

Antagonistic activity of *Bacillus subtilis* B38 and *Pseudomonas fluorescens* B103 against root-rot and wilting fungi in tomato

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ABSTRACT

This experiment was conducted under greenhouse conditions in the experimental farm station of Moshtohor, Fac. Agric., Benha Univ., during 2012 season. The efficient antagonistic bacterial strains *Bacillus subtilis* B38 and *Pseudomonas fluorescens* B103 either individually or in a mixture was evaluated for their activity against soil-borne pathogens of root-rot disease and wilting fungi compare with fungicide on tomato. Damping-off, survived plants, disease severity, enzymatic activity, plant growth characters, phenol compounds, peroxidase activity and tomato yield were recorded. The treatments of tomato plants with fungicide and bioagents (*B. subtilis* B38 and *Ps. fluorescens* B103) significantly decreased the percentage of damping-off and disease severity of infested tomato with *F. oxysporum*, *R. solani* and *S. rolfsii* compared to uninoculated ones, while, the percentages of survived plants significantly increased when tomato was treated with fungicide or bioagents. In addition, data showed that the individual treatment of *B. subtilis* B38 was found more effective than *Ps. fluorescens* B103 for suppression the soil-borne pathogenic fungi. Moreover, inoculations of tomato with either individually or in a mixture exhibited significant increase of phosphatase, chitinase and dehydrogenase activities in tomato rhizosphere. Data also revealed that all growth characters and yield components were significantly increased especially with dual inoculation by *B. subtilis* B38 and *Ps. fluorescens* B103. Also, the values of free, combined and total phenols as well as peroxidase activity were significantly increased when tomato plants were treated with biocontrol agents either individually or in mixed culture. It could be stated that the dual application of *B. subtilis* B38 and *Ps. fluorescens* B103 was found more effective for controlling the soil-borne fungi. Although, this application was still less than chemical control but it was the best schedule for controlling fungal disease incidence in an ecofriendly system.

INTRODUCTION

Biological control employs natural enemies of pests or pathogens to eradicate or control their population. This can involve the introduction of exotic species, or it can be a matter of harnessing whatever form of biological control exists naturally in the ecosystem. The induction of plant resistance using non-pathogenic or incompatible microorganisms is also a form of biological control (Schouten *et al*, 2004).

Fungal plant diseases are considered the most important microbial agents causing serious losses in the agriculture annually. Some fungal diseases that have successfully been controlled using biological agents are pathogens of pruning wounds and other cut surfaces, diseases of leaves and flowers, such as powdery mildew, diseases of fruits and vegetables, such as *Botrytis* and fungal pathogens in the soil (Heydari, 2007). Kapoor and Kar (1989) reported that *Fusarium spp*, *Rhizoctonia solani* and *Sclerotium rolfsii* were considered the most important soil-borne phytopathogenic fungi of tomato. *Fusarium oxysporum* f.sp *lycopersici* can attack the plants at any stage of growth causing great economic losses to farmers. In addition, *Fusarium* root rot has considerably increased in Egyptian soils. Tomato plants have leaf yellowing and wilting, followed by death of the whole plant due to infection with *Fusarium oxysporum* (Kuwait *et al*, 1994).

The isolates of *Bacillus spp*. produce volatile metabolites and inhibit mycelia growth of *Fusarium oxysporum* and reducing *Fusarium* wilt of onion were recognized (Tehrani and

Ramezani, 2003). *Bacillus* species are widely used in the biocontrol of plant diseases for more than 50 years, because they have a well-developed secretory system producing structurally diverse secondary metabolites such as glucanase, protease inhibitors, ribosome inactivating proteins, chitinase and chitinase-like proteins with a wide spectrum of antibiotic activity (Liu *et al*, 2007 and Zhang *et al*, 2008). Fluorescent pseudomonads represent an important group of rhizospheric bacteria that have promising antagonistic potential for use in biological control of soil-borne fungal pathogens (De La Fuente *et al*, 2004). Because of their catabolic versatility, their excellent root-colonizing abilities and their capacity to produce a wide range of antifungal metabolites, fluorescent pseudomonads have received particular attention (Walsh *et al*, 2001)

The inhibition of pathogenic fungi by the tested bacteria could be attributed to the antifungal metabolite production such as, ammonia, siderophore and cell wall degrading enzyme; cellulase, chitinase and proteolytic enzymes, (Chaiharn *et al*, 2008). In addition to the above-mentioned metabolites, other microbial by-products may also play important roles in pathogenic fungi inhibition. For example, hydrogen cyanide (HCN) and volatile antibiotics according to Phillips *et al* (2004). On the other hand, The pot experiments in greenhouse indicated that *B. subtilis* and *Ps. fluorescens* could be a promising agent for biocontrol of Fusarium wilt on cucumber, which might help to minimize the yield loss of cucumber caused by *F. oxysporum f. sp. cucumerinum* in north China (Li *et al*, 2012).

The main objective of this study was concerned with the evaluation of bacterial candidate isolated from local soils for controlling soil-borne diseases on tomato plants under greenhouse conditions.

MATERIALS AND METHODS

Experimental soil

Soil of the experiment was obtained from Qalubeia Governorate. Experimental soil was subjected to mechanical and chemical analyses in Agricultural Consultancy and Analysis Center, Moshtohor Fac. Agric. according to the method described by Page *et al* (1982). Obtained results are presented in Table 1.

Table 1. Mechanical and chemical analyses of the used soil

Mechanical analysis				Chemical analysis			
Sand (%)	Silt (%)	Clay (%)	Textural Class	EC (dsm ⁻¹)	pH	CaCO ₃ (%)	Total N (%)
23.0	10.8	66.2	Clay	4.14	8.2	1.36	0.23

Preparation of pots and tomato seedlings

Hybrid yasmin-775 of tomato cultivar obtained from Technogreen Corporation for Agricultural projects, Cairo, Egypt was used as a host plant for pathogenic fungi. This experiment was carried out in plastic pots 40 cm in diameter under greenhouse conditions. The pots were filled with 10kg of soil for pathogenic fungal infestation experiment.

Bacterial inocula

Two bacterial strains namely *Bacillus subtilis* (B38) and *Pseudomonas fluorescens* which showed pronounced results with all examined characters of antagonistic action of pathogenic fungi were identified by Bahloul, 2013. Bacterial inocula were prepared using poly broth medium (Bourgouin *et al*, 1984). The inocula suspension was approximately adjusted to 2.5×10^8 cfu/ml.

Pathogenic fungi inocula

Three pathogenic fungi namely *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* were isolated from plant rhizosphere and identified by Bahloul, 2013. For preparation of *R. solani* and *S. rolfsii* inocula, bottles containing sorghum grains sand medium were inoculated with 9 mm-diameter disk of *R. solani* or *S. rolfsii* grown on PDA

medium incubated at 28°C for two weeks (Sneh *et al*, 1991). The inoculum was thoroughly mixed with the experimental soil.

Soil infestation with each fungus was done at the ratio of 3.0% (fungal growth: soil, w/w) (Abd-El-Wahab, 2004). Pots which used for control were filled with the experimental soil mixed with the same ratio of fungus-free sand sorghum medium and treated in the same manner.

For preparation of *F. oxysporum*, conical flasks containing 200 ml potato dextrose broth medium were inoculated with 9 mm-diameter disk of *F. oxysporum* grown on PDA medium. Flasks were incubated at 30°C for 2 weeks. After incubation, growth was decanted and mycelial mats were blended in a warring blender. The spores density was counted using a haemocytometer slide then, adjusted to contain about 6×10^7 spores/ml (Khalil, 1992). The inoculum of *F. oxysporum* was used for inoculating pots containing soil at a ratio of 6×10^7 spores/ kg soil. The added inoculum was then covered with a thin layer of soil. Pots were care irrigated and kept under greenhouse conditions for 7 days before planting.

Greenhouse experiment

Seedlings of tomato (yasmin-775) were washed with water and air dried. Seedlings roots were dipped into bacterial cell suspension (2.5×10^8 cfu/ml) of each tested bacterial strains for 60 minutes before transplanting. Sucrose solution (30%) was added as an adhesive agent prior to inoculation. The bacterial inocula were added to the pots three times (every month) throughout the growing season at a ratio of 300 ml. pot⁻¹ (Khalifa, 2005). In fungicides treatments, the seedlings were immersed for 60 minutes in Maxim (3.5%) at a ratio of 2 cm/L water for chemical control of *F. oxysporum* and Rizolex-T (50%) at a ratio of 3g/kg soil for control of *R. solani* and *S. rolfsii*.

Five tomato seedlings were planted in a pot containing infested soil with the pathogenic fungi. Three infested pots with the pathogenic fungi were planted with untreated seedlings (as control). Chemical fertilizers were supplemented with a full dose of inorganic nitrogen fertilizer (50 kg N/fed) as ammonium sulphate (20.5% N), inorganic phosphorus fertilizer (25 kg P₂O₅/fed) as super phosphate (15.5% P₂O₅) and potassium fertilizer (40 kg K₂O/fed) as potassium sulphate (48% K₂O) (Khalifa, 2005). Treatments were distributed in a randomized complete block design (RCBD) with three replicates.

Determinations

Damping-off and survival plants were counted and recorded after 30 days of transplanting for *R. solani* and *S. rolfsii* according the method and scale described by O'sullivan and Kavanagh (1991) while assessment of damping-off and survival plants were recorded after 60 days for *F. oxysporum* according method of Hassan (1992). Also, alkaline phosphatase, chitinase and dehydrogenase activities of experimental soil were determined according to the method described by Tabatabai (1982), Miller (1959) and Glathe' and Thalmann (1970) respectively, after 30 and 60 days. Peroxidase activity and phenolic compounds were estimated according to the method described by Allam and Hollis (1972) and Snell and Snell (1953) respectively, after 60 days transplanting.

A fresh and dry weight of shoots and number of flowers were determined at flowering stage (60 days) using three randomly selected plants. Plant height was also determined after 120 days of transplanting. Fruits were harvested at proper maturity stage (120 days), then counted, weighted and the following data were calculated; Number of fruits/plant, individual plant yield and weight of one fruit.

Statistical analysis

Statistical analysis was carried out according to Snedecor and Cochran (1989). The differences between the means value of various treatments were compared by Duncan's multiple range test (Duncan's, 1955).

RESULTS AND DISCUSSION

Effect of bioagents and fungicide on damping-off, survived plants and disease severity in infested tomato plants with pathogenic fungi

This trial was carried out using the two bioagents (*B. subtilis* B38 and *Ps. fluorescens* B103), individually or in a mixture compared with fungicide to study their effect on controlling of pathogenic fungi (*F. oxysporum*, *R. solani* and *S. rolfisii*). The obtained data listed in **Table 2** indicated that all pathogenic fungi caused damping-off at different ratio in the presence or absence of bioagents and or fungicide. *S. rolfisii* recorded the highest percentages of damping-off and disease severity in absence of fungicide and bioagents. Generally, the treatments of tomato plants with fungicide and bioagents (*B. subtilis* B38 and *Ps. fluorescens* B103) significantly decreased the percentage of damping-off and disease severity of infested tomato with *F. oxysporum*, *R. solani* and *S. rolfisii* compared to un-inoculated ones, while, the percentages of survived plants significantly increased when tomato was treated with fungicide or bioagents.

Also, the fungicide treatments exhibited the highest antagonistic effect since they recorded the lowest percentages of damping-off and disease severity of infested tomato plants with all pathogenic fungi followed by a mixture of *B. subtilis* B38 and *Ps. fluorescens* B103. While, inoculated tomato with *Ps. fluorescens* B103 exhibited the moderate effect for decreasing damping-off and disease severity of infested tomato plants. Moreover, except control treatment the highest percentage of survived plants recorded by the fungicide followed by a mixture of *B. subtilis* B38 and *Ps. fluorescens* B103. While, *Ps. fluorescens* B103 showed the lowest survived plants.

Table 2. Effect of bioagents and fungicide on percentages of damping-off, survival plants and disease severity of infested tomato with pathogenic fungi under greenhouse conditions.

Treatments	<i>F. oxysporum</i>			<i>R. solani</i>			<i>S. rolfisii</i>		
	Damping-off	Survived Plants	Disease severity	Damping-off	Survived Plants	Disease severity	Damping-off	Survived Plants	Disease severity
Control	40.43 ^c	59.57 ^g	30.03 ^b	47.10 ^b	52.90 ^h	39.40 ^a	80.00 ^a	20.00 ⁱ	42.13 ^a
Fungicide	11.33 ⁱ	88.67 ^a	5.96 ^f	15.60 ^{hi}	84.40 ^{ab}	7.30 ^f	15.43 ^{hi}	84.57 ^{ab}	6.70 ^f
<i>B. subtilis</i> B38 (B)	22.33 ^{fg}	77.67 ^{cd}	16.0 ^{de}	26.60 ^{ef}	73.40 ^{de}	16.30 ^{de}	27.77 ^e	72.23 ^e	16.73 ^d
<i>Ps. fluorescens</i> B103 (Ps)	30.00 ^{de}	70.00 ^{ef}	24.33 ^c	30.03 ^{de}	69.97 ^{ef}	25.7 ^c	33.40 ^d	66.60 ^f	26.27 ^c
B + Ps	19.73 ^{gh}	80.27 ^{bc}	14.67 ^{de}	22.90 ^{fg}	77.10 ^{cd}	16.10 ^{de}	21.03 ^g	78.97 ^c	13.30 ^e

In addition, data showed that the individual treatment of *B. subtilis* B38 was found more effective than *Ps. fluorescens* B103 for suppression the soil-borne pathogenic fungi. It could be also stated that the dual application of *B. subtilis* B38 and *Ps. fluorescens* B103 was found more effective for controlling the soil-borne fungi. Similar results in *Bacillus* strains have been isolated from cucumber growing fields in China, such as *B. subtilis* SQR-5 and *B. subtilis* B579 which have a great potential in biocontrol of *Fusarium* wilt of cucumber. In combination with another biocontrol strains *Paenibacillus polymyxa* SQR-21 and *B. subtilis* SQR-5 was found to be a super biofertilizer which could effectively controlled *F. oxysporum* wilt disease of cucumber (Zhang *et al*, 2008 and Chen *et al*, 2010).

These results are in line with the findings Asaka and Shoda (1996) and Sadler (1996) who found that *Bacillus subtilis* strongly suppressed the growth of *Fusarium oxysporum*, *Sclerotium rolfsii* and *Fusarium solani* responsible for tomato wilting and damping off in vitro by producing antifungal antibiotics (basillomycin and subtilisin) against several pathogens.

Also, Sundaramoorthy *et al* (2012) reported that the mixture of *B. subtilis* (EPCO16 and EPC5) and *Ps. fluorescens* (Pf1) were more effective to reduce damping off than using single strain. These findings suggested that synergistic interaction of biocontrol agents may be responsible for the management of chilli wilt disease caused by *F. solani*.

Enzymatic activities in rhizosphere of infested tomato plants with pathogenic fungi

Phosphatase, chitinase and dehydrogenase activities were measured as indexes to detect changes in the microbial functioning in soil as affected by biocontrol agents against pathogenic fungi, (Table 3).

Alkaline phosphatase activity was estimated in soil for its important role in organic phosphorus compounds hydrolysis by microorganisms. The obtained data clearly showed that the alkaline phosphatase activity in rhizosphere of tomato infested with pathogenic fungi treated with various treatments was higher at flowering stage than vegetative one. Also, results emphasized that the lowest values of phosphatase activities were observed with control treatment (control-1), untreated plants (control-2) and fungicide application. This trend of results was observed at vegetative and flowering stages.

Table 3. Effect of biocontrol agents on alkaline phosphatase, chitinase and dehydrogenase activities in rhizosphere of infested tomato plants with pathogenic fungi.

Treatments		Alkaline phosphatase activity ($\mu\text{g p-nitrophenol} \cdot \text{g}^{-1} \cdot \text{dm}^{-1} \cdot \text{h}^{-1}$)		Chitinase activity (mM N-acetylglucose amine/ g dry soil /1 hrs)		Dehydrogenase activity ($\mu\text{gTPF/g dry soil/day}$)	
		Vegetative stage	Flowering stage	Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
Control-1	With <i>F. oxysporum</i>	12.03 ^{nop}	16.33 ^{ijklmno}	1.00 ⁿ	1.83 ^{mn}	69.17 ^{klm}	102.1 ^{ijkl}
Control-2		13.03 ^{mno}	13.37 ^{lmno}	3.20 ^{klm}	4.63 ^{ijk}	66.90 ^{klm}	79.27 ^{klm}
Fungicide		13.93 ^{lmno}	21.37 ^{ghij}	2.80 ^{lm}	2.65 ^m	74.77 ^{klm}	80.43 ^{ijklm}
<i>B. subtilis</i> B38 (B)		17.13 ^{ijklmn}	38.57 ^f	6.46 ^h	8.90 ^g	169.8 ^f	186.4 ^{def}
<i>Ps. fluorescens</i> B103 (Ps)		21.40 ^{ghij}	52.50 ^{cd}	11.03 ^{de}	13.50 ^c	122.7 ^{ghi}	133.4 ^{gh}
B + Ps		26.27 ^g	59.77 ^{ab}	11.93 ^{cde}	15.10 ^b	220.9 ^{bcd}	311.9 ^a
Control-2	With <i>R. solani</i>	7.93 ^p	11.33 ^{op}	1.90 ^{mn}	2.76 ^{lm}	50.83 ^m	62.80 ^{lm}
Fungicide		15.33 ^{klmno}	22.70 ^{gh}	2.36 ^{mn}	1.63 ^{mn}	51.17 ^m	67.40 ^{klm}
<i>B. subtilis</i> B38 (B)		17.60 ^{hijklm}	43.70 ^e	4.83 ^{ij}	6.03 ^{hi}	82.13 ^{ijklm}	180.9 ^{ef}
<i>Ps. fluorescens</i> B103 (Ps)		18.52 ^{hijkl}	50.80 ^d	7.00 ^h	8.10 ^g	100.1 ^{ijkl}	155.6 ^{fgh}
B + Ps		20.13 ^{hijk}	57.10 ^{bc}	8.73 ^g	10.57 ^{ef}	225.2 ^{bc}	214.6 ^{cde}
Control-2		With <i>S. rolfsii</i>	13.03 ^{mno}	19.67 ^{hijk}	4.30 ^{kl}	4.63 ^{ijk}	96.30 ^{ijkl}
Fungicide	13.90 ^{lmnop}		21.97 ^{ghi}	2.00 ^{mn}	4.93 ^{ij}	130.6 ^{ghi}	161.2 ^{fg}
<i>B. subtilis</i> B38 (B)	17.13 ^{ijklmn}		55.47 ^{bcd}	9.20 ^{fg}	13.03 ^c	230.7 ^{bc}	241.2 ^{bc}
<i>Ps. fluorescens</i> B103 (Ps)	21.40 ^{ghij}		59.03 ^{ab}	8.53 ^g	12.43 ^{cd}	155.5 ^{fgh}	183.3 ^{def}
B + Ps	26.27 ^g		63.67 ^a	10.50 ^{ef}	16.57 ^a	235.3 ^{bc}	254.5 ^b

Control-1: healthy plants

Control-2: pathogenic fungi infested plants

In addition, inoculation of tomato plants with biocontrol agent *B. subtilis* B38 and *Ps. fluorescens* B103 individually or in a mixture significantly increased alkaline phosphatase activity compared to un-inoculated treatments. Moreover, the combination of bioagents *B. subtilis* B38 and *Ps. fluorescens* B103 recorded the highest values of alkaline phosphatase activity compared with other treatments. These results could be attributed to synergistic effect occurred in case of dual and mixed inoculation (Abou-Aly, 2009) as well as to the higher ability of bioagent strains to colonize tomato roots. These results also coincide with those obtained by Chebotar *et al* (2001) who reported that the success of inoculated seeds or seedlings with beneficial bacteria usually depends on the colonization potential of the introduced strains.

Dehydrogenase (DHA) activity was determined as a criterion of respiration rate and total microbial activity in the soil. In view of the infested tomato plants with pathogenic fungi, data in Table (2) showed that soil infested with pathogenic fungi and treated with fungicide application caused a decrease in dehydrogenase activity. The higher values of DHA in tomato rhizosphere infected with pathogenic fungi were observed by the application of bioagent *B. subtilis* B38 and *Ps. fluorescens* B103 either individually or in a mixture compared to other treatments. Moreover, the obtained results clearly indicated that inoculation of infested tomato with the mixed culture of bioagent strains gave significant increase of DHA as compared to individual inoculation treatments at vegetative and flowering stages.

Similar trend was obtained by Zaghloul *et al* (2008) who studied the efficiency of soil inoculation with *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* on some soil enzymatic activity and found that *A. chroococcum* combined with *B. megaterium* var. *phosphaticum* gave higher values of dehydrogenase activity compared with individual inoculation treatments. Moreover, Abou-Aly (2009) found that dual inoculation with *Ps. fluorescens*, *P. polymyxa* and *Az. lipoforum* of pepper rhizosphere infested with *F. oxysporum* resulted in higher values of dehydrogenase than that of individual inoculation. Moreover, high activity of DHA at flowering stage is likely due to the difference in multiplication rate of different soil microorganisms which usually maximum values during flowering stage. Such differences could be attributed to the qualitative and quantitative changes in the nature of root exudates during different growth stages.

Growth characters of tomato

The obtained data in Table (4) revealed that growth parameters recorded the lowest values with the treatment of infested tomato plants with pathogenic fungi (*F. oxysporum*, *R. solani* and *S. rolfsii*). Also, application of fungicide and exhibited significant increase of growth parameters compared to untreated tomato plants. In addition, the fungicide application induced high increases of growth parameters as compared with other treatments.

Table 4. Effect of bioagents on growth characters of infested tomato with pathogenic fungi under greenhouse conditions.

Treatments		Plant height (cm)	No. of flowers /plant	Shoots fresh weight g/plant	Shoots dry weight g/plant
Control-1		65.20 ^d	10.60 ^{bc}	86.28 ^d	17.31 ^e
Control-2	With <i>F. oxysporum</i>	40.77 ^e	6.33 ^d	39.53 ^e	8.63 ^f
Fungicide		99.30 ^a	16.67 ^a	103.40 ^{bc}	18.13 ^e
<i>B. subtilis</i> B38 (B)		96.10 ^a	10.00 ^c	88.43 ^d	18.97 ^e
<i>Ps. fluorescens</i> B103 (Ps)		87.57 ^{abc}	10.00 ^c	98.60 ^{cd}	23.27 ^c
B + Ps		90.67 ^{ab}	11.00 ^{bc}	99.30 ^{bcd}	22.43 ^{cd}
Control-2	With <i>R. solani</i>	48.13 ^e	6.66 ^d	38.20 ^e	9.36 ^f
Fungicide		97.00 ^a	12.33 ^b	118.0 ^a	31.30 ^a
<i>B. subtilis</i> B38 (B)		86.27 ^{abc}	11.00 ^{bc}	98.83 ^{bcd}	26.97 ^b
<i>Ps. fluorescens</i> B103 (Ps)		80.33 ^{abcd}	10.00 ^c	98.10 ^{cd}	22.23 ^{cd}
B + Ps		88.13 ^{abc}	10.00 ^c	106.5 ^{bc}	26.83 ^b
Control-2	With <i>S. rolfii</i>	46.57 ^e	6.00 ^d	28.87 ^e	5.00 ^g
Fungicide		85.47 ^{abc}	12.00 ^b	110.5 ^{ab}	30.33 ^a
<i>B. subtilis</i> B38 (B)		65.73 ^d	11.33 ^{bc}	97.50 ^{cd}	23.57 ^c
<i>Ps. fluorescens</i> B103 (Ps)		70.50 ^{cd}	10.67 ^{bc}	90.27 ^d	20.93 ^d
B + Ps		76.93 ^{bcd}	9.66 ^c	105.3 ^{bc}	27.13 ^b

Control-1: healthy plants

Control-2: pathogenic fungi infested plants

The obtained results are confirmed with those obtained by **Abou-Aly (2009)** who demonstrated that soil infestation with *F. oxysporum* significantly decreased root size, root dry weight; plant height and shoot dry weight of pepper as compared with control plants. In addition, he reported that growth characters significantly increased in the infested plants inoculated with PGPR individually or in a mixture form of them.

The beneficial effect of *B. subtilis* B38 and *Ps. fluorescens* B103 as rhizobacteria could be attributed to promotion of plant growth as well as development of healthy plants. Plant growth promoting rhizobacteria (PGPR) including all bacteria which inhabit plant roots that exert a positive effect by various mechanisms which ranging from a direct effect such as increasing of nutrients uptake to an indirect effect such as pathogens suppression. Also, productions of plant growth regulators (PGRs) by PGPR are one of the suggested mechanisms through which these bacteria affecting plant growth and development. Plant growth promoting rhizobacteria (PGPR) can induce rooting, plant growth and disease control in many crops. Although the mechanisms are not completely clarified, plant growth promotion may occur by production of phytohormones such as auxins, gibberellins and cytokinins and by mineralization of nutrients (**Teixeira et al, 2007**).

Phenolic compounds and peroxidase activity in tomato leaves

It is important to mention that the phenolic compounds and peroxidase activity accumulate in tomato plants in response to stress by pathogens play an important role in soil-borne fungi diseases stress resistance especially when applied with biocontrol agents as shown in **Tables (5)**.

Table 5. Phenols and peroxidase activities in infested tomato plants with pathogenic fungi.

Treatments		Phenols, mg / g fresh weight			Peroxidase as absorbance/g fresh leaves
		Total	Free	Combined	
Control-1		6.58 ^f	3.26 ^g	3.32 ⁱ	1.62 ^h
Control-2	With <i>F. oxysporum</i>	13.40 ^{de}	6.03 ^{def}	7.37 ^{cde}	1.95 ^h
Fungicide		8.87 ^f	5.30 ^{efg}	3.57 ⁱ	2.12 ^{gh}
<i>B. subtilis</i> B38 (B)		13.73 ^{de}	6.13 ^{de}	7.60 ^{cd}	3.29 ^{cd}
<i>Ps. fluorescens</i> B103 (Ps)		15.03 ^{bcd}	8.80 ^{bc}	6.23 ^{efg}	3.83 ^b
B + Ps		16.43 ^{abc}	6.30 ^{de}	10.13 ^{ab}	3.96 ^b
Control-2	With <i>R. solani</i>	12.63 ^e	6.53 ^d	6.10 ^{efg}	2.13 ^{gh}
Fungicide		8.33 ^f	4.36 ^g	3.97 ⁱ	2.13 ^{gh}
<i>B. subtilis</i> B38 (B)		14.67 ^{bcd}	10.20 ^a	4.47 ^{hi}	2.76 ^e
<i>Ps. fluorescens</i> B103 (Ps)		14.27 ^{de}	8.90 ^{bc}	5.37 ^{fgh}	3.10 ^d
B + Ps		17.20 ^a	9.83 ^{ab}	7.37 ^{cde}	4.00 ^b
Control-2	With <i>S. rolfisii</i>	16.57 ^{ab}	8.86 ^{bc}	7.71 ^c	2.26 ^{fg}
Fungicide		10.20 ^f	4.93 ^{fg}	5.27 ^{fgh}	2.43 ^f
<i>B. subtilis</i> B38 (B)		14.40 ^{cde}	8.03 ^c	6.37 ^{def}	3.43 ^c
<i>Ps. fluorescens</i> B103 (Ps)		14.23 ^{de}	8.53 ^c	5.70 ^{fgh}	3.23 ^{cd}
B + Ps		16.93 ^{ab}	6.00 ^{def}	10.93 ^a	4.73 ^a

Control-1: healthy plants

Control-2: pathogenic fungi infested plants

Data showed that low values of free, combined and total phenols as well as peroxidase activity were observed with control-1 treatment. Moreover, chemical fungicide treatments exhibited slightly increase of free, combined and total phenols.

However, the values of free, combined and total phenols as well as peroxidase activity were significantly increased when tomato plants were treated with biocontrol either individually or in mixed culture. Therefore, high values of total and combined phenols were observed in combination of *B. subtilis* B38 and *Ps. fluorescens* B103 in comparison with either individually treatment or control.

These results are in harmony with those of **Shehata (2001)** who found positive correlation between level of phenolic compounds and root-rot and wilting infection caused by *S. rolfisii*, *R. solani* and *F. spp* in tomato. Many of these compounds exhibited antimicrobial properties. Therefore, phenols could be playing an important role in disease resistance. Free, combined and total phenols were increased as a result of infection, only in the susceptible and tolerant cultivars. On the other hand, increased peroxidase activity upon infection might be required for an additional deposition of lignin around the lesion court induced by pathogen. Peroxidase is a key enzyme in the biosynthesis of lignin and other oxidized phenols which are highly toxic to the pathogen (**Khatun et al, 2009**). Those findings are also in harmony with those obtained by **Abou-Aly (2009)** who found that application of a biocontrol agent *Ps. fluorescens* induced peroxidase and poly oxidase activity and resulted in significant reduction of pathogen infestation.

Effect of various treatments on the yield of tomato plants

Obtained data in **Table (6)** clearly indicated that soil infestation with *F. oxysporum*, *R. solani* and *S. rolfisii* or root-knot nematode significantly decrease of number of fruits, fruit weight and fruits yield per plant of tomato as compared with control-1 plants. Data also showed that chemical fungicide application significantly induced the number of fruits per plant followed by the mixture of bioagents. Moreover, data revealed that all yield

components were significantly increased especially with plants infested with individual bioagents. Dual inoculation by using *B. subtilis* B38 and *Ps. fluorescens* B103 revealed more increase of all yield components. Similar results were reported by Li *et al* (2012), who estimated *B. subtilis* B068150 as promising bio-agent for controlling *Fusarium oxysporum* infected cucumber plants. The bio-agent also succeeded to prevent yield losses under greenhouse conditions. Inoculation of tomato with biocontrol agents gave an increase of final yield compared with untreated one. This may be due to the high suppressed of soil-borne pathogenic fungi by their activity of antifungal compounds, also ability of bioagents to improve all growth characters by production of plant growth regulator substances (PGRs).

Table 6. Effect of various treatments on yield and yield components of infested tomato plants with pathogenic fungi under greenhouse conditions.

Treatments		Number of fruits/plant	Average of fruit weight (g)	Fruits yield/plant (kg)
Control-1		5.2 ^{ef}	73.90 ^{cdef}	0.587 ^{fg}
Control-2	With <i>F. oxysporum</i>	3.33 ^f	66.13 ^{ef}	0.283 ^h
Fungicide		12.00 ^a	87.97 ^{ab}	1.100 ^a
<i>B. subtilis</i> B38 (B)		6.33 ^e	75.43 ^{cdef}	0.766 ^{de}
<i>Ps. fluorescens</i> B103 (Ps)		7.66 ^{cde}	69.93 ^{def}	0.800 ^{cd}
B + Ps		8.33 ^{bcd}	77.37 ^{bcd}	0.866 ^c
Control-2	With <i>R. solani</i>	3.33 ^f	47.30 ^g	0.200 ^h
Fungicide		10.00 ^b	96.23 ^a	0.966 ^b
<i>B. subtilis</i> B38 (B)		7.66 ^{cde}	65.17 ^f	0.700 ^e
<i>Ps. fluorescens</i> B103 (Ps)		7.00 ^{de}	73.83 ^{cdef}	0.700 ^e
B + Ps		9.33 ^{bc}	80.23 ^{bcd}	0.800 ^{cd}
Control-2	With <i>S. rolfii</i>	1.33 ^g	50.50 ^g	0.100 ⁱ
Fungicide		8.00 ^{cde}	85.50 ^{abc}	0.733 ^{de}
<i>B. subtilis</i> B38 (B)		6.33 ^e	76.93 ^{bcd}	0.533 ^{fg}
<i>Ps. fluorescens</i> B103 (Ps)		6.33 ^e	68.40 ^{def}	0.466 ^g
B + Ps		7.66 ^{cde}	87.70 ^{ab}	0.600 ^f

Control-1: healthy plants

Control-2: pathogenic fungi infested plants

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الملخص العربى

النشاط التضاى لىكترى *Bacillus subtilis* B38 and *Pseudomonas fluorescens* B103 على فطريات عنف

الجذور والذبول فى الطماطم

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تم إجراء هذه التجربة تحت ظروف الصوبة بمركز البحوث والتجارب الزراعية بكلية الزراعة بمشتهر- جامعة بنها خلال موسم 2012م لدراسة التأثير التضاى لىكترى *Bacillus subtilis* B38 and *Pseudomonas fluorescens* B103 على فطريات عنف الجذور والذبول فى الطماطم ومقارنة هذا التأثير بالمبيدات الفطرية . أظهرت نتائج هذه التجربة ان معاملة نباتات الطماطم بالمبيد الفطرى وعوامل المقاومة الحيوية أدى الى انخفاض النسبة المئوية لسقوط الشتلات فى الطماطم المعده بفطريات *F. oxysporum*, *R. solani* and *S.rolfsii* مقارنة بمعاملة الكنترول ، كذلك أظهرت النتائج ان معاملة الطماطم بالمبيد الفطرى ولىكترى المقاومة الحيوية أدى الى زيادة معنوية فى نسبة النباتات السليمة (غير المصابة) ، وأوضحت النتائج ان التأثير الإيجابى لىكترى *Bacillus subtilis* B38 أعلى من لىكترى *Pseudomonas fluorescens* B103 .

عند تلقيح نبات الطماطم بلىكترى المقاومة الحيوية لوحظ زيادة معنوية فى نشاط انزيمات الفوسفاتيز ، الشيتينيز ، الد كيهيدروجينيز فى منطقة الريزوسفير . كذلك اوضحت النتائج أن صفات النمو والمحصول لنباتات الطماطم قد إزدادت معنويا وخصوصا عند التلقيح المزدوج بكل من لىكترى *Bacillus subtilis* B38 ولىكترى *Pseudomonas fluorescens* B103 . وعند تلقيح نباتات الطماطم بلىكترى المقاومة الحيوية لوحظ زيادة معنوية فى محتوى أوراق نباتات الطماطم من الفينولات الكلية والحررة والمرتبطة وكذلك فى نشاط إنزيم البيروكسيديز . وفى ضوء النتائج المتحصل عليها من هذه الدراسة يمكن القول بأنه يمكن إستخدام لىكترى المقاومة الحيوية السابقة الذكر فى مقاومة فطريات أعفان الجذور والذبول فى الطماطم كطرق بديلة و آمنة وصديقة للبيئة لمقاومة مثل هذه الفطريات بدلا من إستخدام المبيدات الفطرية والى تسبب تلوث خطير للبيئة وتؤثر على صحة الإنسان.