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Effect of Certain Biocides on Fusarium Wilt Disease and Their Effect on Gene Expression Alterations in Tomato Plants

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Abstract

This study evaluated the efficacy of two commercial biocides*, i.e.* Bio-Zeid (*Trichoderma album*) and Bio-Arc (*Bacillus megaterium*) against *Fusarium* wilt disease of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*) and *F. solani* (*FS*). Application of these biocides as soil treatment significantly (P=0.05) reduced the percentage of the disease severity of tomato wilt caused by both pathogens under greenhouse conditions at growing seasons 2016 and 2017 when compared to the control treatment. Untreated and treated infected tomato plants (Super strain B cv.) with the tested products were subjected to study the alterations in gene expression using protein profile and four different isozymes(esterase, peroxidase, chitinase and Superoxide Dismutase isozyme) systems. Treated tomato plants showed differences in protein and isozymes banding patterns compared to untreated plants. Two new protein bands at molecular weight 47.2 KD and 16.3 KD were appeared in all infected plants treated with Bio-Zeid and Bio-Arc while, not appeared in control plant. This indicates that these treatments were able to induced new protein bands which increase resistance in tomato plants to *Fusarium* wilt infection. All treatments were able to induced new protein bands patterns which increasing resistance to *Fusarium* wilt, but treatment with Bio-Arc was the best because it induced the higher numbers of new protein bands. At the same time, new esterase band (EST-5a), peroxidase (PRX-4a), chitinase (Chit-5b) and Superoxide Dismutase isozyme (SOD-1a) bands were detected in all treatments while not appeared in the control.

Keywords: *Fusarium wilt, Tomato, Biological control, Trichoderma album, Bacillus megaterium, Protein electrophoresis, Isozymes.*

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop in Egypt and the world and it's the second most consumed vegetable crop next to potato (Srivastava *et al.,* 2010). *Fusarium* wilt disease is a destructive disease of tomato in major growing regions worldwide and causes severe yield loses in field and greenhouse (Girhepuje and Shinde, 2011; Bawa, 2016). This disease caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium solani* and reduces yield in this crop (Ajilogba and Babalola, 2013 and Manikandan *et al*., 2018).

Bio-control agents can induce resistance in plants against many pathogens such as fungi (Bokhari and Perveen, 2012), viruses (Maurhofer *et al.,* 1994) and bacteria (Park and Kloepper, 2000). Jayalakshmi *et al.* (2009) reported that the activity of

both polyphenoloxidase and peroxidase enzymes increased after the treatment with *T. harzianum*. Mohamed *et al.* (2008) found that application of Rhizo-N, Plant guard and culture filtrate of *T. harzianum* and *Chaetomium globosum* as a soil treatment or seed dressing in pot trials increased cumin seed germination and reduced the incidence of cumin wilt disease. The application of *B. subtilis* to the soil increased peroxidases activity in tomato plants at a level comparable to spraying acibenzolar-S-methyl (Araujo and Menezes, 2009).

The ability of Trichoderma to reduce diseases caused by soil borne pathogens is well known and it is related to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogenic fungi and competition for limiting factors in the rhizosphere mainly iron and carbon (Sivan and Chet, 1986 and Benitez *et al*., 2004). Bio-Arc, Bio-zeid and salicylic acid as a foliar treatment or seed soaking lead to provide protection of alfalfa plants against downy mildew, rust, root rot and wilt disease (Morsy *et al*., 2011). Another mechanism has been suggested by Kleifeld and Chet (1992) who found that Trichoderma induced resistance in host plants to fungal attack. There are several evidences which supported that *Trichoderma* spp. were able to induce the defense mechanisms in several plants (Vinale *et al.,* 2008; Brotman *et al.,* 2012). Yedidia *et al.,* (1999) showed that *Trichoderma* initiated increase in peroxidase and chitinase activities.

The objectives of this study were to evaluate the efficacy of Bio-Zeid and Bio-Arc as soil treatment to control *Fusarium* wilt disease of tomato and their effect on gene expression alterations using protein and certain isozymes profiling.

Materials and Methods

Isolation of causal fungal pathogens:

Two isolates of *F. oxysporum* f. sp. *lycopersici* (1 and 2) and *F. solani* isolates (1 and 2) used in this study were isolated from tomato plants, showing typical symptoms of *Fusarium* wilt disease as mentioned by El-Fawy and Ahmed (2015). Identification of the fungal isolates of Fusarium species was carried out by using the morphological characteristics of mycelia and spores as described by Nelson *et al.* (1983) and Leslie and Summerell, (2006) then confirmed by Assiut University Mycological center (AUMC). Pure cultures of all identified fungi were maintained at 4°C PDA slants until use.

Pathogenicity tests

Inocula of the isolated fungi were prepared by Inoculation sterilized bottle 500 ml, containing barely medium (75 gm barely + 25 gm clean sand + 2 gm sucrose + 0.1 gm yeast + 100 ml water) and autoclaved at 121°C for 20°C minute on two consecutive days (Abd El-moneem, 1996). Two isolates of *FOL* (1 and 2) and two isolates of *FS* (1 and 2) were taken from 5-7 days old cultures on PDA medium was aseptically introduced into the bottle and allowed to grow for 3 weeks. Pathogenicity test was carried out by using autoclaved clay loam soil. Batches of autoclaved soil were infested separately with inoculums of

each isolate at the rate 1% (10 gm/kg) of soil dispersed in 30 cm diameter earthern pots, and then pots were planted with four transplantings of tomato from both cultivars (Super Jakal and 745). Four replicates were used for each treatment. In the control treatment sterilized barely medium was mixed with the soil at the rate 1% (10 gm/kg) of soil. Disease severity was recorded after 90 days of transplanting. Disease severity of wilted plants was recorded as described by (Paz-Lago *et al.,* 2000) using the following scale of 0-5 degrees: 0=foliage or not damaged root, 1= wilting or damaged root (20%), 2= wilting or damaged root (40%), 3= wilting or damaged root (60%), 4= wilting or damaged root (80%) and 5= wilting or damaged root (100%) or plant death.

Disease severity (%) = $\sum$ [(n × V) /5×N)] × 100

where, n= number of plants in each infection category, V= numerical values of infection categories, N= total number of plants examined and 5= constant, the highest numerical value.

Effect of Bio-Zeid and Bio-Arc as soil treatment on *Fusarium* wilt disease of tomato under greenhouse conditions:

This experiment was carried out in 2016 and 2017 in the greenhouse of Plant Pathology Dept., Faculty of Agriculture, Assiut University. Inocula of *FOL* (1 and 2) and *FS* (1 and 2) were grown on barley medium (150g barley + 50 g clean sand + 4 g glucose + 0.2 g yeast extract + 200 mL water) in 500-mL flasks and incubated at 25°C for 15 days (Abd El-moneem,1996). Sterilized earthern pots (30 cm in diameter) were filled with sterilized soil and infested by the isolates at the rate of 1% (w/w) one week before planting. Each pot was sown with four transplantings of tomato cultivar (Super Strain B). Two commercial biocides namely, Bio-Zeid (*Trichoderma album*) 10×10^8 spores/g and Bio-Arc (*Bacillus megaterium*) 25×10^8 CFU/g obtained from Biological Control Unit, Plant Pathology Institute preparation contains active ingredient biocides to the recommendations of the Ministry of Agriculture, Egypt. These biocides are produced by the local company producing the organic bio-technology. These biocides were added to the soil pots with irrigated water at concentration of 1 g/L water. These treatments were applied twice (two weeks between each treatment). Four pots were used for each treatment as replicates and untreated pots with the isolates were used as control. Disease severity was recorded after 90 days of transplanting tomato plants as mentioned before.

Biochemical analysis

Proteins or enzymes were extracted using the method reported by (Maria and Gache,2004) by crushing 1.0 g of sample tissue (2-3 leaves from each of control and treatment plants) in 1.0 ml extraction buffer (0.1 M Tris-HCl + 2 mM EDTA, pH 7.8). Then, the samples centrifuged for 25 minutes at 10.000 rpm and 4ºC. 100µl of the supernatant was mixed by 100µl of loading dye for isozymes the samples loaded directly in the electrophoresis. Meanwhile, protein samples were incubated in water path at 100 ^{o}C for five minutes for protein denaturation,

then loaded in the electrophoresis. 100 µl per sample are then loaded directly into the wells with a syringe. Electrophoresis was performed at a constant voltage of 100 V initially for 1 h and 180 V to complete electrophoresis.

Isozymes Staining

The staining protocols were according to Guidkema and Sherman (1980) for peroxidase (E.C.1.11.1.7); for esterase (E.C.3.1.1.2) according to (Tanksley and Orton, (1986); for Superoxide Dismutase (SOD, E.C. 1.15.1.1) was according to Wang, (1996) and Chitinase (E.C.3.2.1.14) Staining gel was done according to (Shen *et al.,* (1991).

Protein staining

Proteins were staining 4 hours with Comassie Brilliant Blue (0.025% Comassie Brilliant Blue R-250, 40% methanol and 7% acetic acid). Then, gels were distained for one hour in a mixture of 50% methanol and 10% acetic acid. Thereafter, the gels transferred to a destainer filled with 7% acetic acid and 5% methanol until the background is clear.

Biochemical data analysis

For isoenzymes: The number of isozyme bands was recorded and their relative mobility (RF) was calculating according to the formula:

$$RF = \frac{\text{Distance travelled by the band}}{\text{distance traveled by the tracking dye}}$$

For protein: Data were obtained by scanning densitometer GS 300 (Hoffer) of protein profiles. The molecular weight of protein bands were determined against protein marker using GS 365 electrophoresis data system program version 3.01 (Microsoft Windows @ version).

Statistical analysis:

Data were analyzed using the MSTAT-C (1991) program version 2.10. Means of treatments were differentiated using Least significant difference (LSD) at P=0.05 (Gomez and Gomez, 1984).

Results

Pathogenicity tests of some Fusarium species isolates on Super Jakal and 745 cultivars under greenhouse conditions during growing.

Data presented in Table (1) show that all the tested isolates of *FOL* and *FS* were able to infect tomato Super Jakal and 745 cultivars and produced typical symptoms of Fusarium wilt disease. The tested isolates significantly reduced the disease severity percentage of (P = 0.05) compared to the control. The highest percentage of disease severity was caused by *FOL* isolate No. 1 on Super Jakal cultivar (78.10%), followed by *FS* isolate No. 2 (43.75%). Meanwhile, *FOL* isolate No. 2 (28.10%) followed by *FS* No. 1 (31.25%) on 745 cultivar.

Table 1. Pathogenicity test of *FOL* and *FS* isolates on Super Jakal and 745 cultivars during growing seasons under greenhouse conditions.

Isolates No.	Disease severity %	
	Super Jakal	745
FOL (1)	78.10	37.50
FOL (2)	34.30	28.10
FS (1)	40.60	31.25
FS (2)	43.75	34.37
Control	0.00	0.00
L.S.D. at 5% for: Isolates (I) Cultivars (C) Interaction (I×C)	10.03 6.35 14.19	

Effect of soil application with Bio-Zeid and Bio-Arc on Fusarium wilt disease in tomato under greenhouse conditions during growing seasons 2016 and 2017.

Results presented in Table (2) indicate that all tested two biocides (Bio-Zeid and Bio-Arc) significantly reduced the percentage of disease severity of tomato wilt disease caused by *FOL* and *FS* both tested seasons compared to the control. Bio-Arc showed higher decreased in percentage of disease severity than Bio-Zeid which it recorded (25 and 9.30%) and (29.10 and 11.70%) with *FOL* and *FS* during growing seasons 2016 and 2017 respectively. During both tested seasons Bio-Zeid was the lowest effect in reducing the percentage of disease severity recorded (34.3 and 28.10%) and (32.20 and 25.7%) with *FOL* and *FS*.

Table 2. Effect of Bio-Zeid and Bio-Arc on Fusarium wilt disease of tomato under greenhouse conditions during growing seasons 2016 and 2017.

Treatments	Disease severity %											
	Season 2016						Season 2017					
	F. o. f. sp. *lycopersici*			*F. solani*			*F. o.* f. sp. *lycopersici*			*F. solani*		
	Iso.1	Iso. 2	Mean	Iso.1	Iso. 2	Mean	Iso.1	Iso. 2	Mean	Iso.1	Iso. 2	Mean
Bio-Zeid	35.37	33.30	34.33	31.22	33.30	32.26	28.10	28.10	28.10	26.52	24.97	25.74
Bio-Arc	25.00	25.00	25.00	27.07	32.27	29.67	18.72	20.30	19.51	23.42	23.40	23.41
Control	56.22	54.15	55.18	60.37	79.12	69.74	45.20	48.40	46.80	54.65	57.77	56.21
Mean	35.39	34.36	34.87	36.95	43.46	40.20	25.34	26.53	25.94	28.87	29.66	29.26
L.S.D. at 5% for Treatments (T) Fungi (F) Isolates (I) Interaction (T×F×I)			4.11 2.91 2.21 6.25						2.79 1.97 2.20 6.22			

Biochemical markers:

Protein profiling:

Protein profiling was done to determine whether some new protein was associated with induced resistance to pathogenic *FOL* (1 and 2) and *FS* (1 and 2) in resistance tomato genotype (Super strain B)

treated with Bio-Zeid and Bio-Arc. SDS-PAGE is used for finding the banding pattern of proteins. It has been found that the banding patterns of protein control and two treatments are variable (Table 3 and Figure 1). The SDS PAGE protein profile of total soluble protein from infected plants treated with two treatments showed differences in band pattern when compared with control. In general, control plant exhibit 20 protein bands, these bands were common in all treatments except protein banding at molecular weight 28.2 KD which disappear in all infected plants with *F. oxysporum* (1). Four Protein bands at molecular weight 47.2KD, 38.2KD, 16.3KD and 15.6KD appeared only in treated plants. Two new protein bands at molecular weight 47.2KD and 16.3 KD were appeared in all infected plants treated with Bio-zeid and Bio-Arc treatments while, not appeared in control plant. This indicates that the two treatments were able to induced new protein banding which increase resistance in tomato plants. New protein band at molecular weight 38.2 KD and 15.6 KD were detected in all plants treatment with Bio-Arec while not appeared in control and Bio-zeid treatment; this indicates that treatment with Bio-Arc increasing resistance to *Fusarium* infection through induced new protein patterns. These results revealed that all treatment were able to induced new protein banding patterns which increasing resistance to Fusarium, Bio-Arc treatment was the best because exhibited higher effect than Bio-zeid.

Table 3. The electrophoretic banding patterns of Protein

No.	MW	Cont.	Bio-Zeid				Bio-Arc			
			FOL (Iso.1)	*FOL* (Iso.2)	*FS* (Iso.1)	*FS* (Iso.2)	*FOL* (Iso.1)	*FOL* (Iso.2)	*FS* (Iso.1)	*FS* (Iso. 2)
1	63.5	+	+	+	+	+	+	+	+	+
2	61.6	+	+	+	+	+	+	+	+	+
3	57.1	+	+	+	+	+	+	+	+	+
4	52.1	+	+	+	+	+	+	+	+	+
5	47.2	-	+	+	+	+	+	+	+	+
6	44.1	+	+	+	+	+	+	+	+	+
7	42.2	+	+	+	+	+	+	+	+	+
8	38.2	-	-	-	-	-	+	+	+	+
9	37.0	+	+	+	+	+	+	+	+	+
10	35.1	+	+	+	+	+	+	+	+	+
11	33.2	+	+	+	+	+	+	+	+	+
12	32.3	+	+	+	+	+	+	+	+	+
13	28.2	+	-	+	+	+	-	+	+	+
14	26.4	+	+	+	+	+	+	+	+	+
15	24.1	+	+	+	+	+	+	+	+	+
16	20.2	+	+	+	+	+	+	+	+	+
17	18.8	+	+	+	+	+	+	+	+	+
18	17.1	+	+	+	+	+	+	+	+	+
19	16.3	-	+	+	+	+	+	+	+	+
20	15.6	-	-	-	-	-	+	+	+	+
21	14.8	+	+	+	+	+	+	+	+	+
22	12.8	+	+	+	+	+	+	+	+	+
23	10.1	+	+	+	+	+	+	+	+	+
24	7.4	+	+	+	+	+	+	+	+	+

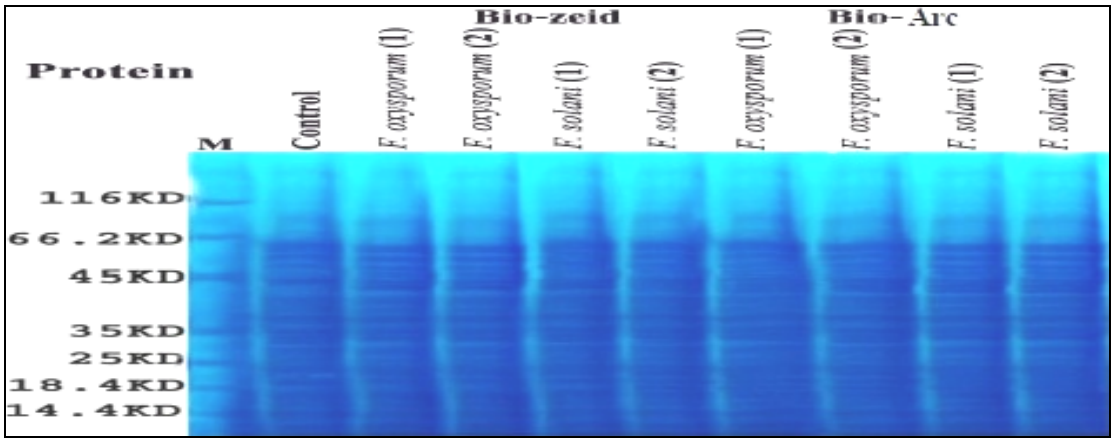

Fig. 1: Electrophoretic pattern of total soluble proteins detected in extracts of tomato plant (Super strain B), cultivated in infested soil with *FOL* (1 and 2) and *FS* (1 and 2) and treated with Bio-Zeid and Bio-Arc.

Biochemical isozyme markers:

Tomato infected plants with *F. oxysporum* (isolate 1 and 2), *F. solani* (isolate 1 and 2), *F. oxysporum* (2) and *F. solani* (2) showing variability in number, relative mobility and density of isozymes compared with control plants.

Esterase isozyme profiles (EST):

The total number of esterase isozymes was nine bands ranged from Rf = 0.32 (EST-1a) to RF= 0.95 (EST-7) in Table (4) and Fig (2a). Infected plants treated with Bio-zeid and Bio-Arc treatments showed variation in number bands compared with control. Seven esterase isozyme bands were common between the control and all infected plants treated with two biocides treatments. New esterase band EST-5a at Rf= 0.77 was appeared in all treatments and appear in the control. Isozyme band EST-1a at Rf= 0.32 appeared only in infected plants treated with Bio-Arc while not appeared in control and Bio-zeid treatment.

Peroxidase isozymes activity (PRX):

The total numbers of peroxidase isozymes were eight bands ranged from PRX-1 (Rf= 0.35) to PRX-8 (RF= 0.92) (Table 4 and Fig.2b). Five out of eight bands were common bands with percentage 62.5% at PRX-1 (Rf= 0.35), PRX -2 (Rf= 0.48), PRX-3 (Rf=0.77), PRX-4 (Rf= 0.85) and PRX-5 (Rf= 0.92) these bands related to tomato plants. Peroxidase isozyme band PRE-1a with Rf= 0.4 induced only in infected plants treated with Bio-Arc but not found in control and Bio-zeid treatment. New isozyme peroxidase bands PRX-2a (Rf= 0.53) and PRX-4a (Rf= 0.8) were detected in infected plants treated with both Bio-zeid and Bio-Arc treatments.

Chitinase isozymes activity (Chit.):

Table (3) and Figure (2c) represents numbers of Chitinase bands detected in tomato plants infected with *FOL* isolates No. 1 and 2 and isolates No. 1 and 2 of *FS* and treated with Bio-Zeid and Bio-Arc by electrophoretic analysis. Data show that different chitinase isozyme bands were found. A total of ten bands were detected with Rf ranged from 0.14-0.97, five of them were common bands between control and all treatments with percentage 50%, while the other bands 50% were

polymorphic bands. Both of Bio-zeid and Bio-Arc treatments induced three new chitinase bands Chit-1a (Rf= 0.23), Chit-5a (Rf= 0.83) and Chit-5b (Rf= 0.89). The two Chitinase bands Chit-1b (Rf= 0.31) and Chit-3a (Rf= 0.54) were expressed only in infected plants treated with Bio-Arc while not expressed in control and infected plants treated with Bio-zeid treatment.

Superoxide Dismutase (SOD, E.C. 1.15.1.1):

Superoxide Dismutase bands SOD-1 (Rf= 0.32), SOD-3 (Rf=0.6) and SOD-5 (Rf= 0.95) were monomorphic and common in control and all treatment plants. Superoxide Dismutase band SOD-1a at Rf= 0.47 was induced in all infected plants treated with both Bio-zeid and Bio-Arc treatments. New isozyme band SOD-3a at Rf= 0.72 was expressed only in infected plant treated with Bio-Arec (Table 4 and Fig. 2d).

Table 4. The electrophoretic banding patterns of four isozymes (EST, PER, Chit and SOD) in extracts of tomato plant (Super strain B), cultivated in infested soil with *FOL* isolates No. 1 and 2 and *FS* No. 1 and 2 and treated with Bio-Zeid and Bio-Arc.

No	IOSZYMES	Rf	Cont.	Bio Zeid				Bio Arc			
				FOL (Iso. 1)	*FOL* (Iso. 2)	*FS* (Iso. 1)	*FS* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)	*FS* (Iso. 1)	*FS* (Iso. 2)
1	EST-1a	0.32	-	-	-	-	-	+	+	+	+
2	EST-1	0.37	+	+	+	+	+	+	+	+	+
3	EST-2	0.42	+	+	+	+	+	+	+	+	+
4	EST-3	0.46	+	+	+	+	+	+	+	+	+
5	EST-4	0.68	+	+	+	+	+	+	+	+	+
6	EST-5	0.72	+	+	+	+	+	+	+	+	+
7	EST-5a	0.77	-	+	+	+	+	+	+	+	+
8	EST-6	0.89	+	+	+	+	+	+	+	+	+
9	EST-7	0.95	+	+	+	+	+	+	+	+	+
No	IOSZYMES	Rf	Cont.	Bio Zeid				Bio Arc			
				FOL (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)
1	PRX-1	0.35	+	+	+	+	+	+	+	+	+
2	PRX-1a	0.4	-	-	-	-	-	+	+	+	+
3	PRX-2	0.48	+	+	+	+	+	+	+	+	+
4	PRX-2a	0.53	-	+	+	+	+	+	+	+	+
5	PRX-3	0.77	+	+	+	+	+	+	+	+	+
6	PRX-4a	0.8	-	+	+	+	+	+	+	+	+
7	PRX-4	0.85	+	+	+	+	+	+	+	+	+
8	PRX-5	0.92	+	+	+	+	+	+	+	+	+
No	IOSZYMES	Rf	Cont.	Bio Zeid				Bio Arc			
				FOL (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)
1	Chit-1	0.14	+	+	+	+	+	+	+	+	+
2	Chit-1a	0.23	-	+	+	+	+	+	+	+	+
3	Chit-1b	0.31	-	-	-	-	-	+	+	+	+
4	Chit-2	0.37	+	+	+	+	+	+	+	+	+
5	Chit-3	0.48	+	+	+	+	+	+	+	+	+
6	Chit-3a	0.54	-	-	-	-	-	+	+	+	+
7	Chit-4	0.71	+	+	+	+	+	+	+	+	+
8	Chit-5a	0.83	-	+	+	+	+	+	+	+	+
9	Chit-5b	0.89	-	+	+	+	+	+	+	+	+
10	Chit-5	0.97	+	+	+	+	+	+	+	+	+
No	IOSZYMES	Rf	Cont.	Bio Zeid				Bio Arc			
				FOL (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)	*FOL* (Iso. 1)	*FOL* (Iso. 2)
1	SOD-1	0.32	+	+	+	+	+	+	+	+	+
2	SOD-1a	0.47	-	+	+	+	+	+	+	+	+
3	SOD-2	0.6	+	+	+	+	+	+	+	+	+
4	SOD-3a	0.72	-	-	-	-	-	+	+	+	+
5	SOD-3	0.95	+	+	+	+	+	+	+	+	+

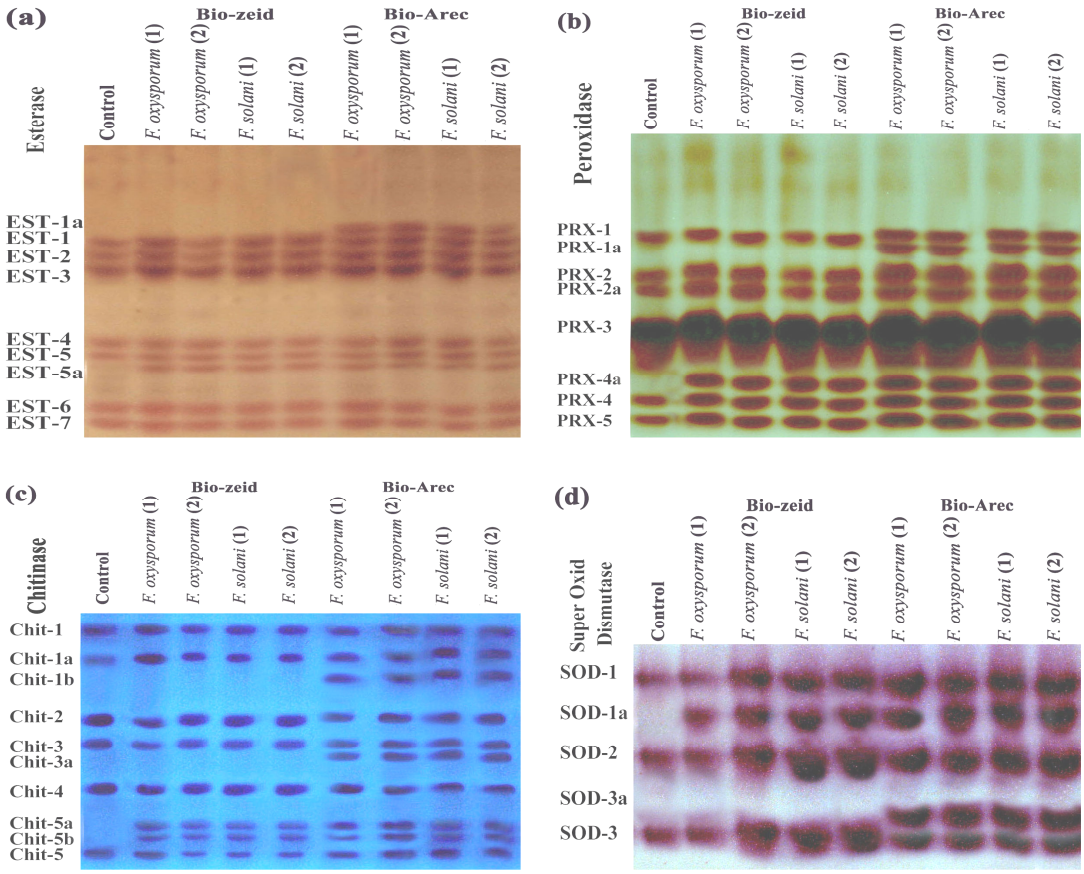

Fig. 2: Electrophoretic pattern of esterase, peroxidase, chitinase and Super Oxide Dismutase isozymes detected in extracts of tomato plant (Super strain B), cultivated in infested soil with *FOL* No. 1 and 2 and *FS* No. 1 and 2 and treated with Bio Zeid and Bio Arc.

Discussion

Tomato wilt disease caused reduction in weight of tomato fruits and productivity (Dursun *et al.,* 2010). This disease is an economically important and a destructive disease of tomato crop in all production areas (Jones *et al.,* 1991 and Jabnoun-Khiareddine *et al.,* 2016).

Application of Bio-Zeid (*Trichoderma album*) and Bio-Arc (*Bacillus megaterium*) to infested soil with pathogenic fungi (*Fusarium oxysporum* f. sp. *lycopersici* or *F. solani*) has a beneficial effect, when added to soil with irrigated water. Generally, soil amendment with these bio-control products was effective in decreasing *Fusarium* wilt disease symptoms on tomato plants. These results are consistent with those reported by Ibrahim (2015) showed that application of biocides (Bio-Arc and Bio-Zeid) as seed dressing reduced percentage of mean infection seed-borne fungi such as *Alternaria* spp., *F. moniliforme*, *F. semitectum*, *F. solani*, *F. oxysporum* and *Rhizoctonia solani*. In this study, Bio-Arc showed higher effect in reducing percentage of disease severity of tomato wilt. Biological control of plant diseases is a result of many different types of interaction among microorganisms and can occur through different mechanisms, which are generally classified

as parasitism, antibiosis, competition, lytic enzymes, and induced resistance (Pal, 2005). The ability of *Trichoderma* to reduce diseases caused by soil borne pathogens is well known and it is related to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogenic fungi and competitions for limiting factors in the rhizosphere mainly iron and carbon (Sivan and Chet, 1986). In another study, El-Metwally *et al.,* (2010) found that foliar treatment of faba bean plants with Bio-Zeid and Bio-Arc led to maximum reduction of chocolate spot disease severity. Abou-Aly *et al*., (2008) showed that *B. megaterium, B. subtilis*, *B. coagulans P. fluorescens* and *Paenibacillus polymyxa* exhibited beneficial mechanisms against *Fusarium oxysporum* f. sp *lycopersici* and *F. solani*. Treated tomato plants with these compounds showed differences in protein and isozymes banding patterns compared to untreated plants. Two new protein bands at molecular weight 47.2 KD and 16.3 KD were appeared in all infected plants treated with Bio-Zeid and Bio-Arc while, not appeared in the control plants. These results are in agreement with those reported by El-Askary *et al.* (2003) and Akladious *et al.*, (2015). They showed that new protein bands induced in infected plants treated with the bio-products act as a protein marker in resistance mechanisms; such response allows plants to become more tolerant to the pathogens. In this study, data revealed that treatments with Bio-Zeid and Bio-Arc able to induced new protein banding patterns which increasing resistance to Fusarium wilt, but treatment with Bio-Arc showed higher effect because it induced the higher numbers of new protein bands. At the same time, new esterase band (EST-5a), peroxidase (PRX-2a and PRX-4a), chitinase (Chit-1a, Chit-5a and Chit-5b) and Superoxide Dismutase isozyme (SOD-1a) bands were detected in all treatments while not appear in the control. Prabhukarthikeyan *et al.,* (2017) showed that two protein bands were appeared in treated tomato plants with bio-formulations while, not appeared in untreated control plants.

Data also, indicate that tomato treated with Bio-Zeid and Bio-Arc, showing variability in numbers, relative mobility and density of esterase, chitinase, peroxidase and Super Oxide Dismutase isozymes compared with control plants. These results are in conformity with the results reported by El-Habbaa *et al.* (2016) they found that application of bio-formulations such as Rhizo-N, Plant guard and Bio-Zeid as a foliar treatment increased activities of peroxidase, polyphenoloxidase and chitinase in grapevine plants compared to the control treatment. In another study, *T. viride* induced higher levels of defense enzymes such as peroxidase, polyphenol oxidase and phenyl alanine ammonia lyase in black gram during pathogenesis by *F. oxysporum* and *A. alteranata* (Surekha *et al*., 2014). The increase in the antioxidant enzymes system (Catalase, Ascorbate peroxidase and Glutathione reductase) was associated with resistance to Fusarium wilt in chickpea (Garcia-Limones *et al.,* 2012). Amer *et al.,* (2014) reported

that application of the bio-control agents could play an important role in inducing partial resistance and exhibit greater potential to protect tomato plants against wilt.

These results revealed that all treatment were able to induced new isozyme bands which increasing resistance to Fusarium, but treatment with Bio-Arc was the best because it induced the highest number of new isozyme bands (12 isozyme bands) compared to Bio-zeid treatment (7 isozyme bands).

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تأثير بعض المبيدات الحيوية على مرض الذبول الفيوزاريومى وعلى إختلافات التعبير الجيني فى نباتات الطماطم

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الملخص:

تم تقييم فعالية معاملة التربة بنوعين من المبيدات الحيوية التجارية وهى البيو زد والبيو أرك ضد الفطرين *Fusarium oxysporum* f. sp. *lycopersici* and *F. solani*. المسببان لمرض الذبول الفيوزاريومى لنباتات الطماطم تحت ظروف الصوبة.

وأظهرت النتائج أن إضافة هذه المركبات الى التربة قللت من مرض الذبول الفيوزاريومى المتسبب عن كلا الفطرين على نباتات الطماطم (صنف سوبر ستربن ب) مقارنة بالكنترول. وأيضا تم دراسة الاختلافات فى التعبير الجينى لنباتات الطماطم المعاملة بالمركبات المختبرة بإستخدام النمط البروتينى وبعض الإنزيمات وهى البيروكسيديز، الاستيريز، الشيتينيز والسوبراكسيديز ديسميوتيز. حيث اظهرت النتائج أن نباتات الطماطم المعاملة بهذه المركبات أظهرت إختلافات فى أنماط البروتين والإنزيمات مقارنة بالنباتات غير المعاملة. حيث وجد إثنيين نمط بروتينى جديد فى الوزن الجزيئى ٤٧,٢ ك. دالتون و ١٦,٣ ك. دالتون ظهرت فى النباتات المصابة المعاملة بالبيو زد والبيو أرك بينما لم تظهر فى النباتات غير المعاملة.

وتشير هذه النتائج أيضا إلى أن المعاملات كانت قادرة على إستحثاث نمط بروتين جديد والذى ساعد فى زيادة مقاومة نباتات الطماطم للمرض. وتبين ان المعاملة بالبيو أرك كانت الأفضل فى إستحثاث العدد الأكبر من أنماط البروتين الجديدة. وفى الوقت نفسه ظهرت انماط جديدة من الإنزيمات وهى الاستيريز (EST-5a), والبيروكسيديز (PRX-4a), والشيتينيز (Chit-5b) و السوبراكسيديز ديسميوتيز (SOD-1a) فى كل المعاملات بينما لم تظهر فى الكنترول.