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Induction of Resistance in Watermelon Plants against Fusarium Wilt using Chemical Inducers and Compost under Greenhouse Conditions

A.M.M. Mahdy*; M.H. Abd-El-Mageed*; Faten M. Abd-El-Latif*; M.M.M. Diab and Nehal M. Saied****

* *Plant Pathol. Dept., Fac. Agric., Benha Univ., Egypt.*

** *Plant Pathol. Dep., N.R.C., Egypt.*

Fusarium wilt of watermelon caused by *Fusarium oxysporum* f.sp. *niveum* is the most severe disease attacking watermelon plants. Induced resistance in watermelon plants using chitosan, salicylic acid and compost was evaluated. All tested isolates of *F. oxysporum* f.sp. *niveum* were able to attack watermelon plants causing damping-off symptoms. The most aggressive isolate was isolated from Nobariya (cv. Giza 1). It caused 71.9, 46.9 and 51.6% damping-off for cvs. Gorma, Giza1 and Aswan hybrid, respectively, 30 days after sowing. Laboratory experiment results revealed that all tested concentrations of chitosan and salicylic acid significantly reduced the disease incidence under greenhouse conditions. Moreover, animal and plant compost at all concentrations significantly reduced the watermelon wilt incidence. Plant compost at 10.0 g/kg soil, chitosan at 8.0 g/kg soil and salicylic acid at 2.0 g/kg soil were tested alone or in combination to study their effect on watermelon wilt incidence and on enzyme activities of watermelon plants. Results showed that the highest reduction in disease incidence was recorded with combined treatments between plant compost and chitosan at concentration of 8.0 g/kg soil or salicylic acid at 2.0 g/kg soil which reduced the dead plant (%) by 92.9, 88.5 and 86.6% for Aswan hybrid, cvs. Gorma and Giza1, respectively. As for enzyme activities, results revealed that the highest increase was recorded with combined treatments between plant compost and chitosan at concentration of 8 g/kg soil or salicylic acid at 2.0 g/kg soil which increased the peroxidase, chitinase and β-1,3-glucanase activities.

Keywords: β-1,3-glucanase, chitinase, chitosan, compost, Fusarium wilt, peroxidase, salicylic acid and watermelon.

Watermelon (*Citrullus lanatus* (Trunb.) Matsum and Nakai) is a widely cultivated vegetable crop that consumed globally as fruits. The fungus *Fusarium oxysporum* Schleicher: Fr. f.sp. *niveum* (E.F. Smith) W.C. Snyder and H.N. Hans is the causal of watermelon Fusarium wilt (Booth, 1971) and was found to be worldwide in tropical and subtropical regions. Watermelon wilt appears during the growing season at different stages of plant growth from seedling to mature stages and may happen earlier to cause pre-emergence damping-off (Sheng *et al.,* 2009 and Lu *et al.,* 2014). Also, Booth (1971) reported that *F. oxysporum* f.sp. *niveum* causes damping-off, stunting of watermelon seedlings and wilt of older plants.

An investigation of this disease is considered important especially in view of its wide prevalence in Egypt particularly in sandy soils where watermelon is an important crop.

Recently, fungicide alternatives are promising methods for controlling plant diseases. Induced resistance is accomplished by the inoculation of plant with an avirulent or non-pathogenic isolates prior to or concomitant with a challenge inoculation with a pathogen. That is called biotic inducers, while chemical inducers include natural or synthetic chemicals, *i.e.* ethephon, acetylsalicylic acid, salicylic acid and chitosan (Suprakash and Chatterjee, 2012; Abd-El-Kareem and Abd-El-Latif, 2012 and Abd-El-Kareem *et al.*, 2013a).

Chitosan applied as seed or soil treatments was reported to control Fusarium wilts in many plant species (Badawy *et al.*, 2005). Chitosan induces host defence responses in both monocotyledons and dicotyledons (Elwagia and Algam, 2014 and Mishra *et al*., 2014). Moreover, salicylic acid ($C_7 H_6O_3$) is an important signalling molecule involved in both locally and systemically induced disease resistance responses. The ability to accumulate salicylic acid has been shown to be essential for systemic acquired resistance and reactions to a biotic stress in plants (Morse *et al.*, 2007; Zawoznik *et al.*, 2007 and Fawzy, 2013).

On the other hand, utilization of composts to minimize organic waste pollution and to reduce the addition of chemical fertilizers and fungicides in crop production is a promising strategy for both the present and the future. Furthermore, many soil borne pathogens can be reduced by application of composts made of different raw materials (Trillas *et al.*, 2006; Abd-El-Kareem *et al.*, 2013b and Zhao *et al.*, 2014).

The present research was designed to study and evaluate the effect of chitosan, salicylic acid and compost, alone or in combination, against watermelon wilt incidence under greenhouse conditions.

Materials and Methods

Isolation, identification and Pathogenicity test:

Isolation trails were carried out from watermelon plants cvs. Gorma, Giza 1 and Aswan hybrid showing wilt symptoms collected from different locations, *i.e.* Kafr El-Shikh (Baltem & Kafr El-Shikh), Ismailiya (Abo-Soyer & Salhiya) and Behira (Nobariya & Badr City). The obtained isolates were purified and identified according to Gilman (1957) and Booth (1971). Three identified isolates of *Fusarium oxysporum* isolated from the three watermelon cultivars of each location were tested for their pathogenic ability on watermelon plants under greenhouse conditions. Experiment was carried out in Plant Pathology- greenhouse at the National Research Centre, Dokki, Egypt. Inocula of *F. oxysporum* isolates were prepared by culturing each isolate in 50 ml potato dextrose broth (PDB) medium in 250 ml Erlenmeyer flasks for 15 days at (25±2°C). Inoculum of each *F. oxysporum* isolate was prepared from the growing upper solid layers which were blended in sterilized water. Colonies forming units (cfu) were adjusted to 10^6 cfu/ml using haemocytometer slide. Soil infestation was carried out at the rate of 50 ml (10^6 cfu/ml)/kg soil (Elad

and Baker, 1985). Plastic pots (30-cm-diam., 5.0 kg soil) containing sterilized sandy -loamy soil were artificially infested individually with the inoculum of the desired isolate at the rate of 50 ml (10^6 cfu/ml)/kg soil (Elad and Baker, 1985). Eight pots were used as replicates for each isolate as well as check treatment (uninfested soil). Disinfected watermelon seeds of cvs. Giza1, Gorma and Aswan hybrid were sown at the rate of 8 seeds/pot. Disease incidence was estimated as percentage of dead plants 15 and 30 days after sowing.

Assessment of dead plants:

Fusarium damping-off and/or Fusarium wilt were measured as percentage of dead plants 15 and 30 days after sowing (Booth, 1971) as follow:

$$\text{Dead plants (\%)} = \frac{\text{Number of dead plants}}{\text{Total number of planted seeds}} \times 100$$

Host range of F. oxysporum isolated from watermelon:

The highest aggressive isolate of *F. oxysporum* causing watermelon wilt incidence was chosen for testing its ability to induce wilt on various plant species belonging to different families. This experiment was carried out under greenhouse conditions. Ten plant species belonging to families *Cucurbitaceae, Solanaceae* and *Leguminoceae* were tested. Three watermelon cultivars, *i.e.* Giza 1, Gorma and Aswan hybrid, in addition to cucumber (*Cucumis sativus* L.) cv. Beit Alpha, Muskmelon (*Cucumis melo* L.) cv. Honeydew, Squash (*Cucurbita pepo* L.) cv. Eskandarany, tomato (*Solanum lycopersicum* L.) cv. Castel Rock, pepper (*Piper nigrum* L.) cv. California, bean (*Phaseolus vulgaris* L.) cv. Giza 3 and pea (*Pisum sativum* L.) cv. Master were tested. Inoculum preparation, soil infestation and assessment of disease incidence were carried out 18, 30 and 45 days after sowing as mentioned before.

In vitro evaluation of the inhibitory effect of chitosan and salicylic acid on the linear growth and spore germination of F. oxysporum f.sp. niveum:

The inhibitory effect of chitosan and salicylic acid (Sigma Company) on the linear growth and spore germination of the *F. oxysporum* f.sp. *niveum* was evaluated under laboratory conditions. Five concentrations of chitosan solutions, *i.e.* 0, 2, 4, 6 and 8 g/l and five concentrations of salicylic acid, *i.e.* 0, 0.5, 1.0, 1.5 and 2.0 g/l (these concentrations are equivalent to be as 0.0, 3.6, 7.2, 10.9 and 14.5 mM) were tested. As for linear growth test, chitosan and salicylic acid solutions were added individually to conical flasks containing sterilized PDA medium before its solidification and mixed gently then transferred in sterilized Petri plates (9-cm-diam.). Plates were individually inoculated with equal disks (6-mm-diam.) taken from 7 days old cultures of *F. oxysporum* f.sp. *niveum*, then incubated at 25±2°C. Linear growth of the fungus was measured, when the control plates reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 plates as replicates. As for spore germination test, spore suspension was prepared by culturing *F. oxysporum* f. sp. *niveum* in Petri plates containing PDA medium for 20 days at 25±2°C. Colony forming units (cfu) containing hyphal fragments, microconidia, macroconidia and chlamydospores were released in

sterilized water using a needle then adjusted to 10^6 cfu/ml using haemocytometer slide. One ml of the suspension was transferred to test tube containing sterilized PDB (broth) medium treated with previous concentrations of chitosan or salicylic acid. Test tubes were incubated for 24 h at 25±2°C. One ml of treated spore suspension (cfu) was examined microscopically and the percent of spore germination was calculated.

Disease control experiments:

The efficacy of the tested chemicals and compost used for controlling damping-off incidence of watermelon was carried out in pot experiment.

Watermelon seeds:

Watermelon seeds (cv. Giza1) were obtained from the Dept. of Vegetable Crop Res., Agric. Res. Centre, Giza. While, Aswan hybrid was obtained from Sakata Company, Japan and cv. Gorma from commercial markets in Egypt.

Evaluation of chitosan and salicylic acid on watermelon damping-off incidence:
Seed treatment with chitosan or salicylic acid:

Watermelon seeds cvs. Giza1, Gorma and Aswan hybrid were soaked individually for 24 hours in each solution of chitosan at concentrations of 0, 2, 4, 6 and 8 g/l or salicylic acid solutions at concentrations of 0.0, 0.5, 1.0, 1.5 and 2.0 g/l then kept between two heavy layers of cottony cheesecloth saturated with tap water for 24 h at 30°C until the beginning of germination. Seeds soaked in tap water served as control.

Soil treatment with chitosan or salicylic acid:

Artificially potted infested soil was treated by chitosan at the rate 0.0, 2.0, 4.0, 6.0 and 8.0 g/kg of soil**,** while**,** salicylic acid was used at the rate of 0.0, 0.5, 1.0, 1.5 and 2.0 g/kg soil.

Effect of soil amendment with compost for controlling Fusarium damping-off:

This study was carried-out in pots contained artificially infested soil with *F. oxysporum niveum***.** Two types of composts (obtained from El-Nile Company, Giza, Egypt), *i.e.* animal and plant composts at concentrations of 0.0, 5.0 and 10.0 g/kg soil were used as soil drench 15 days before sowing to evaluate their effects on Fusarium damping-off incidence of watermelon. Disinfected watermelon seeds were sown at the rate of 8 seeds/pot and 8 pots were used for every treatment. Disease incidence was assessed as mentioned before.

Efficacy of chitosan or salicylic acid alone or in combination with compost on Fusarium wilt incidence of watermelon transplants under greenhouse conditions:

Chitosan at 8 g/kg soil or salicylic acid at 2 g/kg were applied as seed bed (treated peat-moss in trays 84 eyes before seed sowing). Disinfected watermelon seeds, cvs. Giza 1, Gorma and Aswan hybrid were sown in treated seed bed. Latter, watermelon transplants (25-day-old) were transplanted in plastic pots (30-cm- diam.) containing infested soil with the pathogenic fungus and treated with plant compost at the rate of 10.0 g/kg soil. Transplants were planted at the rate of 4 transplants/pot and 8 replicates were used.

Disease assessment:

Wilted plants were recorded 30 days after transplanting and disease incidence (%) was calculated using the following disease index scale: (1) apparently healthy plants; (2) slight chlorosis of lower; slight wilt of plants; (3) necrosis, following of lower leaves, yellow areas on upper leaves; (4) dead plants (Tziros *et al.,* 2007).

Determination of enzymes activities:

Watermelon plants cvs. Giza1, Gorma and Aswan hybrid (20-day after transplanting) representing the different treatments grown under greenhouse conditions were used to determine the activity of some enzymes related to plant resistance, *i.e.* peroxidase, chitinase and *β*-1,3-glucanase.

Extraction of enzymes:

Plant roots (g) were homogenized with 0.1 M sodium phosphate buffer (pH 7.1) (Goldschmidt *et al.,* 1968) at the rate of 1/3 w/v. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was used to determine enzyme activities.

Peroxidase assay:

Peroxidase activity was measured by incubation 0.1 ml of enzyme extract with 4ml of guaiacol solution for one minute at 25°C and absorbance at 470 nm was determined. The guaiacol solution consisted of 3 ml of 0.05 M K. phosphate, pH 7, 0.5 ml of 2% guaiacol and 0.5 ml of 0.3% H_2O_2 (Abeles *et al.,* 1971). Peroxidase activity was expressed as the increase in absorbance at 470 nm/g fresh weight/1 min.

Chitinase assay:

The substrate colloidal chitin was prepared from chitin powder according to the method described by Ried and Ogryd-Ziak (1981). Determination the chitinase activity was carried out according to the method of Monreal and Reese (1969), 1 ml of 1% colloidal chitin in 0.05 M citrate phosphate buffer (pH 6.6) in test tubes, 1 ml of enzyme extract was added and mixed by shaking. Tubes were kept in a water bath at 37°C for 60 min, then cooled and centrifuged before assaying. Reducing sugar was determined in 1 ml of the supernatant by dinitrosalicylic acid (DNS)**.** The reaction was stopped by heating the tubes for 5 min at 100°C. The tubes were cooled and 3 ml distilled water were added before assay. Optical density was determined at 540 nm. Chitinase activity was expressed as mM N-acetylglucosamine equivalent released/g fresh weight tissue/60 min.

β-1,3-glucanase assay:

The method of Abeles and Forrence (1970) was used to determine *β*-1,3-glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars.The method was carried out as 0.5 ml of enzyme extract was added to 0.5 ml of 0.05 M of potassium acetate buffer (pH 5) containing 2% laminarin. The mixture was incubated at 40°C for 60 min. The reaction was stopped by adding 1 ml of dinitrosalicylic acid reagent and heating the tubes for 5 min at 100°C. The tubes were cooled and 3 ml distilled water were added before assay. The optical density was read at 500 nm. *β*-1,3-glucanase activity was expressed as mM glucose equivalent released/g fresh weight tissues/60 min.

Statistical analysis:

Tukey test for multiple comparisons among means was used (Neler *et al.,* 1985).

Results

Isolation, identification and pathogenicity test:

Isolation trails resulted 54 different fungal isolates. Obtained isolates were screened microscopically for genus *Fusarium*. Eighteen isolates were found to be *F. oxysporum* according to Gilman (1957) and (Booth 1971). Thereafter, three identified isolates of *F. oxysporum* isolated from the three watermelon cultivars of each location were tested for their pathogenic ability to induce damping-off and/or wilt diseases on watermelon plants under greenhouse conditions. Results in Table (1) indicate that all tested isolates of *F. oxysporum* were able to attack watermelon plants causing damping-off and/or wilt symptoms (expressed as dead plants, %).

Table 1. Pathogenic ability of *F. oxysporum* isolates to induce damping-off of watermelon plants 15 and 30 days after sowing

Governorate	Isolate	Dead plants (%)					
		Watermelon cultivar					
		Gorma		Giza 1		Aswan hybrid	
		Days after sowing					
		15	30	15	30	15	30
Behira	Nobariya Aswan	18.5 b*	43.8 cd	10.9 d	32.8 c	10.9 c	32.8
	Nobariya Giza 1	28.1 a	71.9 a	18.5 a	46.9 a	20.3 a	51.6 a
	Nobariya Gorma	17.2 b	45.3 cd	15.6 bc	32.8 c	14.1 b	32.8 c
	Bader Aswan	21.9 b	60.9 b	14.1 bc	42.2 b	12.5 b	40.6 b
	Bader Giza 1	17.2b	43.8 cd	14.1 bc	29.7 c	14.1b	32.8 cd
	Bader Gorma	21.9 b	43.8 cd	12.5cd	32.8 c	12.5 b	32.8 cd
Ismailiya	Abo-Soyer Aswan	17.2b	39.1e	15.6 bc	34.4 c	12.5 bc	31.3 cd
	Abo-Soyer Giza 1	14.1 c	31.3 f	10.9d	32.8 c	12.5 b	32.8 c
	Abo-Soyer Gorma	17.2 b	39.1 e	12.5 cd	32. 8 c	14.1 b	32.8 c
	Salhiya Aswan	9.4 d	31.3f	10.9d	14.1 e	10.9 c	18.8 f
	Salhiya Giza 1	12.5 d	40.6 de	10.9d	31.3 c	12.5 bc	23.4 f
	Salhiya Gorma	12.5 d	39.1 e	12.5 cd	23.4c	14.1 b	32.8 c
Kafr El-Sheikh	Baltem Aswan	17.2 b	42.2 de	12.5 cd	31.3 c	14.1 b	31.3 cd
	Baltem Giza 1	9.4 ed	31.3 f	9.4 d	23.4 d	14.1b	42.2 b
	Baltem Gorma	14.1 c	39.1 e	9.4 d	31.3 c	12.5 bc	32.8 c
	Kafr El-Sheikh Aswan	21.9 b	53.1 c	15.6 d	32.8 c	14.1b	28.1 d
	Kafr El-Sheikh Giza 1	21.9 b	39.1 f	12.5c	23.4 d	10.9 c	21.9 f
	Kafr El-Sheikh Gorma	17.2 b	39.1 e	12.5 c	32.8 c	12.5 bc	21.9 f
Control		3.1 f	4.7 g	3.1 e	3.1 f	3.1	3.1 e

***** Figures with the same letter are not significantly different (P= 0.05).

The tested isolates significantly varied in their ability to cause damping-off infection of watermelon. The most aggressive isolate was isolated from Nobariya (cv. Giza 1) which caused damping-off 28.1, 18.5 and 20.3% after 15 days from sowing for cvs. Gorma, Giza1 and Aswan hybrid, respectively. Moreover, dead plant percentages reached 71.9, 46.9 and 51.6% for cvs. Gorma, Giza1 and Aswan hybrid, respectively, 30 days after sowing. Other isolates caused infections reached 9.4-21.9 to 18.2-61.0%, 15 and 30 days after sowing, respectively. The highest infection was recorded in case of cv. Gorma followed by the other two tested watermelon cultivars. The most aggressive isolate (Nobariya - Giza 1) was chosen to use in further studies.

Host range of F. oxysporum isolates:

Host range of *F. oxysporum* isolates was evaluated under greenhouse conditions on different hosts as mentioned before. Results in Table (2) show that among the ten tested plant species, only watermelon cultivars were susceptible to infection with the tested *F. oxysporum* isolates. Cultivar Gorma was the most susceptible cultivar; when percentages of damping-off and wilted plants recorded 26.7, 73.4 and 84.4%, 15, 30 and 45 days, respectively, after sowing. Meanwhile, the tested cultivars of cucumber, muskmelon, squash, tomato, pepper, bean and pea plants were not affected by the tested fungal isolate and no disease symptoms were observed.

Table 2. Host range of *F. oxysporum* on different plant species

Tested plant species	Dead plants (%)		
	Days after sowing		
	Damping-off		Wilt
	15	30	45
Watermelon (Giza 1)	18.8 b*	60.9 b	73.4 b
Watermelon (Gorma)	26.7 a	73.4 a	84.4 a
Watermelon (Aswan)	17.2 b	62.5 b	73.4 b
Cucumber (Beit Alpha)	0.0 c	0.0 c	0.0 c
Muskmelon (Honeydew)	0.0 c	0.0 c	0.0 c
Squash (Eskandarany)	0.0 c	0.0 c	0.0 c
Tomato (Castel Rock)	0.0 c	0.0 c	0.0 c
Pepper (California)	0.0 c	0.0 c	0.0 c
Bean (Giza 3)	0.0 c	0.0 c	0.0 c
Pea (Master)	0.0 c	0.0 c	0.0 c

* Figures with the same letter are not significantly different (P= 0.05).

In vitro evaluation of the inhibitory effect of chitosan and salicylic acid on the linear growth and spore germination of F. oxysporum f.sp. niveum:

The inhibitory effect of chitosan and salicylic acid on the linear growth and spore germination of *F. oxysporum* f.sp. *niveum* was evaluated under laboratory conditions. Chitosan and salicylic acid were tested at five concentrations as mentioned before. Results in Table (3) indicate that all tested concentrations of either chitosan or salicylic acid significantly reduced the linear growth and spore germination of *F. oxysporum* f.sp. *niveum*. Complete inhibition in linear growth was obtained with chitosan at the concentration of 8.0 g/l and salicylic acid at 2.0 g/l. As for spore germination, complete inhibition was obtained with chitosan at concentration of 6.0 g/l and salicylic acid at concentration 1.5 g/l .

Effect of different concentrations of chitosan applied as seed or soil treatment on watermelon damping-off and wilt diseases under greenhouse conditions:

Five concentrations of chitosan were applied, either as seed or soil treatment, to study their effect against Fusarium wilt incidence under greenhouse conditions.

Table 3. *In vitro* evaluation of the inhibitory effect of chitosan and salicylic acid on *F. oxysporum* f.sp. *niveum* linear growth and spore germination

Treatment	Concentration (g/l)	Linear growth (mm)	Reduction (%)	Spore germination (%)	Reduction (%)
Chitosan	2.0	52.5 b*	41.7	35.0 b	59.8
	4.0	27.0 c	70.0	14.0 c	83.9
	6.0	6.0 d	93.3	0.0 d	100.0
	8.0	0.0 d	100.0	0.0 d	100.0
Salicylic acid	0.5	64.0 b	28.9	42.0 b	51.7
	1.0	42.0 c	53.3	19.0 c	78.2
	1.5	14.5 d	83.9	0.0 d	100.0
	2.0	0.0 e	100.0	0.0 d	100.0
Control	0.0	90.0 a	----	87.0 a	----

* Figures with the same letter are not significantly different (P= 0.05).

Results in Table (4) show that all tested concentrations of chitosan applied either as seed or soil treatment significantly reduced the disease incidence 15 and 30 days after sowing. Soil treatment with chitosan was more efficacy than seed treatment. The highest reduction in disease incidence was obtained when the soil was treated with chitosan at 6.0 and 8.0 g/kg soil which reduced the percentage of diseased plants by 60.3, 61.9 and 61.5%, respectively, for all the tested cultivars, *i.e.* cvs. Giza 1, Gorma and Aswan hybrid, respectively, when the soil was treated by 6.0 g/kg soil. Seed treatments with chitosan at concentrations of 6.0 and 8.0 g/kg soil resulted in reducing the disease incidence. Meanwhile, other concentrations were less effective

Table 4. Percentage of watermelon damping-off and/or wilt (dead plants %) as affected by different concentrations of chitosan applied as seed or soil treatments

Chitosan (g/kg)	Dead plants (%) of watermelon cultivar								
	Giza 1			Aswan hybrid			Gorma		
	Days after sowing								
	15	30	R.%**	15	30	R.%	15	30	R.%
Seed treatment (g/l):									
2.0	18.8 b*	50.0 b	25.6	15.6 b	48.4 b	26.2	18.8 b	53.1 b	34.7
4.0	18.8 b	48.4 b	28.0	15.6 b	45.3 bc	30.9	17.2 bc	53.1 b	34.7
6.0	10.9 e	32.8 d	51.2	10.9 d	34.4 d	47.6	12.5 d	35.9 d	55.8
8.0	10.9 e	32.8 d		10.9 d	32.8 d		12.5d	35.9 d	55.8
Soil treatment (g/kg soil):									
2.0	14.1 cd	42.2 c	37.2	12.5 c	40.6 cd	38.1	17.2 bc	45.3 bc	44.3
4.0	12.5 d	37.5 c	44.2	12.5 c	35.9 d	45.3	15.6 c	40.6 c	50.1
6.0	9.4 e	26.7 de	60.3	7.8 e	25.0 e	61.9	12.5 d	31.3 e	61.5
8.0	9.4 e	23.4 e	65.2	7.8 e	25.0 e	61.9	12.5 d	28.1 e	65.4
Control 1	3.1 f	4.7 f	----	0.0 f	3.1f	----	3.1 e	3.1 f	----
Control 2	23.4 a	67.2 a	----	20.3 a	65.6 b	----	31.3 a	81.3 a	----

* Figures with the same letter are not significantly different (P= 0.05).
** Reduction (%) was recorded 30 days after sowing.
- Control 1: Non- infested soil and Control 2: Infested soil.

Effect of different concentrations of salicylic acid applied as seed or soil treatment on the incidence of watermelon wilt and damping-off (dead plants, %):

Salicylic acid solutions at various concentrations were applied either as seed or soil treatment to study their effect on Fusarium incidence of watermelon plants. Results in Table (5) indicate that all concentrations of salicylic acid applied either as seed or soil treatment significantly reduced the disease incidence. Soil treatment with salicylic acid was more efficient than seed treatment. The highest reduction was obtained when the soil was treated with salicylic acid at 1.5 and 2.0 g/kg soil which reduced the disease incidence by 68.3, 66.6 and 70.8 % for cv. Giza 1, Aswan hybrid and cv. Gorma, respectively. Meanwhile, soil treatment with concentrations of 0.5 and 1.0 g/kg soil and seed treatments using 1.5 and 2.0 g/l showed moderate effect.

Table 5. Watermelon damping-off and/or wilt diseases (dead plants, % as affected by different concentrations of salicylic acid applied as seed or soil treatments

Salicylic acid	Dead plants (%) of watermelon cultivar								
	Giza 1			Aswan hybrid			Gorma		
	Days after sowing								
	15	30	R.%**	15	30	R.%	15	30	R.%
Seed treatment (g/l):									
0.5	21.9 b*	39.1b	39.0	18.8 a	35.9 b	45.3	23.4 b	37.5 b	50.0
1.0	18.8 b	35.9 b	44.0	18.8 a	32.8 b	50.0	20.3 c	35.9 b	52.1
1.5	17.2 bc	28.1 c	56.2	15.5 b	26.7 c	59.3	17.2 d	29.7c	60.4
2.0	14.1 d	25.0 cd	61.0	15.5 b	26.7 c	59.3	15.6 e	29.7 c	60.4
Soil treatment (g/kg soil):									
0.5	14.1d	26.7 c	58.3	15.5 b	26.7 c	59.3	17.2 d	35.9 b	52.1
1.0	12.5 de	28.1 c	56.2	14.5 b	26.7 c	59.3	17.2 d	32.8 b	56.3
1.5	10.9 e	23.4 d	63.5	9.4 c	21.9 d	66.6	14.1 ef	25.0 c	66.7
2.0	9.4 e	20.3 d	68.3	9.4 c	21.9 d	66.6	12.5 f	21.9 c	70.8
Control 1	3.1 f	4.7 e	----	0.0 d	3.1 e	----	3.1 g	3.1 d	----
Control 2	21.9 a	64.1 a	----	20.3 a	65.6 a	----	26.7 a	75.0 a	----

* Figures with the same letter are not significantly different (P= 0.05).
** Reduction (%) was recorded 30 days after sowing.
- Control 1: Non- infested soil and Control 2: Infested soil.

Effect of soil amendment with compost for controlling Fusarium wilt of watermelon plants:

This study was carried-out in pots contained artificially infested soil with *F. oxysporum* f.sp. *niveum***.** Two types, *i.e.* animal and plant composts at doses of 5.0 and 10.0 g/kg soil were tested to evaluate their effect on watermelon damping-off and/or wilt (as dead plants %). Results in Table (6) indicate that both types of compost at both the tested doses significantly reduced the disease incidence. The most effective treatment is plant compost at concentration of 10.0 g/kg soil which reduced the dead plants more than 67.4, 68.3 and 71.2% for cv. Giza 1, Aswan hybrid and cv. Gorma, respectively, 30 days after sowing. Meanwhile, other treatments showed moderate effect.

Table 6. Watermelon damping-off and/or wilt diseases (as dead plants %) in response to two compost types

Compost type		Dead plants (%) of watermelon cultivar								
		Giza 1			Aswan hybrid			Gorma		
		Days after sowing								
		15	30	R.%**	15	30	R.%	15	30	R.%
Plant	5.0	10.9 c*	31.3 b	53.4	10.9 bc	25.0 b	61.0	14.1 b	28.1b	65.4
	10.0	7.8 d	21.9 c	67.4	9.4 c	20.3 c	68.3	9.4 c	23.4c	71.2
Animal	5.0	14.1 b	32.8 b	51.2	12.5 b	29.7 b	53.7	15.6 b	32.8b	59.7
	10.0	12.5 bc	28.1 b	58.2	12.5 b	25.0 b	61.0	14.1 b	28.1b	65.4
Control 1***		3.1 e	4.7 d	----	0.0 d	3.1d	----	3.1 d	3.1d	----
Control 2****		23.4 a	67.2 a	----	20.3 a	64.1a	----	31.3 a	81.3a	----

* Figures with the same letter are not significantly different (P= 0.05).
** Reduction (%) was recorded 30 days after sowing.
*** Control 1: Non- infested soil.
**** Control 2: Infested soil.

Efficacy of compost applied as soil amendment and the incidence of chitosan or salicylic acid as seed bed treatments alone or in combination on Fusarium wilt of watermelon:

Plant compost at 10.0 g/kg soil and chitosan at 8.0 g/kg soil or salicylic acid at 2.0 g/kg soil were tested alone or in combination to study their effect on Fusarium wilt incidence and enzyme activities of watermelon plants.

Results in Table (7) show that plant compost, chitosan at 8.0 g/kg soil or salicylic acid at 2.0 g/kg soil applied either alone or in combination significantly reduced the disease incidence with all tested cultivars. The highest reduction was obtained by using the combined treatments between plant compost and chitosan at concentration of 8.0 g/kg soil or salicylic acid at 2.0 g/kg soil which reduced the disease incidence by 92.9, 88.5 and 86.6 % for Aswan hybrid, cvs. Gorma and Giza1, respectively. Meanwhile, single treatments showed moderate effect.

Determination of enzymes activity in watermelon transplants as affected by compost applied as soil amendment and chitosan or salicylic acid as seed bed treatments alone or in combinations:

The effect of plant compost at 10g/kg soil and chitosan at 8 g/kg soil or salicylic acid at 2 g/kg soil applied either alone or in combination on peroxidase, chitinase and β-1,3-glucanase activity was determined. Results in Table (8) reveal that all treatments increased the enzymes activities within all tested cultivars when they were applied each alone or in combination. The highest increase was obtained with combined treatments between plant compost and chitosan at concentration 8 g/kg soil or salicylic acid at 2 g/kg soil which increased the peroxidase, chitinase and β-1,3-glucanase activities, for all tested cultivars. Single treatments showed moderate effect. Meanwhile, plant compost was less effective.

Table 7. Damping-off and Fusarium wilt incidence (dead plants, %) of watermelon as affected by compost applied as soil amendment and chitosan or salicylic acid as seed bed treatments alone or in combinations

Treatment	Dead plants (%)					
	cv. Giza 1		Aswan hybrid		cv. Gorma	
	Disease incidence	R.%	Disease incidence	R. %	Disease incidence	R. %
Single treatment:						
Chitosan (8g/kg soil)	12.5 c*	73.3	12.5 c	71.5	21.9 b	60.0
SA (2g/kg soil)	12.5 c	73.3	14.1 c	67.8	21.9 b	60.0
Plant compost (10 g/kg soil)	21.9 b	53.3	21.9 b	50.0	25.0 b	54.3
Combined treatment:						
Compost + Chitosan (8g/kg soil)	6.3 d	86.6	3.1 d	92.9	6.3 de	88.5
Compost + SA (2g/kg soil)	6.3 d	86.6	3.1 d	92.9	6.3 de	88.5
Control 1**	3.1 d	----	3.1 d	----	3.1 e	----
Control 2***	46.9 a	----	43.8 a	----	54.7 a	----

* Figures with the same letter are not significantly different (P= 0.05).
** Control 1: Non- infested soil.
*** Control 2: Infested soil.

Table 8. Enzyme activities in watermelon plants as affected by compost applied as soil amendment and chitosan or salicylic acid as seed bed alone or in combination

Treatment	Increase in enzyme activities (%)								
	cv. Giza 1			Aswan hybrid			cv. Gorma		
	Po*	Ch**	β-1,3-g***	Po	Ch	β-1,3-g	Po	Ch	β-1,3-g
Single treatment									
Chitosan (8g/kg soil)	180.0	172.0	175.0	163.0	200.0	182.0	140.0	160.0	162.0
SA (2g/kg soil)	180.4	182.0	200.0	195.0	211.0	210.0	154.0	185.0	172.0
Plant compost (10 g/kg soil)	130.0	130.0	154.0	150.0	142.0	134.0	120.0	131.0	132.0
Combined treatment									
Compost + Chitosan (8g/kg soil)	192.0	222.0	214.0	194.0	210.0	220.0	175.0	200.0	201.0
Compost + SA (2 g/kg soil)	194.0	224.0	231.0	210.0	220.0	231.0	178.0	210.0	212.0
Control	5.8	1.0	2.5	6.0	1.2	2.8	4.0	0.8	1.4

* Peroxidase (Po) activity expressed as change in absorbance at 470 nm/g fresh weight/ 1 min.
** Chitinase (Ch) activity expressed as mM N-acetyle glucose amine equivalent released/g fresh weight/60 min.
*** β-1,3-glucanase (β-1,3-g) activity expressed as mM glucose equivalent released/g fresh weight/60 min.

Discussion

Watermelon is a widely cultivated vegetable. Fusarium wilt caused by *Fusarium oxysporum* f.sp. *niveum* is found to be a worldwide soil borne in temperate, subtropical and tropical regions (Sheng *et al.,* 2009).

In the present study, results indicated that all tested isolates of *F. oxysporum* were able to attack watermelon plants causing damping-off and wilt diseases. Also, results showed that among all the tested plant species, only watermelon cultivars were susceptible to infect with the tested *F. oxysporum* isolates. Therefore, it could be concluded that the tested fungal isolates are *F. oxysporum* f.sp. *niveum* (E.F. Smith) W.C. Snyder and H.N. Hans (Booth, 1971; Sheng *et al*., 2009 and Lu *et al*., 2014). Also, results indicated that Nobariya (cv. Giza 1) was the most aggressive isolate on tested watermelon cultivars. In this respect, Nguyen (2013) reported that Fusarium wilt, caused by *F. oxysporum* f.sp. *niveum*, is one of the most severe diseases on watermelon.

Chitosan exhibits a variety of antimicrobial activities against plant pathogens (Benhamou, 2004; Badawy *et al*., 2005; Abd-El-Kareem *et al.,* 2006 and El-Mohamedy *et al.,* 2013). Results of the present study showed that all tested concentrations of chitosan significantly reduced the linear growth of *F. oxysporum* f.sp. *niveum*. Complete inhibition for linear growth and spore germination was recorded when chitosan was applied at concentrations of 6.0 and 8.0 g/l, respectively. In this respect, Kulikov *et al.* (2006) reported that the antimicrobial activity of chitosan increases with the increase in chitosan molecular weight and seems to be faster on fungi and algae than on bacteria. Fungicidal activity of chitosan has been documented against various species of fungi and Oomycetes (Vasyukova *et al.,* 2000 and Rabea *et al.,* 2005). Some of the derivatives also suppressed spore formation at rather high concentrations (Badawy *et al*., 2005). Recently, Palma-Guerrero *et al.* (2009) demonstrated that chitosan is able to permeabilize the plasma membrane of *Neurospora crassa* and kills the cells. In general, chitosan is able to reduce the *in vitro* growth of a number of fungi and Oomycetes (Palma-Guerrero *et al.* 2008). For instance, chitosan was reported to exert an inhibitory action on the hyphal growth of numerous pathogenic fungi, including root and wilt diseases. Chitosan applied as seed or soil treatments was able to control Fusarium wilts in many plant species (Badawy *et al*., 2005). In the present study, results indicated that all tested concentrations of chitosan significantly reduced the disease incidence. The highest reduction in disease incidence on all tested cultivars was recorded when soil was treated with chitosan at concentrations of 6.0 and 8.0 g/kg soil.

Salicylic acid is an important signalling molecule involved in both locally and systemically induced disease resistance responses. The ability to accumulate salicylic acid has been shown to be essential for systemic acquired resistance and reactions to a biotic stress in plants (Morse *et al.,* 2007; Zawoznik *et al.,* 2007; Abdel-Kader *et al*., 2012 and Fawzy, 2013). In the present study, the inhibitory effect of salicylic acid on the linear growth and spore germination of *F. oxysporum* f.sp. *niveum* was evaluated under laboratory conditions. All tested concentrations significantly reduced the linear growth of the fungus. Complete inhibition was

recorded using salicylic acid at concentrations of 1.5 and 2.0 g/l for linear growth and spore germination, respectively. In this respect, Ozgonen *et al.* (2001) found that salicylic acid (SA) completely inhibited the mycelial development of *F. oxysporum* f.sp. *lycopersici* at concentrations ranged from 0.6 mM to 1.0 mM and increased dry weight of plant, length of shoot and root growth of tomato plants. Moreover, Hemeda (2009) reported that complete inhibition of linear growth of *Alternaria alternata*, *Fusarium oxysporum* f.sp. *phaseoli*, *F. solani* f.sp. *phaseoli*, *Macrophomina phaseolina* and *Rhizoctonia solani* was obtained with benzoic, salicylic and sorbic acids at 10 mM.

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). In the present study, results revealed that under greenhouse conditions, all concentrations of salicylic acid significantly reduced the disease incidence when applied as seed or soil treatments. In this respect, Suprakash and Chatterjee (2012) investigated the effect of soil application of salicylic acid (SA) and *Trichoderma harzianum* (TH) on the induction of phenolic accumulation content and defence enzymes in tomato plants infected with *F. oxysporum* f.sp. *lycopersici*. Tomato plants treated with SA showed significant decrease in wilt incidence and increase in the activities of both peroxidase and polyphenoloxidase enzymes.

Many soil borne pathogens can be reduced by application of composts made of different raw materials (Cotxarrera *et al*., 2002) and mature composts can sustain biological control agents (Litterick *et al.*, 2004 and Zhao *et al.*, 2014). In the present study, results indicated that two types of compost, *i.e.* animal and plant at doses of 5.0 and 10.0 g/kg soil significantly reduced the disease incidence under greenhouse conditions. The most effective treatment is plant compost at 10.0 g/kg soil which reduced the disease incidence by 67.2% for all tested cultivars. Also, plant compost at 10.0 g/kg soil and chitosan at 8.0 g/kg soil or salicylic acid at 2.0 g/kg soil were tested, alone or in combination, to evaluate their effect on Fusarium wilt and damping –off incidence and enzyme activities in watermelon plants. Results showed that the highest reduction was obtained with combined treatments of plant compost at 10 g/kg soil and chitosan at 8.0 g/kg soil or salicylic acid at 2.0 g/kg soil which reduced the wilt incidence by 92.9, 88.5 and 86.6% for Aswan hybrid, cvs. Gorma and Giza1, respectively. Meanwhile, single treatments showed moderate effect. As for enzyme activities, the highest increase was obtained with combined treatments between plant compost at concentration of 10 g/kg soil and chitosan at 8.0 g / kg soil or salicylic acid at 2.0 g/ kg soil which increased the peroxidase, chitinase and β-1,3-glucanase activities by 175.0, 200.0 and 201.0%, respectively, for all tested cultivars. Single treatments showed moderate effect.

Chitosan and salicylic acid had different properties, *i.e.* inhibitory effect against the pathogenic fungus and its ability to be potent elicitors of plant defence resistance. In the present study, chitosan and salicylic acid have two properties, *i.e.* antifungal activity and inducing resistance against *F. oxysporum*.

In the present study, chitosan was used to enhance watermelon plant defences. There are different mechanisms for reducing Fusarium wilt on susceptible plants.

Many researchers reported that chitosan induced host defence responses in both monocotyledons and dicotyledons (Elwagia and Algam, 2014 and Mishra *et al*., 2014). These responses include lignification, cytoplasmic acidification, membrane depolarization and protein phosphorylation, chitinase and glucanase activation, phytoalexin biosynthesis, generation of reactive oxygen species (Kuchitsu *et al.*, 1995), biosynthesis of jasmonic acid (Nojiri *et al.*, 1996) and the expression of unique early responsive and defence-related genes (Takai *et al*., 2001). In addition, chitosan was reported to induce callose formation and proteinase inhibitors (Conrath *et al*., 1989).

Moreover, it was found that salicylic acid plays its role in inducing resistance by increase the activity of chitinase, β-1,3-glucanase, peroxidase, polyphenoloxidase and phenylalanine amonia lyase. The increase of enzymes activity was correlated with increased formation of papillae in epidermal cells (Schneider and Ullrich, 1994). 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 (Fernandes *et al*., 2006 and Chen *et al.*, 2006). Furthermore, as for compost treatments Hoitink and Boehm (1999) have postulated the following biological mechanisms of disease control with composts: parasitism against pathogens by beneficial microorganisms, antibiotic production by beneficial microorganisms, competition for nutrients by beneficial microorganisms, activation of disease-resistance genes in plants by microorganisms and improved plant nutrition and vigour, leading to enhance disease resistance. The latter two modes of action have been used to explain instances where disease control resulting from compost amendment of soil was not accompanied by a corresponding reduction in pathogen inoculum. Also, several studies under controlled conditions have demonstrated a suppressive effect of composts on soil borne diseases such as damping-off, root rot, and wilt (Hoitink and Boehm, 1999).

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المقاومة المستحثة في نباتات البطيخ ضد مرض الذبول الفيوزاريومي باستخدام المستحثات الكيماوية والكمبوست تحت ظروف الصوبة

عبده مهدي محمد مهدي* ، محمد هارون عبد المجيد* ،
فاتن محمود عبد اللطيف* ،محمد محمد دياب** ،
نهال محمد سيد**
* قسم أمراض النبات - كلية الزراعة - جامعة بنها - مصر.
** قسم أمراض النبات - المركز القومي للبحوث - مصر.

أحدثت كل العزلات المختبرة من الفطر *Fusarium oxysporum* أعراض الذبول وموت البادرات (بقعوط البادرات) علي نباتات البطيخ وكانت اقوي عزلة هي النوبارية (معزولة من صنف البطيخ جيزة ١) حيث أدت الي نسبة اصابة بمقدار ٥١.٠٦ و ٧١.٩ و ٤٦.٩% بالنسبة لاصناف البطيخ هجين أسوان وجورما و جيزة ١ علي الترتيب.

لم تسبب عزلة الفطر *F. oxysporum* أي اصابة علي أي أنواع أو أصناف نباتية أخري من الفصيلة القرعية او الفصيلة الباذنجانية أو البقولية الا أصناف نباتات البطيخ فقط مما يدل علي أنها عزلة من الفطر *F. oxysporum* f.sp. *niveum*. أدت كل تركيزات الكيتوزان وحامض الساليسيلك و الكمبوست النباتي والحيواني الي انخفاض نسبة النباتات الميتة في نباتات البطيخ .

تم دراسة تكامل المعاملات بين معاملة التربة في الاصص المعداه بالفطر الممرض بالكمبوست النباتي بتركيز ١٠ جم/كجم تربة و معاملة مهد البذرة لشتلات البطيخ بالكيتوزان بتركيز ٨ جم/كجم تربة وحامض الساليسيلك بتركيز ٢ جم/كجم تربة علي النسبة المئوية لنباتات البطيخ الميته وكذلك نشاط الانزيمات المسئولة عن المقاومة في نباتات البطيخ وأوضحت النتائج ما يلي:-

أدي تكامل المعاملات بين معاملة التربة بالكمبوست النباتي بتركيز ١٠ جم/كجم تربة و معاملة مهد البذرة لشتلات البطيخ بالكيتوزان بتركيز ٨ جم / كجم تربة وحامض الساليسيلك بتركيز ٢ جم/كجم تربة الي مكافحة فعالة لمرض الذبول وموت البادرات علي نباتات البطيخ حيث أدت الي انخفاض نسبة حدوث المرض بمقدار ٨٦.٦ و ٩٢.٩ و ٨٨.٥% بالنسبة لاصناف البطيخ جورما و جيزة ١ و هجين أسوان علي الترتيب. بالنسبة لنشاط الانزيمات أدت المعاملات السابق ذكرها الي زيادة معنوية في كل الانزيمات المختبرة (البيروكسيديز والكيتينيز والبيتا جلوكانيز) في كل الاصناف المختبرة.