EFFICIENCY OF *TRICHODERMA VIRIDE* AND *BACILLUS SUBTILIS* AS BIOCONTROL AGENTS AGAINST ROOT ROT CAUSED BY *FUSARIUM SOLANI* IN TOMATO

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Abstract

Tomato (cv.Peto-86) is one of the important crops all over the world. In Egypt and many other countries it is often attacked by several pathogens causing root rot. Trichoderma and Bacillus species are widely the most feasible biocontrol microorganisms that suppress several pathogens. The efficiency of both antagonistic organisms was evaluated *in vitro*. as well as *in vivo* against root rot of tomato. The inoculation with *T. viride* and or *B. subtilis* of tomato plant suppressed *F. salani* as indexed by survival rate. Field experiments, ware carried out at Senowras Fayoum governorate during 2017 and 2018 seasons,. The obtained results showed that, the tested fungicide Topsin-M70 and the two biocontrol agents significantly reduced the incidence of root rot of tomato. The fungicide was the most efficient treatment in this regard compared with the two tested bioagents. Two bioagents treatments favored greater proliferation of rhizophere microflora and dehydrogenase activity in the rhizosphere. It is recommended to use these strains as a common biocontrol application in agriculture.

Key words: Biological control, *Fusarium solani Trichoderma viride*, *Bacillus subtilis* and Tomato.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is considered one of the most economic vegetable crops in Egypt either for exportation or local consumption. It is considered as one of the highest nutritional value. It contains antioxidants like vitamin C as well as Lycopene which is used in cancer treatment (Giovannucci, 1999) and other many chemical compounds and elements which are not found in other Solanaceous crops. Tomato is subjected to the attack of numerous bacterial, fungal and viral diseases. However, diseases caused by fungi are more prevalent and distructive especially soil borne diseases such as root rots and wilt which cause considerable reduction to tomato production. *Fusarium solani* causes a serious damage on tomato plants either in nurseries or in open field (Abdel-Kader, *et al*., 2012, and Muhanna , *et al*. 2016). The natural control of several phytopathogens is based on the presence of antagonistic microorganisms such as those belonging to genera Trichoderma, Pseudomonas and Bacillus (Weller *et al*., 2002 Morsy, *et al*. 2009 Abdel-Kader, *et.al*, 2012, and Muhanna, *et al*. 2016).*Trichoderma* spp. are essential control phytopathogens affecting different crops (Soytong *et.al*, 2005 and Abdel-Kader, *et.al*, 2012,). Also, tomato plants inoculated with *B.subtilis* and / or *T.harzianum* showed low disease incidene and gave a higher yield of tomato (Maude,2006 , Zaghloul *et al*., 2007 and Muhanna *et al*. 2016).

The main goal of this study was to estimate the efficiency of *T.viride* and *B. subtilis* as bio-control agents against *F. solani* the causal pathogen of root rot disease in tomato.

MATERIALS AND METHODS

Source of the tested fungi:

Pure cultures representing an isolate of *Fusarium solani* pathogenic to tomato as well as the bio agents *T.viride* and *B.subtilis* were kindly obtained from the Vegetable Dis.Res. Dept., Plant Pathol. Res. Inst., ARC.

Efficacy of antagonistic bio-agents against *F.solani*:

The antagonistic effect of the analyzed two biocontrol agents against *F.solani* was tested. *T.viride* and *F.solani* were placed and cultured on PDA medium in Petri dishes for 7 days, at 28°C. Then, a disc (0.5-cm-diameter) of the antagonistic fungal colony was cut and taken to the opposite colony of the pathogen. On the other hand, streak of bacterial isolates *B. subtilis* was put on PDA plates medium 2 cm. from the plate's edge and the edge and the plates were incubated at 28±1 °C for 24 hours, a disc (0.5cm) bearing the growth of the tested fungus was placed on the PDA medium at the same distance from the edage against the bio-control agents streak. Four replicates were used in each experiment. All inoculated plates were incubated at temperature 28°C. until the fungal growth covered the surface of any plate. The growth and reduction in mycelia of the pathogenic fungus were measured according to Fokkema, (1973).

Preparation of tomato seedlings:

Pots (25 cm diameter) were sterilized by immersing in 5% formalin solution for fifteen minute and then left for 2 days to insure complete evaporation of formalin. The sterilized pots were filled with sterilized sandy-clay soil [1:1 w/w]. Seeds of tomato cv. Peto-86 were surface sterilized with 0.2% mercuric chloride solution for two minute Then washed twice in sterilized distilled water and dried between folds of sterilized filter paper. The sterilized seeds were sown in the potteries and the plants were left to grow for 15 days. The plants were irrigated when necessary. Then, roots of tomato seedlings were treated by dipping into the suspensions of the each of the fungicide and the bio control agents each alone, for 30 min.

Preparation of antagonists' inoculum

Inoculum of *T.viride* was prepared in a form of conidial suspension (10^6 spores/ml) as described by Sivan *et al*. (1984). Meanwhile. *B. subtilis* inoculum was prepared (10^8 c.f.u /ml) as mentioned by Tylkowska and Szopinska (1998). Tomato seedlings were dipped, thoroughly in the bacterial suspension (10^8c.f.u/ml) or fungal spore suspension (10^6 conidia/ml) prepared in 0.1 g methyl cellulose individually.

Pot experiment:

The pot experiment was designed under the greenhouse conditions using the plastic pots of 25 cm diameter, and containing 5 kg of disinfected loamy clay soil. Soil was infested with *F.solani* grown on barely grain medium at the rate of 2.5% before transplanting. Infested pots were irrigated for 5 days before transplanting. Five tomato seedlings Peto-86 *cv*. were transplanted in each pot; five replicated pots were specified for each treatment in completely randomized block design. The experiment included the following treatments:

1) Non infested soil (control), 2) soil treated with *F.solani* only, *3) F.solani* + *B.subtilis*, 4) *F.solani* + *T.viride*, and 5) *F.solani* + fungicide (Topsin-M70 250g/100L water soluble). NPK (1:1:1) mineral fertilizer was added to soil in pots and irrigated weakly. The pots were kept under greenhouse conditions until the end of the experiment. Evaluation of disease due to dead plants and survival rate of seedlings was determined after 20-45 days of transplanting, respectively, as described by Phillips and Hayman (1970). Also, dry weight of the survived plants was determined 60 days after transplanting.

Field experiment

A field experiment was carried out at Senowras, Fayoum governorate during 2017 and 2018 Growth seasons, to estimate the efficiency of test strains (*T.Viride* and *B.subtilis*) for controlling root rot disease of tomato plants. The chosen field test area was naturally infested with *F.solani*. The experimental design was a complete random block with three replicates. The experimental unit was 10.5 m2 (3.5 x 3 m). Each unit contains twelve rows; each length was 3.5 meters each unit included twelve rows; each was 3.5m in length and 25cm wide. Tomato transplants were at a rate of 15 seedlings within each row. Tomato seedlings were immersed for 30 min. in the conidial suspension (10^6/ml) of *T.viride* or bacterial suspension (10^8 c.f.u/ml) of *B.subtilis* before transplanting individually. A fungicidal treatment was also used. Tomato seedlings were immersed directly for 30 min. in Topsen –M70 suspension. Control plants were treated with water only. The NPK mineral fertilizers were applied as recommended by Ministry of Agriculture and Land Reclamation. The disease incidence of root rot and percentage of healthy survived plants were recorded after 45 days from transplanting.

Dehydrogenises activity was determined according to Morsy (2005). Total microbial count on modified medium, (Bunt and Rovira, 1955) was estimated by using the decimal plate count technique. Plant height, fresh and dry weights of the plant were determined. Fruit weights were also taken.

Statistical analysis: The obtained data were subjected to the proper statistically analyses using the MSTAT statistician software and the comparison was done according to Fisher (1948).L.S.D ($P<0.05$).

RESULTS AND DISCUSSION

Efficacy of antagonistic biocontrol agents against *F.solani:*

Antagonistic effect of *T. viride* and *B.subtilis* was evaluated against *F.solani* in Petri dishes containing PDA medium. Data (Fig 1) show that the two biocontrol agents succeeded to reduce the radial growth of *F.solani*. Trichoderme *viride* was more effective than *B. subtilis* in reducing the radial growth of *F.solani,* It reached 3.1 and 5.2 cm, respectively. Moreover, *T.viride* inhibited the over growth of *F.solani*, comparing with *B. subtilis*.

Trichoderme viride and *B.subtilis* reduced growth percentage by 57.8 and 34.4%, respectively, comparing with the control. This trend reveals essential role of both bio agents in controlling root rot disease of tomato plants. The potential of isolates used can be attributed to their ability to secrete antifungal metabolites or hydrolytic enzymes.or antifungal metabolites. These findings are in harmony with those obtained by Jorjandi *et al.* (2009), Morsy, *et al*. (2009), Bokhari, and Kahkashan (2012), and Muhanna, *et al.* (2016) who studied that Trichoderma spp. Produce Chitinase and B 1, 3 Glucanase. Also, Sarhan, *et al*. (2001), Jorjandi *et al*. (2009) and Mnif, *et al*., (2015) reported that the cell free culture filtrate of *B. subtilis* inhibited the radial growth, mycelial growth, spore germination and germ- tubes elongation of *F.solani*. Moreover, Kohl *et al.* (1991), Jorjandi *et al*. (2009) and Mnif *et al*., (2015) pointed that *B. subtilis* can secrete several antifungal metabolites such as bacitracin, subtilis, bicillin and bacillomycin which inhibited fungal pathogenesis.

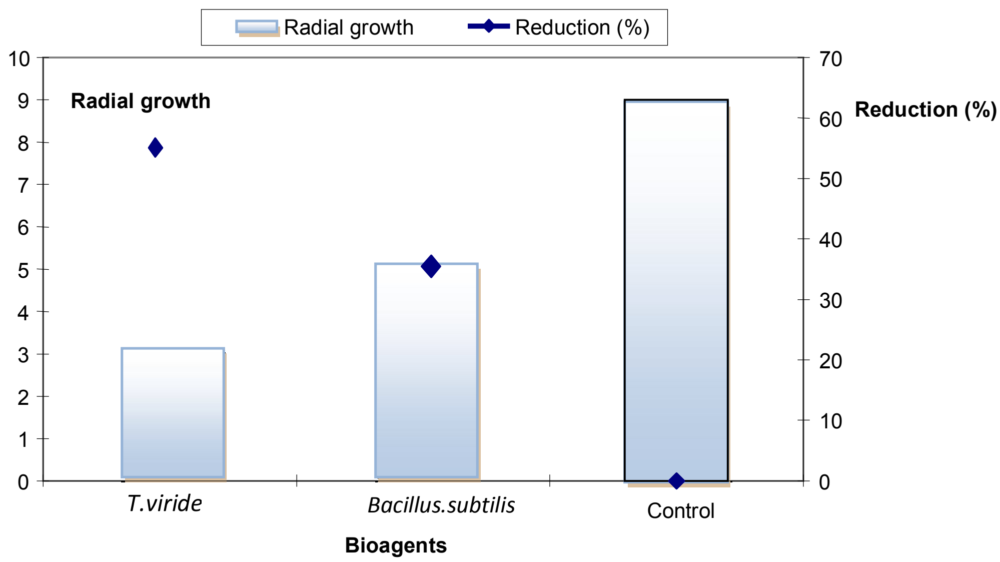

Fig. 1. Effect of *T.viride* and *B. subtils* on the radial growth of *F.solani*

Pot experiment:

A pot experiment *In vivo* was carried out to examine the efficiency of both bio agents to antagonize *F.solani* under greenhouse conditions. Data presented in Table (1) reveal that soil infested with *F.solani* significantly increased infection of tomato seedlings and severely reduced plant survival to (36%) compared to uninfected control (100%) or topsin-M70 treatment (84%).

Trichoderme viride or *B.subtilis*, treatment significantly increased plant survival rate compared with the infested soil, ranging between 72 and 68%, respectively, (Table 1). Similar results were obtained by Morsy, *et al.* (2009), and Muhanna, *et al.* (2016), who cleared that *T.viride* and *B. subtilis* were effective antagonistic against *F.solani*. In addition, plant dry weights in the presence of *T.viride*, or *B. subtilis* were considerably higher than those in the control treatment or plants infected with *F.solani*. The corresponding values of plant dry weights were 2.5, and 2.3 g/plant, respectively, are able to decrease.

Table 1. Influence of *T.viride* and *B.subtilis* against *F.solani* and their effect on seedling dry weight of tomato plant under greenhouse conditions.

Treatments	Dead plants, %	Plant Survival rate %	Plant dry weight (G/plant)
Control	00.0	100	1.7
F.solani	64.0	36.0	1.1
F.solani+B.subtilis	32.0	68.0	2.5
F.solani+T.viride	28.0	72.0	2.3
F.solani+Topsin-M70 rate of (250g/100 L)	16.0	84.0	3.2
LSD at 0.05	3.14	5.15	0.57

Efficiency of *T.viride* and *B.subtilis* against F.solani under field conditions:

Infection and survival percentage

Data in Table (2) reveal that the highest infection of tomato plants with the pathogen was in untreated seedlings whereas, the lowest infection percentage was observed in seedlings treated with topsin-M70 in addition, high percentage of survival rate was recorded.

Table 2. Influence of *T.viride* and *B.subtilis*, each alone on infection with *F.solani* and survival percentage of tomato plants under field conditions during 2017 and 2018 seasons.

Treatments	2017		2018	
	Tomato seedling infection (%)	Tomato seedling survival (%)	Tomato seedling infection (%)	Tomato seedling survival (%)
Control	33.3	66.7	36.1	63.9
B. subtilis	20.0	80.0	22.2	77.8
T.viride	16.6	83.4	16.6	83.4
Topsin-M70 250g/100 L.water	10.0	90.0	11.6	88.4
LSD at 0.05	4.08	9.1	6.3	10.25

Inoculation with *B.subtilis*, significantly increased survival rate compared with the infested soil, being 80.0%, However, higher percentage of survival rates of tomato plants was attained in response to treatment with *T.viride* (83.4%). The Mechanism of action of Bacillus and Tricoderma on pathogens is by attacking pathogenic organisms and linking them by binding sugar and begins the secretion of extracellular proteases and lipase (Maude, 2006 and Bokhari, and Kahkashan, 2012).

Dehydrogenase activity and microbial population:

Dehydrogenase activity (DHA) was recorded after 45 and 60 days after transplanting (Table 3). Low DHA and total microflora values were observed with treatment of Topsin-M70 indicating the antimicrobial activity of this fungicide against the rhizospheric microflora and pathogenic fungi. Meanwhile, inoculation of tomato plants with *T.viride* or *B. subtilis* increased DHA activity and microbial flora in the tomato rhizosphere than Topsin-M70. Similar results were reported by Morsy (2005) and Zaghloul *et.al*. (2007) who estimated high DHA activity and total microbial flora in case of inoculation with *B.subtilis* or *T.harzianum*.

Table 3. Influence of *T.viride* and *B.subtilis* applied individually on dehydrogenase activity and total microbial counts during 2017-2018 seasons in soil.

Treatments	Dehydrogenase activity (µg TPF/g dry soil/day)				Microbial total count (cfux10^6)			
	2017		2018		2017		2018	
	(Days)		(Days)		(Days)		(Days)	
	45	60	45	60	45	60	45	60
Control	35.7	140.8	39.4	152.4	22.0	55.1	27.3	66.7
B.subtilis	54.2	175.1	60.8	186.9	27.0	46.5	35.1	62.8
T.viride	63.9	190.4	70.4	202.5	12.8	43.9	15.4	57.5
Topsin-M70	28.4	137.7	34.7	142.6	19.9	34.2	21.3	42.7
LSD at 0.05					5.39	7.70	5.43	6.39

In general, the values of DHA activity and counts of the microbial flora of various treatments were higher at the flowering stage than the vegetative one. This finding may be due to the difference in multi-application rate of different soil microorganisms which are usually a maximum during the flowering stage. Such difference could be attributed to the qualitative and quantitative changes in the nature of root exudates during different growth stages (Abdel-Jawad, 1998).

Plant Growth parameters

Data presented in Table (4) reveal that low values of some plant growth parameters, (plant height, fresh and dry weights of plants) were found with the control treatment in comparison with other treatments. The growth parameters of tomato plants were significantly increased with the inoculation by *B.subtilis* and *T.viride*, each alone, compared with control.

Table 4. Influence of *T.viride* and *B.subtilis* applied individually on growth characters of tomato plants during 2017-2018 seasons.

Treatments	Plant height (cm)		Fresh weight (g/plant)		Dry weight (g/plant)	
	2017	2018	2017	2018	2017	2018
Control	50.8	54.5	95.2	138.7	13.9	17.2
B.subtilis	65.0	70.6	205.9	337.4	35.8	44.9
T.viride	62.7	65.1	167.5	297.2	26.1	35.5
Topsin-M70	73.2	70.9	356.7	420.9	58.2	64.9
LSD at 0.05	12.6	11.3	9.2	13.9	7.3	4.3

The promotion of tomato growth parameters using *B.subtilis* and *T.viride* isolates may be due to their abilities to produce phytohormones, vitamins and solublizing minerals besides, their role in inhibiting Fusarium growth (Morsy, 2005 ,Zaghloul *et al.2007* Morsy, *et al.*2009, and Muhanna,*et al.*2016). This increase in growth was as a result of disease control and increased hormone production.

Fruits weight

Data in Table (5) show a decrease in the fruit weight of tomato plants of control treatment. However, the significant increase was determined by fungicidal treatment (Table 5).

Table 5. Influence of *T.viride* and *B.subtilis* applied individually on fruit yield of tomato plants during 2017-2018 seasons.

Treatments	Fruits yield (kg/plant)	
	2017	2018
Control	0.12	0.15
B.subtilis	0.15	0.17
T.viride	0.17	0.19
Topsin-M70)	0.20	0.22
LSD at 0.05	0.31	0.25

The treated tomato with *T.viride* only gave higher records of fruit weight than those treated with *B.subtilis*. However, this increment was not significant. Moreover, Topsin-M70 gained significant increase in fruits weight of tomato plants compared to the bio agents.

This result could be referred to the synergistic effect between the microbial populations of the rhizosphere and biocontrol agents. In this respect, Kohl *et al.*(1991), Jorjandi *et al.*2009) and Muhanna , *et al.*(2016) reported that application of selected antagonists (*B. subilis*, *T. viride*) individually significantly increased weight of fruits and the total yield of tomato fruits.

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كفاءة التريكودرما فيردي والباسيلس ساتلس في المكافحة الحيوية لمرض عفن الجذور المتسبب عن فيوزاريوم سولاني على نبات الطماطم

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تعتبر الطماطم (صنف بيتو-86) أحد أهم محاصيل الحقل التي تصاب بالعديد من الأمراض الهامة مثل عفن الجذور. ومن أهم الكائنات الحيوية التي لها قدرة على مقاومة مرض عفن الجذور المتسبب عن فطر فيوزاريوم سولاني فطريات تابعة لجنس التريكودرما والباسيلس ساتلس. وقد تم تقييم كفاءة هذه العزلات في المكافحة الحيوية تحت الظروف المعملية, وبتجربة الأصص أضيفت التريكودرما فيردى والباسيلس ساتليس لأختبار كفاءتهما فى تثبيط نمو فطر فيوزاريوم سولاني وذلك بتقدير نسبة النباتات الباقية في نهاية التجربة المتحملة للأصابة. أجريت تجربة حقلية فى مركز سنورس بمحافظة الفيوم خلال موسمي 2017، 2018 وقد أظهرت النتائج المتحصل عليها أن هذه المعاملات أدت إلى زيادة في أعداد الميكروبات وزيادة نشاط أنزيم الديهيدروجينيز بمنطقة الريزوسفير. كما أن التلقيح بكل من الترايكوديرما فيردي أوالميكروب باسيللس ساتلس أدى إلى انخفاض نسبة الإصابة وبالتالي زيادة نسبة النباتات المتحملة للإصابة بأي منهما. وأوضحت النتائج أيضاً أن المعاملة أدت إلى زيادة في مؤشرات النمو ووزن الثمار مقارنة بالكنترول. وعلى ذلك توصي هذه الدراسة باستخدام هذه العزلات في المقاومة الحيوية لمرض عفن جذور الطماطم المتسبب عن الفطر فيوزاريوم سولاني وكذلك الاستفادة منها فى برنامج المكافحة المتكاملة لعفن جذور الطماطم.