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Effect of some Biotic and Abiotic Applications on Control of Fusarium Wilt of Pepper Plants

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Six different pathogenic isolates of *Fusarium oxysporum* were recovered from diseased pepper plants collected from Dakahlya and Gharbya governorates, in Egypt. The F3 isolate of *F. oxysporum* recovered from Dakahlya samples was more aggressive hence produced 96.67% wilt severity, that being significantly higher than Tanta isolate F4 producing 71.67% severity. Remarkable variation in wilt producing potentials was found among other isolates denoting different pathogenic capabilities. Chemical substances inducing plant resistance against *F. oxysporum* were checked for their effect on linear growth of the pathogen. Dipotassium phosphates, potassium sorbate, salicylic acid, ascorbic acid along with chitosan, clove oil, mint oil and cumin oil were evaluated. *Trichoderma hamatum* and *T. harzianum* were also considered. A positive relation between a given inducer and inhibition of fungal growth *in vitro* was concluded. However, clove oil at low concentration (0.25%) inhibited completely the mycelial growth of pathogen, compared to other inducers that decreased the growth in a range between 42.2 and 91.9%, for the low concentrations. In greenhouse trials, a significant reduction in wilt was recorded. Immersed seedlings in the selecting concentration of inducers, bioagents, biocides and Topsin M70 fungicide were compared. The most effective treatments were Bio-Zeid and salicylic acid giving a similar wilt severity (21.67%), followed by ascorbic acid, Bio-Nagi, *T. hamatum* and *T. harzianum* that decreased severity in an ascending order from 26.67 to 43.33%. Topsin M70 fungicide gave 16.67% wilt severity, being the best control treatment, compared to 91.67% for cumin oil that could be ignored. Field grown plants of pepper showed significant wilt decrease following application with salicylic acid and Bio-Zeid. Ascorbic acid and Bio-Nagi along with *T. hamatum* showed promising results. Moreover, application of salicylic acid and Bio-Zeid increased growth parameters. The study suggested that Bio-Zeid and salicylic acid were the most effective treatments for controlling wilt and increasing yield of pepper. Ascorbic acid, Bio-Nagi and *T. hamatum* were promising in this regard. Further investigations are needed.

Keywords: Bioagents, biocides, disease control, Fusarium wilt, resistance inducers and yield quality.

Pepper (*Capsicum annum* L.) is one of the most important vegetable crops in Egypt. Green and red fruits are used for fresh salad and food commodities containing proteins, vitamins (A and C), and minerals as calcium, phosphorous and irons (Chowdhury *et al*., 2015). Production has been hampered by many diseases of which Fusarium wilt caused by *Fusarium oxysporum* was reported by many investigators to cause great losses in pepper production in worldwide. The disease affects plant at all developmental stage causing wilt syndrome of many crops (Lefebvre and Palloix, 1996; Kucuk and Kivanc, 2003; Abdel-Monaim and Ismail, 2010). The total cultivated area with pepper in Egypt during 2013 reached about 65240 feddan with a total production of 387964 ton, at the rate of 5.95 ton/feddan. In addition, there exist approximately 1000 feddan in protected cultivation producing about 7500 ton with an average of 7.5 ton/feddan (The Yearly Book of Economics and Statistics of the Agriculture Ministry of Egypt, 2014). Though chemical control against such diseases sometimes gives good results, the use of fungicides leads mostly to environmental pollution and the phenomena of resistance to the plant pathogens (Brewer and Larkin, 2005). Biological control is considered an important approach of agricultural biotechnology in recent years for controlling many fungal plant pathogens (Zaher *et al.*, 2013). *Trichoderma* spp. are a major mycoparasites which parasitize a large number of plant pathogens (Liu and Baker, 1980). They produce antimicrobial substances for control of many soil borne diseases such as Fusarium wilt of pepper (Hulang, 1980). Induction of resistance in plants to overcome pathogen infection is a promising approach for controlling plant diseases. Exogenous or endogenous factors could substantially affect host physiology, lead to rapid and coordinated activation of defense gene in plants normally expressing susceptibility to pathogen infection (Mandal *et al*., 2009 and Metwally, 2004). This induced resistance to pathogens can be achieved by the application of various abiotic agents, chemical inducers such as salicylic acid, potassium salts and ascorbic acid (El-Khallal, 2007; Abdel-Monaim, 2010; Akram and Anjum, 2011 and El-Mohamedy *et al*., 2013). Fortunately, application of such inducers under field conditions have increased growth parameters, yield components and quality of fruits in many vegetable plants (El-Mougy, 1995; El-Mougy *et al*., 2004; Karlidag *et al*., 2009 and Zahra *et al*., 2010).The present study was conducted to investigate the effect of some biotic and abiotic agents as resistance inducers in pepper plants against Fusarium wilt pathogen *in vitro, in vivo* and under field conditions and to elucidate their impacts on growth parameters and yield.

Materials and Methods

Isolation and identification of the causal pathogens:

Samples of wilted pepper plants were collected from different governorates in Egypt, washed under tap water and sterilized by immersing in 5% sodium hypochlorite for 2 minutes (Waller, 1981) and subsequently rinsed with sterile distilled water. Thin sections were placed on potato dextrose agar (PDA) medium in Petri dishes and incubated at 25±2°C, for five days. Each of the emerged fungi was picked up and purified by transferring the hyphal tip into PDA plates and identified according to their morphological features using the descriptions of Booth (1971) and Domsch *et al*. (1980) at the Unit of Identification of Micro-organisms, Plant Pathology Research Institute, A.R.C., Giza, Egypt.

Pathogenicity test:

Six isolates were grown in autoclaved bottles (500-ml) containing (25g clean sand and 75g sorghum grains and sufficient amount of water to cover the mixture and autoclaved at 121^{o}C for 30 min) and incubated for 15 days at 25±2°C. Formalin sterilized pots (20 cm. in diameter) were filled with autoclaved clay soil and infested with pathogenic fungus at the rate of 5% (Abou-Zeid *et al*., 2002). Three cultivars Anaheim-M of 35-day old pepper transplants grown in disinfested soil were used as control. The plants were left for three months after transplanting under greenhouse conditions at the Unit of Identification of Micro-organisms, Plant Pathology Research Institute, A.R.C. Giza, Egypt. The wilt disease severity was carried out using a visual 1-6 scale according to Silva and Bettiol (2005) as follows: 1= no symptom; 2= plant showed yellowing leaves, and wilting 1-20%, 3= plant showed yellowing leaves, and wilting 21-40%, 4= plant showed yellowing leaves, and wilting 41-60%, 5= plant showed yellowing leaves, and wilting 61-80% and 6= plant showed yellowing leaves, and wilting 81-100% or dead plant. Virulent group was categorized according to DSI as non-pathogenic (DSI =1), low (DSI ≤ 3.50), moderate (DSI > 3.50 - 4.50), and high (DSI > 4.50). The percentages of disease severity index (DSI) and disease reduction were determined using the formula:

DSI (%) =∑ (grade x number of plants in that grade) / (maximum grade x total number of assessed plants) x 100

In vitro experiments:

Effect of bioagents on linear growth of F. oxysporum isolate:

Trichoderma hamatum and *T. harzianum* obtained from the Identification of Microorganisms Unit, Plant Pathol. Res. Inst., A.R.C were used to study the inhibitory effect of these antagonistic fungi on growth of *F. oxysporum,* the cause of pepper wilt. In this respect, Petri plates (9 cm-diameter) containing PDA were inoculated with disc (5mmØ) of the *Trichoderma* isolates in one side of the plate and another disc (5mmØ) of *F. oxysporum* at the opposite side. Three replicates were used for each particular treatment. Control plates inoculated with the pathogenic fungus only were prepared. Incubation was made at 25±2°C for 7-10 days. The growth of each treatment was determined when the mycelial growth covered the medium surface of any plate. The reduction percentage was calculated using the following formula according to Abada and Ahmed (2014).

$$I = (C- T)/C X100$$

Where;

I = Percent of inhibition in growth of the tested pathogen.

C = Radial growth of the pathogen (mm) in control.

T= Radial growth of the pathogen (mm) in treatment.

Effect of resistance inducers on linear growth of F. oxysporum isolate:

Dipotassium phosphate (K_2HPO_4), and potassium sorbate at three concentrations 2.0, 4.0 and 6.0 % (w/v); salicylic acid and ascorbic acid at concentration of 0.5, 1.0 and 2.0% (w/v) and chitosan at concentration of 0.5, 1.0 and 2.0% (w/v) were prepared. The chemicals were kindly provided by Sigma Company, Cairo-Egypt, to study their effects on growth of *F. oxysporum* isolate (El-Mougy *et al.*, 2004). Chemicals were added individually to conical flasks containing sterilized PDA medium to obtain the desired concentrations, then mixed and dispensed in sterilized

Petri dishes. The discs (5 mm Ø) of *F. oxysporum* were transferred to the center of the PDA plates. Each treatment was replicated 3 times. Control treatment was made by inoculating a disc of *F. oxysporum* onto PDA medium in petri plate without any inducers. Incubation was made at 25±2°C for 7-10 days. The diameters of colonies were measured as mentioned before.

Effect of plant oils on linear growth of F. oxysporum isolate:

The extracted oils from clove, mint and cumin plants were used to study their effect on the growth of *F. oxysporum*. The tested oils were brought from EL Captain Company for extracting natural oils and plants. Cairo, Egypt. Chloroform at a rate 2% was added to each of the concentrated plant oils to increase their solubility (Fahiem, 2010). Concentrations of 0.25, 0.5 and 1.0 % of each oil were made and each was added to melted PDA medium. Then the poured plates were left to set up and an equal disc (5 mm Ø) of *F. oxysporum* was put at the center. Three replicate plates were used for each treatment. Control treatment was in form of inoculated plates without adding any of the tested plant oils (Chloroform only). Inoculated plates were incubated at 25±2°C for 7-10 days. The percentage of growth reduction of *F. oxysporum* was calculated as mentioned before.

In vivo experiments:

Effect of bioagents, resistance inducers, plant oils, biocides and fungicide on the severity of pepper wilt under greenhouse conditions:

The efficacy of chemical inducers selecting according to the best inhibitory concentration against the fungus in concern was experimented under greenhouse conditions. Dipotassium phosphate and potassium sorbate, salicylic acid, ascorbic acid, chitosan, three of plant oils in addition to *T. hamatum* and *T. harzianum* (10^7 spore/ml) Abou-Zeid *et al*. (2003), Bio Arc® and Bio Zeid® are commercial biocides labeled on different crops in Egypt were tested along with Bio Nagi (*Tricoderma asperellum*) at rate of 2.5 g/L (Mahmoud *et al*., 2013), that is first recorded in Egypt by Abou-Zeid and Mahmoud (2012) and still under registration. Types, active ingredients and rate of applications are shown in Table (1).

Table 1. Active ingredients of *Trichoderma* spp., chemical inducers, plant oils, biocides and a fungicide

Tested products	Active ingredients	Types	Rate
T. hamatum	*T. hamatum*	Fungus	$1x10^7$/ml
T.harzianum	*T.harzianum*	Fungus	$1x10^7$/ml
Dipotassium phosphate	K_2HPO_4	Salts	2.0%w/v
Potassium sorbate	$C_6H_7KO_2$	Salts	2.0%w/v
Salicylic acid	$C_7H_6O_3$	Acid	0.5%w/v
Ascorbic acid	$C_6H_8O_6$	Acid	0.5%w/v
Chitosan	Natural polysaccharide	Chemical	2.0%w/v
Clove oil	Eugenol, 90–95%	Oil	0.5%w/v
Mint oil	Menthol, 48%	Oil	0.5%w/v
Cumin oil	Cuminaldehyde	Oil	0.5%w/v
Bio-ARC	*Bacillus megaterium* 6% (w/w)	Biocide	2.5 g/L
Bio-Zeid	*Trichoderma album* 2.5 % (w/w)	Biocide	2.5 g/L
Bio-Nagi	*Trichoderma asperellum*	Biocide	2.5 g/L
Topsin-M (70%)	Thiophanate-Methyl	fungicide	1 g/L

Pepper seedlings (cv. Anaheim-M) were soaked in the solutions of each treatment for 2 hours (El-Mohamedy *et al*., 2014) before planting in pots containing soil infested with *F. oxysporum* at the rate of 5%. Three seedlings per/pot were transplanted and three pots were used for each treatment as replicates. Untreated pepper seedlings were planted in infested soil as control. The wilt severity was determined according to Silva and Bettiol (2005) as mentioned before.

The effect of bioagents, resistance inducers, and fungicide on disease severity and yield under field conditions:

Randomized block design was followed; planting was made on February of two successive seasons 2015 and 2016 in naturally infected field with the causal organism of pepper wilt, Giza Research Station. Seedlings were soaked in solutions of inducers for 6 hr. The field plots (9 m^2) consisted of 3 rows of 3 m long and 1 m in between. One seedling/hill was sown with 50 cm apart between hills. Untreated seedlings were allowed to grow as control. Disease severity was recorded every 30 days interval for 3 months using a visual 1-6 scale according to Silva and Bettiol (2005). At the end of the growing season, 20 plants were randomly chosen from each treatment to determine some yield parameters as plant height (cm), number of branches per plant, number of pods per plant and pod yield (g/plant) in the two successive seasons (2015 & 2016).

Statistical Analysis:

Statistical Analysis was done using analysis of variance, (ANOVA) using Computer Statistical Package Assistant V.7.6 beta according to Silva and Azevedo (2009).

Results

Isolation and identification of the causal pathogen:

Six selected fungal isolates isolated from pepper plants, collected from different governorates i.e. Beni-Sweif, Kafr El-Sheikh, Dakahliya, Gharbiya, Sharkiya and Menofia were purified using the hyphal tip technique and then they were identified at the Unit of Identification of Micro-organisms, Plant Pathology Research Institute, A.R.C., Giza, Egypt, as *Fusarium oxysporum.*

Pathogenicity Test:

Data in Table (2) show the pathogenic ability of the tested isolates on cv. Anaheim-M pepper plants. The isolates varied in their ability to cause wilt on plants under greenhouse conditions.

Table 2. Pathogenicity test of *F. oxysporum* isolates on pepper plants under greenhouse conditions

Isolate No.	Governorates	Locations	DSI %	Virulent group
F1	Kafr El-Sheikh	Sedi-Selem	55.00	L
F2	Beni-Sweif	Beni-Sewif	33.33	L
F3	Dakahliya	Sinbellawein	96.67	H
F4	Gharbiya	Tanta	71.67	M
F5	Sharkiya	Hihia	68.33	M
F6	Menofia	Berkeh-El-Sabaa	63.33	L
L.S.D at 5%			4.64	-

The most aggressive isolate was *F. oxysporum* (F3) isolated from Dakahliya which recorded 96.67% disease severity. Meanwhile, *F. oxysporum* (F2) caused the least disease severity, being 33.33%.

Effect of resistance inducers, plant oils and bioagents on growth of F. oxysporum in vitro:

The inhibitory effect of antagonistic micro-organisms and plant resistance inducers on the growth of *F. oxysporum* was evaluated under *in vitro*. The results revealed that all treatments could drastically reduce the linear growth of the tested isolate (F3) of *F. oxysporum*.

Data in Table (3) indicate that all tested treatments reduced the growth of *F. oxysporum* on PDA plates compared with control. Concerning to chemical inducers and plant oils; it was found a positive relation between the high concentration of the resistance inducers and plant oils the recorded reduction of *F. oxysporum* growth. Clove oil at 0.25% showed complete inhibition of mycelial growth of *F. oxysporum* (100%), followed by chitosan at 0.4% which gave (98.8%) without significant differences. Salicylic acid at 0.1% gave the highest inhibitory effect (92.2 %).

Table 3. The effect of different resistance inducers, plant oils and bioagents on growth of *F. oxysporum* under laboratory conditions

Treatments	Conc. %	Linear growth (mm)	Growth reduction (%)
Di Potassium phosphate	2.0	18.0	80.0
	4.0	13.0	85.5
	6.0	9.0	90.0
Potassium sorbate	2.0	22.0	75.5
	4.0	19.0	78.8
	6.0	10.0	88.8
Salicylic acid	0.05	12.0	86.6
	0.07	9.0	90.0
	0.1	7.0	92.2
Ascorbic acid	0.05	52.0	42.2
	0.07	17.0	81.1
	0.1	14.0	84.4
Chitosan	0.1	8.0	91.1
	0.2	5.0	94.4
	0.4	1.0	98.8
Clove oil	0.25	0.0	100
	0.5	0.0	100
	1	0.0	100
Mint oil	0.25	3.8	57.7
	0.5	3.1	65.5
	1	2.5	72.2
Cumin oil	0.25	5.1	43.3
	0.5	4.8	46.6
	1	4.5	50.0
T. hamatum		8.0	91.1
T. harzianum		10.0	88.8
Control (Chloroform)		90.0	0.0
L.S.D. at 5%		2.62	-

Meanwhile, cumin oil at 1.0% concentration gave the least inhibitory effect (50.0%) compared with control treatment with Chloroform which had no effect on the linear growth of the pathogen. On the other hand, the antagonistic *T. hamatum* isolate showed greater inhibitory effect (91.1%) compared with *T. harzianum* (88.8 %). However, the difference between the two antagonists was not significant.

Greenhouse testing:

Effect of bioagents, resistance inducers, plant oils, biocides and a fungicide on pepper wilt under greenhouse conditions:

All treatments significantly reduced severity of Fusarium wilt under greenhouse conditions as result of soaking pepper seedlings in solutions of inducers at the selected concentrations of dipotassium phosphate, potassium sorbate, salicylic, ascorbic acid and chitosan, along with clove, mint and cumin oils and bioagents *T. hamatum* and *T. harzianum.* Biocides Bio-Arc, Bio-Zeid and Bio-Nagi were experimented and the fungicide Topsin-M70 against pepper wilt caused by *F.oxysporum* compared with the untreated control plants.

Data presented in Table (4) indicate that the most effective treatments were Bio-Zeid and salicylic acid which gave similar disease severity (DSI%), being 21.67%, followed by ascorbic acid, Bio-Nagi, *T. hamatum* and *T. harzianum* which decreased disease reading, being 26.67, 33.33, 38.33 and 43.33% on the average, respectively. Meanwhile, cumin oil was the least effective one where it recorded 91.67% disease severity compared to the control. However, all tested inducers were less effective than Topsin-M70 in controlling pepper wilt which recorded 16.67% disease severity.

Table 4. Effect of bioagents, resistance inducers, plant oils, biocides and fungicide on the disease severity under greenhouse conditions

Treatments	DSI %
T.hamatum	38.33
T.harzianum	43.33
Di Potassium phosphate	50.00
Potassium sorbate	89.47
Ascorbic acid	26.67
Salicylic acid	21.67
Clove oil	56.20
Mint oil	66.00
Cumin oil	91.67
Chitosan	55.00
Bio-Arc	60.00
Bio-Zeid	21.67
Bio-Nagi	33.33
Topsin-M (70%)	16.67
Control	100.00
L.S.D. at 5%	4.73

Field experiments:

Effect of bioagents, resistance inducers, plant oils, biocides and the fungicide Topsin-M on pepper wilt under field conditions:

Data in Tables (5 and 6) show that all biotic and abiotic treatments significantly reduced disease severity compared to control treatment. In this respect, Bio-Zeid and salicylic acid showed the highest efficiency compared to the other treatments where they recorded 9.64 and 8.94% disease severity in the first season, respectively. While, salicylic acid recorded higher efficient than Bio-Zeid in the second season, being 7.79 and 7.92 %. Seedlings soaked in ascorbic acid, Bio-Nagi and *T. hamatum* came next to both of Bio-Zeid and salicylic acid and decreased disease severity to 11.16, 13.44 and 15.66% in the first season and 9.88, 11.91 and 13.87% in the second season, respectively. Cumin showed the lowest effect on wilt disease, so it recorded 29.33 and 26.71%, as compared to control which recorded 37.64 and 33.33% in both seasons, respectively.

Table 5. Effect of bioagents, resistance inducers, plant oils, biocides and fungicide (Topsin-M) on pepper wilt and some plant parameters under field conditions in season 2015

Treatments	DSI (%)	Plant height (cm)	No. of branch/plant	No. of pods/plant	Pod yield (gm)/plant
T.hamatum	15.66	56.21	4.6	23.84	433.81
T.harzianum	24.19	53.57	4.4	18.21	331.43
Di Potassium phosphate	17.88	55.32	5.0	20.46	372.38
Potassium sorbate	28.90	47.79	4.6	17.40	276.67
Ascorbic acid	11.16	58.75	4.5	24.39	443.81
Salicylic acid	9.64	61.70	4.6	25.46	463.33
Chitosan	20.16	52.89	4.5	18.94	344.76
Clove	23.90	60.43	4.6	20.66	369.33
Mint	22.46	56.20	4.7	17.66	297.86
Cumin	29.33	48.60	4.5	15.98	285.81
Bio-Arc	22.85	59.36	4.5	22.03	400.95
Bio-Zeid	8.94	62.89	4.6	26.61	504.29
Bio-Nagi	13.44	58.41	5.0	24.05	437.62
Topsin-M (70%)	6.72	61.59	4.5	28.99	547.62
Control	37.64	41.41	4.4	15.06	234.10
L.S. D at 5%	1.75	2.52	1.24	1.79	15.08

On the other side, a significant increase in plant height, number of branches/plant, number of pods/plant and pod yield (gm)/plant in seedling treatments with some biotic and abiotic inducers (Tables 5 and 6) was recorded. The most effective inducers were Bio-Zeid and salicylic acid followed by ascorbic acid, Bio-Nagi and *T. hamatum*, whereas the lowest treatment was potassium sorbate in both seasons. The results indicated that the highest pod yield/ plant was found in case of treatment with Bio-Zeid followed by those treated with salicylic acid, being 463.33 and 504.29 gm at the first season, as well as, second season salicylic acid recorded the highest effect compared to Bio-Zeid, being 551.56 and 506.77%. While the lowest yield was obtained from cumin treatments, being 285.81 and 299.80% in both seasons.

Table 6. Effect of bioagents, resistance inducers, plant oils, biocides and fungicide (Topsin-M) on pepper wilt and some plant parameters under field conditions in season 2016

Treatments	DSI (%)	Plant height (cm)	No. of branch/plant	No. of pods/plant	Pod yield (gm)/plant
T.hamatum	13.87	59.63	4.7	26.69	474.48
T.harzianum	21.43	56.82	5.0	20.39	362.50
Di Potassium phosphate	15.83	57.62	4.3	22.91	407.29
Potassium sorbate	25.60	51.75	4.6	19.27	302.60
Ascorbic acid	9.88	61.26	4.5	27.30	485.42
Salicylic acid	7.79	65.39	5.0	29.33	551.56
Chitosan	17.86	55.05	4.5	21.21	377.08
Clove	23.33	62.75	4.6	22.76	393.75
Mint	22.82	57.72	4.7	19.95	345.10
Cumin	26.71	51.20	4.5	17.33	299.80
Bio-Arc	20.24	57.66	4.9	24.66	438.54
Bio-Zeid	7.92	64.33	5.0	28.50	506.77
Bio-Nagi	11.91	62.96	4.6	26.92	478.65
Topsin-M (70%)	5.95	64.65	5.0	30.88	598.96
Control	33.33	43.93	4.4	16.44	262.30
L.S.D. at 5%	2.50	4.85	1.54	1.00	21.29

Discussion

Pepper plants are subject to infection with many diseases (Lamb *et al.*, 1999; Utkhede and Mathur, 2005 and Demirci and Dolar, 2006), among which the soil-borne diseases are the most important. Many fungi belonging to genera *Fusarium*, M*acrophomina, Rhizoctonia*, *Pythium, Verticilium* and *Sclerotinia* causing damping-off, root rot and wilt diseases are commonly encountered in the greenhouse, nurseries and fields (Liu and baker, 1980; Fletcher, 1994; Soner *et al.*, 2005 and Goicoechea, 2006).

Results of the present study showed that isolated fungi from wilted pepper plants collected from six Governorates (Beni-Sweif, Kafr El-Sheikh, Dakahliya, Gharbiya, Sharkiya and Menofia) were identified as *Fusarium oxysporum.* The isolate F3 was highly pathogenic towards pepper plant caused 96.67% disease severity. Data are in accordance with those reported by Mushtaq and Hashmi (1997); Sahii and Khalid (2007). Moreover, the results revealed that all tested antagonistic micro-organisms could effectively decrease the linear growth of *Fusarium oxysporum in vitro*. Meanwhile *T. hamatum* isolate, showed superior inhibitory effect (91.1%) against the growth of the pathogenic fungus compared, with *T. harzianum*, being 88.8 %. These results are in accordance with those previously reported by Sahii and Khalid (2007) who examined biocontrol of *Fusarium oxysporum*, the causal pathogen of pepper wilt, using different strains of antagonistic fungi of *Trichoderma* sp., *in vitro*. They observed considerable inhibition of the mycelial growth of pathogen as a response to the effect of *Trichoderma* sp. antagonism. The mechanism of biocontrol of plant pathogens using antagonists may be through competition for space and food or by stimulating host plant by inducing resistance to the pathogen, or antibiosis, as production of low molecular fungi toxic compounds or enzymes (Matar *et al.*, 2009). The mechanism of biological control and the antagonistic relationship of microorganisms to the plant pathogens in general was studied by several authors (Cavagliery *et al.*, 2005; Dubey *et al.*, 2007; Guoj *et al.*, 2004; Matar *et al.*, 2009 and Sahii and Khalid, 2007).

Chitosan, salicylic acid and dipotassium hydrogen phosphate gave the highest inhibitory effect on mycelial growth of *F. oxysporum*. Many investigators used abiotic factors for induction of plant resistance against several diseases. Our results are in agreement with Ragab *et al*. (2012) who revealed that Chitosan caused complete reduction in growth of *F. oxysporum* the causal of pepper wilt (*Capsicum annum* L.). Similar results were recorded by many investigators with various crops. Chitin and chitosan as well the safe materials were reported to induce resistance by Abd-El-Kareem (2002). These findings in general agree with Özgönen *et al*. (2001) who stated that salicylic acid completely inhibited the mycelial development of *F. oxysporum* f. sp. *lycopersici in vitro.* As well as, clove gave complete inhibitory effect on growth of *F. oxysporum* followed by mint, while, cumin oil showed the lowest inhibitory activity in controlling *F. oxysporum*. These results are in agreement with Torre *et al*. (2016) who reported that the best results were obtained with clove oil and its major component eugenol in controlling *F. oxysporum* f. sp. *lycopersici*, and those of Barrerea-Necha *et al*. (2009) who reported that *F. oxysporum* f. sp. *gladioli* was totally inhibited with clove oil. In the present study, it was clear that the tested treatments significantly reduced severity of Fusarium wilt under greenhouse conditions. The most effective treatments were Bio-Zeid and salicylic acid followed by Ascorbic acid, Bio-Nagi, *T. hamatum* and *T. harzianum.* Similar results were obtained by Al-Sohaibani *et al*. (2011) who reported that two biocides, Bio-Arc and Bio-Zeid were significantly effective in controlling root rot disease incidence of sweet basil and also pepper (Abdel-Monaim and Ismail, 2010) who reported that antioxidant compounds (coumaric acid, citric acid, propylgalate and salicylic acid each at 100 and 200 ppm) reduced wilt when used as seedling soaking under greenhouse and field conditions. All tested chemicals significantly increased plant height, plant branching and total yield per plant. Also, El- Hassan *et al*. (2013) reported that using *T. hamatum* delayed the time of infection by *F. oxysporum*, promoted the growth, and increased the dry weight of a susceptible variety of lentil. The percent of mortality was reduced to 33 and 40% when using *T. hamatum*, respectively, compared to 93% in the control treatment. Plant colonization results indicated that *T. hamatum* and its filtrate significantly ($p \leq 0.05$) reduced development of the pathogen in the vascular tissue of lentil to < 30 and < 40% stem colonization, respectively. So, they suggested that potential biocontrol mechanisms of *T. hamatum* towards *F. oxysporum* f. sp. *lentis* were antibiosis by production of antifungal enzymes, complex mechanisms of mycoparasitism, competition for key nutrients and/or ecological niches, growth promotion, and a combination of these effects.

Also, in the present study, all the tested treatments reduced the area of the wilt under field conditions and increased the plant vigor and pod yield per plant in both seasons. In this respect, the most effective inducers were salicylic acid and Bio-Zeid followed by Ascorbic acid, Bio-Nagi and *T. hamatum*, whereas the lowest treatment was potassium sorbate in both seasons. These results are in agreement with Abdel-Monaim and Ismail (2010) who reported that application of SA inhibits ethylene production leading to an increase in fruit number and consequently increases fruit yield per plant. Also, SAR mechanisms might be as a result of induce plant resistance (De Meyer *et al.*, 1998), produce extracellular enzymes and antifungal or antibiotics, which decrease biotic stress on plant, and produce growth promoters substances (Szczech and Shoda, 2004). Also, Al-Sohaibani *et al.* (2011) reported that two biocides, Bio-Arc (*Bacillus megaterium*) and Bio-Zeid (*Trichoderma album)* were significantly effective in controlling root rot disease incidence.

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تأثير بعض التطبيقات الحيوية والغير حيوية على مكافحة الذبول الفيوزاريومي في نباتات الفلفل

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تم عزل ست عزلات من الفطر فيوزاريوم اكسيسبورم المعزولة من نباتات الفلفل التي تم جمعها من محافظات الدقهلية والغربية وكفر الشيخ والمنوفية والشرقية وبنى سويف في مصر. تعد عزلة فيوزاريوم أوكسيسبورم (F3) المعزولة من محافظة الدقهلية هى الأكثر مرضية حيث سببت أعلى شدة إصابة (97.67%) عن عزلة طنطا (F4) التى سجلت شدة إصابة (71.67%). من اللافت للنظر وجود اختلافات بين العزلات في قدرتها علي إحداث مرض الذبول. تم تقييم التأثير التثبيطى من المستحثات المختبرة على النمو الطولي للفطر فيوزاريوم أوكسيسبورم. فوسفات ثنائية البوتاسيوم، سوربات البوتاسيوم وحمض الساليسيليك وحمض الاسكوربيك والشيتوزان و زيوت القرنفل والنعناع والكمون والفطرين ترايكودراما هاماتم، ترايكودراما هارزيانم وكانت هناك علاقة إيجابية بين التركيز العالي للمستحثات المختبرة و تثبيط نمو الفطر فيوزاريوم أوكسيسبورم في المعمل. وقد أعطى زيت القرنفل عند أقل تركيز 25.% تثبيط كامل للنمو الفطرى عن المعاملات الأخرى والتى قللت معدل النمو ما بين 42.2 إلى 91.9٪ عند أقل تركيز. تحت ظروف الصوبة خفضت كل المعاملات المختبرة بشكل معنوى من شدة الذبول الفيوزاريومى بأفضل تركيز من المستحثات والعوامل الحيوية، والمبيدات الحيوية والمبيد الكيماوى (توبسين -أم 70). وكانت المعاملة بالبيوزيد وحمض الساليسيليك هى الأكثر فاعلية والتي سجلت نفس شدة الإصابة 21.67%، يليه حمض الاسكوربيك، بيوناجي، ترايكودراما هاماتم، ترايكودراما هارزيانم والتي قللت شدة المرض ما بين 26.67 إلى 43.33%. في حين أن المبيد الكيميائي توبسين-أم 70 سجل أعلى انخفاض في شدة الاصابة ، 16.67% مقارنة بزيت الكمون الذى سجل 91,67% والذى يجب تجاهله. في التجارب الحقلية على نباتات الفلفل قلت الإصابة بمرض الذبول عند المعاملة بحامض الساليسيليك والبيوزيد يليها حامض الاسكوربيك، بيو ناجي و ترايكودراما هاماتم. كان استخدام حمض الساليسيليك وبيوزيد الأكثر كفاءة لزيادة الصفات المحصولية. لذلك يمكن اقتراح تطبيق البيوزيد وحامض الساليسيليك لأنها تعتبر من أكثر المعاملات كفاءة لمقاومة مرض الذبول وزيادة الصفات المحصولية وكان حامض الأسكوربيك، بيوناجي، ترايكودراما هاماتم من المعاملات المؤثرة فى هذا الصدد. وهذا الموضوع يحتاج لمزيد من الدراسات.