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Promotion of tomato growth and resistance against Fusarium infection using some bioagents

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ABSTRACT

Fusarium sp is representing the one of the most important pathogens in causing a greet losses in tomato crops. Molecular identification show that the causal agent for tomato wilts in Minya governorate is *F. oxysporum*. Salmiya 65010 and Habiba are the most resistant genotype among the tested ones. *T. harzianum* and *Pseudomonas fluorescense* has the greatest inhibitory effect towards *F. oxysporum* growth. On the other hands, culture filtrates of *T. harzianum* and *B. subtilis* have the greatest inhibitory value on the pathogen growth (linear growth and dry weight). Also, *T. harzianum* significantly reduce the disease incidence and severity compared to control plants. It also shows some stimulation on growth parameter of tomato plants such as the plants height (cm), shoot length and root length, fresh weight of plants and dry weight of plant compare with the control.

Keywords: *Fusarium oxysporum*, tomato wilt, bio-agent, Folicure®

INTRODUCTION

Tomatoes (*Solanum lycopersicum L.*) are important fruit vegetables. It is a good source for minerals, fibers and vitamins in human nutrition. It is distributed all over the world in open fields or in protected cultures. Tomato plants are subjected to many pathogen-attacks in all growth stages (Wani, 2011). Because of tomato economic importance, disease-management is needed essential to avoid yield and productivity

losses. Among the most common tomato diseases, Fusarium wilt diseases, specifically, *F. oxysporum f. sp. lycopersici* is the forma specialist that affects tomato plants (Michielse and Rep, 2009). It is representing the fifth most important plant pathogenic fungus in the entire world led to an economic loss around 14 % in tomato crops (Pothiraj et al., 2021).

Fungicides are the most important components in the management of fungal

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diseases. Meanwhile and several hundreds of fungicide formulations are available. These chemicals can suppress infection, but do not cure a plant once infected. A disadvantage of these compounds are that they also affect soil microbiota, and some accumulate in the food chain and for these reasons many of these products are, or will be, banned (**Lopez-Aranda *et al.*, 2016**).

The biological control acts to prevent or reduce plant diseases caused by pathogens, or establishment of the pathogen in the plant. it has advantages of using these methods is that using bioagent are highly specific for a pathogen and hence are considered harmless to non-target species (**O'Brien, 2017**). However, a shift from fungicidal to alternative means of control is underway to ensure environmentally safer measures to accomplish international regulations. Several biological control agents are commercialized against soil borne pathogens and improved plant growth. However, all these agents need some time to suppress disease development comparing to soil chemical disinfections and can provide only a partial control (**Ayala-Doñas *et al.*, 2020**). Unfortunately, there is no single method to provide effective control of *Fusarium* fungi on cultivated plants. Typically, management of pathogenic *Fusarium* species includes crop rotation, sanitation, and judicious use of fungicides (**Aleksandra *et al.*, 2020**).

The recent work aimed to:- 1)isolate and identify the causal agents of tomato wilt. 2) clarify the response of some tomato genotypes toward infection and 3) manage the disease using biological control methods.

MATERIALS AND METHODS

1-Isolation and pathogenicity: Naturally, designed wilted of tomato plants were collected from various location in El-Minya

showing typically wilt symptoms and subjected to isolation trials using PDA medium. Developed fungi were purified by hyphal-tip technique. Pathogenicity test was conducted in the greenhouse of Plant Pathology Dept. Minia Univ. During 2018-2019 using sterilized pots and soil. The pots were infested with the tested isolates grown individually on sorghum grain for 10 days, and then added to sterilized soil 3% weight to weight. After seven days later, tomato seedlings (var. 023 F1 Sakata) 25 days old were single transplanted in either infested or non-infested soil and checked up to 4 weeks for wilt development.

Disease assessment: disease incidence was assayed after 30days of transplantation. The wilt severity (ws) was recorded by 0–4 scale as described by (**Weitang *et al.*, 2004**), where zero representing no infection and four denoting plants completely dead. And wilt severity (ws) was calculated valuing the aquation described by (**Sharma *et al.*, 2006**).

Vascular discoloration scales: The severity of the vascular discoloration was evaluated on a zero to five rating scale suggested by (**Pottorff *et al.*, 2012**).

2- Fungal identification:

Two of the most virulent isolates (Fo8 and Fo2)were used for identification. Five days-old of highly virulent fungal-cultures were subjected to DNA extraction at the Molecular Biology Research Unit, Assiut University using Patho-gene-spin DNA/RNA extraction kit (Intron Biotechnology Company, Korea). Polymerase chain reaction (PCR) and sequencing were performed at SolGent Company, Daejeon, South Korea. The ITS region of rRNA gene was amplified using the universal primers ITS1 (forward) and ITS4 (reverse). Primers have the following

composition: ITS1 (5' – TCCGTAGGTG AACCTGCGG-3'), and ITS4 (5'-TCCTCCGCTTATTGAT ATGC -3'). Purified PCR product of DNA was sequenced using the same manner with the incorporation of ddNTPs in the reaction mixture (White *et al.*, 1990). The obtained sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Analysis of sequences and establishment of phylogenetic trees were done using MegAlign (DNA Star) software version 5.05.

3- reaction of tomato genotypes to infection:

Seedlings of 9 tomato genotypes (023 F1, Banora F1, T-186, Thuraya F1, Salmiya 65010 F1, Al-Quds 448 F1, Gs-12 F1, Habiba F1, and SV320 TD F1) were tested for their reaction to infection by the highest virulent isolate of *F. oxysporum* under greenhouse conditions. Inoculum preparation, soil infestation and transplantation were performed similarly as described above both (Di) and (ws) were evaluated for week after transplanting.

4- Pathogen growth as affected by bioagents:

Four bioagents i.e., *Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces sp* and *Trichoderma harzianum* were kindly provided from Agriculture Microbiology Dept., Fac. Agric., Minia Univ. Bacterial suspension of *B. subtilis* was grown in nutrient broth medium, while *P. fluorescens* was multiplied on King Broth (KB) medium. *T. harzianum* and *Streptomyces sp* were maintained on PD broth medium at 26 °C for one week. The tested bioagent were streaked on the nutrient agar medium at periphery of Petri plates, a 5 mm disk from a 7 days-old culture of *F. oxysporum* was placed near the periphery of Petri plates directly opposite the bioagent (70 mm

distance). Control was medium containing plates were strigh with the tested pathogen only bioagents 5 mm disk bearing the tested pathogen (Juber *et al.*, 2014). Petri dish containing PDA amended with Folicure® in concentration of 250 ppm (25% Tebuconazole, Bayer) was used as a positive control. Three replicates were used for each treatment. Cultures were incubated at 25°C for 7 days. Linear growth of the tested fungus was recorded. Reduction percentage in mycelial growth for *F. oxysporum* was calculated.

5-Pathogen growth as affected by bioagent culture- filtrates

Bioagent culture-filtrates were prepared in 100 ml flasks inoculating 50 ml autoclaved nutrient broth. The flasks were inoculated with 5 mm bioagent disks or 1 ml of bacterial suspension. The inoculated flasks were incubated for 10 days under constant shaking at 100 rpm (25 ± 2°C). The culture filtrates were collected by filtration of the fungal mat through Whatmann filter paper No.1, then centrifuged at 7.000 rpm for 15 min at 5° C. These filtrates were added individually to PD media at ratio of 5, 10 and 20% and then inoculated with 5 mm disk of *F. oxysporum*. Petri dish containing PDA amended with Folicure® (0, 100, 200 and 300) was used as a positive control (Sultana and Hossain 2022). For 7 days of incubated at 25° C, linear growth or dry weight was calculated as mentioned before.

6-Disease incidence as affected by bioagents

Infested pots with Fo8, Fo2 were treated with each of *Bacillus subtilis*, *Pseudomonas fluorescens* (3×10⁸cfu), and *Trichoderma harzianum* (1×10⁶ conidia/ml). After another week the pots were cultivated by tomato (023 F1) seedlings. Treatment with Folicure® (250 ppm) was tested as positive control with recommended dose. All agricultural practices were applied according

to recommendations of Ministry of Agricultural and Land Reclamation where plants were watered and fertilized with NPK 19:19:19 as well as necessary microelements. Either (DI) or (WS) (Souza *et al.*, 2010).

7-Effect of bioagents on tomato growth

Infested pots (20 cm diameter) with Fo 8, Fo 2 were treated with the tested bioagents individually or fungicide Folicure® (250 ppm) as mentioned above. After another week each pot were transplanted by 2 tomato seedlings (023 F1). After 30 days of transplanting, plant height (cm), Root length (cm), Fresh weight (g) and dry weight (g) were recorded. Each treatment was presented by 6 pots as replicates.

Statistical analysis

The protected least significant difference (L.S.D) values at 5 % ($P < 0.05$)

were used to test the differences between treatments (Gomez and Gomez, 1984).

RESULTS

1-Isolation and pathogenicity:

Data in Table (1) show that Isolation process revealed 15 isolate of *Fusarium sp.* Isolate Fo 8 is the highest virulent one. It caused DI% 55.7, DS% 42.2 and vascular discoloration 32.4. Meanwhile, isolates Fo5 Fo10 and Fo12 show also high virulence. Isolate Fo2 was the lowest virulent one. It caused DI% 15.0, DS% 10.4 and vascular discoloration 6.0. The other isolate caused disease incidence ranged from 15.0 to 44.7 and disease severity ranged from 30.3 to 10.4 and vascular discoloration ranged from 0.0 to 23.2. At the same time, some isolates caused wilt symptoms without vascular discoloration such as Fo 1, Fo 5, Fo 9, Fo 12 and Fo 13.

Table (1): Pathogenicity test of *Fusarium oxysporum* isolates expressed as disease severity (DS%), disease incidence (DI%) and vascular discoloration (VD)

Isolates	Disease occurrence			Isolates	Disease occurrence		
	DI%	DS%	VD		DI%	DS%	VD
Fo 1	32.3	13.8	0.0	Fo 9	34.6	25	0
Fo 2	15.0	10.4	6.0	Fo 10	41.4	30.3	16.7
Fo 3	34.7	20.6	16.6	Fo 11	32.6	13.3	16.7
Fo 4	35.8	26.1	17.3	Fo 12	44.7	20.5	0
Fo 5	41.4	25.5	0	Fo 13	32.5	22.2	0
Fo 6	32.6	23.3	16.6	Fo 14	33.4	17.8	16.7
Fo 7	27.6	20.6	23.2	Control	0	0	0
Fo 8	55.7	42.2	32.4	Mean	32.9	20.7	11.6

L.S.D_{5%} A: 4.67 B: 3.3 Ab: 2.91

2-Pathogen Identification

Two fungal isolates of *Fusarium sp* isolate Fo8 and Fo2 were subjected for molecular identification. Data confirmed that both isolates could be identified as *Fusarium oxysporum*. Beside the vascular

discoloration that appears as disease symptoms, it can be *F. o. lycopersici*. Accession number for isolate Fo8 is Sub 12868765 and accession number for isolate Fo2 is Sub 12886795.

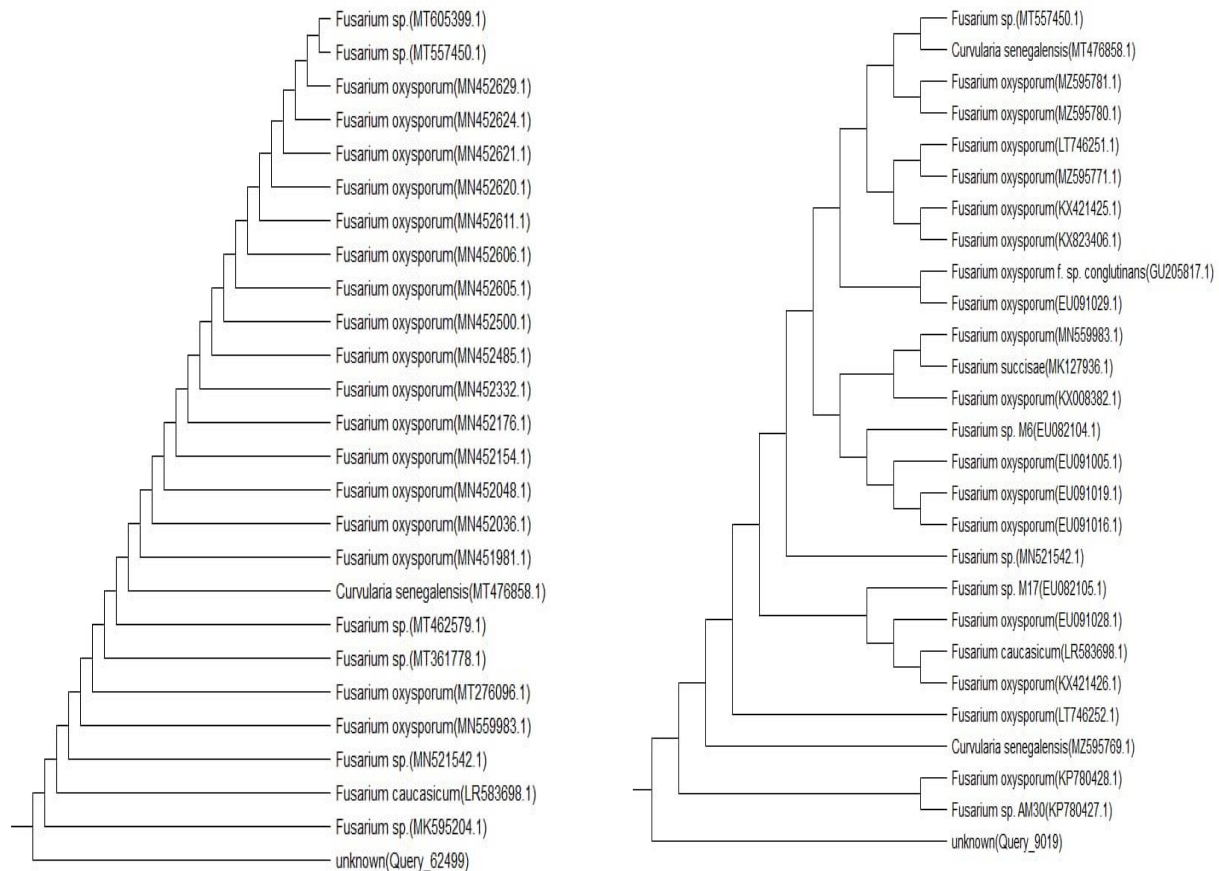


Figure 1: Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (arrowed) aligned with closely related strains accessed from the GenBank in the phylogenetic tree as an outgroup strain. Accession number for isolate Fo 8 and Fo2 is areSub12868765 and Sub 12886795 respectively.

3- reaction of tomato genotypes to infection:

Table (2) clarified the responses of some tomato genotypes toward inoculation by *F. oxysoprum* isolate Fo8 and Fo 2. Data shows that genotype O23F1 and Thuraya is the highest susceptible ones. They show DI and DS 44.9, 24.5 and 39.6, 22.8,

respectively. Meanwhile, Salmiya 65010 and Habiba show the highest resistance ones. The DI and DS was 22.2 and 13.9 in Salmiya 65010 and 22.2 and 19.4 in Habiba, respectively. Generally, Fo8 more is virulent than Fo2. It caused DI ad DS% 40.6 and 26.8 compared with Fo2 that causes 23.8 and 14.2.

Table (2): Responses of some tomato genotypes toward inoculation by *F. oxysporum* isolate Fo8 and Fo 2 expressed as disease incidence (DI%) and disease severity (DS%)

Tomato Genotypes	Disease incidence caused by				Mean	
	Fo 8		Fo 2			
	DI %	DS %	DI %	DS %	DI %	DS %
023 F1	56.8	35.1	33.1	13.9	44.95	24.5
Banora F1	45.4	30.6	21.2	8.3	33.3	19.4
T-186 F1	43.4	25.7	23.2	8.3	33.3	17.5
Thuraya F1	50.6	31.7	28.3	13.9	39.45	22.8
Salmiya 65010 F1	22.2	13.9	22.0	14.4	22.1	14.2
Al-Quds 448 F1	45.7	30.0	23.3	27.8	34.5	28.9
Gs-12 F1	34.7	22.8	20.4	8.3	27.55	15.6
Habiba F1	22.2	19.4	21.2	19.2	21.7	19.3
SV 320 TD F1	44.6	31.7	21.3	13.9	32.95	22.8
Mean	40.6	26.8	23.8	14.2	32.2	20.5

L.S. D_{5%} Genotypes (A) 5.26

Isolates (B) 2.14

AB 12.0

4-Pathogen growth as affected by bioagents:

Table (3) shows that the tested bioagents has ability to reduce the radial growth of both fusarium isolates. The most effecting bioagent for Fo8 is *T. harzianum*. It reduces the radial growth to 6.5 and 8.0 mm for Fo8 and Fo 2 respectively (compared to

45 ml of control). *Streptomyces sp* has the lowest inhibitory effect on the fungal radial growth. It causes about 15.3mm for Fo 8 and 17.2 mm for Fo2 (66.7 and 62.2 inhibition). The fungicide Folicure® show 100% inhibition of radial growth for both tested isolates.

Table (3): Effect of some bioagents, on radial growth (mm) of *F. oxysporum* isolates Fo8 and Fo2 *in vitro*.

Treatments	Growth of Fusarium isolates (mm)					
	Fo8		Fo 2		Mean	
	Radial growth	Inhibition %	Radial growth	Inhibition %	Radial growth	Inhibition %
Control	45.0	0.0	45.0	0.0	45.0	0.0
<i>T. harzianum</i>	6.5	85.6	8.0	81.4	7.75	82.8
<i>B. subtilis</i>	10.3	77.7	10.8	76.6	6.05	77.15
<i>Ps. floresenses</i>	7.2	84.1	9.3	80.0	7.75	82.75
<i>Streptomyces sp</i>	15.3	66.7	17.2	62.2	16.25	64.45
Folicure®	00	100%	00	100%	00	100%
Mean	15.06	62.82	18.06	60.04	34.94	61.43

L.S. D_{5%} Treatment (A) 1.97

Isolates (B) 8.18

(AB) 13.08

4-Effect of Culture Filtrate of Bioagent on the growth of *F. oxysporum*

Data in Table 4 show the effect of culture filtrates of some bioagents on the growth of *F. oxysporum*. The filtrate of *T. harzianum* and *B. subtilis* has the greatest inhibitory effect on pathogen growth comparing o the other bioagents. The radial growth was 39.1 and 37.2 cm and the dray

weight were 391.4 and 363.6 mg respectively. The lowest effect on the pathogen growths was resulted by adding culture filtrate of *Streptomyces* sp. The radial growth was 64.1 cm and the dry weight was 650.9 mg. All tested bioagents had least inhibitory effect on growth of fungus compared to Folicure® fungicide treatment.

Table (4): Effect of culture filtrate of bioagents on dry weight (DW) and linear growth (LG) of Two Isolate of *Fusarium oxysporum*.

Filtrate of		Growth of					
		Fo8		Fo2		Mean	
Bioagents	Conc.	DW (mg)	LG (mm)	DW (mg)	LG (mm)	DW (mg)	LG (mm)
<i>T. harzianum</i>	0%	878.9	90	896.5	90	887.7	90
	5%	435.2	38.1	379.2	40.1	407.2	39.1
	10%	260.9	25.6	280.7	31.2	270.8	28.4
	20%	0	0	0	0	0	0
Mean		393.8	38.4	389.1	40.3	391.4	39.4
<i>Bacillus subtilis</i>	0%	878.9	90	896.5	90	887.7	90
	5%	430.2	45.6	336.7	36.7	383.6	41.2
	10%	156.7	15.2	205.6	19.5	181.2	17.4
	20%	0	0	0	0	0	0
Mean		366.5	37.7	359	36.6	363.1	37.2
<i>Pseudomonas fluorescense</i>	0%	878.9	90	896.5	90	887.7	90
	5%	588.3	59.3	667.4	62.4	627.9	60.9
	10%	340	37.9	490.5	51.3	415.3	44.6
	20%	234.2	25.9	344.6	36.5	289.4	31.2
Mean		355.2	53.3	448.1	60.1	555.1	56.7
<i>Streptomyces spp</i>	0%	878.9	90	896.5	90	887.7	90
	5%	680	66.8	700.	68.3	690.0	67.6
	10%	512.7	50.1	583.4	55.4	548.1	52.8
	20%	490.3	44.3	465.4	48.1	477.9	46.2
Mean		640.5	62.8	661.3	65.5	650.9	64.1
Folicure®	0	878.9	90	896.5	90	887.7	90
	100	496.7	5.5	486.7	5.3	877.7	5.4
	200	263.3	2.2	246.7	2.9	2.5	2.6
	300	0.0	0.0	0.0	0.0	00	00
Mean		409.7	24.4	407.5	24.6	222.6	24.5
Grand Mean		477.8	48.1	502.6	50.6	490.1	49.3
L.S. D at 5 %		170	18.4	165	13.7		

6-Disease incidence as affected by bioagents

Data in Table 6 summarized the effect of 4 bioagents in tomato wilt caused by tow isolates of *F. oxysporium*. It is clearly shows that *T. haresianum* reduced both DI and Ds to 27.8 and 18.1 compared to those in

control plant (61.1 and 44.2 respectively). The lowest protection was recorded in case of using *Streptomyces sp* (55.6 and 33.4). Both bacillus and pseudomonas have a comparable moderate protection effect. The lowest DI and DS was recorded as a result of Folicure treatment for both tested isolates.

Table (5): Effect of some bio-agents on disease severity and disease incidence of tomato plants wilt caused by *F. oxysporum* isolates Fo8 and Fo2 under greenhouse conditions.

Treatments	Disease assessment caused by				Mean	
	Fo8		Fo2			
	DI %	DS %	DI %	DS %	DI %	DS %
Infected +Without bioagent	77.8	55.0	44.4	33.3	61.1	44.2
Infected + <i>Bacillus subtilis</i>	57.0	36.5	33.3	30.5	45.2	34.9
Infected + <i>Ps. fluorescence</i>	60.7	30.8	33.3	16.7	47	23.8
Infected + <i>Tri. Harzianum</i>	33.3	19.4	22.2	16.7	27.8	18.1
Infected + <i>Streptomyces spp</i>	66.7	38.9	44.4	27.8	55.6	33.4
Infected + Folicure®	22.2	16.7	11.1	8.3	16.65	12.5
Not infected	0.0	00	0.0	0.0	0.0	0.0
Mean	53.72	35.93	29.6	20.8		

L.S. D_{5%} Treatment (A) 1.04
 Isolates (B) 2.36
 (AB) 7.13

7-Effect of bioagents on tomato growth

The effect of some biocontrol agents in tomato growth was recorded in Table 6. In general, infection by *F. oxysporium* reduce all growth parameter i.e., plant length, shoot and root length and fresh and dry weight. Treatment infected plant with different bioagents individually has an approximately equal effect in plant height and shoot and root length. Shoot length increased from 22.7 cm in infected plants to about 29 cm in treated ones. The longest root was obtained

after treatment with *T. harzianum*. It is 12.4 cm compared to 7.1 cm in infected plants (17.4 cm in uninoculated plants). The lowest effect was noticed in plants treated with *B. subtilis*. The maximum fresh weight and dry weight are obtained after treatment with *T. harzianum* and *P. fluorescence*. It was 208 and 206 and 150 and 124 mg respectively. Using fungicide folicure at concentration 250 ppm increase all growth parameters comparing with infected plants but still lower than that in control ones.

Table (6) Effect of some biocontrol agents on some vegetative characters of tomato plants.

Treatments	Growth parameters				
	Plant length (cm)	Shoot length (cm)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)
Infected	29.8	22.7	7.13	165	75
Infected + <i>B. subtilis</i>	38.2	29.2	9.0	210	88
Infected + <i>P. fluorescence</i>	40.8	29.6	11.2	260	124
Infected + <i>T. harzianum</i>	41.6	29.2	12.4	280	150
Infected + <i>Streptomyces spp</i>	38.7	28.5	10.2	210	105
Infected + Folicure®	45.6	31.7	13.9	284	165
Control	52.6	35.2	17.4	320	170
Mean	41.0	29.4	11.6	247	125.3
L.S.D 5%	1.35	2.3	1.12	12.27	9.8

DISCUSSION

Tomato wilt disease, incited by *Fusarium oxysporum* is one of the most prevalent, serious diseases of tomato. The pathogen occurs wherever most tomato-growing worldwide exhibited a vascular wilt that can severely affect the crop (Sudhamoy *et al.*, 2009). Molecular identification was carried out to identify the isolated fungi, it show that the causal agent for tomato wilts in Minya governorate is *F. oxysporum*. This pathogen is a main reason to limit successful cultivation of tomato all over the world. (Sidharthan *et al.*, 2019). Till now, all trials to manage this disease have little impact on vascular wilt incidence.

Among the tested genotypes, Salmiya 65010 and Habiba show the highest resistance ones to fungal infection. Using resistant genotype or resistant rootstocks remains the most suitable way to prevent fusarial diseases in tomato production (Biox-Ruiz *et al.*, 2015). however, the breeding tomato genotype resistances to fusarial wilt is cost-effective and environmentally acceptable, but strains of *Fusarium sp* continue to emerge that conflict

the resistance of these cultivars. These conflictant in the control of fusarial wilt disease have led phytopathologist to focus on diverse alternative control approach. (Jogaiah *et al.*, 2018)

Utilization of beneficial microorganisms to soil, seeds or planting materials has been suggested as a sustainable and supplementary tool to manage plant diseases. Bacteria *Bacillus*, *Pseudomonas*, *Rhizobium*, or the fungi *Trichoderma* are the most widely used microorganisms to their antagonistic activity against plant pathogens and plant growth promotion (Raaijmakers *et al.*, 2010). It is important that the screening of bioagent must include *in vitro* and *in vivo* tests because of the influence of environmental and edaphic factors in the mode of action displayed by the antagonist (Ali *et al.*, 2018).

The tested bioagents has ability to reduce the growth of both *Fusarium* isolates. *Trichoderma sp.* is most effected bioagent for both tested isolates. The Actinomycetes has the lowest inhibitory effect on the fugal radial growth. Mousa *et al.*, (2018) found that many bioagents were able to inhibit the

growth of *Fusarium sp* to differing degrees. A range of mechanisms are potentially behind this antagonistic activity and the suppressed of *F. oxysporum*, such as a greater potential for nutrient competition or by the set of products in the media, resulting in inhibition growth rate or *killing F. oxysporum*, or the production of antibiotics that can suppress the growth of pathogens. **Sahu *et al.*, (2019)** isolated a huge number 310 bacterial endophytes from tomato plants has a potential against three soil-borne fungal

Secondary metabolites of *T. harzianum* and *B. subtilis* have the highest inhibitory effect on pathogen growth comparing o the other bioagents. **Eziashi *et al.*, (2006)** found that *Trichoderma sp* produce a variety of extracellular substances that have antifungal activity. **Etebarian (2006)** suggested that the effect of *Trichoderma sp* metabolite exhibited inhibition of mycelia growth of *Macrophomina sp*. Variation antibiotics produced by fungi and bacteria were listed by **Dianez *et al.*, (2007)**.

The present data show that *T. haresianum* reduced both DI and Ds compared to those in control plant. The lowest protection was recorded in case of using *Streptomyces sp*. *Trichoderma spp.* is considered one of the most important filamentous fungi common in soil and plant roots and can be utilized as a bioagent for soil borne fungal and known for their abilities to control plant diseases. **(Tseng *et al.*, 2008)**. **Bargabus *et al.* (2004)** stated that *P. fluorescens* includes triggered systemic resistance beside to the production of siderophores which contributed to inhibition of pathogens. The treated plants with some endophytic bioagents exhibited increase produce of production of defense-related enzymes. Induction of those enzymes in plant cells led to the strengthening of the cell wall through many presses such as

lignifications and suberization which successively acts as a physical barrier to the entry of pathogen in the point of penetration at first interaction **(Singh *et al.*, 2016., Jacobs *et al.*, 2013)**.

In general, infection by *F. oxysporium* reduce all growth parameter i.e., plant length, shoot and root length and fresh and dry weight. Treatment infected plant with different bioagents individually has an approximately equal effect in plant height and shoot and root length. Plant growing can be easily promoted by fungi through several modes, i.e systemic resistance's induction, plant nutrition improvement, and via their toxicity to various pathogens. **Espinoza (2008)** reported that *Rhizopus sphas* fungicidal activity like that of fungicide Captan against wide range of plant pathogens. *Aspergillus sp* are able to produce a lot of bioactive secondary metabolites i.e bioactive proteins, enzymes that may be resulted in plant recovery from harmful effect of *Fusarium* infection **Frisvad, and Larsen (2015)**.

REFERENCES

- Aleksandra, O., Gavrilova, O. and Gagkaeva, T. (2020)** The effect of fungicides on growth of *Fusarium* fungi *in vitro*. *Bio Web of Conf.*, 18: 1-5.
- Ali, A. A. M. (2018)** Role of hydrogen peroxide in management of root rot and wilt disease of thyme plant. *J. Phytopathol. Pest Manag.*, 5: 1-13.
- Ayala-Doñas, A., De Cara-García, M., Talavera-Rubia, M. and Verdejo-Lucas, S. (2020)** Management of soil-borne fungi and root-knot nematodes in cucurbits through breeding for besistance and grafting. *Agronomy*, 10:1-27.

- Bargabus, R. L., Zidack, N. K., Sherwood, J. W., and Jacobsen, B. J. (2004) Screening for the identification of potential biological control agents that induce systemic acquired resistance in sugar beet. *Biol. Control*, 30: 342-350.
- Boix-Ruíz, A., Gálvez-Patón, L., de Cara-García, M., Palmero-Llamas, D., Camacho-Ferre, F., and Tello-Marquina, J. C. (2015)** Comparison of analytical techniques used to identify tomato-pathogenic strains of *Fusarium oxysporum*. *Phytoparasitica.*, 43, 471–483.
- Espinoza, C., González, G. V., Loera, O., Velásquez, B., and Trigos, Á. (2008)** Antifungal activity of several fungi against plant pathogens. *Micologia Aplicada International*, 20(2), 63-67.
- Etebarian, H. R. (2006)** Evaluation of *Trichoderma* isolates for biological control of charcoal stem rot in melon caused by *Macrophomina phaseolina*. *J agric, Sci, Tech.*, 8:243-250.
- Eziashi, E. I., Uma, N. U., Adekunle, A. A. and Airede, C. E. (2006)** Effect of metabolites produced by *Trichoderma* sp against *Ceratocystis paradoxa*. *Biotechnol.*, 5:703-706.
- Frisvad, J. C., and Larsen, T. O. (2015)** Chemodiversity in the genus *Aspergillus*. *Applied Microbiology and Biotechnology*, 99(19), 7859–7877. 62.
- Gomez, K. A. and Gomez, A. A. (1984) Statistical Procedures for Agricultural Research. *John Willey and Sons*. New York, Second Ed. Pp. 680.
- Jacobs, A., Govender, R., and Van-Heerden, S. (2013)** *Fusarium oxysporum* F. sp. *lycopersicirace* 3 causing tomato wilt in South Africa. *Australian Pl. Pathol.*, 8:145-149.
- Jogaiah, S., Abdelrahman, M., Lam-Son, P. T. and Shin-Ichi, I. (2018)** Different mechanisms of *Trichoderma virens*-mediated resistance in tomato against *Fusarium* wilt involve the jasmonic and salicylic acid pathways. *Molecular plant pathology*, 19: 870–882.
- Juber, K. S., Hassan, A. K., and Alhamiri, Y. N. (2014)** Evaluation of biocontrol agents and chemical inducers for managing vascular wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Evaluation*, 4(27).
- Lopez-Aranda, J. M., Dominguez, P., Miranda, L., de los Santos, B., Talavera, M., Daugovish, O. (2016)** Fumigant use for Strawberry production in Europe: the current landscape and solutions. *Int. J. Fruit Sci.* 16, 1–15.
- Michielse, C. B. and Rep, M. (2009)** Pathogen profile update: *Fusarium oxysporum*. *Mol. Plant Pathol.*, 10: 311–324.
- Mousa, M. A. A., Abo-Elyousr, K. A. M., Abdel Alal, A. M. K. and Alshareef, N .O. (2021)** Management *Fusarium* Wilt Disease in Tomato by Combinations of *Bacillus amyloliquefaciens* and Peppermint Oil. *Agronomy*, 11:1-13.
- O'Brien, P. O. (2017)** Biological control of plant diseases. *Australasian Plant Pathology*, 46, 293–304.
- Pothiraj, G., Hussain, Z., Singh, A. K., Solanke, A. U., Aggarwal, R., Ramesh, R. and Shanmugam, V. (2021)** Characterization of *Fusarium* spp. Inciting vascular wilt of tomato and Its management by a chaetomium-based biocontrol consortium. *Front. Plant Sci.* 12:748013.

- Pottorff, M., Wanamaker, S., Ma, Y. Q., Ehlers, J. D., Roberts, P. A., and Close, T. J. (2012)** Genetic and physical mapping of candidate genes for resistance to *Fusarium oxysporum f. sp. tracheiphilum* race 3 in cowpea [*Vigna unguiculata* (L.) Walp. *PLoS ONE*, 7:1-12.
- Raaijmakers, J. M., De Bruijn, I., Nybroe, O. and Ongen, M. (2010)** Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiol Rev* 34:1037–1062.
- Sahu, P. K., Singh, S., Gupta, A., B. Singh, U., Brahmaprakash, G. P. and , Saxena, K. A. (2019)** Antagonistic potential of bacterial endophytes and induction of systemic resistance against collar rot pathogen *Sclerotium rolfsii* in tomato. *Biological control*, 137:1-10.
- Sharma, A., Wray, V., Johri, N B. (2006)** Rhizosphere *Pseudomonas sp.* strains reduce occurrence of pre-and post-emergence damping-off in chile and tomato in Central Himalayan region. *Arch. Microbiol.*, 187:321–335.
- Sidharthan, V. K., Aggarwal, R., and Shanmugam, V. (2019)** *Fusarium* wilt of crop plants: disease development and management in: *Wilt Diseases of Crops and their Management*, eds A. Bhattacharyya, B. N. Chakraborty, R. N. Pandey, D. Singh, and S. C. Dubey . Ppb519–533 (New Delhi: Today and Tomorrow Printers and Publisher).
- Singh, R., Maurya, S. and Upadhyay, R. S. (2016)** The improvement of competitive saprophytic capabilities of *Trichoderma* species through the use of chemical mutagens. *Brazilian J. Microb.*, 47: 10-17.
- Souza, L. T., Michereff, S. J., Laranjeira, D., Andrade, D. E. G., Ferraz, E., Gsa, L. I. M. and Reis, A. (2010)** Reaction of tomato genotypes to races 2 and 3 of *Fusarium oxysporum f. sp. Lycopersici*. *Horticultura Brasileira*, 28: 102-106.
- Sudhamoy, M., Mallick, N. and Mitra, A. (2009)** Salicylic acid-induced resistance to *Fusarium oxysporum f. sp. lycopersici* in tomato. *Plant Physiology and Biochemistry*, 47: 642-649.
- Sultana, F., and Hossain, M. M. (2022)** Assessing the potentials of bacterial antagonists for plant growth promotion, nutrient acquisition, and biological control of southern blight disease in tomato. *PLoS One*, 17(6), e0267253.
- Tseng, S. H. Liu, S. Y. Yang, H. H., Lo, C. T. and Peng, K. C. (2008)** Proteomic study of biocontrol mechanisms of *Trichoderma harzianum* ETS 323 in response to *Rhizoctonia solani*. *J. Agric. Food Chem.*, 56: 694-6922.
- Wani, A. (2011)** An overview of the fungal rot of tomato. *Mycopathology*, 9:33–38.
- Weitang, S., Ligang, Z., Chengzong, Y., Xiaodong, C., Liqun, Z., and Xili, L. , (2004)** Tomato *Fusarium* wilt and its chemical control strategies in a hydroponic system. *Crop protection*, 23: 120-123.
- White, T. J., Bruns, T., Lee, S. and Taylor, J. (1990)** Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A guide to Methods and Applications* (ed. M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White), pp. 315-322. Academic Press: San Diego, U.S.A.

تحفيز نمو نباتات الطماطم ومقاومتها ضد الاصابة بالفيزوزاريوم باستخدام بعض الطرق الحيوية

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يعتبر فطر الفيزوزاريم واحد من اهم الفطريات التي تسبب خسارة هائلة في محصول الطماطم. واطهر التعريف الجزيئى ان الفطر المسبب لذبول الطماطم المعزول من محافظة المنيا هو الفيزوزاريم اوكسيسبورم. وبينت الدراسه ان الطرازين الوراثى بين سالميا وحبيبه هم اكثر الطرز الوراثيه مقاومة للفطر من ضمن 9 طرز وراثيه مختبره. وكان لفطر التريكودرما هارزيانم والبكتريا باسيلس ستلس أكثر تأثير مثبت على نمو الفطر وكذلك كان لراشح مزارعهم نفس التأثير. كما كان لفطر التريكودرما تأثير معنوى في خفض نسبة الاصابه وشدتها بالذبول وأيضا كان له تأثير ايجابى على مقاييس النمو لنباتات الطماطم.