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Efficiency of Some Bioagents and Potassium Humate in Controlling Tomato wilt Disease caused by *Fusarium Oxysporum* f.sp. *Lycopersici*

*** Mona F. Ahmed; ** Nawal A. Eisa; **Gehad M. El-Habbaa; **Ibrahim A. I. El-Fiki and *Seham M. Ragab**
*Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt.
**Plant pathol. Dept., Fac. Agric., Benha Univ., Egypt.
Corresponding author: monafahim78@gmail.com

Abstract

Fusarium oxysporum f.sp. *lycopersici* (FOL) is one of the most important fungi causing vascular wilt disease of tomato plants. Many different agents *i.e., Bacillus subtilis, Trichoderma harzianum, T. viride,* alga *(Sparulina platensis),* Mycorrhiza (*Glomus* sp.) and potassium humate were investigated for their antagonistic effects on the growth of FOL *in vitro*. *T. harzianum* and potassium humate were the most effective treatments, followed by *B. subtilis* and alga extract. The previously mentioned treatments were studied under greenhouse conditions and natural infection in the field. *T. harzianum* and potassium humate were the most effective treatments against FOL followed by *B. subtilis* and alga extract. Mycrrohiza was the lowest effect treatment under greenhouse and field conditions. *T. harzianum* and potassium humate decreased the activities of hydrolyzing enzymes and increased all of total phenols and plant parameters (fresh, dry weight and length/ plant) of treated tomato plants.

Keywords: *F. oxysporum*, Tomato plants, Biological agents, potassium humate, hydrolyzing enzymes, phenols, growth parameters of the plants.

Introduction

Tomato, (*Solanum lycopersicum* L.), is known as one of the most important vegetable crops worldwide. It has been reported that it could be infect by many pathogens like *F. oxysporum* f. sp. *lycopersici* (FOL), *F. solani*, *F. semitectum*, *Rhizoctonia solani*, *Verticillium albo-atrum*, *Microphomina phaseolina*. *F. oxysporum* is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth **(Hirano and Arie, 2006)**. It causes major economic losses by inducing necrosis and wilting symptoms in many crop plants **(Cotxarrena *et al.*, 2002)**.

Many studies have been carried out to find more convenient alternatives and environmentally safer methods than using fungicides to control plant diseases **(Singh and Prithiviraj, 1997, Paul and Sharma, 2002 and Agbevin *et al.*, 2004)**. Using the antifungal plant products has been proven to be an efficient solution in controlling many plant diseases. Also, *in vitro* using of microbial antagonists significantly reduced seed infection caused by *Fusarium oxysporum* **(Sultana and Ghaffar, 2013)**. *Trichoderma harzianum, T. asperellum,* and *T. virens* affect tomato wilt disease under greenhouse conditions where they caused 80-87% inhibition of *F. oxysporum* compared to the control **(Mohammad and Zohreh, 2015)**. It has been reported that *Trichoderma* spp. (28 isolates) confer plant protection against tomato wilt and promote growth of tomato plants under *in vitro* and *in vivo* conditions. *In vitro, T. harzianum* (N-8) inhibited the radial mycelia growth of the tested pathogen effectively. Also, the same isolate caused the least disease incidence while, *in vivo*, *T. harzianum* followed by *T. viride* and *Bacillus subtilis* caused the maximum reduction in seedling and root infections **(Sultana and Ghaffar, 2013 and Barari, 2016,)**.

Abdel-Monaim *et al.*, (2014) reported that potassium humate and alga (*Sparulina platensis*) increased plant growth parameters (plant height and number of branches). Also, using extracts of *Sparulina platensis*, as chemical fertilizer in view of a sustainable agriculture was safe to the environment, human and animal health **(Aghofach *et al.*, 2015)**.

The objective of this study is to evaluate the potential of different bioagents and natural products for reducing the severity of *Fusarium* wilt of tomato *in vitro* and *in vivo*.

Materials and Methods

Isolation, identification and pathogenicity test:

Tomato plants showing typical symptoms of the Fusarium wilt disease were collected from different tomato locations in nine governorates in Egypt (Ismailia, Bani-Sweif, Beheira, Giza, Damietta, Fayum, Sharkiya, Qalyubia, and Minia) during autumn, winter and spring of growing seasons 2012 and 2013. Cuttings (3 cm length) from the stem of tomato plants above the crown area revealing different degrees of vascular discoloration were used for isolation of the wilt fungus **(Katan *et al.*, 1991** and **Amini, 2009)**. The growing fungi were purified using the hyphal tip technique **(Nelson, 1983).** Then, the purified fungal isolates were identified through morphological investigation as reported previously by **Barnett and Hunter, (1998)** at Department of

Mycology and Fungi Disease Survey Research. Plant Pathology Institute, ARC, Giza, Egypt.

Pathogenicity test and Koch's postulates were carried out successfully for each isolate on tomato seedlings (Super Strain B hybrid) in pots (20 cmØ) using sand corn inoculum under greenhouse conditions to confirm their virulence and then the re-isolated pure cultures of FOL were maintained on PDA slants at 4°C. The highest virulent FOL isolate was used for the next trails. Pathogenicity test was carried out under greenhouse conditions of the Plant Pathology Institute, Agricultural Research Center (ARC), Giza, Egypt.

Control of FOL fungus *in vitro*

1-Effect of potassium humate and alga extract on FOL growth.

During this experiment, the tested commercial potassium humate and alga (*Sparulina platensis*) were screened against highest virulent FOL isolate *in vitro* during pathogenicity test as mentioned above. The tested commercial potassium humate was obtained kindly from the Agricultural Wastes Training Center, Moshtohor, Qalyubia Governorate, while, the tested alga (*S. platensis*) was obtained kindly from Microbiology Dept. Soil, Water and Environment Research Institute Agricultural, Research Center, Giza, Egypt.

Potato dextrose agar medium (PDA) was used in the experiment. The antifungal activity of tested treatments accomplished on PDA plates (90 mmØ). Each of humate or alga was tested at concentrations of 1, 2, 3, 4, and 5% by adding 1, 2, 3, 4 and 5 mL of humate solution or alga extract to 99, 98, 97, 96 and 95 mL of melted PDA medium, respectively, to be the final tested concentrations, then poured into Petri dishes in triplicates. After solidification, a disc (3 mmØ) of the tested FOL isolate was placed in the center of each plate. PDA plates treated with Topsin M70 fungicide (700 µg/L medium) served as treated control while, the inoculated plates with FOL agar disc only on the center of the plate served as un-treated control. All plates were incubated at 28°C for 7-10 days, then the reduction percentage was calculated as the following using the formula suggested by **Sirirat *et al.*, (2009)**.

$$\text{Reduction Percentage} = \left(\frac{de - di}{de}\right) \times 100$$

Where, de = mean diameter of growth in control**;** di= mean diameter of growth in treatment.

2-Effect of *Trichoderma harzianum*, *T. viride* and *Bacillus subtilis* on FOL growth.

In this experiment, the effect of some bioagents *i.e., T. harzianum*, *T. viride* and *B. subtilis* (obtained kindly from the Mycology Research and Disease Survey Department, Plant Pathology Institute, ARC, Giza, Egypt) were investigated for their antagonistic effects on the growth of the same highest virulent FOL isolate *in vitro* as mentioned above. Dual culture technique was used for *in vitro* evaluation against the tested FOL isolate. PDA medium was used in this experiment. The antifungal activity of tested treatments accomplished on PDA plates (90 mmØ). PDA medium was poured into Petri dishes, after solidification, a disc (3 mmØ) of each one of the tested *Trichoderma* isolates was placed in one side of each plate. At the same time, a disc (3mmØ) of the tested FOL isolate was placed in the opposite side of the plate. *B. subtilis* was inoculated by streaking onto one side of the prepared Petri dishes and at the same time another disc (3 mmØ) of the pathogen was placed in the opposite side. Each Treatment was replicated three times. On the other hand, PDA plates treated with Topsin M70 fungicide (700 µg/L medium) served as treated control while, the inoculated plates with FOL agar disc only on the center of the plate served as un-treated control. The plates were incubated at 28°C for 7-10 days then the growth reduction was calculated as mentioned above.

Control of FOL fungus *in vivo*

1-Greehouse experiment

The present experiment was carried out on tomato plants (Super Strain B hybrid) in pots (20 cmØ) under greenhouse conditions at Plant Pathology Research Institute Agricultural Research Center Giza, Egypt, during the two successive growing seasons 2014-2015 and 2015-2016. In this experiment, the antagonistic effects of *T. harzianum*, *T. viride*, *B. subtilis*, alga (*S. platensis*), mycorrhiza (*Glomus* sp.), potassium humate and the fungicide Topsin M70 were investigated against the tested FOL isolate as following:

		Treatment	Used concentration
1-	FOL-Inoculated	*T. harzianum*	$1x10^5$ spore/mL
2-		*T. viride*	$1x10^5$ spore/mL
3-		*B. subtilis*	$1x10^6$cfu/mL
4-		Alga (*S. platensis*)	5% (used at 50 mL/L)
5-		Mycorrhiza (*Glomus sp*.)	10 g/Kg soil
6-		Potassium humate	3g/L
7-		Topsin M70	700µg/L
9-	FOL-uninoculated	Control	Water-irrigated

Mycorrhiza (*Glomus* sp.) was used as recommended by Mycology and Fungi Disease Survey Research Department, Plant Pathology Institute, ARC, Giza, Egypt. All treatments were applied as root immersion for 20 min. The experimental treatments were done in randomized complete block design with four replicates (pots). The second treatments were carried out one month after the first treatment with soil drainage.

Preparation the inocula of tested bioagents:

The antagonistic bacterium (*B. subtilis*) was grown on nutrient broth medium **(Abd-Alla *et al.*, 2007)**. The bacterial isolate was incubated in a rotary shaker at 200 rpm for 48h at 28±2°C. The bacterial cells were harvested by centrifugation at 6000 rpm for 10 min, washed twice with 0.05 M phosphate buffer at pH 7.0 and re-suspended in sterilized distilled water. The concentration of bacteria was adjusted to approximately 10^6 cfu/ml using turbidity meter as mentioned by **(Abdel-Kader *et al.*, 2012)**. Meanwhile, the antagonistic fungi were grown on PDA medium and incubated for ten days at 25 ± 2°C. Fungal conidia and mycelium were harvested by scraping the surface of the colonies with a spatula and transferred to sterilized distilled water and filtered through nylon mesh, and then spore suspension was adjusted approximately to 10^5 spore/mL with the aid of a haemacytometer slide, **(Abd-Alla *et al.*, 2007)**.

Preparation the inocula of alga and mycorrhiza:

Alga treatment was prepared for application at rate 50 mL alga extract/L water (5%), meanwhile, mycorrhiza (*Glomus.* sp.) treatment was prepared for application at rate 10 g. /kg soil.

Preparation of Potassium Humate solution:

Potassium humate solution was prepared for application at rate 3 g potassium humate/L water.

Preparation of FOL inoculum:

The inoculum of FOL was prepared as mentioned above in pathogenicity test as following:

The tested fungal isolate was inoculated into autoclaved sand corn medium (25g clean sand and 75g corn covered by enough tap water in 500 mL bottles). The pots (20 cmØ) were filled by 2 Kg of sterilized soil and infested by the prepared inoculum at rate of 3% of soil weight then irrigated regularly for one week before treating with the different treatments and then transplanting the seedlings (4 weeks old of Super Strain B hybrid). Each treatment was replicated 4 times and 4 seedlings were placed in each pot. The control treatment was pots filled by the same soil and inoculated with FOL only.

2-Field experiment

In this trail, tomato plants (Super Strain B hybrid), naturally infected with Fusarium wilt disease under open field conditions at EL-Khanka region, Qalyubia Governorate during the summer growing season 2017 were used. The treatments were arranged in randomized complete block design with three replicates (plots). Each experimental plot included 4 ridges, each one 70 cm wide and 5 m long. Plot area was 10.5 m^2. At the 1st of May, transplanting of thirty-days old of tomato seedlings (Super Strain B hybrid) took place in one side of the ridge in the presence of water at 30 cm a part and each plot contained 27 plants. All agronomic practices endorsed by Ministry of Agriculture, Egypt were carriedout for cultivation of tomato plants, except fungicide application practices. This experiment aimed to evaluate the best-chosen treatments which have inhibitory effects on the fusarium wilt pathogen (FOL) *in vivo*. Treatments were applied individually as root immersion as following: *T. harzianum* (at 10^5 cfu/mL), *B. subtilis* (at 10^6 cfu/mL), potassium humate (at 3g/L), alga (*S. platensis* at 5%), and mycorrhiza (*Glomus* sp.) at 10g/Kg soil and the fungicide (Topsin M70) at recommended dose700µg/L. Plants sprayed with water used as control.

Disease assessments and some vegetative parameters

Two months post transplanting of tomato plants, disease incidence and disease severity percentage were recorded. Also, fresh weight/plant (g.), dry weight/plant (g.) and plant length (cm.) were recorded for each treatment of greenhouse and field trails. Disease incidence (DI%) was calculated and expressed in percentage scale by using the following formula: DI%= (D/T) X 100, where, (D)= Number of diseased plants; (T) = Total observed plants. For assessing disease severity, ascertained number of plants were selected randomly in each replication (plot) to estimate disease severity individually for each one using 0-5 rating scale as described by **Xiao and Subbarao, (1998)** where, 0= no discoloration, 1= 1 to 10% discoloration, 2= 11 to 30% discoloration, 3= 31 to 50% discoloration, 4= 51 to 75% discoloration and 5= 76% to 100% discoloration. Fusarium wilt disease severity% was assessed according to the following formula: Disease severity % = Σ (n X v)/5N) X 100, where, (n) = Number of plants in each category; (v)= Numerical values of symptoms category; (N) = Total number of plants; (5) = Maximum numerical value of symptom category. Efficacy (Ef%) percentage of different treatments was calculated based on mean of disease incidence and disease severity percentage. Efficacy% was calculated for comparing between all tested treatments with untreated control as follows:

Ef % = control – treatment/control X 100.

Biochemical Studies

1- Enzymatic Activity

Two months post transplanting of tomato plants, samples representing the whole plant were taken from each treatment for determining phenolic compounds (conjugated, free and total phenol),

cellulose (CX), polygalacturonase (PG) percentages assessments.

For phenolic contents determination, whole fresh plant samples were extracted separately using the suggested method of A**neja, (2001)**. Phenolic compounds were calculated for each treatment as milligrams of catechol/g fresh weight of leaves. The developed color was measured at 520 nm using spectrophotometer against a reagent blank.

For enzymatic determinations, the crude leaf enzyme extract was prepared as recommended by **Aneja, (2001).** Crude leaf extract was prepared by homogenizing 50 grams of each treatment with 50 mL of distilled water, then filtrated through two layers of cheese cloth and centrifuged at 3000 rpm for 10 minutes. The clear supernatant was used to estimate the pectolytic and cellulolytic enzymes activity. CX activity determination was measured using viscosity method described by **Aneja, (2001)** as follows: 1.2% carboxy methyl cellulose (CMC) substrate was added into phosphate buffer solution at pH 5.6 then 2.5 mL of the crude enzyme sample was added to 5 mL buffer and incubated at 30°C. Viscosity of the reaction mixture was estimated before incubation (zero time), and after 30 minutes incubation. Loss in viscosity was calculated according to this formula:

T0 – T1/T0 – Tw X 100, where, T0 = the time of flow in seconds of the treated mixture at zero time; T1 = the time of flow at a given time interval and Tw = the time of flow at distilled water.

Concerning PG activity determination, 1.2% pectin substrate was added to phosphate buffer solution at pH 5.6 then 2.5 mL of the crude enzyme sample was added to 5 mL buffer and incubated at 30°C. Viscosity of the reaction mixture was estimated before incubation (zero time), and after 30 minutes incubation as mentioned before in CX activity determination.

Statistical analysis

All data were subjected to the proper statistical analysis using the MSTAT statistical software and comparison was made following fishers L.S.D. (0.05). **(Gomez and Gomez, 1984).**

Results and Discussion

Isolation of Fusarium wilt pathogen and frequency of isolated microorganisms:

Results in Table (1) indicate that *Fusarium oxysporum* was the most frequently isolated fungus. It was isolated from all the nine governorates. Fayum isolate showed the highest frequency rate (73.7%) followed by Beheira (70%), Sharkiya (66.7%), Bani-Sweif (57.8%), Qalyubia (55.6%), Ismailia (53%), Minia (47.4%) and Damietta (41.7%) meanwhile, Giza show the least frequency (37.5%). On the other hand, *Microphomina phasolina* was the lowest frequency rate in Damietta (8.2%) followed by Qalyubia (5.5%), Minia and Bani-Sweif (5.3%), Fayum (4.4%), Sharkiya, Beheira, Giza and Ismailia (0.0%). **Selim and Zanaty, (2014)** isolated *Fusarium oxysporum* from five different governorates in the Nile Delta. Four governorates within the Delta in addition to Ismailia in the east were chosen. Fusarium isolates were obtained from the stem of naturally infected tomato plants. Another scientists isolated *Fusarium oxysporum* f. sp. *lycopersici* **Akram *et al.*, (2014), Priyanka *et al.*, (2014), Narendra and Swati, (2015) Barari, (2016) and Mohammed *et al.*, (2016)**.

Table 1. Isolation of tomato fusarium wilt pathogen and frequency of isolated microorganisms.

Isolated microorganism	Isolation localities																	
	Ismailia		Bani Sweif		Beheira		Giza		Damietta		Fayum		Sharkiya		Qalyubia		Minia	
	Fr	%	Fr	%	Fr	%	Fr	%	Fr	%	Fr	%	Fr	%	Fr	%	Fr	%
F. oxysporum	9	53.0	11	57.8	7	70.0	3	37.5	5	41.7	17	73.8	4	66.7	10	55.6	9	47.7
F. solani	4	23.5	1	5.3	1	10.0	1	12.5	0	0.0	2	8.7	0	0.0	2	11.1	2	10.5
F. semitectum	0	0.0	2	10.5	0	0.0	0	0.0	2	16.7	0	0.0	0	0.0	2	11.1	2	10.5
R. solani	4	23.5	4	21.1	1	10.0	1	12.5	2	16.7	2	8.7	1	60.6	3	16.7	5	26.3
V. albo-atrum	0	0.0	0	0.0	1	10.0	3	37.5	2	16.7	1	4.4	1	60.7	0	0.0	0	0.0
M. phaseolina	0	0.0	1	5.3	0	0.0	0	0.0	1	8.2	1	4.4	0	0.0	1	5.5	1	5.3
Total	**17**	**100**	**19**	**100**	**10**	**100**	**8**	**100**	**12**	**100**	**23**	**100**	**6**	**100**	**18**	**100**	**19**	**100**

Fr= frequancy

1-Pathogenicity test

Data in Table 1 indicate that, FOL isolate of Sharkiya gave the highest disease severity and disease incidence percentage (39.7 and 68.7%) respectively, followed by Damietta isolate (25 and 50%), then Giza isolate (21.2 and 43.7%), Beheira isolate (18.7 and 37.5%), Bani Sweif isolate (17.5 and 31.2%), Minia isolate (16.2 and 37.5%), Ismailia isolate (15.0 and 31.2%) and Fayum isolate (13.7 and 25.0%). On the other hand, the lowest recorded disease incidence and disease severity were 12.5 and 25.0% Qalyubia isolate. These results could be discussed in light the findings of **Narendra and Swati, (2015)** and **Barari, (2016)** who tested the pathogenicity of the FOL isolates on PKM1 tomato cultivar and determined that the most virulent isolate was FOL (L-8).

Table 2. Pathogenicity test of FOL isolated from different governorates on tomato plants (Super Strain B hybrid) under greenhouse conditions.

Governorate	Disease incidence%	Disease severity %
Ismailia	31.2	15.0
Fayum	25.0	13.7
Beheira	37.5	18.7
Bani Sweif	31.2	17.5
Minia	37.5	16.2
Giza	43.7	21.2
Damietta	50.0	25.0
Sharkiya	68.7	39.7
Qalyubia	25.0	12.5

Effect of tested bioagents and potassium humate on growth of FOL *in vitro*

Data in Table 2 reveal that all tested bioagents i.e., *T. harzianum*, *B. subtilis* and *S. platensis* and potassium humate in addition to Topsin M70 fungicide reduced effectively the radial growth of tested FOL pathogen. Data in the same table reveal that all tested treatments had clear significant inhibitory effects on FOL growth comparing with control treatment. In this respect, *T. harzianum* at 10^5spore/mL gave the highest reduction percentage (87.7%) followed by *B. subtilis* at 10^6cfu/mL (80.0%) and *Trichoderma viride* at 10^5spore/mL (77.7%), respectively. While, alga extract at 5% and 4% gave 74.4 and 64.4% reduction percentage on pathogen mycelial growth, while, potassium humate gave the lowest reduction percentage (57.7%) at the concentrations of 3, 4 and 5g/L. These results could be discussed in light that *T. harzianum* and *B. subtilis* could be involved for controlling Fusarium wilt disease on tomato **(Abdel-Kader, *et al.* 2012)**. On the other hand, **Alwathnani and Perveen, (2012)** tested some of biological control agents to control wilt disease of tomato incited by FOL *in vitro* and found that, *T. harzianum* inhibited the radial colony growth of the tested pathogen. It is well established that, potassium humate had inhibitory effect on radial growth of the pathogen where, it was evaluated for controlling wilt disease caused by *F. oxysporum* f.sp. *lycopersici* on Super Strain B hybrid and found that potassium humate able to inhibit linear growth of the pathogen (**Abdel-Monaim, *et al.* 2012).** Fulvic acid and humic acid potentially suppressed *Alternaria alternata* and *Fusarium cucumarun* in PDA medium **(Moliszewska and Pisarek, 1996).**

Table 3. Effect of tested bioagents and potassium humate on radial growth of FOL *In vitro*.

Treatment	Concentration	Radial mycelial growth (mm)	Reduction %
Alga extract (*Sparulina platensis*)	1%	85.0 b	5.5
	2%	56.0 d	41.1
	3%	43.0 e	52.2
	4%	32.0 g	64.4
	5%	23.0 h	74.4
Potassium humate	1g/L	73.0 c	18.8
	2g/L	70.0 c	22.2
	3g/L	38.0 f	57.7
	4g/L	38.0 f	57.7
	5g/L	38.0 f	57.7
B. subtilis	10^6cfu/ml	18.0 i	80.0
T. viride	10^5spore/ml	20.0 hi	77.7
T. harzianum	10^5spore/ml	11.0 j	87.7
Topsin M70	700µg/L	0 k	99
Control		90.0 a	0.0
LSD $_{0.05}$		**2.568**	**-**

Effect of tested bioagents and potassium humate on tomato Fusarium wilt disease assessments under greenhouse conditions

Data in Table 4 indicate that all tested biotic and abiotic treatments *i.e., T. harzianum* (10^5spore/mL), *B. subtilis* (10^6cfu/mL), *T. viride* (10^5spore/mL), potassium humate (3g/L), alga extract (*Spirulina platensis*) at 5%, mycrrohiza (*Glomus* sp.) at 10 g/Kg soil and Topsin M70 fungicide at 700µg/L were able to reduce effectively the infection and disease severity% of Fusarium wilt disease on tomato Super Strain B hybrid under greenhouse conditions during the two successive growing seasons 2014-2015 and 2015-2016**.** As shown in the same table, all treatments had clear significant reduction on tomato disease assessments (Fusarium disease severity and disease incidence%) comparing with the control treatment. In this respect, Topsin M70 gave the

highest significant reduction of fusarium disease severity and disease incidence % during the two growing seasons. Topsin M70 followed by *T. harzianum* and potassium humate recorded the highest efficacy% of disease severity (96.22, 84.89 and 84.89%, respectively) and disease incidence % (91.69, 80.95 and 80.95%, respectively). These obtained results confirmed that, Topsin M70 considered good preventive resource for controlling such disease. But, biological agents and potassium humate could be alternatives to the passive use of chemical fertilizers and fungicides in view of a sustainable agriculture that is friendly to the environment, human and animal health. *T. harzianum* tested as biological control agent to control Fusarium wilt of tomato under greenhouse conditions. These results are in harmony with those obtained by **Alwathnani and Perveen, (2012)** where, *T. harzianum* showed seed germination enhancement by 80% and enhanced plant growth parameters significantly in all treatments. **Sultana and Ghaffar, (2013)** reported *in vivo* that the maximum reduction in seedling and root infection was observed with *T. harzianum* treatment followed by *T. viride* and *B. subtilis*. Concerning alga (*Spirulina platensis*), **Aghofach *et al.*, (2015)** mentioned that extracts or powder of *S. platensis* were the most effective in improving tomato plant growth and development parameters. Also, **Layam *et al.*, (2016)** used *S. platensis* as a bio-fortification agent in light the role of zinc and its role for plant protection and nutrition where, *S. platensis* enhance zinc levels in tomato. The obtained results emphasize the application of *S. platensis* to enhance the mineral nutrient in plants which are non-polluting, inexpensive, and utilizing renewable resource to maintain the soil fertility. **Akkopru and Demir (2005)** mentioned that the used *Glomus intraradices* inhibit FOL at the rate of 17.3% and enhanced dry root weight effectively, also **Al-Hamoud and Al-Momany, (2015)** aimed at determining the efficiency of different vesicular arbuscular mycrrohiza fungi (VAM), symbiotic fungi that interact with the root system of higher plants by producing external and internal hyphae, in improving plant resistance against FOL. The enzymatic activity in the soil is strongly connected to the soil organic matter contents, which provides substrate to support microbial biomass, hence, higher enzyme production by Potassium humate **(Yuan and Yue, 2012). Abdel-Monaim *et al.*, (2014)** reported that potassium humate, Alga and effective microorganisms significantly increased growth parameters (plant height and number of branches).

Table 4. Effect of tested bioagents and potassium humate on tomato Fusarium wilt disease assessments *in vivo* under greenhouse conditions during the two successive growing seasons 2014/15 and 2015/16

Treatment	Concentration	Disease severity%				Disease incidence%			
		2014/15	2015/16	Mean	Ef%	2014/15	2015/16	Mean	Ef%
S. platensis	5%	7.5d	6.2c	6.85	79.31	18.7c	12.5c	15.60	76.22
P. humate	3g/L	5.0e	5.0d	5.00	84.89	12.5d	12.5c	12.50	80.95
B. subtilis	10^6 cfu/mL	7.5d	6.2c	6.85	79.31	18.7c	12.5c	15.60	76.22
T. viride	10^5 cfu/mL	10.0c	11.2b	10.60	67.98	25.0b	25.0b	25.00	61.89
T. harzianum	10^5 cfu/mL	5.0e	5.0d	5.00	84.89	12.5d	12.5c	12.50	80.95
Mycorrhiza	10 g/Kg soil	11.2b	11.2b	11.20	66.16	25.0b	25.0b	25.00	61.89
Topsin M 70	700µg/L	1.2f	1.3e	1.25	96.22	5.7e	5.2d	5.45	91.69
Control		33.7a	32.5a	33.10	0.00	68.7a	62.5a	65.60	0.00
LSD at 0.05		**0.461**	**0.588**			**0.877**	**0.670**		

The same letters mean no significant difference **Ef%= Efficacy %**

Effect of tested bioagents and potassium humate on tomato Fusarium wilt disease assessments *in vivo* under open field conditions

Data in Table 5 indicate that all tested biotic and abiotic treatments recorded high significant difference of Fusarium wilt disease severity and disease incidence % and the same trend were found in respect of its efficacy. Topsin M70 gave the highest significant reduction in disease severity and disease incidence where, they were 1.0 and 1.07%. Concerning efficacy of treatments, Topsin M70 followed by *T. harzianum* and humate treatment gave the highest efficacy while, mycorrhiza treatment gave lowest one. **Abdel-Monaim *et al.*, (2012)** evaluated potassium humate to control wilt disease caused by FOL on tomato Super Strain B hybrid. All treatments significantly reduced disease severity and incidence % as well as, increased plant height, fresh and dry weight of survival plants growing in pots infested with the causal pathogen compared with control. **Aghofach *et al.*, (2015)** mentioned that the use of extracts of *S. platensis* showed significant effects on the parameters of growth and development of tomato plants, increased the height, diameter compared to control plants. Thus, they can be alternative to the massive use of chemical fertilizers in view of a sustainable agriculture that is friendly to the environment, human and animal health, also **Ozgonen *et al.*, (2001)** inoculated *Glomus etunicatum* (GE) in pot experiment and the results indicated that GE increases the growth of tomato plants and could be used against Fusarium wilt of tomatoes.

Table 5. Effect of tested bioagents and potassium humate on tomato Fusarium wilt disease under field conditions during the growing season 2016/17.

Treatment	Concentration	Disease assessments			
		Disease severity%	Efficacy %	Incidence %	Efficacy %
S. platensis	5%	3.7c	82.7	7.4c	81.8
Potassium humate	3 g / L	2.9d	86.4	7.3c	81.8
B. subtilis	10^6cfu/mL	3.7c	82.7	7.5c	81.8
T. harzianum	10^5spore/mL	2.9d	86.4	7.4c	81.8
Mycorrhiza	10g/ kg soil	5.1b	76.1	11.1b	72.7
Topsin M70	700 µg/L	1.0	95.3	1.07d	97.4
Control		21.4a		40.7a	
LSD at 0.05		0.586		0.510	

The same letters mean no significant difference

Effect of tested bioagents and potassium humate on some vegetative parameters of treated tomato which naturally infected with fusarium wilt disease *in vivo* under greenhouse conditions

Data in Table 6 reveal that treatments *i.e.,* Topsin M70 (700 µg/L), *T. harzianum* (10^5 spore/mL), potassium humate (3g/L), *B. subtilis* (10^6 cfu/mL), and mycrrohyiza (10g/kg soil) had a great positive effect on some vegetative parameters of tomato plants which naturally infected with FOL under open field conditions comparing with control treatment. In this respect, Topsin M70 treatment recorded the highest significant fresh and dry weight/plant, and length of the plant (430.0 g, 60.0 g and 75.0 cm), respectively, followed by *T. harzianum* treatment (422.0 g, 51.0 g and 72.0 cm), respectively. Meanwhile, potassium humate treatment recorded 415.0 g,50.0 g and 71 cm of the three parameters respectively, followed by *B. subtilis* which recorded 395.0 g, 45.0 g and 69.0 cm respectively. On the other hand, alga (*S. platensis*) recorded 265.0 g, 32.0 g and 32.0 cm respectively and mycorrhiza recorded the lowest vegetative parameters (195.0 g, 20.0 g and 66.0 cm). Generally, all treatments increased the studied vegetative parameters of the tomato plants. In this respect, **Aghofach *et al.*, (2015)** mentioned that the use of extracts or powder of *S. platensis* showed significant effects on growth parameters and development of tomato plants and increased the height, diameter, biomass of aerial parts, as well as the number of fruits compared to control plants. The aqueous extract and powder of *S.platensis* were the most effective in improving tomato plant growth and development parameters. Thus, they can be alternative to the passive use of chemical fertilizers in view of a sustainable agriculture that is friendly to the environment, human and animal health. **Ozgonen *et al.*, (2001)** inoculate *Glomus etunicatum* (GE) in pot experiment. GE was found to be able to increase the weight of the dry plant, length of the shoot and root irrespective whether FOL infected the tomato plants or not. Colonization the roots by GE was determined to be 62.3% when the FOL as absent and as 53.2% when the plants were infected. Results indicate that GE increases the growth of tomato plants and could be used against *Fusarium oxysporum* f.sp. *lycopersici* wilt of tomatoes. **Nikitas *et al.*, (2002)** reported that mycorrhiza treatment increased fresh and dry weight as well as mean plant height in tomatoes by 96, 144 and 21% compared to the controls. **Abdel-Monaim *et al*., (2014)** reported that potassium humate, alga and effective microorganisms significantly increased growth parameters (plant height and number of branches). **Abdel-Monaim *et al*., (2012)** evaluated potassium humate for controlling wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici* on Super Strain B hybrid, all treatments significantly increased plant height, fresh and dry weight of survival plants growing in pots infested with the causal pathogen compared with control.

Table 6. Effect of tested bioagents and potassium humate on some vegetative parameters of treated and naturally infected tomato plants with Fusarium wilt disease *in vivo* under greenhouse conditions during the growing season 2015-2016.

Treatment	Concentration	Fresh weight (g)	Dry weight (g)	Plant length(cm)
S.platensis	**5%**	265c	32d	66.5bc
Potassium humate	3 g / L	415ab	50bc	71.0abc
B.subtilis	10^6cfu/ml	395b	45c	69.0bc
T. harzianum	10^5cfu/ml	422a	51b	72.0ab
Mycorrhiza	10gr/kg soil	195d	20e	66.0c
Topsin M70	700 µg/L	430a	60a	75.0a
Control		127.7e	7.7f	34.7d
LSD at 0.05		21.414	5.796	5.851

Effect of tested bioagents and potassium humate on some bio-constituent contents of treated tomato and naturally infected with Fusarium wilt disease *in vivo* under field conditions

Data in Table (7) indicate that all treatments decreased hydrolyzing enzymes activities (PG and CX) compared with the control treatment. In this respect, Topsin M70 treatment most effective treatment in reducing the activity of PG and CX enzymes (6.4, 8.3%), respectively, followed by potassium humate treatment which recorded 15.4, 09.3%, respectively, then *T. harzianum* (17.3, 18. 5%). On the other hand, mycorrhiza was the least effective treatment in reducing the activity of PG and CX enzymes where the recorded activities % were 36.5, 28.8% respectively. In this respect, **Dwivedi and Singh (2015)** reported that *Fusarium oxysporum* f. sp. *lycopersici* produced cellulolytic enzyme that increased with the increase in age of the culture. **Retig and Chet, (1974)**, reported that activities of polygalacturonase and cellulase increased in catechol – treated and resistant tomato plants. After inoculation with *Fusarium oxysporum* f. sp. *lycopersici* race 2. The catechol- treated and resistant plants remained symptomless, while susceptible plants developed symptoms of disease. It is therefore suggested that increased activity of cell wall degrading enzymes in inoculated plants does not necessary cause the development of disease symptoms.

Data in the same table indicate that, all treatments affected generally the content of phenols comparing with the control treatment. Potassium humate, *T. harzianum* and *B. Subtilis* recorded the highest content of total phenols (5.389, 5.372, 5.217 mg/g), respectively. However, mycorrhiza recorded the lowest content of total phenols (3.555mg/g). **Mohd *et al.*, (2012)** considered the high content of phenols is a good indicator of defense mechanisms in treated plants with *T. harzianum*.

Table 7. Effect of tested bioagents and potassium humate on some bio-constituent contents of treated tomato and naturally infected with Fusarium wilt disease under greenhouse conditions during the growing season 2015-2016.

Treatment	Concentration	Enzyme activity (%)		Phenolic contents (mg/50g)		
		*PG	**CX	Free phenols	Conj. phenols	Total Phenols
S. platensis	5%	25.0	23.0	0.402	3.377	4.779
P. humate	3 g/L	15.4	09.3	0.652	4.737	5.389
B. subtilis	10^6cfu/mL	24.9	24.7	0.953	4.264	5.217
T. viride	10^5cfu/mL	29.1	26.8	0.552	4.187	4.739
T. harizanum	10^5cfu/mL	17.3	18.5	0.961	4.411	5.372
Mycorrhiza	10 g/kg soil	36.5	28.8	0.377	3.178	3.555
Topsin M70	700 µg/L	6.4	8.3	0.599	4.871	5.470
Control		76.0	62.2	0.368	2.860	3.228

*PG= Polygalacturonase
**Cx= Cellulase

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كفاءة بعض عوامل المقاومة الحيوية وهيومات البوتاسيوم في مكافحة الذبول الوعائي في نباتات الطماطم

منى فهيم أحمد[2] نوال عبد المنعم عيسى [1] جهاد محمد دسوقي الهباء [1]

إبراهيم عبد المنعم ابراهيم الفقى [1] سهام سمير محمد رجب[2]

[1]قسم أمراض النباتات – كلية الزراعة –جامعة بنها .

[2]معهد بحوث أمراض النباتات – مركز البحوث الزراعية– الجيزة

تصاب نباتات الطماطم في الحقل والصوبة بالعديد من الأمراض ويعتبر فطر *Fusarium oxysporum* من أهم الفطريات التي تسبب الذبول الوعائى على نباتات الطماطم وقد تم استخدام بعض عوامل المقاومة الحيوية وهيومات البوتاسيوم لمكافحة مرض الذبول الوعائي في نباتات الطماطم. وأوضحت النتائج أن كلا من *Trichoderma harzianum* يليه *Bacillus subtilis* ، *Trichoderma viride* هي أفضل معاملات التضاد فى المعمل . بينما كان كلا من *Trichoderma harzianum*، Potassium humate هي الأفضل تأثيرا للحد من شدة الإصابة يليها في ذلك المعاملة ببكتيريا *Bacillus subtilis* والطحلب وذلك تحت ظروف الحقل والصوبة. ومن ناحية أخرى كانت الميكوريزا الأقل تأثيرا تحت ظروف الحقل والصوبة.

كما أوضحت النتائج ان هذه المواد الحيوية المختبرة كان لها تأثيرا فعال على *Fusarium oxysporum* حيث أدت إلى تناقص الإنزيمات المحللة بينما أدت أيضا إلى زيادة نسبة الفينولات . ومن جهة أخرى كان لها تأثيرا أخر فعال على نباتات الطماطم حيث إنها أدت لزيادة الوزن الرطب والوزن الجاف وزيادة طول النباتات المعاملة بها مقارنة بالكنترول المصاب بالفطر وغير المعامل.