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Induction of systemic resistance against Tomato wilt disease under greenhouse conditions in Egypt

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Fusarium oxysporum is a widespread soil borne plant pathogen, which causes vascular wilt disease. The objective of the present work was to evaluate the inhibitory activity of some biocides and alternatives of fungicide on the growth of *F. oxysporum* the causal pathogen of wilt disease. In this study used the antagonistic activities of Trichoderma and bacterial isolates in controlling tomato wilt as well as other fungicide Topsin-M (70%), biocides (Bio-Arc, Bio-Zeid) and different inducers (dipotassium hydrogen phosphate, potassium sorbate, salicylic acid, ascorbic acid, clove oil, mint oil, chitosan) in vivo. Among all treatments in greenhouse studies showed that, Bio-Zeid and Bio-Arc were the most effective for controlling wilt disease. Meanwhile, *T. harzianum,* mint and ascorbic acid were the same effective for controlling wilt disease compared to control. This study suggested that the use of Bio-Zeid and Bio-Arc were the most effective for controlling wilt disease followed by *T. harzianum,* mint and ascorbic acid as safety alternative important treatments. Soaking roots of tomato seedling for two hours in most of biotic and abiotic inducers before transplanting in soil infested with the pathogenic fungi increased peroxidase, polyphenol oxidase and chitinase activities at 14 days post inoculation to values more than 7 days

Keywords: Wilt disease, *Fusarium oxysporum*, inducers, Biocides, Alternatives.

INTRODUCTION

Tomatoes (Solanum lycopersicum L., syn., Lycopersicon esculentum Mill) are an important crop of the plant not only for its economic value but also for its nutritional value. It has antioxidant compounds, such as vitamin C and carotenoids, which are grown in all countries in both protected fields and culture. Egypt production of tomato was placed fifth with a global tomato production, which constituting 4.86% of all global production in 2014, as well as, it lead the Africa tomato production, which was 43.11% of total global production. Recently, tomato production of Egypt was increased and reached 8.3 million ton from 528854.94 feddan with average production of 15.69 ton/ feddan (FAO, 2017).Tomatoes play an important role in human health because it is a rich source of lycopene, which is used in the treatment of prostate cancer (Giovannucci, 1999). They are consider one of the most important plant crops in Egypt and are used in food and industry (Abdel Karim et al., 2006). Tomato plants are infected with many fungal pathogens; wet conditions increase disease attacks and affect fruit maturity (Mersi et al., 2009; Abdel Moneim, 2010). Fusarium oxysporium can cause severe damage to tomatoes, and is very difficult to control because the fungus is carried by the soil and the seeds that carry it and continue in the soil. (Dohroo, 1988). Controlling such diseases mainly depend on fungicides treatments (Rauf, 2000). However, applications of fungicides cause risk to human health and increase environmental pollution. Therefore, Alternative to other fungicides control methods i.e., biological control agents, plant resistance factors and essential oils were used to control the plant fungal diseases due to the dangerous and toxic fungicides to all leaving organisms and environmental pollution (Punja et al., 2017; Ragab et al., 2009; Hammer et al., 1999). The aim of this study is to control the wilt disease on tomatoes using biotic and abiotic treatments used in the laboratory and greenhouse.

MATERIALS AND METHODS

Isolation and identification of wilt disease pathogen:

Samples of diseased tomato were collected from different locations at different governorates throughout Egypt for isolation the causal pathogen of wilt disease. The purified fungi were identified according to their morphological characters using microscope (Leslie and Summerell 2006; Nelson, 1983).

Pathogenicity test of the isolated fungi under greenhouse conditions: The pathogenic potential of 18 isolated fungi *Fusarium oxysporum* was tested on tomato susceptible cultivar (Super Strain B.) in pot experiment under greenhouse conditions at the Unit of Identification of Microorganisms, Plant Pathology Research Institute, Agricultural Research Center (A.R.C.), Giza, Egypt. Three tomato seedlings 4-5 weeks old were transplanted into plastic pots containing infested soil with *F. oxysporum* isolates. The wilt disease incidence was carried out using a visual 1- 6 scale according to (Silva and Bettiol, 2005).

Isolation of the antagonistic microorganisms:

The antagonistic microorganisms (fungi and bacteria) were isolated from healthy tomato plants rhizosphere which collected from different locations at different governorates throughout Egypt. The most effective antagonistic bacteria and fungi isolates were purified and identified using Biolog-System technique (Bochner, 1989; Jones et al., 1993; Harris and Gudmested 1996) belonging to the Identification of Microorganisms Unit, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Effect of biocides, fungicide, and the most effective bioagent isolates, against *F. oxysporum* on tomato plants (Super strain B cv.) under greenhouse conditions.

The experiment was carried out to study the antagonistic activity of the best effective bacterial and fungal isolates, which tested in vitro to evaluate their ability to protect tomato plants (Super Strain B cv.) against infection with wilt disease caused by F. oxysporum was grown on sorghum grain sand medium (Akram and Anjum, 2011; Sneh et al., 1991). The antagonistic fungi, bacteria, biocides Bio Arc® and Bio Zeid® and fungicide Topsin-M were used as a recommended dose in Table (1). All tested treatments were applied as seedling root dipping for 2 h before transplanting in plastic pots at the rate of 3 seedling/pot following three replicates for each treatment along with check treatment (un- infested soil). The wilt disease incidence was carried out using a visual 1 - 6 scale according to (Silva and Bettiol, 2005).

Identification of the microorganisms

The most effective antagonistic bacteria and purified fungal isolates were identified using Biolog-System technique (Anonymous, 1993 and 2010), belonging to the Identification of Microorganisms Unit, PPRI, ARC, Giza, Egypt.

Effect of different inducers against *F. oxysporum* on tomato plants (Super strain B cv.) under greenhouse conditions.

Effect of soaking tomato seedlings in different inducers on controlling wilt disease under greenhouse conditions. Tomato seedlings (cv. Super Strain B) were soaked for 2 hours before planting (Abdel-Monaim et al., 2012) in the best concentration of each tested inducers dipotassium hydrogen phosphate and potassium sorbate 8.0%, chitosan 0.1%, salicylic acid, and ascorbic acid 0.1%, as well as the clove and mint 0.5%. Tomato seedlings were planted in infested soil. The wilt disease incidence was carried out using a visual 1-6 scale according to (Silva and Bettiol, 2005).

Determination of oxidative enzymes and hydrolytic enzymes in tomato root under greenhouse conditions.

Samples from the most effective treatments in decreasing disease severity from each group such as (T14) *Trichoderma koningii* and (T15) *Trichoderma harzianum,* (B5) *Acinetobacter calcoaceticus*, (B40) *Acinetobacter genospecies,* salicylic acid, ascorbic acid, dipotassium hydrogen phosphate, chitosan, clove oil, mint oil, Bio- Arc®, Bio- Zeid® and Topsin-M (70%) in addition to the infected and uninfected control that were chosen for assaying the different oxidative enzymes (peroxidase, polyphenol oxidase) and hydrolytic enzymes (chitinase). The collected root tissues from each particular treatment at 7 and 14 days post inoculation with the different materials were homogenized immediately using liquid nitrogen Ojha and Chatterjee (2012). One gram of powdered sample was extracted with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged at 4°C for 20 min at 4000 rpm. The crude extract was used to estimate the peroxidase, polyphenol oxidase and chitinase activities (Anand et al. 2007).

Peroxidase activity (PO)

Peroxidase assay (based on oxidation of pyrogallol to purpyrogallin in the presence of H2O2) was determined according to (Hartee, 1955). Enzyme activity was expressed as the change in the absorbance of the reaction mixture min-1 g-1 on a fresh weight according to (Hammerschmidt et al., 1982).

Polyphenol oxidase activity (PPO)

Activity of PPO was determined following (Mayer *et al.* 1965) method. The activity was expressed as changes in absorbance at 495 nm min-1 g-1 fresh weight of tissue.

Chitinase activity

Chitinase activity was assayed according to (Miller, 1959). The enzyme activity was expressed as µmoles GLc NAc g/mL at 575 nm-1 g-1 fresh weight of tissue.

Preparation of Dinitrosalicylic (DNS) Reagent

Quantities of 1 g of DNS, 200 mg of crystalline phenol and 50 mg of sodium sulphite were dissolved simultaneously in 1% solution of NaOH by stirring. The reagent was stored in a stopper bottle at 4°C. The reagent deteriorates during storage due to atmospheric oxidation of the sulphite present.

Statistical analysis:

Statistical analysis were carried out using F-test for significance at p≤0.05 and computing of Least Significant Difference (LSD) test, values to separate means in different statistical groups according to described method by (Sneddecor and Cochran 1980).

Table 1. Active ingredients of fungicide, biofungicides, chemical structure inducers and the most active *Trichoderma* isolates.

Tested products	Active ingredients	Rate
Bio Arc®	*Bacillus megaterium* 6% (w/w)	2.5 g/L
Bio Zeid®	*Trichoderma album* 2.5 % (w/w)	2.5 g/L
Topsin-M (70%)	Thiophanate-Methyl	1.5 g/L
Salicylic acid	C7 H6 O3	0.05-0.1%w/v
Ascorbic acid	C6H8O6	0.05-0.1%w/v
Dipotassium hydrogen phosphate	K2 H PO4	2.0- 8.0 %w/v
Potassium sorbate	C6H7KO2	2.0- 8.0 %w/v
Chitosan	Natural polysaccharide	0.1-0.8 %w/v
Mint	Menthol, 48%	0.5% w/v
Clove	Eugenol, 90–95%	0.5% w/v
Trichoderma koningii	Fungi	1x107spore/ml
Trichoderma harzianum	Fungi	1x107spore/ml
Acinetobacter calcoaceticus	Bacteria	108 cfu cell/ml
Acinetobacter genospecies	Bacteria	108 cfu cell/ml

RESULTS

Isolation and identification of wilt disease pathogen:

The isolated fungi, which showed wilt symptoms of tomato plants collected from various locations at different twelve governorates, were purified and identified as *F. oxysporum*.

Pathogenicity test of the isolated fungi under greenhouse conditions:

Tables (2) showed that, all tested pathogenic fungi isolate of *F. oxysporum* were clearly varied in their virulence onto tomato plants under greenhouse conditions. The isolate no.14 from (Kleen) was more significantly aggressive than the other isolates which gave the highest disease severity of yellow and wilt (5.66). While the least virulent isolate was isolate no.1 (Sadaat) which gave the lowest disease severity of yellow and wilt (2.33).

Effect of biocides, fungicide, and the most effective bio agent isolates, against *F. oxysporum* on tomato plants (Super strain B cv.) under greenhouse conditions.

Data in the Table (3) indicated that treated tomato seedling with bacterial isolates decreased the disease severity of wilt disease compared to control. B5 and B40 were the most effective for controlling wilt disease which recorded the least disease severity (3.33). However, T15 was the highly effective antagonists in controlling wilt disease since it recorded the least disease severity (3.0), followed by T14 which recorded (3.33). On the other hand, among all treatments, Bio-Zeid and Bio-Arc recorded the least disease severity which being 2.0 and 2.33, respectively compared with fungicide Topsin-M was recorded 1.33.

Effect of different inducers against *F. oxysporum* on tomato plants (Super strain B cv.) under greenhouse conditions.

Results in the Table (4) indicated that dipotassium hydrogen phosphate and potassium sorbate at 8% concentration were effective in controlling the wilt disease where they recorded 3.66 and 5.33, respectively. While salicylic acid and ascorbic acid at 0.1% concentration which recorded 3.66 and 3.0, respectively. In addition to plant oils clove, and mint at 0.5% concentration recorded that 3.66 and 3.0, respectively. Meanwhile, chitosan at 0.1% concentration recorded that 4.0 compared to control.

Table 2. Pathogenicity test of *F. oxysporum* on Tomato plants under greenhouse conditions using scale (0-6).

Isolates	Governorate	Localities	DSI1	VG2
1	El-Monofia	Sadaat	2.33	L
2	El- Faiuom	Faiuom	3	L
3	El- Behaira	Etay-El Baroud	4.33	M
4	El-Minya	Samalout	3.33	L
5	El-Sharkia	Menia-Elamh	3.66	M
6	El-Monofia	Shebin -El kom	3.66	M
7	El-Sharkia	Hehia	3.66	M
8	El-Minya	Dayrout	3.33	M
9	Giza	Bdrasheen	3.33	M
10	Giza	Giza	4	M
11	El wady- El Gadid	El wady- El Gadid	4	M
12	Beni-Swief	Beni-Swief	3.66	M
13	Beni-Swief	Naser	3.66	M
14	Kafr-El Sheikh	Kleen	5.66	H
15	Kafr-El Sheikh	Sakha	4.66	H
16	El-Garbia	Tanta	5.33	H
17	El-Qualubia	Miet-Kinana	4.33	M
18	El-Qualubia	Toukh	3.33	L
Control			1	NP
L.S.D at 5% (0.947)				

[1]Disease severity index (DSI),[2]Virulent group (NP = Nonpathogenic, L = low, M = moderate, H = high).

Table 3. The effect of biocide, fungicide, bacterial and Trichoderma isolates on the growth of *F. oxysporum* causing wilt of tomato seedling under greenhouse conditions using scale (1-6).

Isolate No.	DSI
B5	3.33
B7	3.66
B38	4.33
B49	4.33
B40	3.33
T3	3.66
T8	3.66
T10	4.33
T14	3.33
T15	3.0
Bio-Arc	2.33
Bio-Zeid	2.0
Topsin-M (70%)	1.33
Control infect	5.5
Control uninfected	0
L.S.D at 5%	0.294

Table 4. The effect of different inducers on the growth of *F. oxysporum* causing wilt of tomato plants under greenhouse conditions.

Inducers		DSI
Salt	Di Potassium hydrogen phosphate	3.66
	Potassium sorbate	5.33
Acid	Salicylic acid	3.66
	Ascorbic acid	3.0
Plant Oils	Clove	3.66
	Mint	3.0
Natural polysaccharide	Chitosan	4.0
Control infected		5.5
Control uninfected		0
L.S.D at 5%		0.383

Identification of the antagonistic microorganisms.

The most effective antagonistic bacteria and fungi isolates were identified as *Acinetobacter calcoaceticus* (B5), *A. genospecies* (B40), *Trichoderma koningii* (T14) and *T. harzianum* (T15) by using Biolog-System technique.

Enzymatic Studies

Effect of soaking tomato roots with biotic and abiotic resistance inducers on enzyme activities in soil infested with *F. oxysporum* under greenhouse conditions

In greenhouse conditions trial, tomato roots were soaked in suspensions of biotic and abiotic inducers and transplanted in pots infested with *F. oxysporum*. The activities of oxidative enzymes; peroxidase, polyphenol oxidase, and hydrolytic enzyme (Chitinase) were determined 7 and 14 days after soaking with inducers. Data in Table (5) revealed that, treated tomato roots with biotic and abiotic inducers resulted an increase in peroxidase activity compared with untreated ones. The highest increased of peroxidase activity was recorded at 7 days in infected tomato plants with *F. oxysporium* and treated with salicylic acid followed by Bio-Zeid®, *T. harzianum* and Mint oil compared with other treatments. On the other hand, the maximum increased of peroxidase activity at 14 days was recorded in infected tomato plants and treated with Bio-Zeid® followed by Topsin-M (70%) and Bio-Arc®. Meanwhile, the least activity of peroxidase was recorded with *T. koningii* compared other treatments. Meanwhile, the least activity of peroxidase was recorded with chitosan.

2. Polyphenol oxidase activity

Data in Table (6) indicated that the maximum increase in polyphenol oxidase activity was recorded at 7 days in inoculated tomato roots with *F. oxysporium* and treated with salicylic acid followed by mint oil and ascorbic acid. Meanwhile the maximum increase in polyphenol oxidase activity was recorded at 14 days in treated tomato roots with and soaked with Bio-Zeid® treatment followed by ascorbic acid and Bio-Arc®. On the other hand, the least activity of polyphenol oxidase was recorded at 7 and 14 days in infected roots with *F. oxysporium* and soaked with *A. calcoaceticus* and *A. genospecies*, respectively compared with other treatments.

3. Chitinase activity.

Data in Table (7) reveal all biotic and abiotic agents increased the chitinase activity after 14 days in roots inoculation with *F. oxysporium* more than those after 7 days of inoculation. The maximum increased in chitinase activity was recorded at 7 days in inoculated roots with *F. oxysporium* and treated roots with Bio-Zeid®, and clove oil. Meanwhile salicylic acid, dipotassium hydrogen phosphate and Topsin-M (70%) were the best treatments which recorded the maximum increased in chitinase activity after 14 days from roots inoculated with *F. oxysporium.*

Table 5. Effect of different biotic and abiotic inducers on peroxidase activity of tomato roots inoculated with *F. oxysporium*.

Treatment	Enzyme activity (as optical density)	
	7d	14 d
Acinetobacter calcoaceticus	3.838	3.257
A. genospecies	5.037	3.277
Trichoderma koningii	3.662	3.231
T. harzianum	5.330	3.522
Dipotassium hydrogen phosphate	4.267	4.088
Salicylic acid	6.431	3.562
Ascorbic acid	3.415	4.454
Clove oil	3.672	3.685
Mint oil	5.132	4.518
Chitosan	2.877	3.642
Bio-Arc®	4.403	5.200
Bio-Zeid®	5.928	5.862
Topsin-M (70%)	4.385	5.477
Control infected	3.908	3.077
Control uninfected	2.800	2.446
L.S.D at 5%	0.503	0.261

Table 6. Effect of different biotic and abiotic inducers on polyphenol oxidase activity in inoculated roots with *F. oxysporium*.

Treatment	Enzyme activity (as optical density)	
	7d	14 d
Acinetobacter calcoaceticus	0.837	0.554
A. genospecies	1.846	0.300
Trichoderma koningii	1.515	1.423
T. harzianum	1.952	1.315
Dipotassium hydrogen phosphate	2.561	3.900
Salicylic acid	3.897	2.782
Ascorbic acid	3.105	4.387
Clove oil	1.972	3.187
Mint oil	3.537	3.774
Chitosan	1.300	1.892
Bio-Arc®	2.195	4.185
Bio-Zeid®	3.017	5.064
Topsin-M (70%)	2.141	3.513
Control infected	1.321	1.079
Control uninfected	0.500	0.315
L.S.D at 5%	0.139	0.129

Table 7. Effect of different biotic and abiotic inducers on chitinase activity in inoculated roots with *F. oxysporium* at different periods under greenhouse conditions

Treatment	Enzyme activity	
	7d	14 d
Acinetobacter calcoaceticus	0.0071	0.0212
A. genospecies	0.0092	0.0398
Trichoderma koningii	0.0057	0.0172
T. harzianum	0.0107	0.0265
Di Potassium hydrogen phosphate	0.0271	0.0691
Salicylic acid	0.0146	0.0811
Ascorbic acid	0.0146	0.0434
Clove oil	0.0354	0.0363
Mint oil	0.0336	0.0376
Chitosan	0.0132	0.0310
Bio-Arc®	0.0172	0.0336
Bio-Zeid®	0.0429	0.0438
Topsin-M (70%)	0.0412	0.0460
Control (infected)	0.0261	0.0279
Control (uninfected)	0.0163	0.0279
L.S.D at 5%	0.0075	0.0048

Meanwhile, the least activity of chitinase was recorded at 7 and 14 days in infected roots with *F. oxysporium* and soaked with *T. koningii* compared with other treatments.

DISCUSSION

Fusarium oxysporum is a pathogenic plant disease that spreads widely, which causes vascular wilt disease. The pathogenic causal factors of tomato wilt were isolated from the sterilization of the surface of the diseased tomato plant. These results correspond to with (Pasco et al., 2017) who reported Fusarium wilt of tomato from F. oxysporum f. sp. lycopersici is a serious problem limiting the tomatoes production all over the world. Twenty-three isolates from F. oxysporum, from tomato plants showing wilt symptoms in different areas in Dakahlia Governorate, Egypt (Leslie and Summerell 2006). The pathogenicity test was performed on tomato plants under greenhouse conditions, indicating that all tested F. oxysporum isolates have a pathogenic effect and vary in their virulence against tomato plants. The isolation of Kleen-14 was significantly more aggressive than other isolates that gave the highest severity of the disease while Sadaat-1 isolation was at least one. This agreement with (Nusret and Steve 2004) which suggested fungus tested F. oxysporum f. sp. lycopersici, have a pathogenic effect on tomatoes which is 31.3% disease severity. Bio-Zeid and Bio-Arc the most effective for controlling wilt disease which recorded the least disease severity. However, Trichoderma harzianum, T. koningii, Acinetobacter calcoaceticus and A. genospecies were the highly effective bioagent treatments which recorded the lowest disease severity. The results obtained in the greenhouse are consistent with those obtained Ismail (2017 unpublished), who reported that the tested biocides (Bio-Zeid, Bio-Arc) were able to inhibit growth F. solani. Seed treatment with biocides reduced the percentage of root rot disease when tomatoes occurred compared to control. Bio-Zeid was most effective compared to other treatments. Biological control has emerged as one of the most promising alternatives to the fungicides. Biological management of Fusarium wilt of tomato using biological control agents (B.C. As) i.e. Trichoderma harzianum (Basco et al., 2017), meanwhile (Wahba et al., 2016) reported that T. harzianum recorded 100% growth reduction on Fusarium semitectum. The most effective bacteria and fungi were identified from healthy tomato plants in different populations throughout Egypt, such as Acinetobacter calcoaceticus, A. genospecies, Trichoderma koningii and T. harzianum using Biolog-system. These results in agreement with (Bochner, 1989; Druzhinina et al., 2006) they indicated that the antagonistic bacterial and fungal isolates were subjected to characterization and identification according to their physiological characteristics using the Biolog system according to the ability of these isolates to utilize different carbon sours and amino acids. The results of (Khalifa et al., 2016; Ragab et al., 2017) emphasized these obtained results revealed that the fungal isolates were identified as Trichoderma harzianum. In greenhouse trial dipotassium hydrogen phosphate, salicylic acid and ascorbic followed by plant oils and chitosan were effective in controlling wilt disease. These results agreement with (Abdel-Moneim et al., 2011; Akram and Anjum 2011; El-Mohamedy et al., 2013; Gunes et al., 2007; Sharma et al., 2016) they reported that, application of chemical inducers as resulted a great reduction in percentage of wilt disease of tomato plant under greenhouse conditions. Soaking tomato roots with different biotic and abiotic inducers might stimulate some defense mechanisms such as affecting the activity of oxidative enzymes (Peroxidase and Polyphenol oxidase) and hydrolytic enzymes (Chitinase), related to plant defense against pathogen infection F. oxysporium compared with infected and uninfected control at 7 and 14 days after inoculation with inducer treatments. These results are in agreement with those reported by (Yedidia et al., 1999; Ramamoorthy et al., 2002; El-Khallal, 2007; Latha et al. 2009; Abd-El-Khair et al., 2011; Seo et al., 2012 ; Surekha et al., 2014) they reported that, the use of Trichoderma spp., Bacillus spp., Pseudomonas spp. and Serratia marcensens as bioagents induction of defense enzymes such as chitinase, peroxidase and polyphenol oxidase, which play an important role in plant defense mechanisms against pathogens infection in treated bean plants. Also, biocides (Bio-Zeid, Bio-Arc and Plant guard), and plant extracts (Eucalyptus globules, Allium cepa and Mentha viridis) gave the highest increase in peroxidase, polyphenloxidase and chitinase activity (Ayoubi and Soleimani 2015; Ismail 2017). Oxidative enzymes such as peroxidase and polyphenol oxidase enhance formation of lignin, oxidation of phenols to more toxic quinones, while other oxidative phenols contribute in formation of defense barriers for reinforcing the cell structure (Conti et al., 1974; Yamamoto and Tani 1978; Arora, 1979; vdiushko et al., 1993). Peroxidase activity was recorded the highest increased by inoculated with F. oxysporium and soaking tomato roots with (salicylic acid, Bio-Zeid®, T. harzianum and Mint oil) and (Bio-Zeid®, Tobssin and Bio-arc®) at 7 and 14 days after inoculation with inducer treatments, respectively. Meanwhile, the least activity of peroxidase was recorded with T. koningii compared other treatments. Results obtained in the current study in agreement with those reported by Yoshida et al., (2003) who reported that peroxidase plays an important role in biosynthesis of ethylene, regulation of auxin and plant cell wall components viz., lignin, suberin and wall thickening as part of defense response to pathogens particularly fungi. Peroxidases are involved in the lignification, polymerization of hydroxy proline-rich glycoproteins, and regulation of cell wall elongation. The maximum increase in polyphenoloxidase activity was recorded by infected soil with F. oxysporium and soaking tomato roots in (salicylic acid, mint and ascorbic acid) and (Bio-Zeid®, ascorbic acid and Bio-Arc®) at 7 and 14 days after inoculation with inducer treatments, respectively. On the other hand, the least activity of polyphenol oxidase was recorded at 7 and 14 days in infected roots with F. oxysporium and soaked with A. calcoaceticus and A. genospecies, respectively compared with other treatments. These results agreement with (Avdiushko et al., 1993; Yedidia et al., 2003; Newman Sally et al., 2011) they mentioned that polyphenol oxidase involved in the lignification and catalyzing the oxygen dependent oxidation of phenols to quinines, protection of tissue from damage and infection by pathogenic fungi. Chitinase activity was increased considerably with the increase in the inoculation period. The highest value of enzyme activity was recorded up to the 14 days in infected tomato plants with F. oxysporum upon treatment with salicylic acid, dipotassium hydrogen phosphate and Topsin-M (70%) These results are in line with (Schlumbaum et al. 1986; Ham et al., 1991; Leah et al., 1991; Velazhahan et al., 2003) they reported that chitinases hydrolyze chitin which is major component of fungal cell walls, leading to direct inhibition of growth of several fungi. Chitosan affects various physiological responses like plant immunity, defense mechanisms involving various enzymes such as, polyphenol oxidase, tyrosine ammonia lyase and antioxidant enzymes (Katiyar et al., 2015).

CONCLUSION

The present study showed that Bio-Zeid and Bio-Arc were the most effective for controlling tomato wilt followed by *Trichoderma harzianum*-T15, mint and ascorbic acid. Biological control and abiotic inducers is a promising alternative to synthetic fungicides and is now becoming a critically needed component of integrated disease management system. Enzymes activity was increased Peroxidase, Polyphenoloxidase and Chitinase activities at 14 days post inoculation to values more than 7 days.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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