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Root exudates from muskmelon (*Cucumis melon*. L) induce autotoxicity and promote growth of *Fusarium oxysporum* f. sp. *melonis*.

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ABSTRACT

Experiments were conducted to test the influence of allelochemicals from root exudates of muskmelon (*Cucumis melon* L.) plants on the growth of *Fusarium oxysporum* f. sp. *melonis*. It was observed that the root exudates of muskmelon significantly promoted mycelial growth, spore germination of *Fusarium oxysporum* f. sp. *melonis* and incidence of Fusarium wilt as determined in bioactivity test and pot experiments. The results of field experiments indicated that the disease incidence and the quantity of the fungi were higher in sole replant cropping. The autotoxic compounds from muskmelon root exudates were identified by HPLC and seven phenolic compounds were identified: gallic acid, phthalic acid, syringic acid, salicylic acid, ferulic acid, benzoic acid and cinnamic acid, all of which significantly reduced muskmelon seed germination and inhibited seedling growth at higher concentration (0.5 mmol·L^{-1}) when compared with the control. The maximum inhibition in seed germination was cinnamic acid, followed by benzoic acid and ferulic acid. Salicylic acid, syringic acid, gallic acid and phthalic acid showed stimulatory effects (0.05 mmol·L^{-1}), while cinnamic acid, benzoic acid and ferulic acid inhibited all parameters.

Key words: Root exudates, muskmelon, Allelopathy, *Fusarium oxysporum* f. sp. *melonis*, Fusarium wilt, HPLC analysis, Autotoxicity.

INTRODUCTION

The infestation by soil-borne pathogens and autotoxicity are the main restrictions in worldwide muskmelon (*Cucumis melon* L.) production (40). *Fusarium oxysporum* f. sp. *melonis* (Leach & Currence) Snyd. & Hans., causes Fusarium wilt of muskmelon, which is an economically important disease universally. The fungi damage host plants through penetration of hyphae into host vascular tissues, secretion of hydrolytic enzymes related to pathogenesis and mycotoxin production in the progression of infection (7,9,40) and disease symptoms can be observed at all developmental stages of the plant. This vascular wilt disease is one of the most difficult adversities to control because the fungi are soil-borne and remain viable in the soil on nonhost crop residues and roots grown in rotation as chlamydospores for decades (7). Root exudates are the largest direct inputs of plant chemicals into the rhizosphere environment which initiate and manipulate biological

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interactions between roots and soil micro-organisms, and thus play an active role in root-microbe communication (3,4,38). Root exudates can influence plant growth not only directly by acting as autotoxins but also by affecting the soil microbial community (1,5,11, 23). The mechanism of resistance of muskmelon to *F. oxysporum* f. sp. *melonis*, is the allelopathic suppression caused by root exudates of muskmelon (40). Previous experiments have shown that the roots of some plant species release allelochemicals that can inhibit the same plant species and induce autotoxicity (5,14,33). Autotoxicity, a form of intraspecific allelopathy, refers to a process in which a plant species releases the toxic chemicals that inhibit or delay germination and growth of other plants of the same species, which is important in agricultural applications, such as the replant failure of horticultural crops and growth reduction in some fruit vegetables during fruit enlargement (5,32). Under field conditions, additive or synergistic effects among allelochemicals become more influential, especially at low concentrations (2,30). Among all allelochemicals, pheolic compounds are the most abundant under field conditions and are known to affect seed germination, seedling growth, sell division and fungal activity, could accumulate in soil by root exudation, decomposition of plant residues, microbial transformation into other toxic chemical or by environmental factors such as the water deficit, high irradiances, UV radiation, pathogen attack, fungicide and herbicide application, nutrients deficiency and so on (18,21). Autotoxicity is a complicated problem in agricultural production, which needs more studies such as the identification of the phytotoxic substances in the root extracts and exudates, their fluctuation in rhizosphere and the elucidation of the possible role of micro-organisms in the phytotoxicity (32,34).

The purposes of this research are to evaluate the influences of root exudates produced by muskmelon susceptible to the pathogen *F. oxysporum* f. sp. *melonis*, to separate and identify the compounds from muskmelon root exudates that are responsible for muskmelon autotoxicity, and to determine the phytotoxicity of these compounds to muskmelon seed germination and seedling growth.

MATERIALS AND METHODS

Collection of root exudates

Root exudates were collected at bud stage with root soaking method. Muskmelon seedlings were uprooted from soil and the roots were first washed with tap water (5 repeats) and then with distilled water (5 repeats) to remove soil. The seedlings were placed in a beaker containing 500 ml of sterile double-distilled water for 24 h (16 h light/8 h dark) at 28 ^{0}C to 32 ^{0}C. The distilled water was collected and filtered using glass syringes fitted with a Gelman Acrodisc CR-PTFE 0.2-μm filter and evaporated using a rotary evaporator (Yarong Model RE-52A, Shanghai, China) at 40 ^{0}C to a volume of 50 ml. The compounds were subjected to HPLC analysis and bioassay.

Mycelium growth

Fusarium oxysporum f. sp. *melonis* was isolated and identified by Institute of Plant Immunology Lab of Shenyang Agricultural University. Root exudates of muskmelon were selected for bioassays at 0.05, 0.1, 0.25, 0.5 and 1.0 g·mL^{-1} (1 g·mL^{-1}means that 1 ml distilled water contains the exudates obtained from 1 g fresh root). The mycelium growth

of *F. oxysporum* f. sp. *melonis* was evaluated using the growth rate method. Root exudates filtered through filter were added to the PDA media in a ratio of 1:4 during the preparation of PDA plates. The 0.5 cm diameter pure cultured *F. oxysporum* f. sp. *melonis* spots were then inoculated into the plates and incubated at 25 ^{0}C in darkness. Each treatment was replicated three times. The colony diameter was measured with the cross method after 3, 5 and 7 days respectively. The intensity of allelopathic effect was expressed in RI values calculated according to the following formula (27):

$$RI = 1 - C/T \ (T \geq C) \text{ or } RI = T/C - 1 \ (T < C),$$

where C is the control data, T is treatment data. RI values range from +1 to -1, RI > 0 means stimulation, while RI < 0 means inhibition.

Spore germination

Spore suspension of *F. oxysporum* f. sp. *melonis* (at least 20 - 30 spores per microscopic field) was prepared from 7 days-old culture. One drop of about 0.1 ml of spore suspension was placed in a cavity glass slide containing a drop (about 0.1 ml) of different concentration (0.02, 0.04, 0.08, 0.16, 0.32 g·mL^{-1}) of root exudates from muskmelon. These slides were kept in moist chamber prepared by putting two folds of filter paper in both sides of Petri dishes. These Petri dishes were incubated at 24 ± 2 □ for 8, 16 and 24h. Each treatment was replicated five times. The percent of spore germination was calculated as following (13):

$$\text{Percent of spore germination} = \frac{\text{Number of spores germination}}{\text{Total Number of spores examined}} \times 100$$

Pot experiment

The pure cultured *F. oxysporum* f. sp. *melonis* spots were suspended in the flasks containing 250 ml of Richard media (KH_2PO_4 1 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.5 g L^{-1}, KNO_3 3 g L^{-1}, KCl 0.5 g L^{-1} and $Fe_2(SO_4)_3$ 0.01 g L^{-1}) at 28 ^{0}C for 7 days till the liquid media were brown-pink in color. The number of spores was counted by using a haemocytometer, and the spores were diluted with sterile distilled water to reach 3.0×10^5 CFUml^{-1} and homogenized in a blender. The *F. oxysporum* f. sp. *melonis* suspension was used to infect the muskmelon plants.

Root exudates were selected for pot experiment at 0.5, 1.0, 2 g·mL^{-1}. 200 g of soil was amended with 30 ml of root exudates of each treatment in a container (diameter 9 cm and volume 300 ml). Each treatment and the non-amended root exudates (control) were replicated six times. Uniformly growing seedlings at 4-leaf stage were selected and 15ml of *F. oxysporum* f. sp. *melonis* suspension was injected into the rhizosphere (2 cm away from stem bases) to reach the soil pathogenic level around 10^4 CFUml^{-1}. The treatments were arranged in a random pattern in growth chamber with a 16 h light/8 h darkness photocycyle at the temperature regime of 21/15 ^{0}C. Relative humidity was maintained at 70%. After the onset of disease symptom, plant infection was evaluated every 5 days. Severity of symptoms was assessed using the following indice: 1. plant without symptoms; 2. very slight browning of hypocotyl; 3. some wilting of plant; 4. wilting of entire plant; 5. died plant. Disease severity index was calculated as following:

$$\text{Disease Severity Index} = \frac{\sum (\text{Rating number} \times \text{Number of plants with rating})}{\text{Total Number of plants} \times \text{highest rating}} \times 100\%$$

Field experiment

Experiments were conducted in a greenhouse of a field experiment base (70 m length, 8 m width and 3.5 m height) in Shenyang agricultural university during three successive vegetation seasons (2010-2012) and for each year muskmelon was sown in each vegetation seasons. Plants affected by Fusarium wilt pathogen were evaluated visually and the numbers of wilted plants and the total numbers of plants were recorded in mid-August of three years. Disease incidence was calculated as following:

Disease incidence (%) = (Number of Infected Plants/Total Number of Plants) × 100%

To quantify the inoculum density of *F. oxysporum* f. sp. *melonis* in experiment fields, 10 soil samples were collected from each field before plantation and after harvest in every year (2010-2012). For each soil sample, approximately 300 g of soil were collected from a depth of 15 cm. Soil samples from the same treatment were combined into one sample and mixed thoroughly. A 10 g sub-sample of soil was added to 90 ml of autoclaved water and the resulting soil suspension was mixed for 5 min using a magnetic stirrer. 1 ml of this suspension was plated onto each ten Petri dishes of Komada's medium (20,40). Three replicates of soil dilutions were prepared for each field sampled. All plates were incubated at 25 ^{0}C for 5 days, and the total number of colonies of *F. oxysporum* was counted.

HPLC analysis

Analysis was performed in an HPLC system (Agilent 1200, USA) with an XDB-C18 column (4.6 mm×250 mm). The mobile phase consisted of acetic acid (A) and 2% methanol (B) with a gradient elution. The root exudates and standard compound were used in the following gradient system: (1) from 0.0 to 10 min, 95% A plus 5% B at a flow rate of 0.5 ml/min; (2) from 10 to 25.0 min, 95% A plus 5% B at a rate of 0.8 ml/min; (3) from 25.0 to 36.0 min, 85% A plus 15% B at a rate of 0.8 ml/min; (4) from 36.0-45.0 min, 65% A plus 35% B at a rate of 1 ml/min; (5) from 45.0 to 50.0 min, 65% A plus 35% B at a rate of 1.2 ml/min. Detection was performed at 280 nm. The injection volume was 20 μL and the column temperature was maintained at 25 ^{0}C. The mixture of standard compounds was chromatographed. Retention times for the standard compounds and the major peaks in the exudates were recorded. Analyses of the compounds were based on retention time (minutes) and the phenolic compounds of root exudates were identified and quantified with reference to the standards (14,30).

Germination bioassays

Muskmelon seeds were surface sterilized with a solution of distilled water-clorox (95 ∶ 5) for 10 min, and then rinsed with distilled water. Twenty muskmelon seeds were placed in sterilized 3.5-cm glass Petri dishes containing double layered filter paper, then 5 ml solution of phenolic compounds (0.00, 0.05, 0.1 and 0.5 mmol·L^{-1}) was transferred to

the Petri dishes as per treatments; distilled water was used as a control. Four replications were used for each concentration and dishes were placed in a lighted chamber at 24 ^{0}C. After 3 days, the numbers of seeds germinated in each dish were counted and the radicle length, hypocotyl length and fresh weight were recorded to calculate the germination rate, germination index (GI) and germination potential by using the following formula:

$$\text{Germination rate (\%)} = (\text{Number of germinated seeds} / \text{Total seeds number}) \times 100$$

$$\text{Germination potential (\%)} = \frac{\text{Number of seeds germinated on 3rd day}}{\text{Total seed number}} \times 100$$

$$\text{Germination index (GI)} = \sum (Gt/Dt)$$

Where, Gt: Number of seeds germinated on day *t* and Dt: The number of days from the beginning of the experiment.

The bioassay results were expressed in RI value as per Williamson (27).

Growth bioassay

Muskmelon seeds sterilized with 10% H_2O_2 were directly sown in nursery substrates. Average day/night temperatures were 25/20 ^{0}C. The seedlings were transplanted at 2-leaves growth stage into a container (15 × 15 × 10 cm) filled with 0.8 L of Enshinutrient solution (30) and maintained at a relative humidity of 95%-100%. Phenolic acid dissolved in ethanol were added into the nutrient solution at concentration of 0.00, 0.05, 0.1 and 0.5 mmol·L^{-1}. The final concentration of ethanol in each solution including the control was 0.1% (v/v). Each treatment had 8 plants and was replicated tree times. 21 days later, plant height, shoot and root dry weight were measured at the end of the experiment.

Statistical analysis

The data were processed by Microsoft Excel. Analysis of variance was performed using the Data Processing System software (DPS) and the significant differences among treatments were determined by Fisher's least significant difference test at $P<0.05$.

RESULTS AND DISCUSSION

Mycelium growth

It was revealed from the results (Table 1) that root exudates of muskmelon at different concentrations were stimulatory to mycelium growth of *F. oxysporum* f. sp. *melonis* in the first two incubation stages as compared with the control (0 g·mL^{-1}). However, from 0.05 g·mL^{-1} to 0.25 g·mL^{-1} concentrations, the root exudates showed significant stimulatory activity than other concentrations and 0.25 g·mL^{-1} concentration was most stimulatory (RI value: 0.138). The longer the mycelium was incubated with root exudates, the less stimulatory effects were resulted. At 1.0 g·mL^{-1} concentration, the stimulatory effects on mycelium growth were minimal.

Table 1. Effects of muskmelon root exudates on mycelium growth of *Fusarium oxysporum* f. sp. *melonis*

Concentration ($g \cdot mL^{-1}$)	3 d		5 d		7 d	
	Colony diameter (mm)	RI value	Colony diameter (mm)	RI value	Colony diameter (mm)	RI value
0.05	34.74 bc	0.078 c	57.55 b	0.067 c	66.74 cd	0.039 c
0.1	36.15 b	0.114 b	58.93 ab	0.089 b	69.16 b	0.073 b
0.25	37.17 a	0.138 a	60.40 a	0.111 a	71.08 a	0.098 a
0.5	34.32 c	0.067 d	56.77 c	0.054 d	66.97 c	0.042 c
1.0	33.23 cd	0.036 e	54.57 d	0.016 e	65.47 de	0.020 d
CK	32.03 d	-	53.71 d	-	64.13 e	-

Different small letters within each column show significant differences with the control at 0.05 levels.

Spore germination

The application of root exudates from muskmelon showed significant effect on the spore germination of *F. oxysporum* f. sp. *melonis*. Current results showed that all concentrations of root exudates of muskmelon caused stimulatory effects on the spore germination (Figure 1) except 0.02 $g \cdot mL^{-1}$, which showed an inhibitory effect. However, the 0.04 $g \cdot mL^{-1}$ concentration exhibited the least stimulatory effect on the spore germination compared with control in the incubation time of 24 h. The maximum stimulatory effect on the spore germination was found at 0.32 $g \cdot mL^{-1}$ followed by 0.16 $g \cdot mL^{-1}$and 0.08 $g \cdot mL^{-1}$.

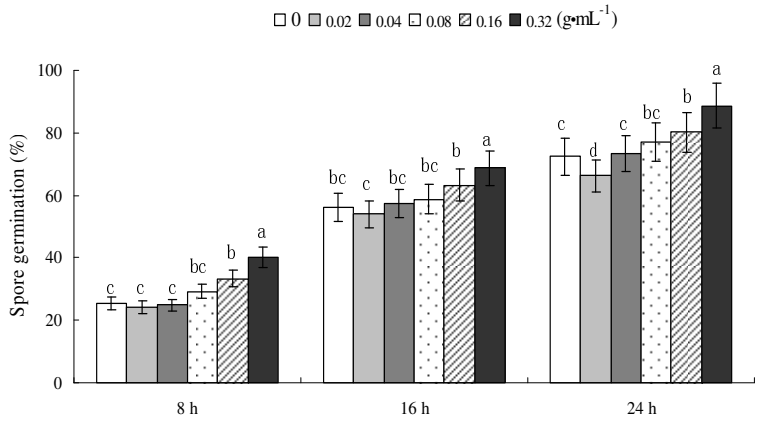

Figure 1. Effects of root exudates of muskmelon from different treatments on the spore germination of *Fusarium oxysporum* f. sp. *melonis* at different periods in the normal system. Treatments included 0.02 $g \cdot mL^{-1}$, 0.04 $g \cdot mL^{-1}$, 0.08 $g \cdot mL^{-1}$, 0.16 $g \cdot mL^{-1}$, 0.32 $g \cdot mL^{-1}$ concentration and the control. The different small letters represent the significant differences from control at 0.05 levels.

Fusarium *oxysporum* f. sp. *melonis* infection

The influence of root exudates on the microbiological quality of pathogen-infested soil was studied, and it was found that amended root exudates of muskmelon soil had improved microbiological qualities. The different concentrations applied to test plants exhibited higher disease infection to *F. oxysporu*m f. sp. *melonis* (Table 4). The 1.0 g·mL^{-1} concentration provided the best promotive action. On the 7th days after treatment, disease severity index of 1.0 g·mL^{-1} concentration was 9.26% lower than the control, but on the 27th day after treatment, the disease severity index of the control reached to 80.95%, while that of 1.0 concentration was 92.59%.

Table 2. Effects of root exudates from muskmelon on the disease index of Fusarium wilt

Concentration (g·mL^{-1})	Disease severity index (%)				
	7 d	12 d	17 d	22 d	27 d
0.5	7.41 c	24.07 bc	51.85 bc	70.37 bc	85.19 bc
1.0	9.26 b	33.33 a	59.26 a	81.48 a	92.59 a
2.0	12.96 a	25.93 b	55.56 ab	74.07 b	88.89 ab
CK	9.23 b	20.37 c	48.15 c	62.96 c	80.95 c

Different small letters within each column show significant differences with the control at 0.05 level.

Field experiment

In 2010 experiment conducted in greenhouse, Fusarium wilt symptoms were observed in 7.6% of muskmelon plants. However, in the experiment conducted in spring of 2011, 38.6% of muskmelon plants were dead. At last observation in the experiment of 2012, wilting was observed in 53.8% of muskmelon plants (Figure 2). These results indicated that the disease incidence was higher in sole replant cropping. In the samples taken from the top 15-cm layer during the three years at two different times, the quantity of the fungi, before planting, was found to vary from 10 to 59 CFU g^{-1} soil in 2010, between 120 to 381 CFU g^{-1} soil in 2011 and between 450 to 685 CFU g^{-1} soil in 2012 (Figure 3).

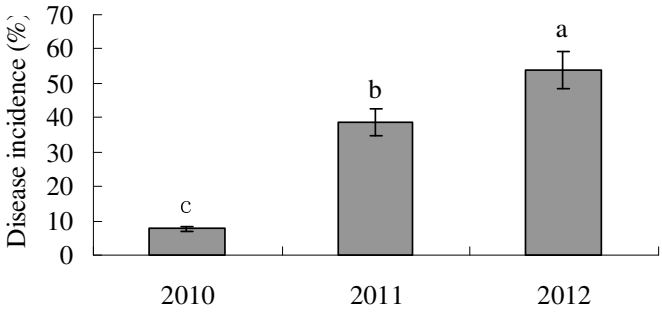

Figure 2. Effects of replant of muskmelon on Fusarium wilt incidence during the three vegetative seasons from 2010 to 2012. For each year the experiments were performed in an environment-controlled greenhouse at 25℃ day/20℃ night. The different letters represent the significance between pairs of mean values at $P < 0.05$.

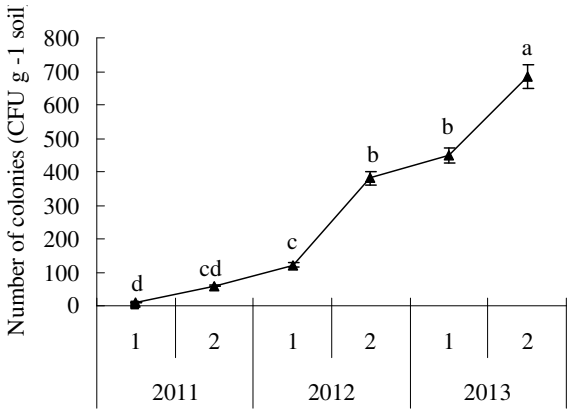

Figure 3. Number of colonies of *F. oxysporum* recovered from the soil surveyed for three years. 1 and 2 refer to the soil samples collected before plantation (mid-April) and after harvest (mid-September) in every year (2010-2012). The different letters represent the significance between pairs of mean values at $P < 0.05$.

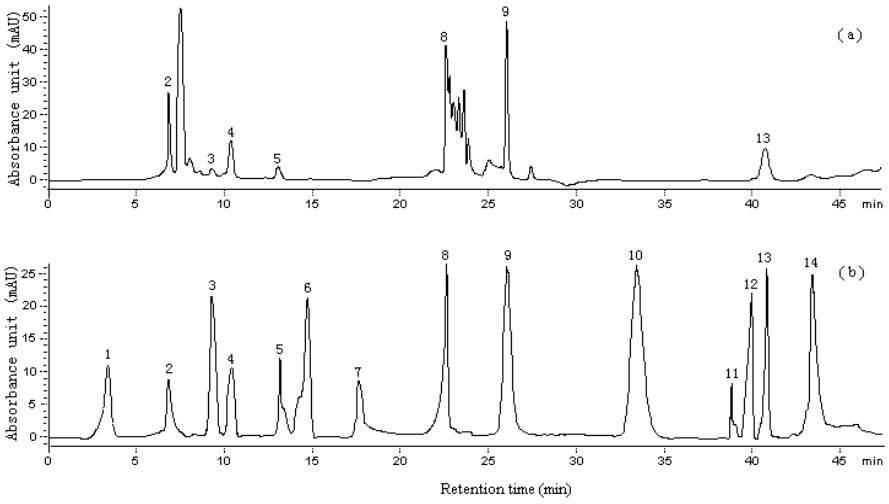

Figure 4. The chromatogram of phenolic acids detected with HPLC both in root exudates of muskmelon and standard chemicals. Figure (a) represented the chromatogram of root exudates of melon. The peaks from left to right in Figure (b) represent the following standard compounds (retention time): 1, p-coumaric acid (3.35 min); 2, gallic acidm (7.01 min); 3, phthalic acid (9.62 min); 4, syringic acid (10.58 min); 5, salicylic acid (13.63 min); 6, p-hydroxybenzoic acid (14.83 min); 7, vanillic acid (17.84 min); 8, ferulic acid (23.05 min); 9, benzoic acid (26.55 min); 10, caffeic acid (33.67 min); 11, methyl 2,5-dihydroxybenzoate (38.73 min); 12, vanillin (39.77 min); 13, cinnamic acid (41.88 min); 14, L - phenylalanine (43.47 min).

HPLP Analysis

Figure 4 depicts typical HPLC chromatograms of root exudates from muskmelon and the fourteen standard chemical phenolic compounds. Retention times of the components were observed and the peak at 7.01 min was identified as gallic acid, the peak at 9.62 min was identified as phthalic acid, the peak at 10.58 min was identified as syringic acid, the peak at 13.63 min was identified as salicylic acid, the peak at 23.05 min was identified as ferulic acid, the peak at 26.55 min was identified as benzoic acid and the peak at 41.88 min was identified as cinnamic acid.

Bioassays

The results in Table 3 indicated that treatment with cinnamic acid had the greatest inhibitory effect, followed by benzoic acid and ferulic acid. All phenolic acids in the laboratory significantly reduced muskmelon seed germination and seedling growth at higher concentration (0.5 $mmol \cdot L^{-1}$) when compared with the control. At low concentration (0.05 $mmol \cdot L^{-1}$), gallic acid, phthalic acid, syringic acid, salicylic acid promoted seed germination, while cinnamic acid, benzoic acid and ferulic acid inhibited all parameters. The maximum inhibition in seed germination was found in treatment with cinnamic acid at 0.5 $mmol \cdot L^{-1}$, germination rate, germination potential and germination index were only 44.17%, 12.50% and 3.41%.

It was revealed from the result (Table 4) that phenolic acids exhibited an allelopathic promotion at lower concentration (0.05 $mmol \cdot L^{-1}$) and inhibition at higher concentration on muskmelon growth. Salicylic acid and syringic acid showed stimulatory effects on the growth of muskmelon from 0.05 $mmol \cdot L^{-1}$ to 0.1 $mmol \cdot L^{-1}$ and gallic acid, phthalic acid, promoted the indices of plant height and shoot weight at low concentration (0.05 $mmol \cdot L^{-1}$), while at both concentrations (0.1 and 0.5 $mmol \cdot L^{-1}$) inhibited the seedling growth of muskmelon. However, cinnamic acid, benzoic acid and ferulic acid inhibited the root and shoot growth of muskmelon at concentrations greater than 0.05 $mmol \cdot L^{-1}$ and the inhibitory effect is concentration-dependent.

Several studies have demonstrated that root exudates enhance or decreases soil-born pathogens by providing substrates for saprophytic growth and influences soil microorganism communities to produce positive or negative feedback to the plants (3,4, 10). Hao *et al.* (13) reported the phenolic acid in root exudates from watermelon significantly increased the spore germination and sporulation of *F. oxysporum* f. sp *niveum* (8). The allelopathic effects on pathogens differed with allelochemicals and their concentrations. This study yielded similar conclusions, where root exudates from muskmelon showed stimulatory effects on the growth and development of *F. oxysporum* f. sp. *melonis* and may thus increase diseases of Fusarium wilt. The results are in agreement with earlier studies reporting that the resistance of muskmelon was reduced by root decaying substance (4,21). Continuous cropping of melon may results in poor performance of this crop because secretions accumulating in the soil have profound effects on pathogen dynamics, thus inducing high disease incidences.

Autotoxic effects typically are the result of an interaction of a mixture of compounds rather than due to a single compound (28,32). The exudates from cucumber contained eleven phenolic acids such as benzoic, p-hydroxybenzoic acid, myristic, stearic acids. In the present study, seven phenolic compounds were identified by HPLC analysis

Table 3. Effects of phenolic acids on germination rate, potential and index of muskmelon seed

Treatments	Concentration ($mmol \cdot L^{-1}$)	Germination rate (%)	RI value	Germination potential (%)	RI value	Germination index	RI value
Gallic acid	0.00	86.67a	–	82.50ab	–	24.10ab	
	0.05	87.50a	0.009a	84.17a	0.020a	25.21a	0.044a
	0.1	80.83b	-0.067b	74.17b	-0.101ab	21.50b	-0.108b
	0.5	79.17b	-0.089c	70.83c	-0.141b	19.26b	-0.201c
Phthalic acid	0.00	86.67b	–	82.50ab	–	24.10b	
	0.05	91.67a	0.055a	88.33a	0.066a	27.68a	0.129a
	0.1	80.00c	-0.079b	78.33b	-0.051b	25.31b	0.048ab
	0.5	75.00d	-0.137c	66.67c	-0.192c	20.53c	-0.148b
Syringic acid	0.00	86.67b	–	82.50b	–	24.10a	
	0.05	90.00a	0.037a	87.50a	0.057a	22.54b	-0.065a
	0.1	85.83b	-0.012b	81.67b	-0.010b	20.31bc	-0.157b
	0.5	76.67c	-0.115c	68.33c	-0.171c	18.34c	-0.239c
Salicylic acid	0.00	86.67b	–	82.50b	–	24.10b	
	0.05	90.83a	0.046a	88.33a	0.066a	27.87a	0.135a
	0.1	88.33ab	0.019b	85.83ab	0.039ab	25.66b	0.061ab
	0.5	80.83c	-0.067c	76.67c	-0.071b	20.22c	-0.161b
Ferulic acid	0.00	86.67a	–	82.50a	–	24.10a	
	0.05	86.67a	0.000a	76.67b	-0.071a	19.63b	-0.185a
	0.1	72.50b	-0.163ab	50.83c	-0.384b	15.22bc	-0.368b
	0.5	70.83c	-0.182b	44.17d	-0.464c	7.75d	-0.678c
Benzoic acid	0.00	86.67ab	–	82.50a	–	24.10a	
	0.05	89.17a	0.028a	83.33a	0.010a	21.35b	-0.114a
	0.1	70.83b	-0.182b	63.33b	-0.232b	17.54c	-0.272b
	0.5	69.23c	-0.201b	40.83c	-0.505c	11.68d	-0.515c
Cinnamic acid	0.00	86.67a	–	82.50a	–	24.10a	
	0.05	76.67b	-0.115a	66.67b	-0.192a	10.77b	-0.553a
	0.1	60.83c	-0.298b	44.17c	-0.464 b	7.88c	-0.673b
	0.5	44.17d	-0.490c	12.50d	-0.848c	3.41d	-0.858c

Different small letters within each column show significant differences with the control at 0.05 levels.

of muskmelon root exudates: gallic acid, phthalic acid, syringic acid, salicylic acid, ferulic acid, benzoic acid and cinnamic acid.

The phenolic compounds found in root exudates have been reported to reduce seed germination, seedling growth and dry weight and the concentration of phenolic compounds ranging from 0.001 $mmol \cdot L^{-1}$ to 1.0 $mmol \cdot L^{-1}$ was potentially allelopathic to plant growth (10,15,25) as shown in the present study. Result reported in this study demonstrated that the seven phenolic compounds identified by HPLC inhibited seed germination and plant growth of muskmelon at the concentrations greater than 0.5 $mmol \cdot L^{-1}$ and cinnamic acid had the greatest inhibitory effect, followed by benzoic acid and ferulic acid. The results are in agreement with earlier studies reporting that plant growth of muskmelon were greatly inhibited by autotoxic substances released from powdered root tissue at a rate of 1 g per seedling (2,33,34). It also has been reported that seed germination is stimulated by low concentrations of phenolic compounds. Results of this study has similar conclusions, that gallic acid, phthalic acid, syringic acid and salicylic acid stimulates seed germination and growth of muskmelon at low conc (0.05 $mmol \cdot L^{-1}$).

Table 4. Effects of phenolic acids on plant height, shoot and root dry weight of muskmelon seedling

Treatments	Concentration ($mmol \cdot L^{-1}$)	Plant height (cm)	RI value	Shoot (g)	RI value	Root (g)	RI value
Gallic acid	0.00	38.4ab	–	2.02b	–	0.41a	–
	0.05	39.3a	0.023a	2.11a	0.043a	0.39ab	-0.049a
	0.1	36.7b	-0.044b	1.87c	-0.074ab	0.33b	-0.195b
	0.5	34.2c	-0.109c	1.82c	-0.099c	0.32b	-0.220b
Phthalic acid	0.00	38.4a	–	2.02a	–	0.41a	–
	0.05	38.6a	0.005a	2.03a	0.005a	0.37b	-0.098a
	0.1	37.5ab	-0.023b	1.97ab	-0.025b	0.35bc	-0.146b
	0.5	32.6b	-0.151c	1.76b	-0.129c	0.33d	-0.195c
Syringic acid	0.00	38.4b	–	2.02b	–	0.41b	–
	0.05	41.0a	0.063a	2.14a	0.056a	0.48a	0.146a
	0.1	39.6ab	0.030ab	2.02b	0.000ab	0.39bc	-0.049b
	0.5	33.5c	-0.128b	1.77c	-0.124b	0.36c	-0.122c
Salicylic acid	0.00	38.4b	–	2.02b	–	0.41b	–
	0.05	42.2a	0.090a	2.35a	0.140a	0.52a	0.212a
	0.1	40.4ab	0.050ab	2.01b	-0.005b	0.41b	0.000b
	0.5	35.6c	-0.073b	1.83c	-0.094c	0.38c	-0.073c
Ferulic acid	0.00	38.4a	–	2.02a	–	0.41a	–
	0.05	36.7ab	-0.044a	1.97ab	-0.025a	0.36b	-0.122a
	0.1	31.0b	-0.193b	1.77b	-0.124ab	0.36b	-0.122a
	0.5	26.3c	-0.315c	1.66c	-0.178b	0.31c	-0.244b
Benzoic acid	0.00	38.4a	–	2.02a	–	0.41a	–
	0.05	37.1ab	-0.034a	2.00a	-0.010a	0.37b	-0.098a
	0.1	29.3b	-0.237b	1.65b	-0.183ab	0.36b	-0.122ab
	0.5	24.6c	-0.359c	1.57c	-0.223b	0.30c	-0.268b
Cinnamic acid	0.00	38.4a	–	2.02a	–	0.41a	–
	0.05	33.6b	-0.125a	1.71b	-0.153a	0.32b	-0.220a
	0.1	20.2c	-0.474b	1.37c	-0.322b	0.26c	-0.366b
	0.5	18.0d	-0.531c	0.87d	-0.569c	0.21d	-0.488c

Different small letters within each column show significant difference from the control at 0.05 levels.

The composition of root exudates depends on plant species and cultivar, developmental stage, plant growth substrate, and stress factors. Besides, root exudates composition is also influenced by the rhizosphere microflora itself (16,26). Identification of the allelopathic compounds which can contribute to controlling replant disease of muskmelon is becoming urgent as well as pivotal for understanding the mechanism of autotoxicity. Further studies will focus on allelopathic effects of phenolic compounds of melon root exudates on the phytotoxicity of soil pathogens and soil enzymes activities and microbial communities.

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