum*

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ABSTRACT

Salicylic acid, which is biosynthesized inside plant and is often found and accumulated in soil due to plant debris decaying, is considered as a signaling substance during plant–microbe interactions. It is involved in the cycling of biogeochemistry and related to plant resistance to biotic and abiotic stress. The antibiotic effect of salicylic acid on *Fusarium oxysporum* f.sp.*niveum* (FON) was studied to investigate the relationships between the salicylic acid and the fungus in the ecological interaction of plant–microbe. Results showed that the biomass, colony diameter, number of conidium germination and conidium production of FON were decreased by 52.0%, 25.7%, 100% and 100% at concentrations of 800 mg $L^{-1}$. However, mycotoxin yield was increased by 233%, pectinase activity raised by 168.0% and cellulase activity increased by 1325% compared to control at higher concentrations. It was concluded that salicylic acid as an allelochemical greatly inhibited FON growth and conidia formation and germination, though stimulated mycotoxin production and activities of hydrolytic enzymes by FON.



## 1. Introduction

Comparatively high economic incentive leads to an increase in acreage of watermelon production in China. Long-term monoculture of watermelon results in frequent incidence of watermelon fusarium wilt due to accumulation of pathogen, *Fusarium oxysporum* f.sp.*niveum* (FON) (Booth, 1971; Joffe, 1986), a causal agent responsible for watermelon fusarium wilt. The pathogen is the most important soilborne pathogen in watermelon, limiting production on many areas of the world (Martyn, 1996).

*Fusarium* species are harmful fungi that cause vascular diseases of plants, such as watermelon, cucumber, tomato, pepper, muskmelon, bean and cotton (Mckeen and Wesley, 1961; Armstrong and Armstrong, 1981; Nelson et al., 1981; Gordon and Martyn, 1997). Fusarial fungi damage host plants through penetration of hyphae into host vascular tissues, secretion of hydrolytic enzymes related to pathogenesis, mycotoxin production in the progression of infection (Gaumann, 1957; Fuchs et al., 1965; Booth, 1971; Joffe, 1986; Bacon et al., 1996; Abbas et al., 1997; Pavlovkin et al., 2004). More than 50% of the isolates of the known *Fusarium* species are toxigenic and produce deleterious secondary metabolites (Marasas, 1984).

Large amounts of work have been done on the effect of *Fusarium* sp. on watermelon and other plant hosts. These studies have focused on how the pathogen invades and damages host in the interaction of plant–microbe. Effect of host plants on pathogen has been little studied. In fact, invasion of pathogen into host plant is closely associated with host aspects, such as root exudates and decaying residues of host plants. It is well-known that many root exudates and decaying residues are phytotoxic due to some allelochemicals in the root exudates and decaying residues. Compounds have been isolated and identified mainly as organic acids, especially phenolic acids such as cinnamic, vanillic, coumaric, ferulic and salicylic acid (Ohno et al., 2001; Asao et al., 2002; Yu et al., 2003; Hao et al., 2006; Lee et al., 2006). In contrast, information on allelopathic action of physiologically active components from root exudates or decaying residues of plants on microorganisms, particularly pathogenic microbes, has been hardly available.

Studies have shown that root exudates might initiate and manipulate biological and physical interactions between roots and soil organisms, and thus play an active role in root–microbe communication (Bais et al., 2004). Almost no study had been reported on influence of a specific allelochemical component on specific pathogen, though artificially applied chemicals, such as

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ferulic, caffeic and vanillic acid, which were added to soil to test an effect of phenolic acids on microbial biomass and populations have been reported (Sparling et al., 1981; Blum and Shafer, 1988; Blum, 1997). Nicol et al. (2003) have reported that gingseng root exudate stimulates growth of *Phytophthora cactorum* and *Pythium irregulare*. Salicylic acid, a carbon-based secondary metabolite, has been considered as a signal molecule, sometimes cross-talking and forming passway interactions with other signaling molecules, such as jasmonic acid and ethylene, in plant–pathogen interactions (Reymond and Farmer, 1998; Lam and del Pozo, 1999; Muller and Chua, 1999).

Salicylic acid is an important signaling molecule involved in both locally and systemically induced disease resistance responses (Pieterse and Loon, 1999). The ability to accumulate salicylic acid has been shown to be essential for systemic acquired resistance and reactions to abiotic stress in plants (Vernooij et al., 1994; Morse et al., 2007; Robert-Seilaniantz et al., 2007; Zawoznik et al., 2007). Salicylic acid is an essential component of the plant resistance to pathogens and also plays an important role in mediating plant response to some abiotic stress (Jing et al., 2007). Pathogenic microorganisms may induce defense responses of plants when plants are treated with some exogenous chemicals such as salicylic acid during pathogen or environmental stress attacking the hosts (Eraslan et al., 2007; Gunes et al., 2007; Guo et al., 2007; Shinya et al., 2007; Zhang et al., 2007). The levels of salicylic acid in the cucumber hypocotyl and root increases significantly following inoculation of the leaves with a pathogenic microorganism capable of inducing systemic acquired resistance (Kubota and Nishi, 2006). Salicylic acid activates antioxidant defense responses of sweet cherry fruit, which may play a role in the resistance against *Penicillium expansum* (Xu and Tian, 2008). Exogenous salicylic acid is able to induce antioxidant enzyme activities, formation of pathogenesis-related proteins such as β-1,3-glucanase and chitinase, and expression of antioxidant enzyme genes in some plant leaves (Hwang et al., 1997; Lu and Chen, 2005; Wen et al., 2005; Yao and Tian, 2005; Fernandes et al., 2006; Chen et al., 2006). Salicylic acid is synthesised by plants in response to challenge by a diverse range of phytopathogens and is essential to the establishment of both local and systemic acquired resistance (SAR). Salicylic acid application induces accumulation of pathogenesis-related (PR) proteins. Mutations leading to either reduced salicylic acid production or impaired salicylic acid perception enhance susceptibility to avirulent and virulent pathogens (Loake and Grant, 2007). Spraying of salicylic acid on faba bean leaves helps to reduce or prevent the harmful effects produced after bean yellow mosaic virus infection. Salicylic acid treatment improves plant health by increasing the photosynthesis rates, pigment contents and levels of other parameters studied similar to control values. It also increases plant resistance against bean yellow mosaic virus, which induces chloroplast number, reduction in percentage of infected plants, decrease in disease severity and virus concentration of plants treated with salicylic acid prior to virus inoculation (Radwan et al., 2008). However, little is known of the direct effect of exogenous salicylic acid on pathogen itself e.g. on biomass, colony growth, conidia germination, sporulation, mycotoxin production, activities of pectinase, proteinase, cellulase and amylase of *in vitro* watermelon pathogen, FON.

The objective of this study was to assess the possible direct effect of salicylic acid on in vitro *F. oxysporum* f.sp.*niveum* growth and relevant properties (between their interactions). Here, we tested the hypothesis that an exogenous signal allelochemical, salicylic acid, would inhibit FON, serving as a signal element or a chemoattractant in the process of ecological plant–microbe interaction. This would be expected to help understand the mechanism of fusarium wilt diseases and to control pathogen in practice.

## 2. Methods and materials

### 2.1. Pathogen strains and chemicals

*F. oxysporum* f. sp. *niveum* (FON, coded NJAUS-1) was isolated from infected watermelon in a greenhouse plot, by the Laboratory of plant–microbe interactions, Nanjing Agricultural University, China. Salicylic acid and the other main chemicals used in the experiment were obtained from Sigma Co. (St Louis, Mo, USA).

### 2.2. Measurement of FON growth

A 5-mm agar plug taken from a 7-d-old PDA (potato dextrose agar) culture was inoculated on the center of the plate and was incubated at 28 °C for 7 d. Colony diameter was measured in three directions on each plate after incubation for 3 and 7 d.

### 2.3. Assessment of conidial germination

To determine the effect of salicylic acid on conidial germination, FON was grown in 2% water agar. A 5-mm agar plug taken from a 7-d-old PDA culture was inoculated in a liquid culture and incubated in 28 °C for 7 d. The broth was filtered to collect conidia. Conidial suspension was diluted to $\leqslant$1000 conidia per mm with sterile distilled water. The diluted suspension (0.1 ml) was spread on plates and incubated at 28 °C for 3 d. The number of colonies was counted daily for the 3 d.

### 2.4. Determination of sporulation

Sporulation was determined following growth of FON (as described above) in Bilay and Joffe's medium (Booth, 1971) with minor modifications (4.0 g CMC-Na instead of 15 g CMC (carboxymethyl cellulose), pH adjusted to 4 with 2 $M^{-1}$ HCl). After incubation for 7 d, 0.1 ml of culture broth, diluted to $10^{-5}$–$10^{-7}$, was spread onto PDA. Plates were incubated at 28 °C in the dark for 4 d, after which colonies were counted and converted to the number of conidia in a liquid culture.

### 2.5. Measurement of biomass production and enzyme activity

FON was grown in 100 ml conical flasks filled with 30 ml potato dextrose broth adjusted to pH 4.5 with 2 $M^{-1}$ HCl and was inoculated with a 5-mm agar plug taken from a 7-d-old PDA culture. Cultures were incubated in a rotatory shaker (170 rpm) at 28 °C for 7 d. Fungal biomass (dry weight) was determined after filtration and drying at 80 °C for 12 h, when constant weight was achieved. Culture filtrate was used for enzyme assays.

Protease activity was assayed as described by Tseng and Mount (1974). One unit of enzyme activity was defined as a 0.001 increase in absorbance per minute under the assay conditions. Pectinase activity (mainly polygalacturonase) was assayed using the DNS (3,5-dinitrosalicylic acid) method (Tokuioshi et al., 2005). One unit of enzyme activity was defined as the amount of β-galacturonic acid hydrolyzed from pectin per minute under the assay condition. Cellulase activity was also determined by the DNS method (Berlin et al., 2005). One unit cellulase activity was defined as the amount of enzyme that produced 1 μmol reduced sugar per minute under the above assay condition. Total amylase activity was assayed by DNS method (Murado et al., 1997). One unit of amylase activity was defined as the amount of enzyme that releases 1 mg reducing sugars (glucose equivalents) per minute under the above assay conditions.

### 2.6. Extraction and assay of mycotoxin

Mycotoxin was determined following growth in Richard's medium (Gaumann, 1957), as described above but with a 12 h photoperiod with fluorescent light for 35 d. Broth was acidified to pH 2 with 2 $M^{-1}$ HCl, mixed with equal volume of ethyl acetate, vigorously shaken for 2 min, settled for 30 min and the organic phase removed. After repeating this procedure 5 times, the organic phase was centrifuged for 10 min at 5000 rpm. The supernatant was dried and condensed at $\leqslant$40 °C. The dried residue was redissolved in 5 ml of ethyl acetate and the $A_{268}$ was determined by UV spectrophotometry (UV-120-02 spectrophotometer, Shimadzu, Japan).

### 2.7. Experimental design and statistical analysis of data

Based on our preliminary experiment, studies were carried out using five concentrations of salicylic acid (St Louis, Mo, USA): 0, 100, 200, 400, and 800 mg $L^{-1}$. Control was treated with sterilized distilled water instead of a solution of salicylic acid. Salicylic acid solution was filter-sterilized by a 0.22 μm of pore membrane (Millipore). Data were analyzed by Microsoft Excel™. The values were represented as means of three replicates (mean ± SE) for each treatment. Duncan's multiple range test was applied when one-way ANOVA revealed significant differences ($P \leqslant 0.05$). All statistical analysis was performed with SPSS Base Version 11.5 statistical software (SPSS Inc. Chicago, IL).

## 3. Results

### 3.1. Inhibition of salicylic acid on growth and biomass of FON

Inhibition of FON growth by salicylic acid was observed. Even at the lowest concentration 100 mg $L^{-1}$ of salicylic acid, FON growth was strikingly suppressed at 7 d when FON was incubated in liquid culture. Dried weight of mycelia in treatments of 800 mg $L^{-1}$ was 0.1007 ± 0.0124, while control was 0.2100 ± 0.0418 g (Fig. 1). Hyphal growth (colony diameter) of FON was stimulated at low concentration of salicylic acid (200, 400 mg $L^{-1}$) with the diameter increasing from 7.0 ± 0.62 cm (control) to 8.0 ± 0.36 cm, while it was inhibited at high concentration of salicylic acid (800 mg $L^{-1}$) with the diameter declining to 5.2 ± 0.03 cm on plates (Fig. 1).

### 3.2. Inhibitory effect on conidia germination and sporulation

Severe repression of conidia germination and sporulation by FON was obtained in a concentration-dependant manner. Dramatic inhibition of conidia germination was found at all concentrations (100–800 mg $L^{-1}$), especially at 800 mg $L^{-1}$, where germination was inhibited completely (Fig. 2). Number of conidia formed in liquid culture in all treatments was strongly inhibited even at the lowest concentration of 100 mg $L^{-1}$. Sporulation was fully suppressed at the highest concentration of 800 mg $L^{-1}$ (Fig. 2).

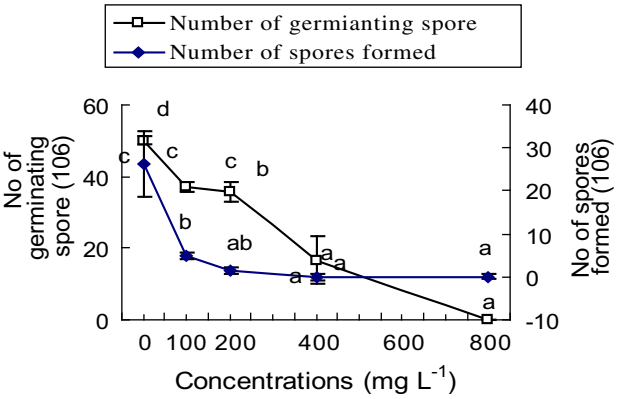


**Fig. 2.** Effect of SA on conidia germination of FON after 4 d of culture on plates and on FON sporulation in liquid culture. Bars represent standard errors of three replicates. Different letter for each datum point indicates significant difference at $P < 0.05$.

### 3.3. Stimulatory effect on mycotoxin production

Mycotoxin yield of FON in liquid culture was increased by salicylic acid and raised with increasing concentrations. The increase in mycotoxin production was very big at high concentration (400, 800 mg $L^{-1}$), though it was little at low concentration of salicylic acid (100, 200 mg $L^{-1}$). The yield at the highest concentration of (800 mg $L^{-1}$) Fusaric acid was elevated from 183.8 ± 30.1(control) to 611.4 ± 88.7 μg $ml^{-1}$ (Fig. 3).

### 3.4. Impact on enzymes activities in relation to pathogenesis

Strong stimulation of pectinase activity in liquid culture by salicylic acid was found; though at higher concentrations little further increase was found (slight rise amplitude of the activity was displayed). The activity at concentration of 800 mg $L^{-1}$ was 0.067 ± 0.0043, while control was 0.025 ± 0.0004 U.$ml^{-1}$ $min^{-1}$ (Fig. 4a). Proteinase activity of FON in liquid culture was lower than in control at low concentrations of salicylic acid (100, 200 mg $L^{-1}$) but the activity was greatly stimulated by salicylic acid at higher concentrations (400, 800 mg $L^{-1}$), (Fig. 4b). Sharp rise of FON cellulase activity treated with salicylic acid in liquid culture was observed. However, further increase of activity was scarcely found (Fig. 4c). Amylase activity by FON in liquid culture was hardly affected by salicylic acid, although small amounts of fall tendency was found, which the activities at treatments were almost no difference compared to control (Fig. 4d).

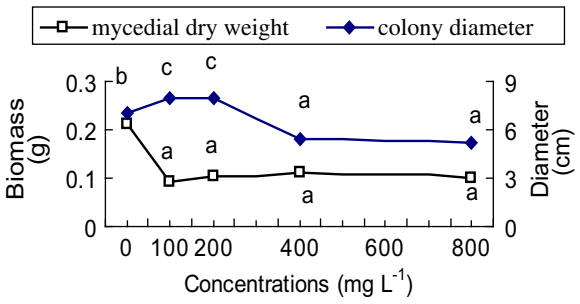


**Fig. 1.** Effect of SA on FON biomass and colony growth after 7 d of liquid culture. Bars represent standard errors of three replicates. Different letter for each datum point indicates significant difference at $P < 0.05$.

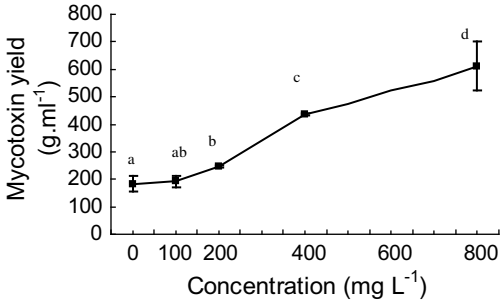


**Fig. 3.** Effect of SA on mycotoxin production by FON in liquid culture. Bars represent standard errors of three replicates. Different letter for each datum point indicates significant difference at $P < 0.05$.

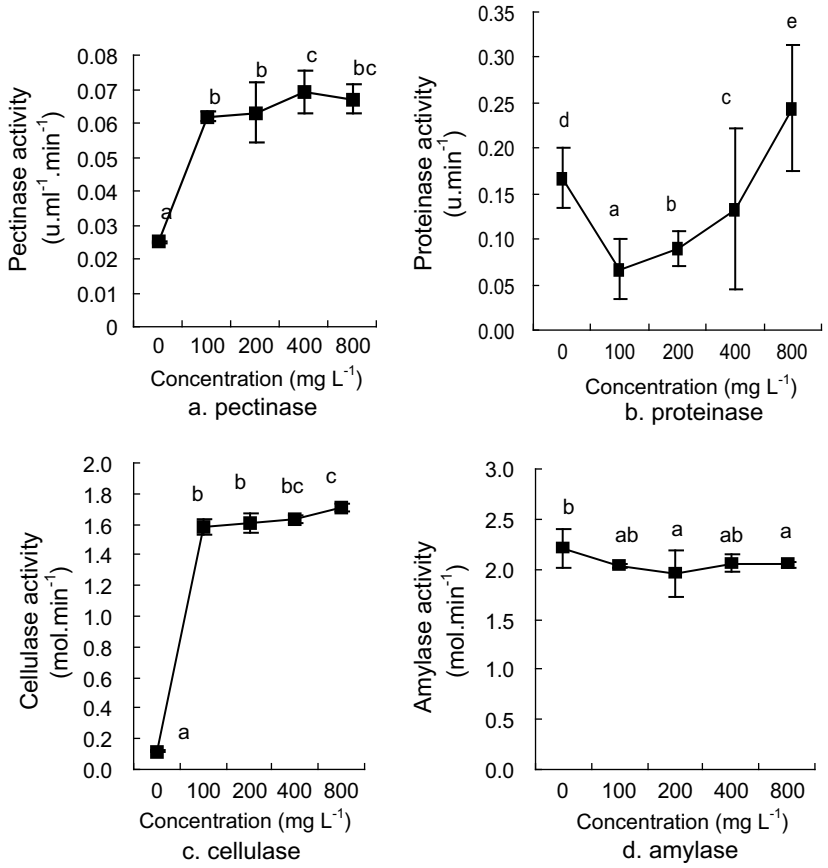


**Fig. 4.** Effect of salicylic acid at different concentrations on enzymes related to pathogenesis of FON in liquid culture. Bars represent standard errors of three replicates. Different letter for each datum point indicates significant difference at $P < 0.05$.

## 4. Discussion

As an antibiotic against microorganism, salicylic acid may be characterized as having two main effects on watermelon wilt pathogen. One is the inhibition of hyphal growth, sporulation and conidia germination; another is the stimulation of mycotoxin production and enzymes related to pathogenesis.

In the current study, salicylic acid caused strong inhibition of hyphal growth and biomass. The dry weight of mycelia at the highest concentration of salicylic acid (800 mg $L^{-1}$) in liquid culture was decreased by 52.0% (Fig. 1). This result confirmed a report that the mycelial growth of the *F. oxysporum* f.sp.*albedinis* is inhibited by cell wall-bound phenolics in resistant cultivars of date palm roots (Modafar and Boustani, 2001). The obtained result was also in agreement with the study that salicylic acid with a concentration of 270 mg $L^{-1}$showed direct fungitoxicity on *Monilinia fructicola* and significantly inhibited mycelial growth of the pathogen *in vitro* (Yao and Tian, 2005). Salicylic acid is an essential component of the plant resistance to pathogens and also plays an important role in mediating plant response to some abiotic stress (Jing et al., 2007). Plant resistance to biotrophic pathogens is classically thought to be mediated through salicylic acid signaling (Loake and Grant, 2007). Inside plant, pathogen-derived salicylic acid is synthesised from chorismate by isochorismate synthase when plant is attacked by pathogen (Loake and Grant, 2007). From the present results, pathogen-induced salicylic acid not only enhances the plant resistance to pathogen but directly inhibits the growth of the pathogen as well.

Suppression of colony growth on plate was found, though low concentration of salicylic acid stimulated colony growth of FON (Fig. 1). Colony growth, in fact, is also mycelial growth on solid plate. The present result was in accordance with salicylic acid inhibiting mycelial growth of *Eutypa lata* (Pers. Fr.) Tul. in a solid as well as in a liquid culture medium in a concentration-dependent manner with the threshold value being 13.8 mg $L^{-1}$(Amborabe et al., 2002). This result also confirms that salicylic acid is involved in the direct repressing of pathogen in the plant–fungus interaction.

In the present study, both sporulation in liquid culture and conidia germination of FON on plates were greatly suppressed by salicylic acid even at the lowest concentration of 100 mg $L^{-1}$. No conidia germination on the plate and sporulation in liquid culture were observed at the highest concentration, namely full inhibition of conidia germination and formation (Fig. 2). The results were consistent to salicylic acid with a concentration of 270 mg $L^{-1}$ showing direct inhibition on *M. fructicola in vitro* (Yao and Tian, 2005). It is well-known that both sporulation and conidium's germination are important preconditions for pathogenic microorganisms' survival and attacking hosts. From the results, it is clear that accumulation of salicylic acid is unfavorable for *F. oxysporum* f.sp.*niveum* because of suffering from such multiple suppressions, including mycelial growth, biomass, colony growth, conidium's germination and formation. The current results were completely in accordance with accumulation of salicylic acid in infected tissues to resist further damage when the host was attacked by pathogen (Zhang et al., 2007; Shinya et al., 2007). We also believe that decreased sporulation and conidial germination of FON by salicylic acid would be one of the mechanisms on plant resistance against pathogen.

As a powerful attack, mycotoxin production is formed when FON attacks hosts, which is a fatal pathogenic factor causing plants

wilt. Mycotoxin is a well-known phytotoxin produced by several fusarium species, particularly pathogenic strains of *F. oxysporum*, the cause of wilt diseases in a great variety of plants, such as watermelon, cucumber, tomato, beans and cotton (Gaumann, 1957). Mycotoxin is a wilt toxin on tomato plants infected with *F. oxysporum* f.sp. *lycopersici* and the toxic concentration needed to cause wilting is about 150 mg $L^{-1}$ (Devid, 1969). Toxins produced by pathogens are primary determinants of pathogenesis when they act as the key elements in infection initiation and symptom development. They are secondary determinants when they only modify the intensity of symptoms (Lepoivre, 2003). Moderate fusaric acid (a fusarial mycotoxin) doses induce apoptosis in saffron while high fusaric acid doses stimulate necrosis (Samadi and Shahsavan, 2006). In the present study, mycotoxin in different treatments in liquid culture was produced at high rates at the highest concentration of 800 mg $L^{-1}$, which was increased by 233% compared with control (Fig. 3). This suggested salicylic acid stimulated mycotoxin production of FON. However, there was little stimulation of mycotoxin production at low concentrations of salicylic acid (100–200 mg $L^{-1}$). It seems conflicting with the above descriptions that salicylic acid strongly repressed FON growth. We analyzed that there might be different mechanisms between mycelia growth and conidia formation and germination and mycotoxin production. Furthermore, it is impossible to produce so high concentration of endogenous salicylic acid inside the plants. The optimal concentration to inhibit growth of fungal pathogen was mostly 140–270 mg $L^{-1}$ salicylic acid (Zhang et al., 2007; Shinya et al., 2007; Gunes et al., 2007). Therefore, the current result was confirmed by others who reported control of fusarium wilt disease in practice (Shinya et al., 2007).

Other equally important virulence factors for FON are hydrolytic enzymes related to pathogenesis. Pectinases and cellulases of phytopathogenic fungi stimulate the infection process in many plant diseases. They facilitate the penetration of the fungus into the plant by a hydrolytic cleavage of polymers (pectic substances and cellulose), which constitute the plant cell walls (Fuchs et al., 1965). Fusarial fungi damage host plants through invasion by hyphae into host vascular tissues, secretion of hydrolytic enzymes related to pathogenesis and mycotoxin production in the progression of infection (Fuchs et al., 1965; Booth, 1971; Joffe, 1986). In the current study, great stimulation of enzyme activities in relation to pathogenesis in liquid culture was found. Pectinase activity by FON at the highest concentration of salicylic acid was increased by 168.0%. Proteinase activity treated with salicylic acid was initially reduced by 59.8% at lowest concentration (100–200 mg $L^{-1}$) but then was elevated by 46.1% at the highest concentration. The highest cellulase activity treated with salicylic acid was raised by 1325.0% at the highest concentration of 800 mg $L^{-1}$. Amylase activity was hardly changed compared to control, though general trends of decrease were found (Fig. 4).

Although salicylic acid could stimulate the pathogenic hydrolytic enzymes activities of FON, it seems to contradict the suppressions of the fungus growth mentioned above, in fact, no such high concentration of salicylic acid is reached in plants and in application for control disease whether induced by pathogen attack or artificially applied (Zhang et al., 2007; Shinya et al., 2007; Gunes et al., 2007). Even endogenous salicylic acid accumulation in plants could generate, sustain or amplify the biotic oxidative stress (Zawoznik et al., 2007). Increased amounts of salicylic acid were detected in the roots and hypocotyl of cucumber plants (*Cucumis sativus*) following inoculation of the leaves with the anthracnose pathogen, *Colletotrichum lagenarium* (Kubota and Nishi, 2006). The concentrations of salicylic acid in the internodes immediately below the infected leaves increased to more than 1 mg $kg^{-1}$ fresh weight. In contrast, the concentrations of salicylic acid in stems distant from, or above the infected leaves increased to 0.1–0.3 mg $kg^{-1}$. Salicylic acid was increased in the upper stem 2 d after inoculation, followed by the hypocotyl with an increase detected 4 d after inoculation. An initial increase in the salicylic acid levels was detected in the stem, followed by a rise in salicylic acid levels in the root from a basal level of approximately 0.3 mg $kg^{-1}$ to more than 1 mg $kg^{-1}$. The increased level of salicylic acid in the lower leaves was less than 0.1 mg $kg^{-1}$ (Kubota and Nishi, 2006). As a matter of fact, it is impossible that excess salicylic acid would have stimulated FON pathogenisis due to the elevated virulence factors because the concentration of salicylic acid inside the plant is often low and the growth of FON is fully suppressed by even 100–200 mg $L^{-1}$ salicylic acid so that the FON can not produce mycotoxins and hydrolytic enzymes.

Our results further clarified that salicylic acid inhibited growth, sporulation and conidia germination of FON and were completely consistent to many known reports mentioned above, although further proved to stimulate mycotoxin production and pathogenically hydrolytic enzymes' activity. The findings of this study also implied that excessive salicylic acid production inside plants induced by the fungus pathogen or artificially added in practice would have adverse effect on the plant, which needs to be further investigated in the future. Suitable amounts of salicylic acid should be used to control the pathogen in field crops. Salicylic acid (100 mg $L^{-1}$) may be regarded as a secondary defense line in a combination of *C. laurentii* and salicylic acid, which could reinforce the biocontrol efficacy of *C. laurentii* by induction of the fruit natural resistance (Yu et al., 2007). Salicylic acid as an inducer in conjunction with antagonistic bacterium enhances the suppression of crop diseases (Rajkumar et al., 2008). Even application of 13 800 mg $L^{-1}$ salicylic acid 3 d before inoculation with bean yellow mosaic virus restores the metabolism of infected faba bean leaves to the levels of healthy controls (Radwan et al., 2008). Salicylic acid alleviated pathogen-induced oxidative stress in harvested sweet cherry fruit by activating antioxidant defense responses of sweet cherry fruit, which may play a role in the resistance against *P. expansum* (Xu and Tian, 2008). It is ascribed to salicylic acid activating resistance through inhibition of some antioxidant enzymes, catalase and peroxidase, leading to production of elevated amounts of $H_2O_2$ accumulation. Meanwhile salicylic acid can play a role in protection of the chlorophyll molecules indirectly through improvement of carotenoid molecules (Radwan et al., 2008).

In summary, salicylic acid is one signaling molecule characterized by its overt antibiotic effect on FON. The finding demonstrated that hyphal growth and conidia formation and germination of FON were strongly inhibited by salicylic acid, although great stimulation of mycotoxin production and hydrolytic enzymes activities related to pathogenesis was induced. Different mechanisms would be suggested on hyphal growth and virulence factors of FON. It need not worry about the risk of the increased virulence factors by much higher concentrations of salicylic acid to plant because salicylic acid inside the plant is often much lower than the adversely acting concentration and the growth of FON is fully suppressed by even 100 mg $L^{-1}$ salicylic acid before FON could produce mycotoxins and hydrolytic enzymes. This may explain the possible mechanism of watermelon fusarium wilt from the point view of chemical interactions.

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