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Full Length Research Paper

Induced Systemic Resistance in Tomato Plants Against Fusarium Wilt Disease

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Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is the most serious diseases attacking tomato plants. The objectives of the study were to determine the effect of SA, H_2O_2, ethephon and mannitol as resistance inducers in tomato plants against *Fusarium o.* f. sp*. lycopersici* and to provide new strategies to control the wilt disease of tomatoes. For that, the effect of these four chemicals inducer, at different concentrations (50, 100, 200 ppm), on severity of the tomato wilt as well as fungal growth and spore formation was investigated. These inducers were applied with two methods, seed soaking and seedling soaking. Generally, all the tested chemical inducers reduce disease severity, the highest reduction in diseases severity was obtained with mannitol and SA at 200 ppm followed by H_2O_2 at 200 ppm, while Ethephon lower treatments for reduce disease severity. Seed soaking in mannitol gave the highest reduction of damping- off and wilt caused by the tested *Fusarium* isolates. Also, this inducer recorded the highest reduction in the area under wilt progress curve (AUWPC) from 1125 and 1024 in control to 156.7 and 124.2 in treatment. Seed soaking in these chemicals induced systemic resistance in seedling stage, whoever seed soaking in mannitol at 200 ppm gave the highest protection against to infection caused by both *Fusarium* isolates (92.14 and 92.72% protection). On the other hand, seed soaking in these inducers at all concentrations increased of seed germination, seedling height, fresh weight and dry weight. *In vitro* studies, all resistance inducers inhibited growth of both Fusarium isolates especially at high concentration (200 ppm). Hydrogen peroxide exhibited the highest effective to suppress dry weight and leaner growth of both tested fungal isolates followed by SA, however SA gave the highest reduction of spore formation followed by H_2O_2. Mannitol showed the lowest effect in all aspects. It could be suggested that mannitol and salicylic acid used as seed soaking or seedling soaking could be used for controlling wilt disease of tomato plants since they are safe, low cost and effective against the disease.

Keywords: Mannitol, salicylic acid, ethephon, H_2O_2, wilt disease, *Fusarium oxysporum* f. sp. *lycopersici*, tomato

INTRODUCTION

Tomato, *Solanum lycopersicon*, L., is one of the most popular and widely consumed vegetables all over the world. Its yield is drastically reduced by fungal diseases. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is a serious problem for tomato production in many areas which cause yield reduction of up to 25% (Fravel et al., 2005).

This pathogen enters tomato through the roots and causes yellowing of the oldest leaves, often on only one side of the plant. The yellowed leaves gradually wilt and die and the infected plants can be severely stunted. Agrochemicals are commonly used to control this pathogen, but are relatively expensive and subjected to various environmental constraints. The use of tomato resistant cultivars is one of the most practical and cost efficient strategies for managing tomato wilt disease (Horinouchi et al., 2007).

The latter mechanism protects the plant not only at the site of penetration (local protection) but also systemic protection could be achieved either biotically or abiotically (Galal and Abodou, 1996 and Galal, et al., 1997). Morsy (2005) found that lentil seed soaking in 0.5 mM of H_2O_2 reduced of damping-off caused by *Rhizoctonia solani* and *Fusarium oxysporum* under greenhouse condition and

increased of survival plants and seed yield under field conditions.

Mannitol at 1 mM reduced of strawberry fruit rots caused by *Botrytis cinerea* and increased of fruit yield under greenhouse and field conditions (Saber et al., 2003)

Salicylic acid (SA) has been used successfully to control some plant disease such as root rot/wilt of sesame (Abdou et al., 2001), root rot of wheat (El-Bana et al., 2002), root rot/wilt of lupine (Ali et al., 2007 and Abdel-Monaim, 2008), Fusarium wilt of tomato (Zgnen, 2001 and El- Khallal, 2007) and Fusarium wilt of chickpea (Nighat-Sarwar et al., 2005).

Also, several researches have on the use of ethylene releasing compound ethephon (2- chloroethyl phosphonic acid) for induction of resistance. Ethephon play many roles of plant defense against many diseases. Metwally (2004) reported that faba bean seed treated with SA at 7 mM was the most effective to reduce of damping-off caused by *Rhizoctonia solani* followed by ethephon at 800 ppm. Belhadj et al. (2008) found that treated grapevine foliar cuttings (*Cabernet sauvignon*) with ethylene-releasing ethephon increase in the number of pathogenesis-related protein (*CHIT4c*, *PIN*, *PGIP*, and *GLU*) gene copies and in an enhancement of phytoalexin biosynthesis by inducing the *PAL* and *STS* genes that correlated with the accumulation of stilbenes (antimicrobial compounds). Moreover, ethephon treatment triggered the protection of grapevine detached leaves and grapevine foliar cuttings against *Erysiphe necator*, the causal agent of powdery mildew (64% and 70%, respectively). These studies emphasize the major role of ethylene in grapevine defense.

The objectives of the study were to determine the effect of SA, H_2O_2, ethephon and mannitol as resistance inducers in tomato plants against *Fusarium o.* f. sp. *lycopersici* and to provide new strategies to control the wilt disease of tomatoes.

MATERIAL AND METHODS

Isolation and Identification of the Causal Organisms

Roots of tomato plants were collected from wilted tomatoes grown in field in different areas of Minia and New Valley Governorates, Egypt. Roots from diseased plants were washed with tap water. Small pieces of vascular tissues were surface sterilized in 0.5% NaOCl for 30-60 seconds, then placed in Petri dishes containing potato-dextrose agar medium (PDA). The dishes were incubated at 22 ^{o}C for 5-7 days. The isolates were identified based on published descriptions (Gilman, 1957; Barnett and Hunter, 1972 and Nelson et al., 1983) of morphological and cultural characteristics mycelium, conidiophores, conidia and colony morphology.

Selection of Isolates to Chemical Inducer Tests

Ten isolates of *Fusarium oxysporum* f. sp. *lycopersici* were tested for their pathogenicity to tomato seedlings (three isolates obtained from Minia governorate, FT1 to FT3, and seven isolates obtained from New Valley governorate, FT4 to FT10). All cultures were grown on potato dextrose agar medium (PDA) at 25 ^{o}C prior to inoculation. Spore suspensions were prepared from 3-week-old cultures by adding 10 ml of sterile distilled water to each Petri dishes and scraping the cultures with a rubber spatula. Using a haemocytometer, the inoculum concentration was adjusted to 2-3 x 10^4 conidia per milliliter. Tomato seedlings of the Supermarmand cultivar were inoculated at the 3 rd and 4 th true leaf stage. These tomato seedlings were uprooted and inoculated by using the root-dip technique. Roots were washed with running water, and placed for 60 minutes in conidia suspension (Tae-Kim *et al.,* 2001). The inoculated seedlings were transplanted to pots (30 cm- in diam.) containing sterilized sandy-clay soil. Three replications of five plants for each isolate were used. The plants were kept in greenhouse for 8 weeks after inoculation, disease severity was determined according to disease index: 0 = neither root discoloration nor leaf yellowing, 1 = 1-25% root discoloration or one leaf yellowed, 2 = 26-50% root discoloration or more than one leaf yellowed, 3= 51-75% root discoloration or vascular discoloration plus one leaf wilted, 4= up to 76% root discoloration or more than one leaf wilted and 5= completely dead plants. For each replicate a disease severity index (DSI) similar to that one described by Liu *et al.* (1995) was calculated as follows:

$DSI= \sum d/(d_{max} X n)X100$

Whereas: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

Disease severity recorded each 10 interval days for 60 days. The mean of area under wilt progress curve (AUWPC) for each replicate was calculated by formula suggested by Pandy *et al*. (1989) and calculated as follows:

AUWPC= D [1/2 (Y1+Yk) + (Y2+Y3+……..+Yk-1)]

Where D= Time interval; Y1= First disease severity; Yk= Last disease severity; Y2, Y3,……Yk-1= Intermediate disease severity.

Effect of Seedling Soaking in Chemical Inducers on Wilt Disease Development

Hydrogen peroxide (H_2O_2), Ethephon (Eth), Salicylic acid (SA) and Mannitol were tested separately at 50, 100 and 200 ppm for controlling tomato wilt diseases caused by the two pathogenic *F. oxysporum* f. sp. *lycopersici* isolates (FT1 and TF7) under artificial inoculation. Tomato seedlings (cv. Supermarmand) were soaking in

the tested solutions for 6 hr. (100 seedlings per 100 ml test solution), then inoculated with spore suspension as before for 1 hr. five seedling per treatment were sowing in plastic pot. Three pots were used for each test as replicates. Area under wilt progress curve was recorded as mentioned before.

Effect of Seed Soaking in Chemical Inducers on Germination, Seedling Growth and Damping-off and Wilt Diseases Development

Healthy tomato seeds cv. Supermarmand were soaking in the tested inducers at different concentrations (50, 100, 200 ppm) for 6 hr., then sown in plastic pots containing sterilized sand-loamy soil. Five seeds sown in pot and three pots were used for each test as replicates. After 45 days, percentage of seed germination, seedling height, fresh weight and dry weight were recorded.

Also, five tomato treated seeds were sown in pot containing soil infested with individually the tested isolates (FT1 and FT7). Damping-off and wilt were recorded 3 and 6 weeks after planting, respectively.

Effect of Seed Soaking in Different Chemical Inducers on Disease Resistance after Transplanting

Healthy seeds of tomato cv. Supermarmand were rinsed three times with distilled water, then air dried were soaked for 6 hr in tested solutions of chemical inducers at different concentrations (50, 100 and 200 ppm). Five seeds were sown in 30-cm diameter pots containing sterilized sandy-loamy soil then irrigated as needed. After 45 days, the seedlings were removed from pots and inoculated with spore suspensions of tested isolates as before then the inoculated seedlings were transplanted in pots

Five seedlings were sown per pot and three pots were used for each test as replicates. Disease severity recorded each 10 interval days for 60 days. The mean of area under wilt progress curve (AUWPC) for each replicate was calculated as before.

Effect of Chemical Inducer on growth and Sporulation of the Tested F. o. f. sp. lycopersici isolates in vitro

Hydrogen peroxide, salicylic acid, ethephon and mannitol were used separately at 50, 100 and 200 ppm for studding their effect on dry weight, linear growth and sporulation of the tested *F. o.* f. sp. *lycopersici* isolates.

A-Dry Weight

A disk (7 mm in diam.) from the actively edge of 7-days culture was cut and added into 250 ml –flasks containing 50 ml of stylized Czapeck's liquid medium amount with the tested inducer chemicals. After 7 days of inoculation, the culture medium was passed through filter paper and fungal mass was dried at 80 ºC for 24 hr. then weighed. Three replicates were used in this study.

B-Linear Growth

Flask (250 ml) containing 200 ml Czapeck's medium were prepared and amended with each concentration of each compound, then each flask was poured in 10 sterilized Petri-dishes (9 cm diam.) In control, dishes non-amended medium was used. Dishes were inoculated in the center with disks (7 mm in diam.) of the tested isolates and incubated at 25 ºC for 7 days. Linear growth of isolates was measured.

C- Sporulation

Spore production was estimated after measuring the fungal linear growth on solid medium as follows:

One disk (7 mm diam.) from each fungal culture, for each treatment, was placed in 1 ml sterilized water in tube. Tubes were shaken for 2 min., then kept 1 hr. Haemocytometer slide was used for counting the spores, the number of spores was counted in 16 squares (1/400 mm^2), chosen at random and the average of three slides was calculated (Tzeng and DeVay, 1989).

Statistical Analysis

In all experiments the least significant difference (LSD) at 0.05 confidences was determined according to Gomez and Gomez (1984).

RESULTS

All obtained isolates were showed significant various ability to cause wilt symptoms on artificial inoculated tomato cv. Supermarmand. Data in Table 1 show that the highest disease severity was caused by isolate FT7

Table 1. Pathogenicity tests of *Fusarium oxysporum* f. sp. *lycopersici* isolates collected from different location to tomato cv. Supermarmand

***Fusarium oxysporum* f. sp. *lycopersici* isolates**	**Locations**	**% Disease severity**
FT1	Minia	90.3*
FT2	Minia	55.7
FT3	Minia	45.0
FT4	Kharga	85.2
FT5	Kharga	65.1
FT6	Kharga	50.0
FT7	Kharga	95.7
FT8	Dakhla	86.0
FT9	Dakhla	24.4
FT10	Dakhla	38.8
LSD at 0.5		4.25

*Values are means of 3 replicates

Table 2. Effect of tomato seedling soaking in different chemical inducers on area under wilt progress carve caused by *Fusarium oxysporum* f. sp. *lycopersici* isolates under green house conditions.

Treatments	**Concen. (ppm)**	**Area under wilt progress carve caused by;**					
		***F. oxysporum* f sp. *lycopersici* isolate FT1**		***F. oxysporum* f. sp. *lycopersici* isolate FT7**		**Mean**	
		AUWPC	**% Protection**	**AUWPC**	**% Protection**	**AUWPC**	**%Protection**
	50	716.0*	36.36	624.4	39.02	670.2	37.63
H_2O_2	100	544.0	51.64	455.3	55.54	499.7	53.49
	200	325.0	71.11	301.3	70.58	313.2	70.85
	Mean	528.3	53.04	460.3	55.05	494.3	54.00
	50	825.3	26.64	736.7	28.06	781.0	27.32
Salicylic acid	100	354.7	68.47	295.3	71.16	325.0	69.75
	200	225.0	80.00	199.5	80.52	212.3	80.24
	Mean	468.3	58.37	410.5	59.91	439.4	59.11
	50	865.0	23.11	784.3	23.41	824.7	23.25
Ethephon	100	651.7	42.07	546.6	46.62	599.2	44.23
	200	525.3	53.30	423.5	58.64	474.4	55.85
	Mean	680.7	39.50	584.8	42.89	632.8	41.11
	50	631.0	43.91	654.3	36.10	642.7	40.19
Mannitol	100	329.3	70.73	514.8	49.73	422.1	60.72
	200	156.7	86.07	124.2	87.87	140. 5	86.92
	Mean	372.3	66.90	431.1	57.90	401.7	62.61
Control		1125.0	-	1024.0	-	1074.5	-

*Values are means of 3 replicates
LSD at 0.05 for:
Treatments (A) = 31.29
Concentrations (B) = 24.55
Isolates (C) = ns
Interactions (AxBxC) = 119.44

(95.7%) followed by isolate FT1 (90.3%). While isolate FT9 gave the lowest disease severity (25.4%).

Effect of Seedling Soaking in Chemical Inducers on Wilt Disease Development

Tomato seedlings soaking in tested chemical inducers reduced significantly area under wilt progress curve (AUWPC) caused by *F. oxysporum* f. sp. *lycopersici* isolates FT1 and FT7 compared with control (Table 2). The reduction in AUWPC increased with increasing of chemical inducers concentration. The overall results reveal that seedling soaking in mannitol at 200 ppm gave the pest protection against two Fusarium isolates followed by salicylic acid at 200 ppm, whereas Eth.

Table 3. Effect of tomato seeds soaking in different inducers on seed germination, seedling height, fresh weight, dry weight growing in pots.

Treatments	Concen. (ppm)	% Germination	Seedling height (cm)	Fresh weight (gm/seedling)	Dry weight (gm/seedling)
	50	75.3*	21.1	4.682	1.534
H_2O_2	100	80.3	25.8	5.065	1.756
	200	83.7	28.4	7.736	2.355
	Mean	79.77	25.1	5.828	1.882
	50	77.3	19.0	4.888	1.787
Salicylic acid	100	80.5	23.7	5.377	1.950
	200	84.8	25.6	7.441	2.257
	Mean	82.65	24.65	6.409	2.104
	50	78.4	22.2	6.423	1.983
Ethephon	100	83.3	24.5	7.322	2.280
	200	86.0	27. 5	8.414	2.467
	Mean	82.57	23.35	7.386	2.243
	50	80.2	26.6	5.212	1.353
Mannitol	100	86.8	27.9	6.826	1.633
	200	87.9	31.4	7.271	1.946
	Mean	84.97	28.63	6.436	1.644
Control		70.3	17.5	4.243	1.155

*Values are means of 3 replicates

LSD at 0.05 for:	Germination	Seedling height	Fresh weight	Dry weight
Treatments (A) =	3.71	0.715	0.715	0.12
Concentrations (B) =	2.84	1.079	0.512	0.08
Interactions (AxB) =	5.69	2.155	1.021	0.16

treatment gave the lowest effect. The mean protection against to wilt disease caused by Fusarium isolates were 86.92 and 80.24% in case of seedling soaking in mannitol and SA at 200 ppm, respectively.

Effect of Seed Soaking in Chemical Inducers on Seed Germination and Seedling Growth

Data present in Table 3 show that seed soaking in mannitol at 200 ppm gave the highest seed germination (87.9%) followed with Eth. (86.0%) compared with 70.3% in control. Also, seedling developing from seed soaked in mannitol solution at 200 ppm recorded the highest seedling height (31.4 cm/ seedling) followed by H_2O_2 at 200 ppm (28.4 cm /seedling) compared with 17.5 cm / seedling in control. On the other hand, seed soaking in Eth. and H_2O_2 at 200 ppm recorded the highest fresh and dry weight per seedling (8.414 and 7.736 gm fresh weight/seedling and 2.467 and 2.355 gm dry weight/seedling, respectively compared with 4.243 gm fresh weight and 1.155 gm dry weight in control.

Effect of Seed Soaking in Chemical Inducers on Damping-off and Wilt Diseases Development:

Data in Table 4 show that all tested concentrations of chemical inducers significantly reduced damping-off and wilt diseases caused by both of tested Fusarium isolates and the protection against to infection increased by increasing in concentration. More protection against to infection caused with Fusarium isolates were obtained when seed soaking in mannitol at 200 ppm (6.2 and 5.9 % damping -off and 6.7 and 5.9 % root rot compared with 28.2 and 32.8 % damping – off and 43.4 and 46.5% wilt in control, respectively. Seed soaking in SA came next mannitol where recorded 7.3, 5.6 % damping-off and 11.5, 10.1% root rot caused by both tested Fusarium isolates, respectively. On the other hand, seed soaking in H_2O_2 and Eth. were recorded the lowest protection against to infection caused by any Fusarium isolates.

Table 4. Effect of seed soaking in different chemical inducers on damping-off and wilt caused by *F. o.* f. sp. *lycopersici* isolates in pots.

Treatments	Concen. (ppm)	*F. oxysporum* f. sp *lycopersici* (isolate FT1)		*F. oxysporum* f. sp *lycopersici* (isolate FT7)	
		% Damping-off	% Wilt	% Damping-off	% Wilt
	50	21.1*	32.5	23.3	30.5
H_2O_2	100	14.3	22.5	16.3	18.1
	200	10.4	14.4	13.2	10.5
	Mean	15.27	23.13	17.6	19.7
	50	19.6	23.0	17.6	20.2
Salicylic acid	100	11.5	18.6	9.0	14.3
	200	7.3	11.5	5.6	10.1
	Mean	12.8	17.7	10.73	14.87
	50	22.7	35.0	25.1	33.2
Ethephon	100	15.3	25.1	18.3	24.1
	200	9.5	19.0	11.5	17.6
	Mean	15.83	26.37	18.3	24.97
	50	15.3	19.5	12.3	17.5
Mannitol	100	9.0	14.4	6.4	10.5
	200	6.2	6.7	4.1	5.9
	Mean	10.17	13.53	7.6	11.3
Control		28.2	43.4	32.8	46.5

*Values are means of 3 replicates

LSD at 0.05 for:		Damping-off	Wilt
Treatments (A)	=	1.17	0.92
Concentrations (B)	=	0.97	1.17
Isolates (C)	=	**	**
Interactions (AxBxC)	=	ns	3.92

Effect of Seed Soaking in Different Chemical Inducers on Developing Resistance Against Wilt Disease in Tomato Plants after Transplanting

Data presented in Table 5 show that tomato seedlings resulting from seeds soaking in tested chemical inducers have systemic resistance against to wilt disease caused by *F. oxysporum* f. sp. *lycopersici* isolates FT1 and FT7). The results also reveal that the high concentration of all the tested inducers were more effective than the lowest ones.

The most effective inducers were mannitol and salicylic acid especially at 200 ppm, whoever seed soaking in mannitol at 200 ppm reduce AUWPC from 1101.8 and 1268.4 to 86.6 (92.14% protection) and 92.4 (92.72 % protection) in both tested Fusarium isolates and seed soaking in SA at 200 reduce AUWPC from 1101.8 and 1268.4 in control to 188.4 (82.9% protection) and 168.7 (86.7% protection). On the other hand seed soaking in Eth gave the lowest protection against to infection with *F. o.* f. sp. *lycopersici* isolates (the mean of protection at all tested concentration were 50.02 and 64.23% in case of both test Fusarium isolates, respectively).

Effect of Chemical Inducers on Growth and Sporulation of the Tested Isolates in vitro

Data in Table 6 show that the highest inhibitory effect was noticed when 200 ppm of H_2O_2 was added to liquid and solid medium followed by SA at 200 ppm. Concerning the lowest concentrations, an inhibitory effect for mannitol was recorded against the both tested isolates. The growth parameters of *F. o.* s. sp. *lycopersici* isolates i.e. linear growth and dry weight were decreased to 183.96 and 166.23 g and 40.20, 45.12 mm under the effect of H_2O_2 at 200 ppm respectively. Added of mannitol to solid and liquid medium was recorded the lowest inhibitory effect of both Fusarium isolates especially at low concentration (50 ppm), while recorded 420.14, 454.23 mg and 86.13 and 81.67 mm compared with 90. 90 mm and 481.08 and 512.49 mg in control, respectively.

On the other hand, all chemical inducers greatly inhibited spore formation of both Fusarium isolates. The highest effect was clear in case of SA at 200 ppm, where reduce of spore formation from 82.37 and 72.63 $x10^5/cm^2$ in control to 5.73 and 4.97 x 10^5 /cm^2. Hydrogen peroxide

Table 5. Response of tomato seedlings to infection with *F. o.* f. sp. *lycopersici* growing from seed treated with different resistance chemical inducers

Treatments	Concen. (ppm)	Area under wilt progress carve caused by; *F. oxysporum* f. sp. *lycopersici* (FT1) AUWPC	% Protection	*F. oxysporum* f. sp. *lycopersici* (FT7) AUWPC	% Protection
	50	525.5*	52.31	435.3	65.68
H_2O_2	100	346.6	68.54	355.2	72.00
	200	242.1	78.03	219.3	82.71
	Mean	371.4	66.29	336.6	73.46
	50	418	62.06	443	65.04
Salicylic acid	100	302.3	72.56	258.9	79.59
	200	188.4	82.9	168.7	86.7
	Mean	302.9	72.51	290.2	77.11
	50	715.5	35.06	635.0	49.94
Ethephon	100	523.2	52.51	425.8	66.43
	200	413.4	62.48	300.5	76.31
	Mean	550.7	50.02	453.77	64.23
	50	430.2	60.95	396.4	68.75
Mannitol	100	225	79.58	186.4	85.30
	200	86.6	92.14	92.4	92.72
	Mean	247.27	77.56	225.07	82.26
Control		1101.8	-	1268.4	-

*Values are means of 3 replicates
LSD at 0.05 for:
Treatments (A) = 12.95
Concentrations (B) = 8.80
Isolates (C) = **
Interactions (AxBxC) = 28.15

Table 6. Effect of different chemical inducers on dry weight, linear growth and spore formation of *Fusarium oxysporum* f. sp. *lycopersici* isolates FT1 and FT7 grown on Czapek's medium.

Treatments	Concen. (ppm)	Dry weight (mg) FT1	FT7	Mean	Linear growth (mm) FT1	FT7	Mean	Sporulation X 10^5/cm^2 FT1	FT7	Mean
	50	313.25*	344.97	329.11	60.83	62.8	61.82	27.97	20.17	24.07
H_2O_2	100	247.10	305.11	276.12	52.64	53.21	52.93	17.40	14.17	15.79
	200	183.96	166.23	175.10	40.20	45.12	42.66	13.27	8.77	11.02
	Mean	248.11	272.10	260.11	51.22	53.71	52.47	19.55	14.37	16.96
	50	321.12	324.28	322.70	80.47	71.67	76.07	35.97	26.07	31.02
Ethephon	100	303.12	314.59	308.86	62.97	56.00	59.49	28.33	24.57	26.45
	200	233.05	234.11	233.58	57.60	54.00	55.80	20.00	15.67	17.84
	Mean	285.76	291.00	288.38	67.01	60.56	63.79	28.10	22.10	25.10
	50	420.14	454.23	437.19	86.13	81.67	83.90	48.23	37.47	42.85
Mannitol	100	400.01	380.00	390.01	79.63	76.00	77.82	40.53	33.20	36.87
	200	382.00	373.59	377.80	74.27	64.00	69.14	32.77	26.97	29.87
	Mean	400.72	402.61	401.66	80.01	73.89	76.95	40.51	32.55	36.53
	50	304.18	382.14	343.16	63.70	65.00	64.35	19.23	13.43	16.33
Salicylic	100	262.47	336.08	299.28	54.67	52.10	53.39	12.33	7.73	10.03
acid	200	206.17	228.19	217.18	48.93	48.60	48.77	5.73	4.97	5.35
	Mean	257.61	315.47	286.54	55.77	55.23	55.50	12.43	8.71	10.57
Control		481.08	512.49	496.79	90.00	90.00	82.37	72.63	77.50	75.07

*Values are means of 3 replicates

LSD at 0.05 for:		Dry weight	Linear growth	Sporulation
Treatments (A)	= 8.95	3.14	1.09	
Concentrations (B)	= 6.45	3.65	0.98	
Isolates (C)	= **	ns	**	
Interactions (AxBxC)	=20.12	8.47	3.08	

came next SA, while reduce of spore formation to 13.27and 8.77 x10^5/cm^2 at 200 ppm concentration of both Fusarium isolates, respectively. Also, added of mannitol to solid medium gave the lowest inhibitory effect on Sporulation of both *Fusarium* isolates compared with the other chemical inducers.

DISCUSSION

Fusarium wilt disease caused by *F. oxysporum* f. sp. *lycopersici* is considered as the most serious disease attacking tomato plants (Ozbay and Newman, 2004 and Fravel et al., 2005). Fungicides could successfully control most of plant diseases; however they have hazardous effects on human health, and environment; so alternatives of these fungicides are needed (El-Mougy et al., 2004). There are several fungicidal alternatives commercially used for induction of plant resistance against diseases. In this concern, many chemical inducers were reported as plant resistance inducers in many root fungal diseases (Benhamou et al., 1994; Kataria et al., 1997; Abdou et al., 2001; Zgnen et al., 2001; El-Bana et al., 2002; Morsy, 2005; Nighat - Sarwar et al., 2005; Ali et al., 2007; El- Khallal, 2007; Abdel-Monaim, 2008; and Mandal et al., 2009).

From the present investigation, it is evident that all tested chemical inducers e. g. H_2O_2, salicylic acid, ethephon and mannitol induce protection in tomato plant against Fusarium wilt disease caused by *F. oxysporum* f. sp. *lycopersici*. However, induced resistance was increased with increasing of concentrations. Tomato seedlings and/or seeds soaking in mannitol at 200 ppm showed highly resistance to damping-off and wilt diseases caused by both Fusarium isolates. Also, the chemical inducers generally, increase the seed germination and stimulated seedling growth, and that stimulation increased as chemical inducers concentration. Seed soaking in mannitol gave the highest seed germination (87.9%), seedling height (31.4 cm/ seedling) compared with 70.3% and 17.5 cm /seedling in control, respectively. On the other hand, seed soaking in Eth. at 200 ppm gave the highest fresh weight (8.414 gm /seedling) and dry weight (2.467 gm/seedling) compared with 4.243 gm fresh weight /seedling and 1.155 gm/seedling dry weight in control.

The role of chemical inducers in induced resistance has been reported in different researches. Application of salicylic acid resulted in accumulation of pathogenesis-related proteins (PRs), which have been defined as plant proteins that are induced in pathological and related situations (Raskin, 1992; Okey and Sreivasan, 1996, Hassan et al., 2006). Salicylic acid was initially proposed to bind to catalase and ascorbate peroxidase. The binding of SA to suck enzymes might lead to the formation of a phenolic radical involved in lipid peroxidation. Lipid products can active defense gene expiration (Farmer et al., 1998). Anderson (1988) confirmed that SA was responsible for the accumulation of phytoalexins in viable tissues.

Also, many researches have been reported on the use of ethylene releasing compound ethephon for induced resistance (Sallam, 1997 and Metwally, 2004). Abdel-Kareem (1998) found that cucumber seed soaking in ethephon induced resistance to powder mildew, such reaction was accompanied by increasing of free phenol content, activation of peroxidase activity and an increase of protein with Mw 69 KD and Mw 33 KD.

On the other hand, data strongly suggest that H_2O_2 directly or indirectly, plays as a signal for inducing systemic resistance as proposed by Levine et al. (1994). Generation of active oxygen species in the plant cell wall and in the plasma membrane is often considered to be a defensive oxidative barrier to phytopathogenic fungi (Merzlyak et al., 1990 and Galal and Abdou, 1996). Pellinen et al. (2002) examined the role of H_2O_2 as a connection between O_3 and subsequent defense gene activation and cell death in birch. They showed that both O_3 and H_2O_2 induce further H_2O_2 accumulation and necrotic formation within birch leaf tissue preferably around the growing lesion. Also, in higher plants the generation of the anion superoxide and /or hydrogen peroxide appears to be involved in several processes at the plasmalemma level or in the wall surrounding the cell (Vianello et al., 1990; Peng and Kuc, 1992; Chen and Asada, 1992). These molecules are either toxic or simulative living cells causing variety of forms of cell damage including lipid peroxidation, induction of enzymes and membrane damage. Such effect may explain why these compounds are the biological trigger keys responsible for cell death and hypersensitive reaction (Doke and Ohashi, 1988).

The *in vitro* effect of chemical inducers on radial growth, mycelial dry weight and spore formation was considered to be a good indicator for its direct effects. The present investigation showed that the chemical inducers tested significantly inhibited the radial growth, mycelial dry weight spore formation of both *F. oxysporum* f. sp. *lycopersici* to different degrees depending on inducers concentrations. The inhibitory effect performed by chemical inducers in the present study almost agrees with that found by Amborabe et al. (2002); Hilal et al. (2006), Mostafa (2006); Ali et al. (2007).

Nesci et al. (2003) introduced possible explanations on the antimicrobial of resistance inducers including: (1) they may inhibit the functions of several enzymes by the oxidized compounds, possibly through reaction with sulfohydryl groups or through more nonspecific interactions with the proteins, (2) they dissolve in membrane lipids and interfere with membrane functions, including transport of nutrients, (3) they also interfere with the synthesis of protein, RNA and DNA, (4) they destroy the membrane potential similar to other week organic acids and/or (5) the site (s) and number of hydroxyl

groups on the phenol compounds (salicylic acid and hydroquinone) are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation associated with increased toxicity. In addition, Scalbert (1991) has found that the more highly oxidized phenols are the more inhibitory effect to the pathogen.

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