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Comprehensive Management for Wilt Disease Caused By *Fusarium Oxysporum* In Tomato Plant

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Abstract

The plant growth promoting from different sources were tested to enhance plant growth and suppress plant diseases in tomato plant, these include rhizobacteria (*Bacillus subtilis*, *Serratia marcescens*), Cyanobacteria (*Nostoc muscorum, Anabaena oryzae*), a plant water extract (Salix, Artemisia), antagonistic fungal species (*Trichoderma* (T) *harzianum* and *Ganoderma* (G) *lucidum*). The current study was carried out at, experimental farm station of Botany and Microbiology Department, Faculty of Science, Al-Azhar University; to investigate the efficient antagonistic these inducers against *Fusarium* wilt disease in tomato plant under filed experiment. Disease symptoms, disease index, phytochemicals and antifungal protein as well as isozyme markers as response to induction SR in tomato plants were recorded. The results demonstrated that *F. oxysporum* f. sp Lycopersici challenged plants treated with *T.harzianum* as well as *G. lucidum* extracts which showed the highest significant reduction in percent disease infection (PDI) with 4.16%, followed by treatment with A. *oryzae* as well as *Artemisia* water extract showed (8.33%), then *S. marcescens* as well as *N. muscorum* extracts showed (25%). Also, *Salix* water extract as well as *B. subtilis with* (33.33%) compared with control infected plants (83.33%). Considerable increase in all tested phytochemical parameters of tomato plants were obtained due to use of the tested elicitors than control infected plants. The beneficial effects of the tested inducers were extended to increase not only salicylic acid (SA), Abscisic acid (ABA), Indole acetic acid (IAA) and Gibberellin (GA_3), but also the activities of peroxidase and polyphenol oxidase enzymes in comparison with control. a new pattern of pathogenesis related proteins (PRS) were produced, also the results appeared that tomato plants treated with inducers show variability in number, relative mobility and density of polypeptide bands of peroxidase and polyphenol oxidase isozymes according to the type of elicitors used.

Key words: Tomato plant – *Fusarium oxysporum* - Plant growth promoting rhizobacteria – Cyanobacteria - *Ganoderma* lucidum - iso-zymes – Biotic and abiotic.

Introduction:

Fusarium wilt of tomato considered one of the most serious diseases of tomato in field as well as greenhouse-grown tomatoes worldwide (**Amini and Sidovich, 2010**). The fungus can be found as soil borne, air borne or on plant residue and can be transmitted through any part of the plant **(Summeral *et al*., 2003).** The wilt caused by *F. oxysporum* is appear as wilt plants, yellowed leaves and significantly decreasing the quantity and the quality of the crop (**Ajigbola and Babalola, 2013** and **Akram et al., 2013**). Pathogenic problems can be decreased or elimination through exogenous application of biotic or abiotic elicitors that induce resistance which can be categorized either as systemic acquired (SAR)

or induced systemic resistance (ISR). Induced resistance (SAR and ISR) involves the synchronized action of multiple genes and/or defense signaling pathways. Although the downstream components are similar in SAR and ISR mechanisms, upstream components differ, mainly involving the salicylic acid and jasmonic acid/ethylene pathways for SAR and ISR induction, respectively (**Pieterse and Van Loon, 2007).** A number of plant species have been reported to possess natural substances that are toxic to several plant pathogenic fungi (**Goussous *et al.* 2010**). In addition, **Sivaprakasam *et al.* (2011)** find that aqueous extract of fruiting bodies from *G. lucidum* show strong antifungal activity against fungal pathogens including *F. oxysporum*. Plant growth promoting rhizo-bacteria (PGPR) have various mechanisms one of which is regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. In addition, PGPR show synergistic and antagonistic interactions with microorganisms within the rhizosphere which indirectly enhance the growth of plant (**Vejan *et al.*, 2016**). Also, may be producing various active compounds which induced resistance against plant pathogens (**Ragaa and Mostafa,2013**). Cyanobacteria have also been studied for the control of plant pathogenic fungi, particularly soil borne diseases (**Tassara *et al.*, 2008**). Dipotassium hydrogen phosphate application in plants for induction of resistance is based on the activity of enzymes related to the cell wall structure (**Olivieri *et al* 2012**).

2 Materials and methods

Plant material:

For the present study, well identified Four Weeks-Tomato seedlings (*Solanum Lycopersicon* L. cv. Castle rock II PVP) were kindly obtained from agricultural research center (ARC), ministry of agriculture, Giza, Egypt.

Isolation and maintenance of *F. oxysporum*:

Fusarium oxysporum was isolated from symptomatic diseased tomato plants according to **(Katan *et al.*, 1991),** and identified morphologically macroscopic and microscopic according to **(Nelson *et al.*, 1983 and Leslie and Sumerell, 2006)**. then pathogen was confirmed by pathogenicity test according to **(Hibar *et al.*, 2007)**. Maintenance of stock culture, *Fusarium oxysporum* was preserved on slants at 0^{o}c on molt extract agar medium (MEA).

Source and application methods of elicitors:

Tow bacterial strains *Bacillus subtilis*, and *Serratia marcescens* were obtained kindly from Bio-log Technique at Bio-fertilizer production unit, Soil, Water and Environment Research institute, Agricultural Research centre (ARC), Giza, Egypt. The inoculum suspensions were approximately adjusted to 10^9 CFU/ml culture (colony forming unit). Tow strains of Cyanobacteria (*Nostoc muscorum, Anabaena. oryzae*) were obtained kindly from the Microbiology Department; Soils, Water and Environment Research Institute (SWERI), Agricultural Research, Centre (ARC). The inoculum suspensions were approximately adjusted to 10^6 CFU/ml culture. The plant water extract of *Salix*, *Artemisia* obtained According to **(Adebolu and Oladimeji, 2007)** dried leaves of both plants obtained from Agriculture Research Centre (ARC) Giza Egypt. *Trichoderma harzianum* from the Microbiology Department; Soils, Water and Environment Research Institute (SWERI), Agricultural Research, Centre (ARC). Also, the fruiting bodies of *Ganoderma lucidum* was obtained kindly from Dr. Younis and extracted according to the method described by **Younis Younis *et al.*, 2014.** Finally salicylic acid 10 mM (sigma company) and Di-potassium hydrogen phosphate (K2HPO4) (sigma company) at concentration 100Mm were prepared.

Greenhouse experiment:

Tested elicitors were applied 7 days before inoculation with F. oxysporum. The experiment was done in the garden of Faculty of Science, Al Azhar University Nasr city Egypt in April 2017. Complete block design was used with ten treatments and two controls (each has eight replicates). Treatments include; 1) healthy control (no fungus); 2) tomato seedlings inoculated with *F. oxysporum* that served as infected control; 3) *B. subtilis + F. oxysporum;* 4) *S. marcescens + F. oxysporum;* 5) *N. muscorum + F. oxysporum;* 6) *A. oryzae + F. oxysporum;* 7)*Artemisia extract + F. oxysporum;* 8)*Salix extract + F. oxysporum;* 9) *salicylic acid + F. oxysporum;* 10) *Dipotassium hydrogen phosphate + F. oxysporum;* 11) *T. harzianum + F. oxysporum;* 12) *G. leucidum + F. oxysporum* . Treatments were kept in the greenhouse under room temperature receiving water as required. Tomato plants symptoms were followed and stage one determined after 30 days after F. oxysporum inoculation and second stage was determined after 50 days.The plant samples were collected carefully for studying metabolic and biochemical indicators of resistance in tomato.

Disease symptoms and disease index:

Disease symptoms were assessed after 60 days inoculation and the disease index was evaluated according to **(leath *et al*., 1989)** with slight modifications using score consisting of five classes: 0: (no symptoms), 1: (slight yellow of lower leaves), 2: (moderate yellow plant), 3: (wilted plant), 4: (plants severely stunted and destroyed). Disease index (DI) was calculated using the five-grade scale according to the formula: DI= (1n1+ 2n2 + 3n3 + 4n4)100/4nt. Where n_1-n_4 the number of plants in the indicated classes, and Nt total number of plants tested. Protection % = A–B/A × 100% Where, A = PDI in infected control plants B = PDI in infected- treated plants.

5-Metabolic and biochemical indicators of resistance in tomato:

Determination of total soluble proteins (mg/100g of dry wt) according to the method of **(Lowery *et al*., 1951)** using casein as a standard protein. Total soluble carbohydrate was extracted according to **(Said *et al*., 1964)** and determined using anthrone technique according to **(Umbriet *et al*., 1969)**. Determination of phenolic compounds (mg/100g of dry wt) was carried out according to that method described by **(Daniel and George, 1972)**. Enzymes extracted according to **(MuKherjee and Choudhuri, 1983).** SOD activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described by **(Marklund and Marklund, 1974)**. Peroxidase activity enzyme was determined according to the method adopted by **(Srivastava, 1987).** The activity of polyphenol oxidase enzyme was determined according to the method adopted by **(Matta and Dimond, 1963)**. Determination of endogenous hormones (IAA, GA and ABA) in the terminal buds of the treated plants as well as the control were carried out as described by **(Knegt and Brunima, 1973)** Protein finger print was analyzed using Sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS - PAGE) according to **(Studier, 1973).** Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted to identify isozyme variations among the studied plants using two isozyme systems. **Peroxidase (Px)** isozyme was determined according to the method **(Brown, 1978)**. **Polyphenoloxidase (PPO)** isozyme was determined according to the method **(Baaziz *et al*., 1994)**.

D- Statistical analyses:

Experimental data were subjected to one-way analysis of variance (ANOVA) and the differences between means were separated using Duncans multiple rang test and the (L.S.D) at 5% level of probability using M-state software **(Snedecor and Cochran, 1982)**.

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Results:

1. Identification of causal pathogen:

Fungus isolate was obtained from infected tomato leaves and stem showing wilt

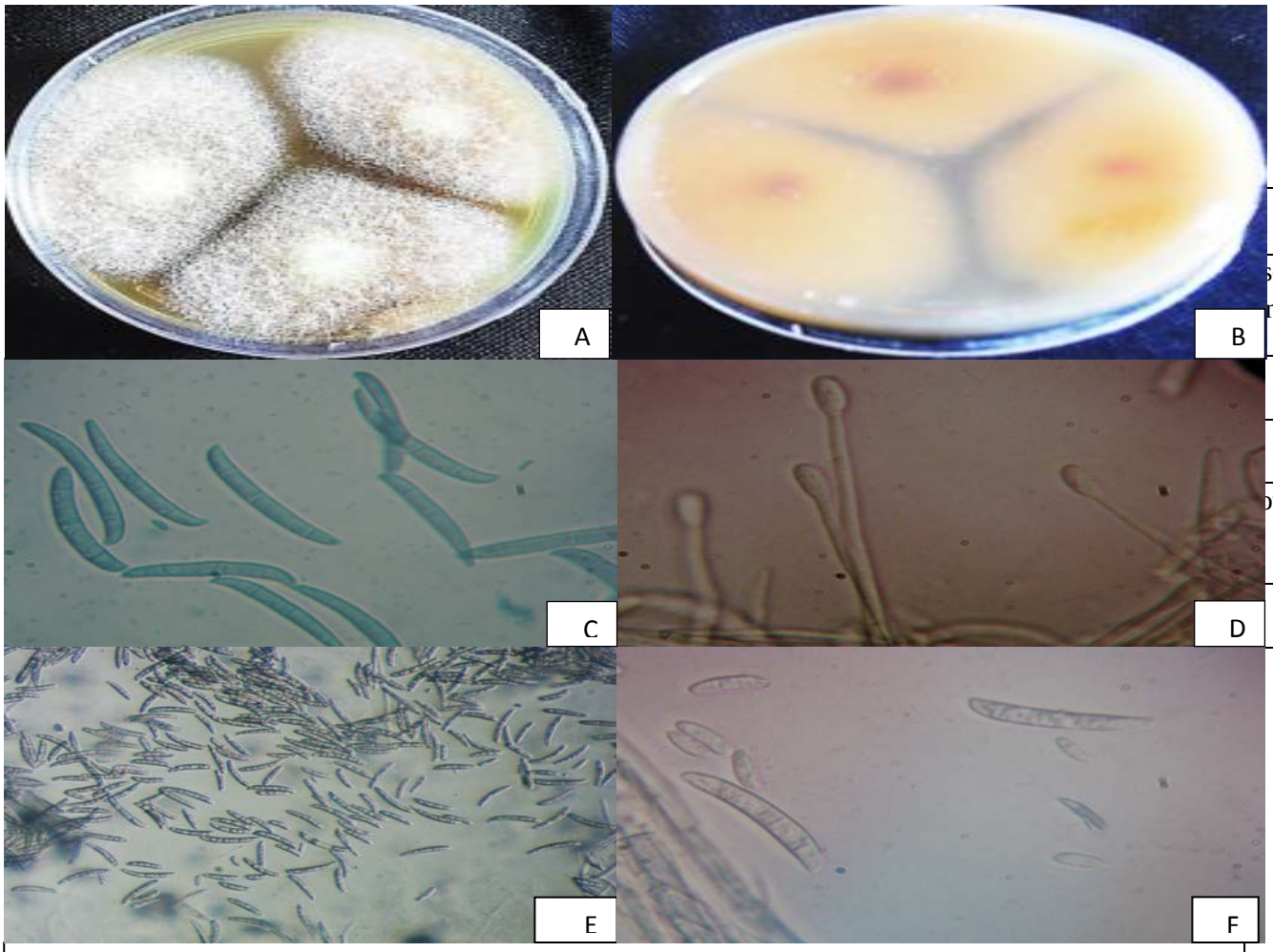


Figure (1)

A. Colony of *Fusarium oxysporium* on MEA**. B**. Reverse colony of *Fusarium oxysporium* on MEA.**C.** light microscope showing stained conidia of *Fusarium oxysporium* (Mag. power 20×40x). **D.** light microscope showing conidiophore of *Fusarium oxysporium* (Mag. power 20×40x). **E**. light microscope showing conidia of *Fusarium oxysporium* (Mag. power 20×10x). **F.** microscope showing conidia of *Fusarium oxysporium* (Mag. power 20×40x)

2. Effect of biotic and abiotic agents on disease index:

Also data in table (2) showed that application of *S. marcescens* was the best inducer which gave highly protection percent (96.29%), followed by *G. lucidum* (92.19%) and *N. muscorum* (88.88%). Also the application of *A. oryzae*, *B. subtilis*, Di-potassium hydrogen phosphate, *Salix* extract, salicylic acid, and *T. harzianum* gave the same protection percent (85.18%), finally *Artemisia* (55.55%).

Table 2: Effect of tested biotic and abiotic agents on disease index of tomato plants infected with *F. oxysporum*:

Treatment	Classes					DI (disease index) (%)	Protection (%)
	0	1	2	3	4		
Control healthy	6	2	0	0	0	6.25	-
Control Infected	0	0	1	3	4	84.37	0
Infected + *B. subtilis*	6	0	2	0	0	12.5	85.18
Infected + *S. marcescens*	7	1	0	0	0	3.125	96.29
Infected + *N. muscorum*	5	3	0	0	0	9.375	88.88
Infected + *A. oryzae*	4	4	0	0	0	12.5	85.18
I*nfected + *Artemisia	2	4	0	0	2	37.5	55.55
Infected + *Salix*	4	4	0	0	0	12.5	85.18
Infected + Salicylic acid	4	4	0	0	0	12.5	85.18
Infected + Di-P.H.P.	4	4	0	0	0	12.5	85.18
Infected + *T. harzianum*	4	4	0	0	0	12.5	85.18
I*nfected + *G. l. f. B.	6	2	0	0	0	6.25	92.19

Key of table: *B: Bacillus, S: Serratia, N: Nostoc, A: Anabaena,* Di-P.H.P.: DI-potassium hydrogen phosphate, *T: Trichoderma,* and *G. l. f. B.: Ganoderma leucidum* fruiting bodies.

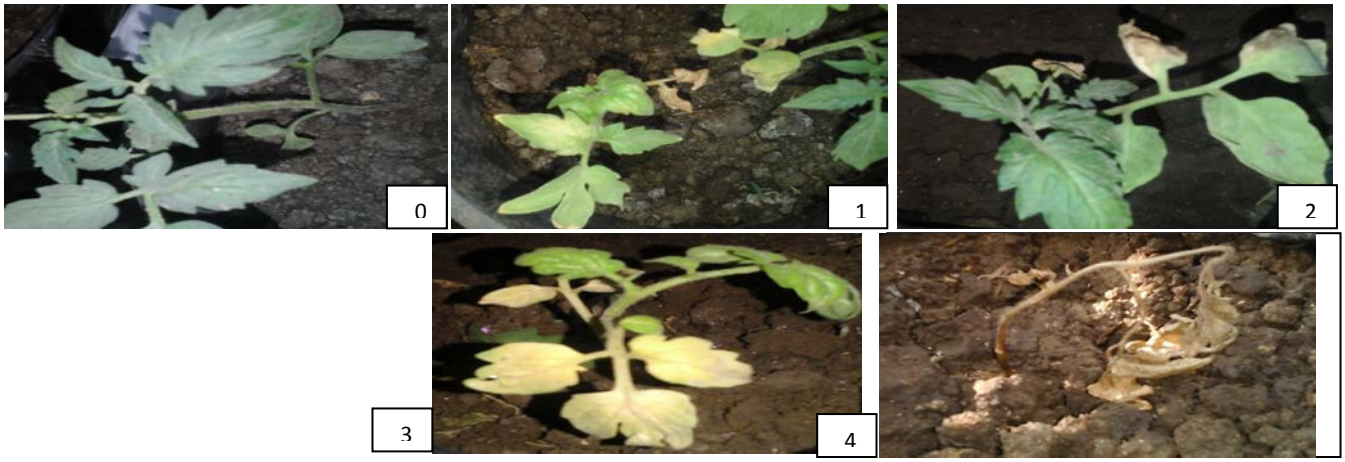


Figure (2): classes of wilt disease symptoms caused by *Fusarium oxysporum* on tomato plant

3- Biochemical indicators of resistance in tomato:

3.1. Total soluble carbohydrates: Data listed in table (3) revealed that, total soluble carbohydrate contents of shoot and roots in tomato plants were significantly decreased due to *fusarium* infection during two stages of growth. It was found that application of all biotic and abiotic agents caused highly significant increase in total soluble carbohydrate in shoot and root during both stages compared to infected control.

Table 3: Effect of tested biotic and abiotic agents on carbohydrate content of infected tomato plants:

Treatment	Total soluble carbohydrate content mg/g d. wt. (g)			
	Shoot		Root	
	Stage1	Stage2	Stage1	Stage2
Control healthy	25.37	65.65	15.89	21.00
Control Infected	10.96	21.45	8.85	8.06
Infected + *B. subtilis*	26.81	69.66	11.68	30.92
Infected + *S. marcescens*	23.55	48.89	13.00	17.51
Infected + *N. muscorum*	21.57	52.32	12.47	23.83
Infected t+ *A. oryzae*	23.98	68.56	13.56	17.06
Infected + *Artemisia*	26.67	52.66	16.19	20.02
Infected + *Salix*	26.23	72.16	13.55	17.93
Infected + salicylic acid	38.55	70.96	19.63	23.07
Infected + Di-P.H.P.	33.92	56.33	22.78	28.92
Infected + *T. harzianum*	46.92	63.54	29.82	38.24
Infected + *G. l. f. B.*	65.24	93.75	56.47	64.03
LSD 0.05	10.30	6.35	5.31	6.17

Key of table: *B: Bacillus, S: Serratia, N: Nostoc, A: Anabaena,* Di-P.H.P.: DI-potassium hydrogen phosphate, *T: Trichoderma,* and *G. l. f. B.: Ganoderma leucidum* fruiting bodies. d. wt: dry weight.

3.2. Total soluble protein contents: The results in Table (4) showed that, total soluble protein contents of shoot and roots in tomato plants were significantly decreased due to *fusarium* infection during two stages of growth. It was found that application of all biotic and abiotic agents caused highly significant increase in total soluble protein in shoot and root during both stages compared to infected control.

Table 4: Effect of tested biotic and abiotic agents on Total soluble protein content of tomato plants infected with *F. oxysporum*:

Treatment	Total soluble protein content mg/g d. wt. (g)			
	Shoot		Root	
	Stage1	Stage2	Stage1	Stage2
Control healthy	21.71	26.80	12.59	19.28
Control Infected	12.94	15.57	8.35	12.86
Infected + *B. subtilis*	20.74	24.81	11.59	18.03
Infected + *S. marcescens*	19.89	23.04	16.27	19.05
Infected + *N. muscorum*	23.21	25.28	12.40	17.90
Infected t+ *A. oryzae*	21.81	29.77	15.92	20.08
Infected + *Artemisia*	19.52	28.07	18.25	21.82
Infected + *Salix*	17.69	21.27	12.80	18.28
Infected + salicylic acid	19.95	24.52	16.15	21.70
Infected + Di-P.H.P.	21.44	26.85	17.86	20.25
Infected + *T. harzianum*	21.81	24.87	14.23	17.27
Infected + *G. l. f. B.*	22.02	24.12	12.85	16.68
LSD 0.05	1.69	1.85	1.97	3.52

Key of table: *B: Bacillus, S: Serratia, N: Nostoc, A: Anabaena,* Di-P.H.P.: DI-potassium hydrogen phosphate, *T: Trichoderma,* and *G. l. f. B.: Ganoderma leucidum* fruiting bodies. d. wt: dry weight.

3.3. Total phenols: The results in Table (5) indicate that, all tested inducers significantly increased total phenols compared with control. The highest increase in shoots was recorded by *G. lucidum* then (*T. harzianum*, Salix, Artemisia, Di-potassium hydrogen phosphate and *N. muscorum*) respectively. Then *S. marcescens*, *B. subtilis* and finally salicylic acid. Also all treatments, increased total phenols of

infected tomato root. The highest increase was induced by *S. marcescens* and *B. subtilis* respectively followed by (*A. oryzae,* salicylic acid, Di-potassium hydrogen phosphate and *T. harzianum*) followed by (*G. lucidum*, Artemisia and finally Salix.

Table 5: Effect of tested biotic and abiotic agents on Phenolic compounds of infected plant:

Treatment	Phenolic compounds mg/100g d.wt(g)			
	Shoot		Root	
	Stage1	Stage2	Stage1	Stage2
Control healthy	1.019	1.976	1.145	0.286
Control Infected	1.586	2.081	1.448	0.573
Infected + *B. subtilis*	2.351	2.764	3.425	1.486
Infected + *S. marcescens*	2.541	3.466	3.510	1.930
Infected + *N. muscorum*	3.130	3.687	1.805	0.976
Infected + *A. oryzae*	2.924	2.604	3.166	1.220
Infected + *Artemisia*	3.487	3.966	1.769	0.632
Infected + *Salix*	3.524	4.005	1.503	0.800
Infected + salicylic acid	2.162	2.634	2.938	1.057
Infected + Di-P.H.P.	3.362	3.472	2.533	0.994
Infected + *T. harzianum*	3.726	4.406	2.435	0.822
Infected + *G. l. f. B.*	4.009	4.475	2.148	0.802
LSD 0.05	0.0815	0.0868	0.1272	0.4699

Key of table: *B: Bacillus, S: Serratia, N: Nostoc, A: Anabaena,* Di-P.H.P.: DI-potassium hydrogen phosphate, *T: Trichoderma,* and *G. l. f. B.: Ganoderma leucidum* fruiting bodies. d. wt: dry weight

3.4. Antioxidant enzymes activity:

Results of the present work (table 6) indicated that, tomato plants infected with *F. oxysporum* recorded insignificant increases in PPO activity in shoots compared to healthy tomato plants at the both stages of growth. All applied inducers caused significantly increased PPO activity compared with infected control throughout the first stage of growth except Salix extract and Artemisia recorded insignificantly increasing. While at the second stage all inducers have insignificant increase except B. subtilis was significantly increased.

For Peroxidase (POD) activity it was found that all tested agent stimulated POD activity of shoots. Applied of *T. harzianum*, *A. oryzae*, Di-potassium hydrogen phosphate, salicylic acid, *G. lucidum*, Artemisia and S. marcescens were significant increase POD activity, followed by N. *muscorum*, Salix extract and B. subtilis which recorded insignificant increasing compared with infected control during the experiment. Also results in (table 6) revealed that, tomato plants infected with *Fusarium* gave highly significant increases in Superoxide dismutase SOD activity related to healthy tomato plant during second stage of growth but insignificant during the first. It was found that all inducers showed, significant increasing in SOD activity throughout the second stage of growth as the order A. oryzae, G. *lucidum*, *T. Harzianum*, S. marcescens, Salix extract, Di-potassium hydrogen phosphate, Artemisia, N. muscorum, salicylic acid and finally *B. subtilis*. But at the first stage insignificant increasing was recorded and the highest was A. oryzae followed by G. lucidum, S. marcescens, *T. Harzianum*, salix extract, Di-potassium hydrogen phosphate, salicylic acid, Artemisia, N. muscorum and finally *B. subtilis*.

Table 6: Effect of tested biotic and abiotic agents on antioxidant enzymes activity of infected plant:

Treatment	Polyphenoloxidase (PPO) ug/g. f.wt of shoot		Peroxidase (POD) ug/g. f.wt of shoot		Superoxide dismutase (SOD) ug/g. f.wt of shoot	
	Stage1	Stage2	Stage1	Stage2	Stage1	Stage2
Control healthy	0.14	0. 2	0.31	0.17	0.07	0.08
Control Infected	0.20	0. 5	0.39	0.43	0.37	0.86
Infected + *B. subtilis*	1.38	3.1	0.51	1. 10	0.50	1.50
Infected + *S. marcescens*	0.69	0. 81	0.57	1.61	0.87	2.41
Infected + *N. muscorum*	0.73	1.3	0.55	1.18	0.52	1.86
Infected + *A. oryzae*	0.42	0.8	1.07	1.90	0.96	2.99
Infected + *Artemisia*	0.24	0. 7	0.61	1.20	0.60	1.89
Infected + *Salix*	0.28	0.6	0.54	1.02	0.81	2.36
Infected + salicylic acid	0.36	1. 2	0.59	1.41	0.65	1.68
Infected + Di-P.H.P.	0.53	0. 7	0.98	1.20	0.74	2.27
Infected + *T. harzianum*	0.40	1. 1	1.10	1.92	0.85	2.48
Infected + *G. l. f. B.*	0.76	1. 8	0.92	1.74	0.91	2.53
LSD 0.05	0.128	1. 34	0.18	0.768	0.767	0.450

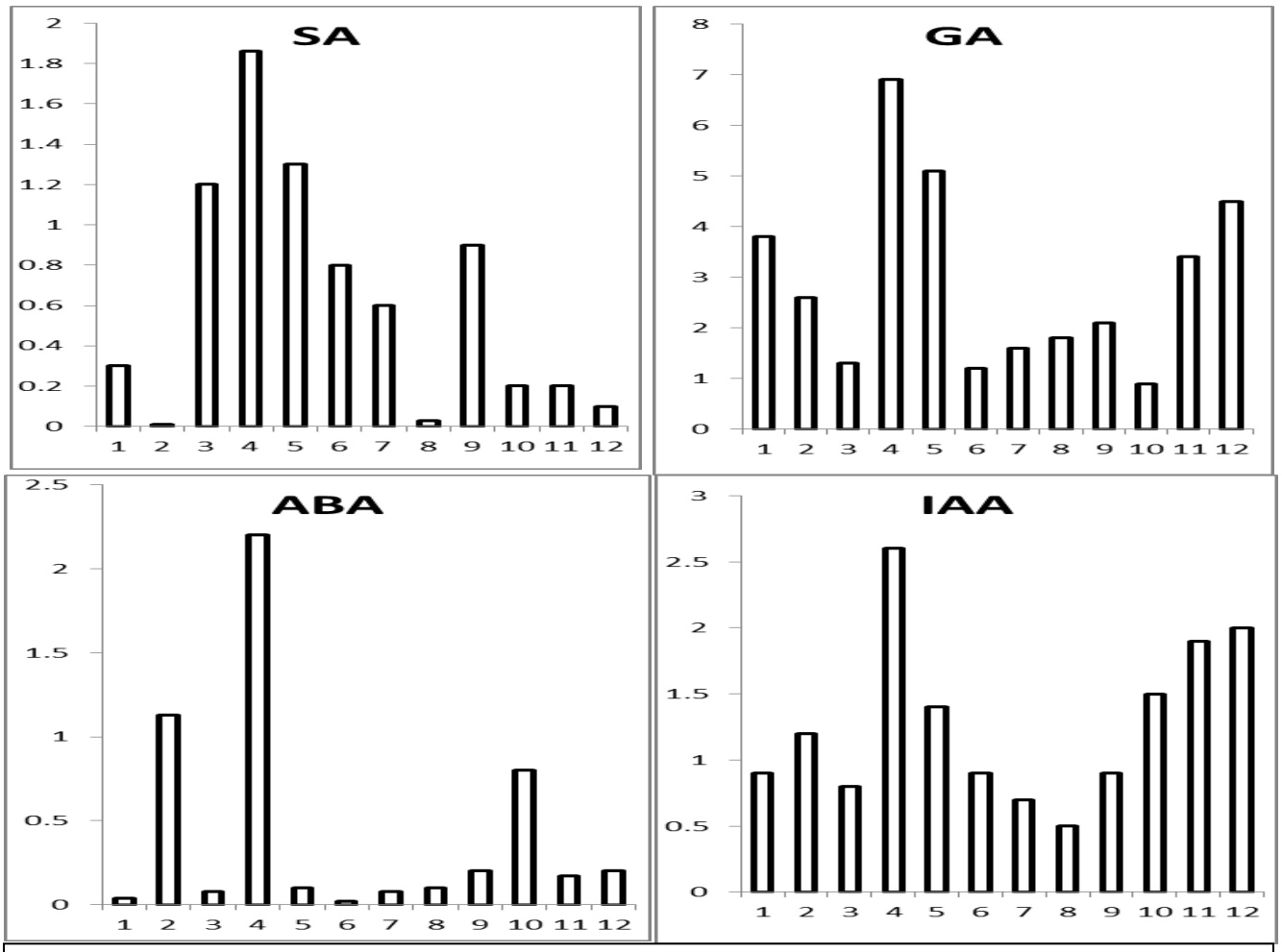
Key of table: *B: Bacillus*, *S*: Serratia, *N: Nostoc*, *A: Anabaena*, Di-P.H.P.: DI-potassium hydrogen phosphate, *T: Trichoderma*, and *G. l.* f. B.: *Ganoderma leucidum* fruiting bodies. f.wt.: fresh weight.

Endogenous hormones:

Results shown in (chart 1) and revealed that contents of GA3 and SA were markedly decreased in *fusarium* infected plants than that of healthy ones. At the same time, a marked increase in the contents of ABA and IAA was observed in infected plants as being compared with healthy ones. Concerning the effect tested elicitors on the challenged plants with *F. oxysporum;* it was found that contents of GA were generally decreased due to the application of different elicitors except *S. marcescens*, *N. muscorum*,

T. harzianum and G. lucidum fruiting bodies. Contents of SA were decreased due to using of all elicitors which the same result of ABA except *S. marcescens*. Also application of *S. marcescens*, *N. muscorum*, Di-potassium hydrogen phosphate and T. harzianum recorded increasing in IAA.

Chart 1: Effect of tested biotic and abiotic agents on antioxidant enzymes activity of infected plant:



1: Control healthy,2: Control Infected,3: Infected + B. subtilis,4: Infected + S. marcescens,5; Infected + N. muscorum,6:nfected + A. oryzae,7: Infected + Artemisia,8: Infected + Salix,9: Infected + salicylic acid,10: infected + Di-potassium hydrogen phosphate,11: Infected + T. harzianum,12: Infected + G. lucidum fruiting bodies. Unit is (mg/100g fresh weight).

4.1. Detection of the elicited antifungal protein as response to induction SR:

Data listed in Table (7 and 8) and Fig (3) showed that tomato plants treated with tested inducers and infected with ***F. oxysporum*** showed variation in number, molecular weight of protein bands. The variability analysis among tested inducers appeared 149 protein bands.

The most prominent specific polypeptide alteration (polymorphic bands) ranged molecular weight from 272.262 to 44.882 KD with percentage 18.3 %. These bands may be related to tested inducers. The unique bands ranged molecular weight from 44.269to 39.836KDa with percentage 1.34%. The prominent polypeptide bands in all inducers (monomorphic or common polypeptide) were ranged molecular weight from 51.504, to 4.189. kDa with percentage 80.53 %. These bands may be related to tomato plant.

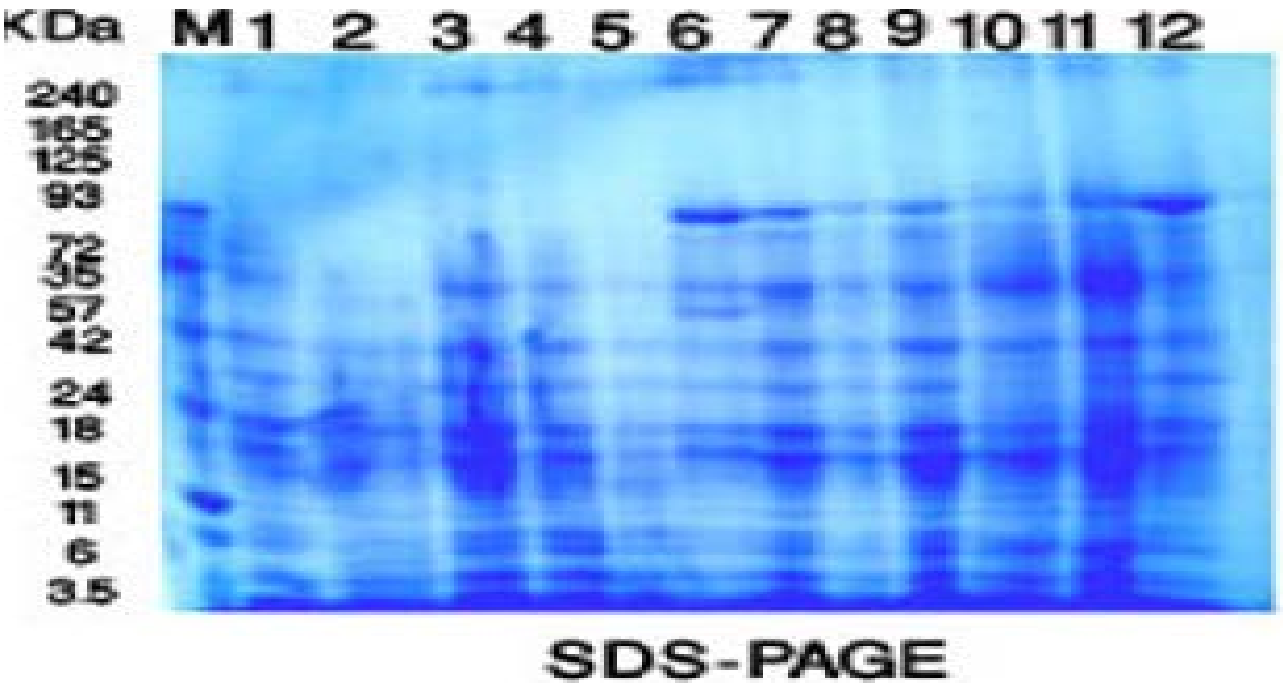
Table (7): Protein fractions in leaf of *fusarium* infected tomato plants treated with biotic and abiotic agents using SDS-PAGE

MW	1	2	3	4	5	6	7	8	9	10	11	12	Frequency	Polymorphism
272.262	-	-	+	+	-	+	+	-	-	+	+	+	0.583	Polymorphic
96.111	-	-	-	-	-	+	+	+	+	+	+	+	0.583	Polymorphic
79.633	-	-	-	-	-	+	+	+	+	+	+	+	0.583	Polymorphic
51.504	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
44.882	-	-	+	-	-	-	+	-	+	+	+	+	0.500	Polymorphic
44.269	-	+	-	-	-	-	-	-	-	-	-	-	0.083	Unique
39.836	-	-	-	-	-	+	-	-	-	-	-	-	0.083	Unique
31.527	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
22.973	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
15.990	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
13.494	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
11.652	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
8.110	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
6.273	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
4.874	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic
4.189	+	+	+	+	+	+	+	+	+	+	+	+	1.000	Monomorphic

Table (8): polymorphism of Protein fractions in leaf of *fusarium* infected tomato plants treated with biotic and abiotic agents using SDS-PAGE

Bands	1	2	3	4	5	6	7	8	9	10	11	12	Polymorphism %
Mono	10	10	10	10	10	10	10	10	10	10	10	10	80.53
Poly	0	0	2	1	0	3	4	2	3	4	4	4	18.13
Unique	0	1	0	0	0	1	0	0	0	0	0	0	1.34
Total bands	10	11	12	11	10	14	14	12	13	14	14	14	100

(Figure 3): Protein fractions in leaf of *fusarium* infected tomato plants treated with biotic and abiotic agents using SDS-PAGE



Key: 1: Control healthy,2: Control Infected,3: Infected + *B. subtilis*,4: Infected + *S. marcescens*,5; Infected + *N. muscorum*,6:nfected + *A. oryzae*,7: Infected + *Artemisia*,8: Infected + *Salix*,9: Infected + salicylic acid,10: infected + Di-potassium hydrogen phosphate,11: Infected + *T. harzianum*,12: Infected + *G. lucidum* fruiting bodies.

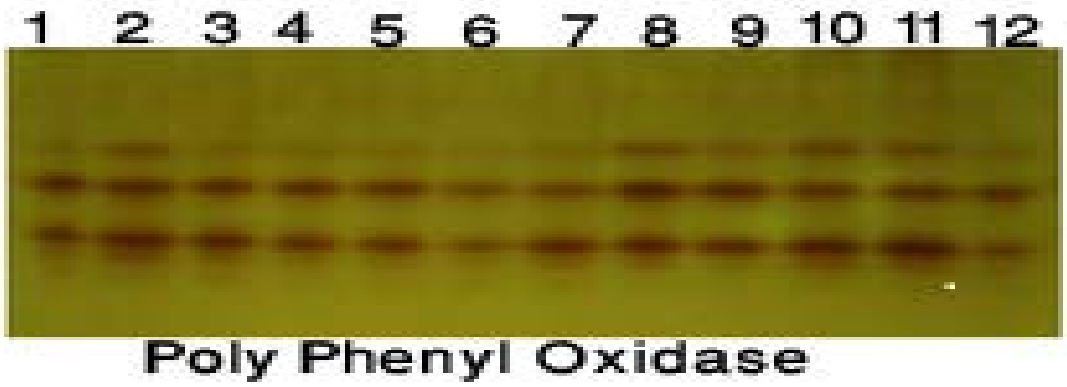
4.2. Biochemical iso-zymes markers:

4.2.1. Polyphenol oxidase isozyme: Tested inducers and ***F. oxysporum*** -infection treatments appeared variation in number, relative mobility and density polypeptide bands compared with healthy ones. *T. harzianum***,** Di-potassium hydrogen phosphate and Salix extract were the more effective followed by *S. marcescens*, and **salicylic** acid which gave (4) Isozymes with strong density bands. While *B. subtilis*, *A. oryzae*, and Artemisia gave the

same number of bands (3isozyme*). G. lucidum* was the least effective which gave 2 bands (Table**9** and **fig4)**.

Table (9): Polyphenol oxidase isozyme of *fusarium i*nfected tomato plants treated with biotic and abiotic

Poly Phenyl Oxidase Groups	Relative Mobility (R.M)	1	2	3	4	5	6	7	8	9	10	11	12
PX1	0.417	+	+	-	+	-	-	-	+	+	+	+	-
PX2	0.589	+	+	+	+	+	+	+	+	+	+	+	+
PX3	0.863	+	+	+	+	+	+	+	+	+	+	+	-
PX4	0.811	+	+	+	+	+	+	+	+	+	+	+	+



4.2.2. Peroxidase isozyme: Applied inducers and ***F. oxysporum*** -infection treatments

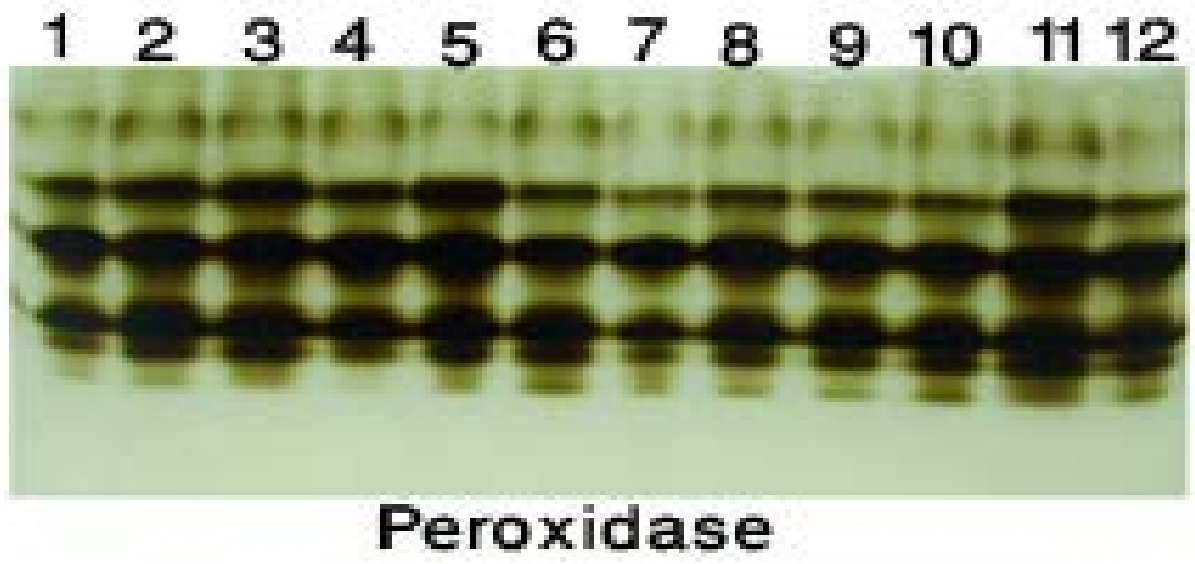
Key: 1: Control healthy,2: Control Infected,3: Infected + *B. subtilis*,4: Infected + *S. marcescens*,5; Infected + *N. muscorum*,6:nfected + *A. oryzae*,7: Infected + *Artemisia*,8: Infected + *Salix*,9: Infected + salicylic acid,10: infected + Di-potassium hydrogen phosphate,11: Infected *+ T. harzianum*,12: Infected + *G. lucidum* fruiting bodies.

potassium hydrogen phosphate and *G. lucidum* were the least effective which gave the lowest number of bands (5 Isozyme) with moderate density.

Table (10): Peroxidase isozyme of *fusarium* infected tomato plants treated with biotic and abiotic

Peroxidase Groups	Relative Mobility (R.M)	1	2	3	4	5	6	7	8	9	10	11	12
PX1	0.188	-	+	+	+	+	+	-	+	+	-	+	-
PX2	0.360	+	+	+	+	+	+	+	+	+	+	+	+
PX3	0.531	+	+	+	+	+	+	+	+	+	+	+	+
PX4	0.760	+	+	+	+	+	+	+	+	+	+	+	+
PX5	0.877	+	+	+	+	+	+	+	+	+	+	+	+
PX6	0.943	+	+	+	-	+	+	+	-	-	-	-	-
PX7	0.970	-	-	-	-	-	+	+	+	-	+	+	-

(Figure5): Polyphenol oxidase isozyme of *fusarium* infected tomato plants treated with biotic and abiotic:



Key: 1: Control healthy,2: Control Infected,3: Infected + *B. subtilis*,4: Infected + *S. marcescens*,5; Infected + *N. muscorum*,6:nfected + *A. oryzae*,7: Infected + *Artemisia,8:* Infected + *Salix,*9: Infected + salicylic acid,10: infected + Di-potassium hydrogen phosphate,11: Infected + *T. harzianum*,12: Infected + *G. lucidum* fruiting bodies.

Discussion:

Induced resistance can be activated by microorganisms as rhizobacteria in some plants, while in other plants; the same kind of defense can be induced by certain groups of chemicals (Prasad **and Naik, 2003**). The objectives of this study were induction of systemic resistance in Tomato plants against wilt disease caused by ***F. oxysporum*** infection. The first standard to govern the occurrence of systemic resistance in tomato plants, reduction disease index. Data

obtained in the present study showed that application of all inducers reduced disease index present which recorded highly protection percent effect against *fusarium* wilt disease. These results agree with **Chandra *et al*, 2007 and Farrag *et al*, 2017** how showed Application of ***S. marcescens***, ***B. subtilis*** ,*T. harzianum*, ***N. muscorum*** as well as, *G. lucidum* showed highly significant reduction in percent disease infection compared with control plants infected with *fusarium oxysporum*. This result may be explained by **Vejan *et al*., 2016** which reported that PGPR show synergistic and antagonistic interactions with microorganisms within the rhizosphere which indirectly boosts plant growth rate or through production of phytohormones (**Bhardwaj D. *et al*., 2014**) or through salicylic acid (SA) accumulation (**Van Loon *et al*., 1998**) which linked with pathogenesis-related (PR) protein accumulation, mainly PR-1 (**Dong, 1998**). New induced proteins were found in plants treated with tested inducers have been defined as pathogenesis related proteins, they implicated in plant defense because of their anti-pathogenic activities (**Van-Loon *et al*., 1994**).

Also these results could be demonstrated by **Shi *et al*., 2013** who decided that *G. lucidum* fruiting bodies have bioactive compounds Investigations on the mechanisms of disease suppression. Plant extracts have suggested that the active principles may either act on the pathogen directly (**Amadioha 2000**) or induce systemic resistance in host plants resulting in a reduction of the disease development **(Kagale *et al.* 2004)**. These results indicated that **salicylic acid** showed highly significant reduction in percent disease which explained by **Vlot *et al*., 2009** who proved that SA play important roles in lignin biosynthesis and regulate plant responses to pathogen attacks. Our results showed that Di-potassium hydrogen phosphate highly significant reduction in percent disease which can be demonstrated by two mechanisms: the first is a direct toxic action on the pathogen and the second in indirect action due to phosphate anion activates plant defense responses (**Moor *et al*., 2009**).

Tested biochemical parameters were highly affected by application of tested elicitors as total soluble carbohydrate, protein and phenols could be act as indicators of resistance (**Couee *et al*., 2006, Sudhakar *et al*., 2007, Rakib and Mustafa 2013, Osman *et al*., 2016 and Attia *et al* 2017**). However SOD, PO and PPO activities were greater in the infected plants treated with tested inducers compared to control plants which agree with **(Harish *et al*., 2009 and Attia, *et al* 2017).** On the other hand this study indicated that the activities of tested isozymes in challenged plants treated with inducers were higher than that in infected control which might be the potential factor for induction of SAR against ***F. oxysporum*** according to previous findings. Also, biotic inducers increased many PR-proteins

such as isozymes of peroxidase and Polyphenol oxidase, (**Anand *et al*., 2009 and Sharaf *et al* 2016**). Finally the present study indicated that all tested biological, Natural and chemical inducers Induce resistance in tomato plant against wilt disease caused by *F. oxysporum*.

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