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Evaluation of some fungitoxicants for controlling tomato early blight disease

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Abstract

Three different fungitoxicants, *i.e.*, *Trichoderma harzianum*, *Bacillus subtilis* and Roxil Plus WP50 fungicide, were investigated against tomato early blight disease caused by *Alternaria solani* fungus *in vitro* and *in vivo*. By using dual culture technique, the highest reduction percentage of *A. solani* growth was recorded with *B. subtilis* followed by Roxil treatments, while Roxil followed by *T. harzianum* treatments recorded highest reduction percentage of sporulation. Meanwhile, by using poisoned food technique the highest reduction percentage of *A. solani* growth was recorded with Roxil followed by *B. subtilis* treatments, while *B. subtilis* recorded highest reduction percentage of sporulation. Three fungitoxicants were investigated as foliar spray for their abilities for controlling early blight disease on naturally infected tomato plants under open field conditions. Roxil fungicide followed by *B. subtilis* treatments caused the highest significant reduction in disease incidence and disease severity percentage during the two successive growing seasons 2013/14 and 2014/15. Concerning to yield parameters, Roxil and *T. harzianum* treatments were significantly increased total yield weight and average weight of tomato fruit. Moreover, all tested treatments increased clearly total phenol content, peroxidase, polyphenoloxidase and chitinase activities, as well as, vitamince C contents as compared to control treatment.

Keywords: Early blight disease, *Alternaria solani*, *Trichoderma harzianum*, *Bacillus subtilis*, tomato.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is known as certainly one of the most crucial vegetable crops which have tremendous popularity around the world. In 2014, Egypt was ranked 5th in the world with 8.3 million tons of tomatoes produced (FAO, 2014). Early blight caused by the *Alternaria solani* (Ellis and Martin) fungus is one of the most common destructive tomato diseases, attacking tomato or potato plants wherever are grown nearly every season. The causal fungus is pathogenic on tomato and potato crops and has also been reported on other hosts such as *Brassica* sp. (Hooker, 1986). This disease has great economic importance where, it infects and destroys the entire canopy of tomato plants, limiting photosynthetic activity and affecting tomato productivity in the quantity and quality up to 79% (Tewari & Vishunavat, 2012). For controlling early blight disease of tomato, several management strategies were done to manage such disease. Among the most truly effective and old method for disease control is using of fungicides. It is thought that chemical fungicides are most suitable choice to manage Alternaria diseases (Arain et al., 2012; Mesta et al., 2011). Fungicides based on copper and mancozeb are good effective resource for controlling alternariosis on tomatoes and inhibiting mycelium growth and sporulation of *Alternaria solani* Nees (Patel et al., 2005). Recently, *Trichoderma* spp. and *Bacillus subtilis* are considered ecofriendly biocontrol agent and has been marketed commercially as biopesticides, biofertilizers and soil improvements (George et al., 2004, Harman et al., 2004). A number of investigators confirmed the action of *Trichoderma* sp. against the pathogen growth due to its ability to producing of extracellular enzymes, antifungal metabolites and antibiotics (Montealegre et al., 2010; El-Katatny et al., 2006). The action of *B. subtilis* against the pathogenic fungi could be in the form of a competitor for nutrient and space as well as producer of hydrolytic enzymes where hydrolytic enzymes (protease, glucanase, chitinase, lipase and amylase) capable to degrade the cell wall of these pathogenic fungi (Cazorla et al., 2007; Detry et al., 2006; Konsoula & Liakopoulou-Kyriakides, 2006; Manjula et al., 2004), in addition to, product of several ribosomal and non-ribosomal peptides that act as antibiotics such as iturins, surfactins and zwittermycin (Stein, 2005; Asaka & Shoda, 1996). The main objectives of this study were to evaluate two bioagents, *i.e. T. harzianum* and *B. subtilis* and one fungicide, *i.e.* Roxil Plus 50WP against the causal pathogen of early blight of tomato *in vitro* and *in vivo*, and to study total phenols, peroxidase, polyphenoloxidase and chitinase activities in tomato as biochemical changes after treatment.

Materials and methods

Isolation and identification of causal pathogen: Isolation of the causal pathogen of early blight pathogen was done from naturally infected tomato plants which showed typical symptoms of early blight disease and cultivated in the crop farm site of Faculty of Agriculture, Moshtohor, Benha University, Egypt during growing season 2013. In this respect, infected leaves with advanced margins of early blight lesions were picked and cut into small bits with about 5 mm for isolation and purification of the causal fungus according to Naik et al., (2010). The isolated target fungus

was identified on the basis of cultural and microscopic morphological characteristics and pigmentation on medium and mycelial growth pattern on PDA plates according to the key given by Ellis (1971) and Barnett and Hunter (1972) and identified as *A. solani***.** Pathogenicity test and Koch's postulates were carried out successfully on tomato seedlings (cv. Super Strain B hybrid) under laboratory conditions to confirm its pathogenicity and re-isolated pure cultures of *A. solani* was maintained on PDA slants at 4^{O}C.

Isolation and identification of antagonistic microorganisms: Isolation of target bioagents (*T. harzianum* and *B. subtilis*) was done from the rhizospheric soil of tomato plants that exhibited high insusceptible level against early blight disease according to Waksman (1922) using soil dilution plate method. The isolated target microorganisms *i.e. T. harzianum* (Johnson et. al., 1960) and *B. subtilis* (Lochhead, 1940), which grew in separate colonies on the prepared dilution plates, were selected, sub-cultured and identified according to their morphological, cultural characters (Bergy & Holt, 1993; Rifai, 1969; Comm, 1955). Identification was confirmed at the Mycology and Plant Disease Survey Research Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt as well as confirmed in Department of Plant Pathology, Faculty of Agriculture, Moshtohor, Benha University, Egypt.

Effect of some fungitoxicants on *A. solani* using dual culture technique *in vitro*: Dual culture technique was used for in *vitro* evaluation of two isolated target antagonistics *i.e. T. harzianum* and *B. subtilis* cultures and Roxil Plus 50WP (metalaxyl+copper hydroxide) fungicide against isolated *A. solani* (Morton & Strouble, 1955). Potato dextrose agar (PDA) medium was used in this experiment. The antifungal activity of tested treatments accomplished on PDA plates (90 mm Ø). Concerning to *T. harzianum* treatment, aseptically, a PDA disc inoculum (0.5 mm) of young active culture of *T. harzianum* was placed on PDA plates at a distance of 20 mm from the plate margin. At the opposite direction, at distance of 20 mm from the plate margin, a PDA disc inoculum (0.5mm) of young active culture of *A. solani* was placed on. Concerning to *B. subtilis* bioagent treatment, by a similar way, single streaks technique according to Wang et al., (2003) with some modifications was used, where, single streak of *B. subtilis* bacterium was drawn by a loop at a distance of 20 mm from the margin of the plate, and then, the plates were incubated for 24h (this time is enough for the colonies to be visible) at 27°C. At the opposite direction of Bacillus streak, an equal disc inoculum (0.5mm Ø) of young active culture of *A. solani* was placed on at distance of 20 mm from the margin of the plate. In respect of Roxil fungicide treatment, a 0.5 mm well was created near the edge (20 mm apart) of each medium plate using the sterilized cork borer, and then recommended dose of Roxil Plus 50WP fungicide (2 g/L) was prepared and poured into the well. At the opposite direction at distance of 20 mm from the margin of the plate, a PDA disc (0.5mm) of young active culture of *A. solani* was placed on. Plates containing *A. solani* inoculum disc only on the center of the

plate represented as control treatment. The inoculated plates were incubated at 27^0C and daily observed until the radius growth of *A. solani* inoculum covered whole plate in control.

Effect of some fungitoxicants on *A. solani* using poisoned food technique *in vitro*: Poisoned food technique was used for *in vitro* evaluation of two isolated target bioagents filtrates *i.e. T. harzianum* and *B. subtilis* and Roxil fungicide against *A. solani* (Dhingra & Sinclair, 1985). *Trichoderma harzianum* was grown on geliotoxin fermentation medium for 7 days at 27°C (Brian & Hemming, 1945). *Bacillus subtilis* was grown for 3 days on liquid nutrient glucose broth medium at 30°C under complete darkness to stimulate toxin production (Dowson, 1957). The medium and growth bulk of each antagonistic bioagent was homogenized and filtered through Whatman No.1 paper and filtrate was sterilized through Millipore filter (0.4 µm) (EL-Abyad et. al., 1983). Aliquots of PDA media were prepared and poured onto sterilized petri dishes. Then, 5 ml of each of tested filtrate poured onto the platted PDA dishes and gently rotary shaken. Roxil fungicide was added to sterile molten PDA medium in a recommended dose (at a rate of 2 g/L) at 45°C before pouring into plates. After mixing thoroughly, medium was poured into the presterilized plates. After solidifying of the poured media, and aseptically, a PDA disc (0.5mm) of young active culture of *A. solani* placed onto the center of each poured dish. Plates containing *A. solani* agar disc only on the center of the plat represented as control treatment. All plates were incubated at 27°C and daily observed until the *A. solani* inoculum covered whole plate in control.

Radius growth and sporulation measurements: The inhibition activity of tested bioagents and fungicide in both techniques (dual culture and poisoned food) were determined by measuring linear growth (mm) of the target pathogen (*A. solani*) and data was expressed as percent inhibition over control using formula suggested by Sundar et al., (1995). Sporulation was recorded by cutting 1 cm^2 agar disc from the margin of *A. solani* colony of each treatment and transferred to a vial containing 10 ml of sterile distilled water; the suspension was continuously shaken for 5 min, after which time, the density of spores/ml was counted by a haemocytometer according to EL-Abyad, et al., (1983).

Effect of some fungitoxicants on tomato early blight disease *in vivo*: The present experiment was carried out on tomato plants (cv. Super Strain B hybrid) naturally infected with early blight disease under open field conditions at the Experimental Station, Faculty of Agriculture, Moshtohor, Benha University, Egypt during the two successive summer growing seasons 2013/14 and 2014/15. The experimental treatments were laid out in randomized complete block design with three replicates (plots). Each experimental plot included 4 ridges, each of 70 cm wide and 3.75 m long. Plot area was 10.5 m^2. On the 1^{st} of April, transplanting of thirty-days old of Super Starian B hybrid tomato seedlings took place in one side of the ridge in the presence of water at 30 cm apart and each plot contained 48

plants. All agronomic practices endorsed by Ministry of Agriculture, Egypt were carried out for cultivation of tomato plants, except fungicide application practices. The tested treatments of antagonistic bioagents (*T. harzianum* and *B. subtilis*) and the fungicide Roxil Plus 50WP which have inhibitory effects on the early blight pathogen (*A. solani*) *in vitro* tests were subjected as well as to *in vivo* test to determine their biocontrol efficacy against early blight disease. Treatments were applied individually as foliar spray as following: *T. harzianum* at concentration of 1×10^5 spores/ml, *B. subtilis* at concentration of $0.5x10^8$ cell/ml and Roxil fungicide at recommended dose (2 g/L). Preliminary screening revealed that abovementioned concentrations were the most effective. Plants sprayed with water used as control. Application of all treatments were carried out three times, the first at the initiation of the disease symptom (30-days after transplanting) while, the second and third were done at 15 days intervals.

Disease assessments and yield parameters: At 80-days after transplanting of tomato plants, disease incidence and disease severity percentage were recorded. As well as, total yield weight expressed as ton/feddan (feddan = 1.038 acre), fruits number/plant and average weight of fruit (g) were recorded for each treatment of each growing season. Disease incidence (DI %) was calculated and expressed in percentage scale by using the following formula: DI%= (D/T) X 100, where, (I) = Number of diseased plants; (T) = Total observed plants. For assessing disease severity, ten plants were selected randomly in each replication (plot) due to recording disease severity individually for each one using 0-5 rating scale described by Pandey et al., (2003) where, 0 = No symptoms, 1 = < 10% of surface area of leaf, stem and fruit infected by early blight, 2 = 11-25% of foliage of plant covered with a few isolated spot, 3 = Many spot coalesced on the leaves, covering 26-50% of surface area of plant, 4 = 51-75% of surface area of the plants infected, fruits also infected at peduncle end, defoliation and blightening started, sunken lesions with prominent concentric ring on stem, petioles and fruits and 5 = < 75% surface area of the plants part blighted, severe lesion on stem and fruit rotting on peduncle end. Early blight 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 as previously mentioned was calculated based on mean of disease incidence and disease severity percentage during the two seasons 2013/14 and 2014/15. Efficacy-I % (Ef-I %) calculated for comparison all tested treatments with untreated control, while Efficacy-II % (Ef-II %) calculated for comparison all tested biofungicides with Roxil Plus fungicide (Mahmoud et al., 2013) as follows:

$$\text{Ef-I\%} = \frac{\text{disease incidence or disease severity \% in control treatment} - \text{disease incidence or disease severity \% in biofungicide or fungicide treatment}}{\text{disease incidence or disease severity \% in control treatment}} \times 100$$

$$\text{Ef-II\%} = \frac{\text{disease incidence or disease severity \% in biofungicide treatment efficacy \%}}{\text{disease incidence or disease severity \% in Roxil Plus fungicide treatment efficacy \%}} \times 100$$

Also, efficacy (Ef) percentage of different treatments was calculated based on mean of each yield parameter during the two seasons as follows:

$$\text{Ef-I\%} = \frac{\text{yield parameter in biofungicide or fungicide treatment - yield parameter in control treatment}}{\text{yield parameter in control treatment}} \times 100$$

$$\text{Ef-II\%} = \frac{\text{yield parameter in biofungicide treatment efficacy \%}}{\text{yield parameter in Roxil Plus fungicide treatment efficacy \%}} \times 100$$

Biochemical change assessments: Samples representing the tenth plant leaf were taken apically from each particular treatment for determining of total phenols contents and oxidative enzymes assessments. For total phenol contents determination, leaves samples were extracted separately by using the method suggested by kâhkônen et al., (1999). The total phenol contents in extracts was determined by Folin – Ciocateu method as modified by Singleton and Rossi (I965), and were calculated for each treatment as milligrams of gallic acid per one gram dry weight (mg GA/DW) according to standard curve of gallic acid. The crude leaf enzyme extract was prepared as recommended by Ni et al., (2001). Crude leaf extract was prepared by homogenizing each particular treatment with 0.1 M of phosphate buffer (pH 7.0) at rate of 2.0 ml/g fresh weight and centrifuged under cooling ($4^{O}C$) at 10.000 rpm for 10 min. The clear supernatant was taken as crude extract for assaying the peroxidase, polyphenoloxidase and chitinase activities. The supernatant was collected and stored at -20ºC until use. The activity of peroxidase enzyme was measured as described by Vetter (I958), and was calculated for each treatment as the change in absorbance at 430 nm per minute per gram fresh weight (Δ_{430}/min/g FW). Polyphenoloxidase activity was determined according to a modification of Ishaaya (I971), and was calculated Δ_{405}/min/g FW. Chitinase activity was determined by the sensitive method of Waterhouse *et al.*, (1961), and was expressed as µg N-acetylglucoseamine × 10^3 /min/gm fresh weight (µg NAGA X 10^3/g FW). Determination of vitamin c in tomato was done calorimetrically by using 2,6-dichlorophenol-indophenol dye method outlined by (A.O.A.C., 1975), and was expressed as µg ascorbic acid per gram fresh weight (µg A.A./g FW). As well as, increase percentage of all determined enzymes and vitamin C were calculated using the following formula:

Increase (%) = (value of treatment - value of control / value of control) × 100%

Statistical analysis: The presented data were laid out in randomized complete block design in triplicates and were statistically analyzed for the least significant difference (L.S.D.) according to Gomez and Gomez (1984). Also, combined statistical analysis of the two seasons was done.

Results and Discussion

Effect of some fungitoxicants on *A. solani in vitro*: Three different fungitoxicants were tested against *A. solani* for their antifungal properties on radius growth and sporulation. Data in Table 1 reveale that, all treatments significantly reduced the radius growth and sporulation of *A. solani* in comparing with control in both techniques. Where, in dual culture technique, *B. subtilis* recorded highest significant reduction in radius growth (41.9 mm) followed by Roxil fungicide (54.0 mm) and *Trichoderma harzianum* (64.3 mm) in comparison with control treatment (90 mm). In respect of sporulation, Roxil fungicide treatment (1.5) followed by *T. harzianum* (2.3) caused highest significant reduction, respectively. According to poisoned food technique results, *T. harzianum* filtrate caused lowest significant reduction (57.3 mm) in comparison with control in respect of radius growth. Meanwhile, there were high significant differences between *B. subtilis* filtrate and Roxil fungicide. Concerning to sporulation, Roxil fungicide treatment gave highest significant reduction (1.4) followed by *B. subtilis* (2.2) treatment comparing with control (3.5) treatment. Meanwhile, *T. harzianum* treatment (3.3) significantly differenced from control. In general, it is believed that chemical fungicides are the best option to control Alternaria diseases (Arain et al., 2012; Mesta et al., 2011). These results could be discussed in light that fungicides based on copper are good preventive resource for inhibiting the mycelium development and sporulation of *A. solani* (Patel et al., 2005). The inhibitory effect of *B. subtilis* to *A. solani* could describe in light that *B. subtilis* produce hydrolytic enzymes, *i.e.* protease, glucanase (Cazorla et al., 2007), chitinase (Manjula et al., 2004), lipase (Detry et al., 2006) and amylase (Konsoula & Liakopoulou-Kyriakides, 2006) that are capable to degrade the cell wall of broad spectrum of fungal pathogens (Saha et al., 2012), in addition to, production of several ribosomal and non-ribosomal peptides that act as antibiotics such as iturins, surfactins and zwittermycin (Stein, 2005; Asaka & Shoda, 1996). Also, Trichoderma species due to its antagonistic activity are considered as potential biological control agents against numerous plant pathogenic fungi (Mohamed and Haggag, 2006). Raziq and Ishtiaq (2010) confirmed that different fungicides and Trichoderma species effectively reduced the growth *of A. solani* under laboratory condition. Many researchers confirmed that *Trichoderma* sp. control the pathogen growth due to the production of extracellular enzymes, antifungal metabolites and antibiotics (Montealegre et al., 2010; El-Katatny et al., 2006). Also, in the present study, *T. harzianum* showed varied degree of inhibition against *A. solani* inoculum. This may be due to mycoparasitism or secretion of antibiotics in PDA plates (Tapwal et al., 2015).

Effect of some fungitoxicants on tomato early blight disease *in vivo*: Data illustrated in Table 2 reveal that all tested bioagents and Roxil had a great significant effect in decreasing the early blight disease incidence and disease severity percentage caused by *A. solani* on tomato plants during the two growing

seasons 2013/14 and 2014/15 in comparing with control treatment under open field conditions. In this respect, Roxil fungicide treatment followed by *B. subtilis* scored highest significant decrease in disease incidence percentage (31.67 and 40.0%, respectively) and disease severity percentage (10.0 and 16.67%, respectively). A same trend was observed in concerning to efficacy (Ef-I %), where, Roxil fungicide treatment followed by *B. subtilis* scored highest treatment efficacy in comparing with control. Concerning treatment efficacy percentage in comparing with Roxil fungicide treatment (Ef-II %), *B. subtilis* scored the highest treatment efficacy in this respect. These obtained results confirmed that various fungicides based on copper and copper oxychloride have been used to control tomato early blight and considered good preventive resources for controlling such disease (Mate et al., 2005; Patel et al., 2005).

Table 1: Effect of some fungitoxicants on growth of *A. solani* fungus, the causal of tomato early blight *in vitro*.

Treatments	Dual culture Technique		Poisoned Food Technique	
	Radius growth (mm)	Sporulation X 10^3	Radius growth (mm)	Sporulation X 10^3
T. harzianum	64.3	2.3	57.3	3.3
B. subtilis	41.9	3.2	26.7	2.2
Roxil	54.0	1.5	20.3	1.4
Control	90.0	3.5	90.0	3.5
L.S.D at 1%	6.53	0.20	1.37	0.09

Table 2: Effect of some fungitoxicants on tomato early blight disease assessments *in vivo* under field conditions during the growing seasons 2013/14 and 2014/15.

Treatments	Disease incidence percentage					Disease severity percentage				
	2013/14	2014/15	Mean	Ef-I%*	Ef-II%**	2013/14	2014/15	Mean	Ef-I%*	Ef-II%**
T. harzianum	73.33	56.67	65.00	21.06	34.22	25.33	26.67	26.00	53.85	65.47
B. subtilis	46.67	33.33	40.00	51.42	83.56	20.00	13.33	16.67	70.41	85.61
Roxil	36.67	26.67	31.67	61.54	100.00	11.33	8.67	10.00	82.25	100.00
Control	86.67	78.00	82.34	0.00	0.00	60.00	52.67	56.34	0.00	0.00
L.S.D at 5%	15.952	9.210	8.491			12.547	8.071	9.616		

*Ef-I%: Efficacy-I - calculated based on mean of disease incidence and disease severity percentage in two seasons for comparison all tested treatments with control. **Ef-II%: Efficacy-II - calculated based on mean of disease incidence and disease severity percentage in two seasons for comparison all tested biofungicides with fungicide.

On the other side, recently ecofriendly biocontrol agents, *B. subtilis* and *Trichoderma* sp. have received much attention by both conventional and organic farmers to suppress early blight in tomato and various plant diseases (Romero et al., 2007; Zitter et al., 2005; Gardener & Fravel, 2002). The role of *B. subtilis* in decreasing disease severity might be due to its ability to competition for nutrient and space, as well as the production of inhibitory compounds and hydrolytic enzymes which are capable to degrade the cell wall against of broad spectrum of fungal pathogens (Saha et al., 2012). Also, *B. subtilis* produces several peptides that act as antibiotics (Stein, 2005; Asaka & Shoda, 1996) and it secretes also hydrolytic enzymes, *i.e.* protease, glucanase (Cazorla et al., 2007), chitinase (Manjula et al., 2004), lipase (Detry et al., 2006) and amylase (Konsoula & Liakopoulou-Kyriakides, 2006).

Effect of some fungitoxicants on tomato yield parameters: As regard to the effect of applied treatments on total tomato yield, data in Table 3 reveal that most of treatments significantly increased tomato yield/feddan and yield components during the two successive growing seasons 2013/14 and 2014/15. Concerning tomato yield weight/feddan, Roxil fungicide treatment (47.14 ton) followed by *T. harzianum* treatment (40.74 ton) gave highest significant increase in tomato yield weight/feddan in comparing with control treatment (21.45 ton) with no significant differences among them during the two seasons. The same trend was observed in respect of treatment efficacy compared with control (Ef-I %). Concerning to Ef-II %, *T. harzianum* treatment (75.09%) followed by *B. subtilis* treatment (27.91%) gave highest treatment efficacy % in comparing with Roxyl fungicide treatment (100.0%). *Bacillus subtilis* treatment had slight increase in tomato yield/feddan whether in comparing with control (Ef-I %) where recorded 33.43% or in comparing with Roxyl fungicide treatment (Ef-II %) where recorded 27.91%. Meanwhile, *B. subtilis* treatment scored highest significant increase in tomato fruit no/plant during the two successive growing seasons. Calculated Ef-I % obviously reveal that *B. subtilis* (72.53%) followed by Roxil fungicide (40.45%) treatment gave highest efficacy in comparing with control treatment. Meanwhile, calculated Ef-II % reveal that *B. subtilis* (179.31%) followed by *T. harzianum* treatment (80.89%) scored highest efficacy in comparing with Roxyl fungicide treatment. As regard to average weight of tomato fruit, *B. subtilis* treatment decreased the average weight of tomato fruit non-significantly in comparing with control treatment during the two growing seasons, while significantly decreased in comparing with Roxyl fungicide treatment during the same seasons. Data in the table obviously reveal that Roxil fungicide treatment had a great significant effect in increasing average weight of tomato fruits during the two growing seasons. Furthermore, the pre-mentioned treatment gave highest treatment efficacy in comparing with control treatment. On the other hand, *T. harzianum* recorded the second position after Roxyl fungicide treatment in comparing with control or in comparing with Roxyl fungicide treatment. Obtained data confirmed that chemical fungicides are the most effective method to control Alternaria diseases (Arain et al., 2012; Mesta et al., 2011) and fungicides based on copper are good preventive resource for controlling the development of alternariosis on tomatoes and improving yield parameters of treated plants (Patel et al., 2005). A possible alternative to synthetic chemical fungicides is to exploit the antimicrobial activities of ecofriendly biocontrol agents, *Trichoderma* spp. and *B. subtilis*. Commercially, they have been marketed as biopesticides, biofertilizers and soil amendments (Harman et al., 2004). In this respect, Niknejad et al., (2000) and Zaghloul et al., (2007) reported that application of selected antagonists (*B. subtilis*, *T. harzianum*) has considerably increased tomato yield parameters *i.e.* number of fruits/plant, weight of fruits

and the total yield of tomato fruits. On the other hand, the adversed effect of *B. subtilis* application on some yield parameters could be discussed in light that large numbers of bacterial cells on the stigmatic surface and/or any antibiotics or other metabolites produced by them could lead to reduced fruit weight and increased time to fruit ripening (Gupton & Spiers, 1994; Darnell & Lyrene, 1989; Garvey & Lyrene, 1987).

Table 3: Effect of some fungitoxicants on tomato yield parameters under field conditions during the growing seasons 2013/14 and 2014/15.

Treatments	Total yield weight (ton/feddan)					Fruits no/plant					Average weight (g) of fruit				
	2013/14	2014/15	Mean	Ef-I%	Ef-II%	2013/14	2014/15	Mean	Ef-I%	Ef-II%	2013/14	2014/15	Mean	Ef-I%	Ef-II%
T. harzianum	41.66	39.82	40.74	89.93	75.09	25.33	20.67	23.00	32.72	80.89	85.66	100.34	93.00	44.59	78.06
B. subtilis	31.17	26.06	28.62	33.43	27.91	32.47	27.33	29.90	72.53	179.31	50.00	49.66	49.83	-22.53	-39.44
Roxil	48.32	45.96	47.14	119.77	100.00	25.67	23.00	24.34	40.45	100.00	98.04	104.08	101.06	57.12	100.00
Control	23.55	19.35	21.45	0.00	0.00	18.33	16.33	17.33	0.00	0.00	66.92	61.72	64.32	0.00	0.00
L.S.D at 5%	10.366	8.103	7.462			1.595	2.330	1.965			29.206	23.250	21.554		

Table 4: Effect of some fungitoxicants on tomato biochemical changes.

Treatments	Total Phenols		Peroxidase		Polyphenoloxidase		Chitinase		Vitamin C	
	mg GA/DW	Increase%*	Δ_{430}/min/g FW	Increase%	Δ_{405}/min/g FW	Increase%	µg NAGA X 10^3/g FW	Increase%	µg A.A./g FW	Increase%
T. harzianum	979.3	56.19	470.33	117.75	429.0	20.27	2132.0	146.10	508.00	292.79
B. subtilis	1423.3	127.00	624.00	188.89	363.3	1.85	1764.3	103.66	309.67	139.44
Roxil	1133.3	80.75	562.00	160.19	439.3	23.16	2087.7	140.99	204.33	57.99
Control	627.0	0.00	216.00	0.00	356.7	0.00	866.3	0.00	129.33	0.00
L.S.D at 5%	121.29		46.68		28.95		101.34		23.83	

* Increase (%) = value of treatment - value of control / value of control × 100%

Effect of some fungitoxicants on tomato biochemical changes: As regard to the effect of applied treatments on tomato biochemical changes, data in Table 4 reveal that most of treatments significantly increased all biochemical measurements during the two successive growing seasons 2013/14 and 2014/15 comparing with control treatment. Concerning total phenol contents and peroxidase enzyme activity, the same table shows that *B. subtilis* treatment exhibited the highest significant increase in total phenols (127.0%) and peroxidase activity (188.89%) followed by Roxil fungicide treatment (8.75% and 160.19%, respectively). In respect of polyphenoloxidase enzyme activity, Roxil fungicide followed by *T. harzianum* treatment scored the highest significant increasing percentage (23.16% and 20.27%, respectively). The same trend was observed regarding to chitinase enzyme activity where, *T. harzianum* and Roxil fungicide treatments (146.10 and 140.99, respectively) gave highest significant enzyme activity comparing with control. Regarding to vitamin C content tomato fruits, data in the table obviously reveal that *T. harzianum* followed by *B. subtilis* treatments recorded highest significant contents of vitamin C (292.79% and 139.44%, respectively). The present results declare that, all tested treatments caused significant increase in total phenols compared with control treatment. These results are in agreements with those of Runeckles (1964), Khaled et al., (2000) and EL-Rafai et al., (2003), they stated that phenolic compounds play a marked role in disease resistance and immunity of plant, and so, leaves of treated plants

contained higher phenols than untreated plants. Similar confirmation was reported by (Khashaba, 1980) who stated that resistance of cotton to fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *vasinifectum* depends upon the production of phenolic compounds which are extremely toxic to the pathogen. Also, these results could be discussed in light the findings of Chérif et al., (2007) and Mohamed et al, (2007) who reported that, resistance might be exhibited on treated plants due to the accumulation of various phenolic and oxidative enzymes (peroxidases and polyphenoloxidases), activation of key enzymes in the phenylpropanoid and isoflavonoid pathways which may play an essential role in resistance to pathogenic attack in plants and biological control of diseases. Concerning to vitamin C, results are in harmony with George et al., (2004) who found that ascorbic acid increased by using biological control agents (*T. harzianum* or *B. subtilis*) and fungicides.

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