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Using Bioproducts Made with Native Microorganisms to Limit the Damage for Some Sugar Beet Cultivars Seeds

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Abstract: Several diseases have been reported to affect sugar beet plants. Damping-off and root-rot diseases of sugar beet are the most important diseases that attack both seedlings and adult plants causing serious losses in crop productivity and quality. *Rhizoctonia solani* is one of the main causal organisms for pre, post emergence damping-off and root-rot diseases. In laboratory experiments using PDA medium, seven different biocontrol agents (*Trichoderma harzianum, Pseudomonas fluorescens, Pseudomonas putida, Brevibacillus brevis, Bacillus megaterium, Paenibacillus polymyxa* and *Streptomyces sp.*) showed an inhibitory effects on the linear growth of *R. solani*. *T. harzianum* was the most effective bioagent in reducing the linear growth of *R. solani* followed by *P. fluorescens, P. putida, B. megaterium, P. polymyxa, B. brevis* and *Streptomyces sp* respectively. Under greenhouse conditions, the most effective biocontrol agent was *B. brevis* against *R. solani*, followed by *Streptomyces sp., B. megaterium, T. harzianum, P. polymyxa, P. putida* and *P. fluorescens* respectively. Bioagents treatments resulted in significant increase in soluble protein contents with significant increase in the phenol content, polyphenol-oxidase, peroxidase and chitinase.
The different growth parameters, plant root length, dry root weight, leaves dry weight and number of leaves per plant were higher in case of the treated seedlings with antagonistic organisms than with untreated seedlings. Results revealed that the most effective biocontrol agent was *Streptomyces sp.* concerning the number of survival plants, followed by *P. fluorescens, P. putida, B. megaterium, T. harzianum, B. brevis* and *P. polymyxa* respectively. The growth parameters results indicated that significant differences were observed among most of the variables measured for the biocontrol treatments compared with the untreated control.
Key words: Sugar beet, *Beta vulgaris L., Rhizoctonia solani*, root-rot, damping-off, biocontrol, Soluble protein, phenol, polyphenol-oxidase, peroxidase.

Introduction

Sugar beet (*Beta vulgaris* L.), is grown for sugar production and is considered the second important sugar crop in Egypt, after sugar cane (*Sacchurum officinarum* L.). It received a great attention in Egypt because of its strategic and industrial values as a sugar crop. The importance of sugar beet crop to agriculture is not only confined to sugar production, but also it is well known to be adopted to poor, saline, alkaline and calcareous soils. Thus, it can be economically grown in the newly reclaimed land of Egypt as it is one of the most tolerant crops to salinity. It also makes the soil in good condition for the benefit of the following cereal crop. According to the statistics made in 2010 by ministry of agriculture and land reclamation, the amount of sugar beet that was produced in Egypt was 7840304 tons, only 4% of this production is produced in outside the Nile valley,

(Egyptian agricultural statistics Report, 2011). Worldwide, 40% of produced sugar come from sugar beet, whereas in Egypt, we find only 25% are produced from sugar beet. The strategy of increasing the sugar production in Egypt depends on expansion of sugar from sugar beet cultivation in newly reclaimed soil. Under Egyptian environmental conditions, sugar beet plants are attacked by numerous foliar and root diseases which have always been major limiting factors for sugar beet production. Sugar beet diseases can be divided into two groups. The first group is the diseases caused by infectious microorganisms including fungi, bacteria, viruses, and nematodes. The second group includes physiological diseases caused by non-infectious physical or chemical factors such as adverse environmental factors, nutritional and physiological disorders. Some of the infectious diseases, caused by soil-borne pathogens of sugar beet plants cause damping-off and the root rot of plants just before or after emergence. This is one of the most prevalent diseases of sugar beet crop in Egypt, which leads to a major economic loss. Therefore, damping-off and root rot diseases caused by Rhizoctonia on sugar beet were the focus of the proposed research. With the prohibition of pesticides such as methyl bromide for the control of soil borne plant diseases, attention has shifted to alternative synthetic fungicides and non-chemical control measures for controlling soil borne diseases. The use of synthetic fungicides to control pathogenic fungi; however, is increasingly restricted due to their harmful effects on human health and the environment (1). The increasing demand for production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control strategies. Using composts for suppressing soil borne diseases in particular has increased in recent years due to their contribution to the recycling of agricultural waste. They are also reported to provide a cheap and effective means to suppress soil borne diseases when used as soil amendments (2-9).

Antagonism between soil microorganisms is a common phenomenon. Many articles were concerned with the existence and importance of the mechanisms of antagonism (10) (11), (12; 13), (14; 15). Biological control of Rhizoctonia diseases has been demonstrated in some cases and represents an additional strategy that may provide effective and sustainable management for these important diseases. Biocontrol can be an effective mean of control in many instances where chemical control is not available or not practical (16). Several microbial antagonists, some of which are available in commercial formulations, have shown potential for control of *R. solani* on sugar beet or other host crops. *T. harzianum* and *Trichoderma* (*Gliocladium*) virens have successfully suppressed *R. solani* in several pathosystems (17-20).

New bio-formulation systems involved *Trichoderma* spp. were mixing vermiculite and powder bran (21), wheat bran (22) and colonization in peat and sand mixture (23). *T. harzianum,* grown on solid substrates had varying degrees of success against white rot of onion caused by *Sclerotium cepivorum* (24), and root-rot of cucumber caused by *P. ulimum* (25). Several biocontrol organisms have shown potential for control of *R. solani* on sugar beet or other host crops. *T. harzianum* Rifai have successfully suppressed *R. solani* in several pathosystems (26-28), *P. fluorescens* (29-33) and *P. polymyxa* Prazmowski (34) have all shown some potential for disease control under the conditions studied (35). However, many of these organisms have not been studied for suppression of Rhizoctonia disease of sugar beet and most have only been evaluated under limited conditions and have not been compared with each other in the same pathosystem.

The objective of this study were: Evaluate the efficacy of several biocontrol organisms in the control of Rhizoctonia root-rot and damping-off diseases of sugar beet; The effects of several biocontrol organisms on plants growth characters of sugar beet plants and Evaluate the effects of microbial antagonists on populations of *R. solani* in soil.

Material and Methods

An isolate of *R. solani* recovered from infected Sugar beet seeds were used as the source of pathogen inoculum in all experiments. This isolate caused considerable root-rot and Damping-off in green house trials. PDA medium were autoclaved at 121^{o}C for 15 min, inoculated after cooling with a single disk of five mm diameter from the full-grown *R. solani* plates. The fungal culture of *R. solani* was incubated at room temperature (21-25 C°) for 7 days and stored in a refrigerator until used.

Biocontrol treatments

Isolates of antagonistic fungi, bacteria and actinomycetes were kindly provided by Prof. Dr. Wafaa Mohamed Haggag, Plant Pathology Department, National Research Centre (NRC). Each isolate was sub

cultured on suitable medium, i.e., PDA for fungi, nitrate glucose agar medium and starch nitrate agar medium for bacteria and actinomycetes, respectively. In this experiment, the efficacy of different biocontrol agents were evaluated against the root-rot on sugar beet plants. These biocontrol agents were *T. harzianum, P. fluorescens* and *P. putida, B. brevis and B.megaterium, P. polymyxa* and *Strepromyces sp*.

Effect of antagonistic organisms on mycelial growth of *R. solani* under laboratory conditions:

The effect of the antagonistic organisms *T. harzianum*, *P. fluorescens*, *P. putida*, *B. brevis*, *B. megaterium*, *P.polymyxa* and *Strepromyces sp*. on the linear growth of the pathogenic fungus *R.solani* was evaluated under laboratory conditions. The biological control agents were tested against *R. solani in vitro* using potato dextrose agar (PDA) medium. Petri dishes (9 cm diameter), each contained 15 ml of PDA medium. Five mm diameter disc of a seven days old fungal growth of the tested *R. solani* isolate was placed 10 mm from the edge of the plate. On the opposite side of the petri plate a five mm disc of the tested antagonistic fungus or streak of the tested bacteria or actinomycetes was placed. Plates inoculated with pathogenic fungus only were used as control (36). Five replicates for each treatment were used. All Petri dishes were incubated at 25°C and kept under observations and data were recorded when the growth of the pathogenic fungus completely covered the medium surface in control treatment, the liner growth of *R. solani* in different treatments was measured. The efficiency of antagonistic bioagents was calculated according to the following formula (37).

Antifungal produce by biocontrol agents:

Activities of hydrolysis enzymes were assessed in culture supernatant of the tested microorganisms using spectrophotometer. Protease activity was measured in dimethyl casein (5 mg/ ml in 20 mM phosphate buffer pH 7.0) as substrate using auto analyser. The release of alanine was measured and used as a basis for the expression of protease activity (I U = 1 µmol alanine/ min/ g) (38). Exo-glucanase activity was detonated using glucose oxidase-O-dianisidine reaction (Sigma Chemicals, glucose determination kit 510-A) which specifically measures glucose produced from laminarin hydrolysis. ß-1,3-Glucanase activity was assayed by incubating 0.2 ml of culture filtrate in 50 mM potassium acetate buffer (pH 5.5) with 50 µl of enzyme solution appropriately diluted in the same buffer. Reaction mixtures were incubated at 37°C for 30 min and were stopped by boiling for 5 min. One unit of ß-1,3-glucanase activity was defined as the amount of enzyme that releases 1 µmol of reducing sugar equivalents (expressed as glucose) per min under the standard assay conditions. The activity of exo-chitinase, was measured as the release of N-acetylglucoseamine from chitin and one unit (U) of enzyme activity was defined as the amount of enzyme that release 1 µmol of reducing groups/ min/ ml of the filtrate (39).

Biological control of *Rhizoctonia solani* under greenhouse conditions

The effect of different biocontrol agents in controlling sugar beet damping-off and root rot was carried out under greenhouse conditions during 2014 season. Some of Polyethylene bags (20 cm) contained infested soil and some contained sterilized soil.

The inoculam of *T. harzianum* was prepared by growing the fungal isolate on sterilized liquid PDA medium incubated in conical flask (500 ml) on shaker at 25° C for 7 days (180 rpm). The inocula of *P. fluorescens* and *P. putida* were prepared by growing the isolate on sterilized liquid King's medium incubated in conical flask on shaker at 25° C for 7 days (180 rpm). The inocula of *B. brevis*, *B. megaterium* and *P. polymyxa* were prepared by growing the isolate on sterilized liquid Nutrient medium, incubated in conical flask on shaker at 25° C for 7 days (180 rpm). The inoculum of *Strepromyces sp*. was prepared by growing the isolate on sterilized liquid Starch medium, incubated in conical flask on shaker at 25° C for 7 days (180 rpm). The biocontrol agents, which showed high reduction of mycelial growth of *R. solani* in laboratory assays, were used for greenhouse experiment. Beta-max and Diamond cultivars seeds were disinfected with 2% sodium hypochlorite solution for two minutes then dried and soaked for 12 hour in liquid medium of biocontrol agents in Petri dishes. The treated seeds were air-dried and five seeds were sown in the rate of 5 seeds/pot. Irrigation was performed when necessary. The percentage of pre- and post-emergence damping-off was calculated after 7 and 30 days, respectively. Uninoculated soil served as control. Ten pots were used for each particular treatment.

Chemical analysis:

Thirty days after plant growth, three top leaves per plant were separately collected, frozen for 36 h, dried and powdered. Generally, 100 mg dried sample were used for analysis. Phenol, peroxidase, polyphenol oxidase and chitinase activities in the sugar beet plants that was treated by soaking sugar beet seeds in biocontrol agents were determined. Total protein was extracted from t leaves and the supernatant prepared according to (40). Total phenolic compounds was determined according to the Folin-Ciocalteu method described by Sun *et al* (41). Peroxidase activity was measured according to the methods described by Alam *et al* (42). Polyphenoloxidase (PPO) was measured according to the methods described by Oktay *et al* (43). The chitinase activity was determined by the colorimetric method (44).

Plant growth parameters measurements

Some growth parameters were measured in this experiment. Samples were taken during the growth period after 120 days and 150 days of sowing, the plants were removed, placed in plastic bags, labeled and transferred to the laboratory for data recording. To study the relationship between biocontrol agents and propagules of the pathogen in soil and disease severity, the population of *R. solani* propagules were counted as described by Lumsden and Locke (45).

Statistical analysis

The means and standard deviations of the data were calculated and statistically analyzed using the analysis of variance (ANOVA) and Duncan's multiple range tests ($P \leq 0.05$). Version 20.0 of SPSS was used for statistical analysis (SPSS Inc., Chicago, IL, USA).

Results

In vitro*, antifungal activities of the biocontrol agents against *Rhizoctonia solani

The effect of the antagonistic organisms; *T. harzianum*, *P. fluorescens*, *P. putida*, *B. brevis*, *B. megaterium*, *P. polymyxa* and *Streptomyces sp*, on mycelial growth of *R. solani* was studied under laboratory conditions. Data illustrated in fig. (1) indicated that all biocontrol agents reduced the percentage of mycelial growth of *R. solani*. Data indicated that *T.harzianum* showed the highest percentage of growth reduction (75.72 %), followed by *P. fluorescens* (63.16 %), *P. putida* and *B.megaterium* isolates showed a moderate percentage of growth reduction *P. putida* (59.33 %), *B.megaterium* (59 %). *P. polymyxa* (54.33 %), *B. brevis* (54.36 %) and *Streptomyces sp*. (53.16 %) as compared with the control.

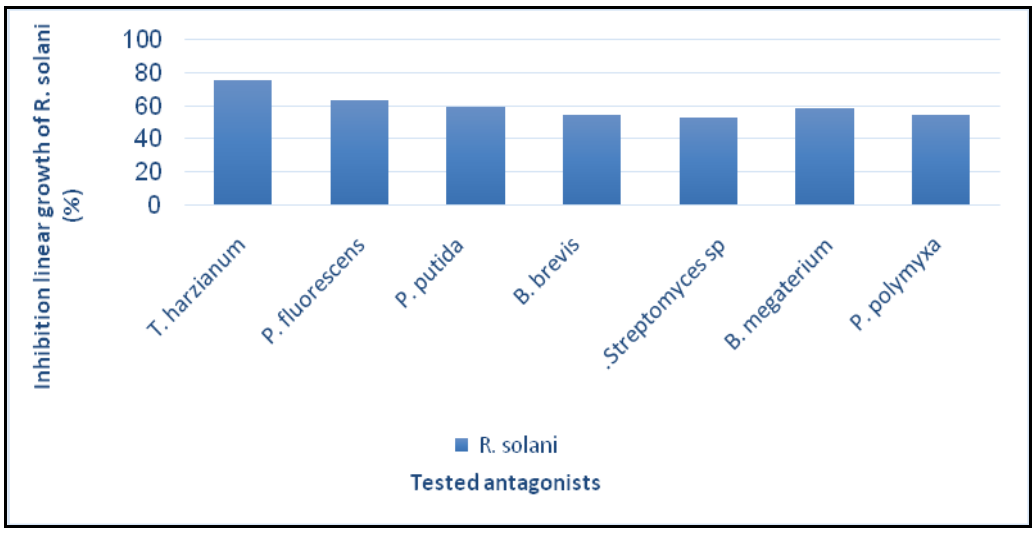


Fig. (1). Effect of antagonistic organisms on the linear growth of *R. solani* under laboratory conditions

The antagonistic reaction between the bioagent microorganisms and the pathogenic fungus illustrated as zone of inhibition is presented in Fig.2 &3 . Data indicated that *T. harzianum* isolate showed the highest antagonistic effect as it overgrew the pathogen and covered the entire medium surface. Data showed that, the largest zone of inhibition was found by the bioagent *P. fluorescens* (23.6 mm) followed by *P. polymyxa* (10.24 mm). *B. brevis* showed a moderate zone of inhibition reaction for the pathogenic fungal, *B.brevis* (7.44 mm). *B. megaterium*, *P. putida*.

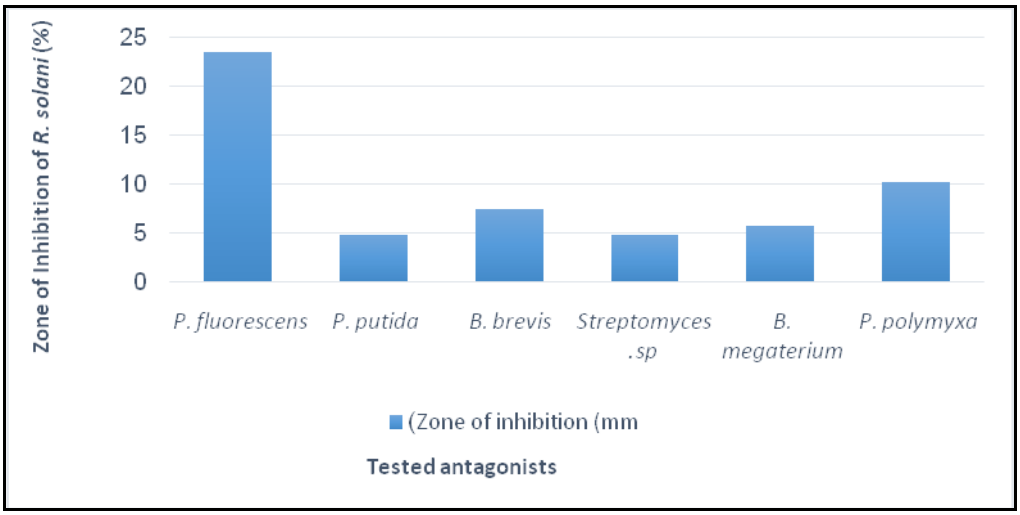
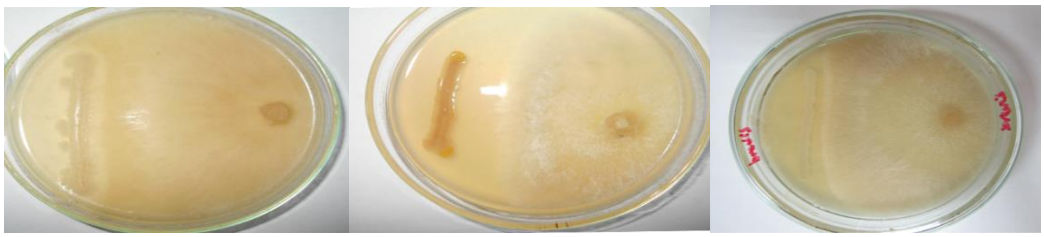


Figure (2) The antagonistic reactions of bioagents microorganisms against *Rhizoctonia solani in vitro*

Trichoderma harzianum

P. polymyxa *P.fluorescens* *B.brevis*



Bacillus megaterium *Pseudomonas putida* *Streptomyces sp.*

Fig. 3 Inhibition assay showing the antifungal activity of bioagents against *R. solani in vitro*

Antifungal produce by biocontrol agents:

As shown in Table (1). Results showed production of high levels of protein, the data on soluble protein were significantly different between the biocontrol agents. The bioagent *B. brevis* showed highest soluble protein content, which was followed by *P. polymyxa, P. putida* and *T. harzianum.* The bioagent *P. fluorescens* showed a moderate soluble protein content followed by *Streptomyces sp.* The lowest soluble protein content were recorded in *B. megaterium* bioagent. Data showed that *Streptomyces sp.* showed the highest chitinase production followed by *T. harzianum, B. megaterium* and *P. polymyxa*. The bioagent *B. brevis* showed a moderate chitinase production followed by *P. putida* and *P. fluorescens* showed a lowest chitinase production.

Data showed that the bioagent *P. polymyxa* showed the highest β-1,3 glucanase production followed by *Streptomyces sp., T. harzianum* and *B. megaterium. B. brevis* showed midrates β-1,3 glucanase production followed by *P. putida* and the bioagent *P. fluorescens* showed the lowest β-1,3 glucanase production.

Table (1) Total protein and antifungal Enzymes produced by biocontrol agent

Bioagents	Cell concentration (OD 610 nm)	Total soluble protein ; Conc. (mg/l)	Antifungal products (Unit/ ml)		
			Chitinase	β-1,3 glucanase	Protease
P. fluorescens	0.867 b	4.4143 cd	0.02 b	0.03 b	0.0 b
P. putida	1.324 b	7.2991 ab	0.03 b	0.04 b	0.0 b
B. brevis	0.878 b	8.3531 a	0.07 b	0.05 b	0.25 b
P. polymyxa	0.898 b	7.6582 ab	0.43 b	3.12 a	0.21 b
Streptomyces sp.	0.732 b	3.8752 d	2.54 a	2.43 a	0.56 b
B. megaterium	0.765 b	3.7569 d	0.67 b	0.56 b	0.0 b
T. harzianum	2.432 a	5.8767 bc	1.87 a	2.43 a	1.98 a

Greenhouse experiments

The effect of different biocontrol agents against *Rhizoctonia solani* under greenhouse conditions

The effects of certain biocontrol agents *T. harzianum, P. fluorescens, P. putida, B. brevis, B. megaterium, P.polymyxa* and *Streptomyces sp*, on damping-off and survival of sugar beet seedlings grown in soil infested with *R. solani* were studied under greenhouse conditions (Fig.4). This experiment was performed using two sugar beet cultivars, Beta-max 13 and Diamond cultivars. Data indicated that all tested biocontrol agents reduced damping-off as compared with the check under greenhouse conditions with subsequent increase in number of survival plants. The most effective biocontrol agent in reducing percentage of pre-, post-emergence damping-off was *B. brevis.* It reduced infection caused by *R. solani* in Beta-max 13 cultivar, the percentage of pre-, post-emergence damping-off were (6 %, 4%) respectively, followed by *B. megaterium* (8 %, 4%). The bioagent *Streptomyces sp.* showed a moderate effect in The percentage of pre-, post-emergence damping-off (10 %, 2%), followed by *P. polymyxa* (10 %, 8%) and *T. harzianum* (12 %, 6%). *P. fluorescens* (14 %, 10%) and *P. putida* (16 %, 6%) was the least effective biocontrol agents in reducing pre-, post-emergence damping-off percentage as compared with control (infested soil with *R. solani*) which has recorded the highest percentage of pre-, post-emergence damping-off reached (24 %, 22%).

Survival plants results had the same trend. Data showed that the most effective biocontrol agent was *B. brevis* against the pathogen and reduced fungal infection caused by *R. solani* in Beta-max 13 cultivar, the survival plants were (90 %), followed by *Streptomyces sp.* (88 %) and *B. megaterium* (88 %). The bioagent *T. harzianum* showed a moderate effect of survival plants followed by *P. polymyxa* (82 %). *P. putida* (78 %) and *P. fluorescens* (76 %) had the least effect of survival plants percentage. Soil infested with *R. solani* only showed the lowest percentage of survival plants (54 %). In case of susceptible cultivar (Diamond), data showed that all recorded parameters were generally lower than Beta-max 13 cultivar. Data showed that the most effective biocontrol agent was also *B. brevis*, the survival plants were (86 %), followed by *Streptomyces sp.* (82 %) and *B. megaterium* (82 %). The bioagent *P. polymyxa* (80 %) showed a moderate effect of survival plants, followed by *T. harzianum* (78 %). *P. putida* (72 %) and *P. fluorescens* (72 %) had the least effect of survival plants

percentage. Soil infested with *R. solani* only showed the lowest percentage of survival plants (30 %). Statistical analysis showed that there were significant differences between the studied antagonistic biocontrol agents as compared with control for all recorded parameters pre-, post-emergence damping-off, survival plants of sugar beet.

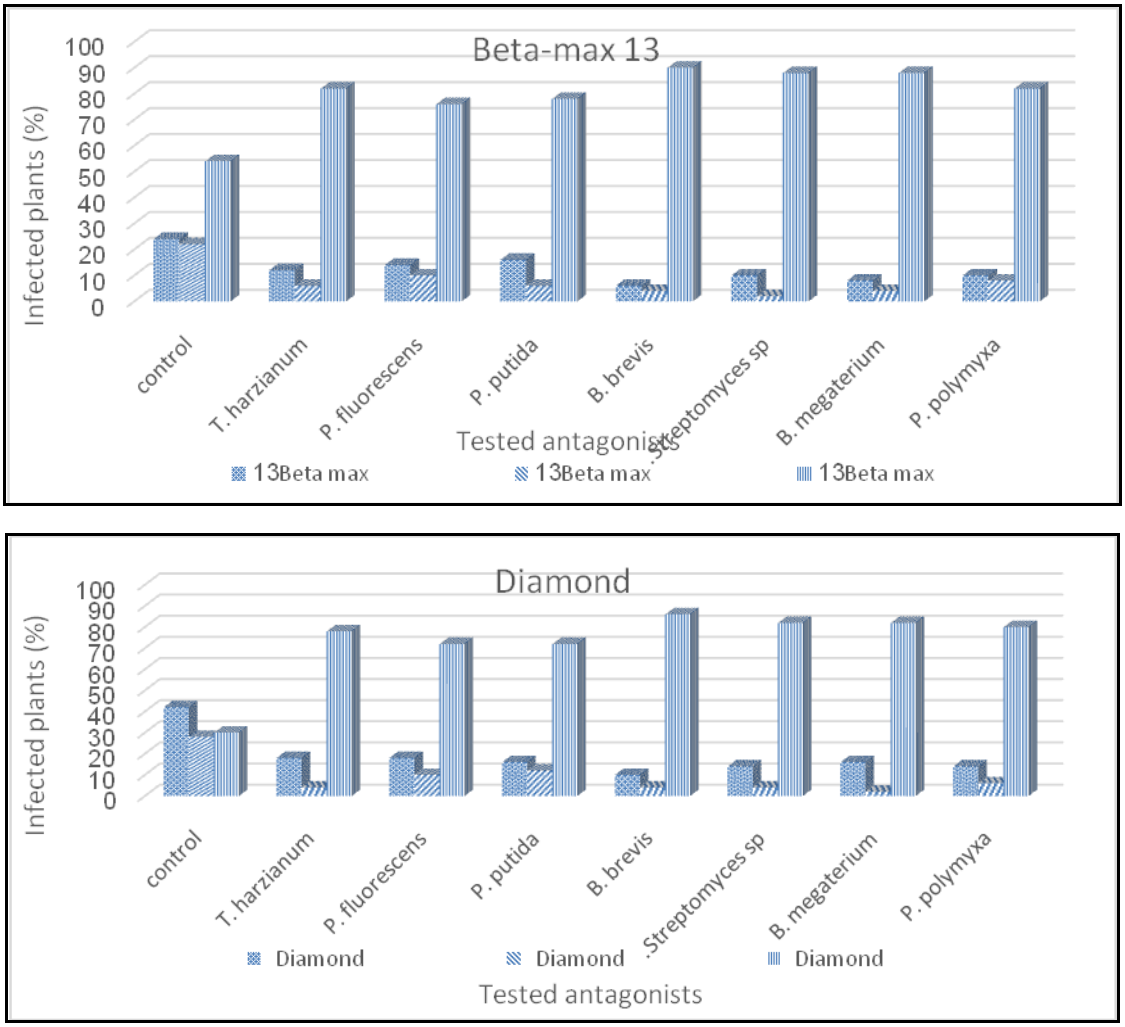


Fig. (4) Effect of different biocontrol agents on damping-off caused by *R. solani* of two sugar beet cultivar under greenhouse conditions during 2014

The effect of different biocontrol agents on sugar beet plants under greenhouse conditions

This experiment was performed using two sugar beet cultivars: Beta-max 13 and Diamond cultivars. Data in table (2) showed that all tested biocontrol agents led to significant differences in the percentage of germination of treated seeds under greenhouse conditions with subsequent increase in number of survival plants. Data indicated that the antagonistic biocontrol treatments significantly Increased percentage of germination of sugar beet plants. In case of Beta-max 13 cultivar, the most effective biocontrol agents in reducing percentages of pre-, post-emergence damping-off were *Streptomyces sp.* (2 %, 0%), *P. fluorescens* (2 %, 2%) and *B. megaterium* (2 %, 3%), followed by *T. harzianum* (4 %, 1%), *P. putida* (4 %, 0%), *B. brevis* (4 %, 2%) and *P. polymyxa* (4 %, 4%). The control showed the highest percentage of pre-, post-emergence (10 %, 2%). Data showed that the most effective biocontrol agent was *Streptomyces sp.* with percentage of survival plants (98 %), followed by *P. fluorescens* (96 %) and *P. putida* (96 %) and *B. megaterium* (96 %). The bioagent *T. harzianum* (94 %) showed a moderate effect on the percentage of survival plants.

In case of sensitive cultivar (Diamond cultivar), data showed that all recorded parameters were generally lower than Beta-max 13 cultivar. Data showed that the most effective biocontrol agents were *B. megaterium* (92 %) and *T. harzianum* (92%) on the percentage of survival plants, followed by *P. fluorescens* (90%) and *Streptomyces sp.* (90 %), *P. polymyxa* (88 %) showed a moderate effect on the percentage of survival plants.

Table (2) Effect of different biocontrol agents on damping-off sugar beet plants under greenhouse conditions during 2014

Treatment	Beta max13			Diamond		
	Damping-off		Survival plants %	Damping-off		Survival plants %
	Pre- %	Post- %		Pre- %	Post- %	
Control	10c	2bc	88e	10d	4b	86d
T. harzianum	4b	2bc	94c	6b	2a	92a
P. fluorescens	2a	2bc	96b	6b	4b	90b
P. putida	4b	0a	96b	8c	6c	86d
B. brevis	4b	2bc	94c	6b	2a	92a
Streptomyces sp.	2a	0a	98a	6b	4b	90b
B. megaterium	2a	2bc	96b	4a	4b	92a
P. polymyxa	4b	4d	92d	8c	4b	88c

Chemical analysis:

Soluble protein contents:

Analysis of variance for soluble protein contents in biocontrol assay showed that there were significant differences between biocontrol treatments compared with untreated control (Table 3). Data indicated that soaking sugar beet seeds in the solutions of tested biocontrol agents *T. harzianum*, *P. fluorescens*, *P. putida, B. brevis, B. megaterium*, *P. polymyxa* and *Streptomyces sp*. significantly elevated the protein contents as compared with untreated control. In Beta max13 cultivar, soaking sugar beet seeds in the biocontrol agents elevated the soluble protein contents over the control. The highest total protein content was observed in *P. putida* treatment (33.2 mg/g), whereas the lowest was recorded for *Streptomyces sp.* at (21.9 mg/g) as compared with the control (12.3 mg/g). Significant differences were found among all tested biocontrol agents and control treatments. In Diamond cultivar, the level of protein contents in all tested biocontrol agents and control treatment was significantly different. The highest protein content was observed in *P. polymyxa* treatment (33.8 mg/g), whereas the lowest was recorded for *Streptomyces sp.* at (18.8 mg/g) as compared to the control (11.4 mg/g).

Table (3) Soluble protein contents (mg/g) of two sugar beet cultivars treated with antagonistic micro-organisms before planting in *R. solani* infested soil under greenhouse conditions

Treatment	Soluble protein	
	Beta max13	Diamond
Control	12.3 e	11.4 f
T. harzianum	26.4 c	25.6 d
P. fluorescens	29.9 b	28.9 c
P. putida	33.2 a	32.8 a
B. brevis	31.8 a	30.8 b
Streptomyces sp.	21.9 d	18.8 e
B. megaterium	25.8 c	27.9 c
P. polymyxa	32.8 a	33.8 a

Effect of biocontrol agents on phenol content:

Data in table (4) indicated that soaking sugar beet seeds in the solutions of tested biocontrol agents *T. harzianum*, *P. fluorescens*, *P.putida, B. brevis, B. megaterium*, *P. polymyxa* and *Streptomyces sp*. significantly elevated the phenolic compounds as compared with untreated control. In Beta max13 cultivar, soaking sugar beet seeds in the biocontrol agents elevated the phenolic compounds over the control. The highest total phenols was observed in *P. polymyxa* treatment (1.23 mg/g fresh weight), whereas the lowest was recorded for *Streptomyces sp.* at (0.65 mg/g fresh weight) as compared with the control (0.076 mg/g fresh weight). Significant differences were found among all tested biocontrol agents and control treatment. In Diamond cultivar, the level of phenol compounds in all tested biocontrol agents and control treatment was significantly different. The highest total phenols was observed in

P. polymyxa treatment (0.956 mg/g fresh weight), whereas the lowest was recorded for *Streptomyces sp.* at (0.67 mg/g fresh weight) as compared with the control (0.12 mg/g fresh weight).

Table (4) Phenol content (mg/g fresh Tissue weight) of two sugar beet cultivars treated with antagonistic micro-organisms before planting in *R. solani* infested soil under greenhouse conditions

Treatment	Phenol activity	
	Phenol Content mg/g of fresh Tissue	
	Beta max13	Diamond
Control	0.076 b	0.12 c
T. harzianum	0.667 ab	0.77 ab
P. fluorescens	0.78 a	0.789 ab
P. putida	0.956 a	0.87 a
B. brevis	0.917 a	0.943 a
Streptomyces sp.	0.65 ab	0.67 b
B. megaterium	0.876 a	0.78 ab
P. polymyxa	1.23 a	0.956 a

Effect of different biocontrol agents on polyphenol-oxidase activity and peroxidase activity in sugar beet plants:

As shown in Table (5), the tested biocontrol agents increased polyphenol-oxidase activity and peroxidase activity compared with untreated control in the two tested cultivars. polyphenol-oxidase activity was higher in the Beta max 13 cultivar than Diamond cultivar. All tested biocontrol agents elevated polyphenol-oxidase activity over the control treatment. The highest polyphenol-oxidase activity and in Beta max13 cultivar was recorded in *P. polymyxa* treatment 1.43 unit/g fresh weight, and the lowest polyphenol-oxidase activity was recorded in *B. megaterium* treatment 0.570 unit/g fresh weight as compared with control treatment 0.226 unit/g fresh weight. In Diamond cultivar, the highest polyphenol-oxidase activity was recorded in *P. polymyxa* treatment 1.32 unit/g fresh weight, and the lowest polyphenol-oxidase activity was recorded in *B. megaterium* treatment 0.65 unit/g fresh weight as compared with control treatment 0.32 unit/g fresh weight. The highest total peroxidase activity was observed in *P. polymyxa* treatment 2.43 unit/g, and the lowest peroxidase activity was recorded in *Streptomyces sp.* treatment 0.87 unit/g as compared with untreated control 0.21 unit/g. In Diamond cultivar, all tested biocontrol agents also elevated peroxidase activity over untreated control. The highest total peroxidase activity was recorded in *P. polymyxa* treatment 2.54 unit/g, and the lowest total peroxidase activity was recorded in *Streptomyces sp.* treatment 0.98 unit/g as compared with untreated control 0.23 unit/g.

Table (5) Polyphenol-oxidase activity and Peroxidase activity of two sugar beet cultivars treated with biocontrol agents before planting in *R. solani* infested soil under greenhouse conditions

Treatment	Polyphenol oxidase activity		Peroxidase activity H_2O_2 µmo $l.g^{-1}$ dm	
	Beta max13	Diamond	Beta max13	Diamond
Control	0.226 c	0.32 c	0.21 b	0.23 b
T. harzianum	0.89 ab	0.87 ab	1.56 ab	1.27 ab
P. fluorescens	0.98 ab	0.87 ab	1.57 ab	1.76 ab
P. putida	1.23 a	1.23 a	2.41 a	2.43 a
B. brevis	1.21 a	1.13 a	2.43 a	2.32 a
Streptomyces sp.	0.76 ab	0.87 ab	0.87 ab	0.98 ab
B. megaterium	0.57 ab	0.65 ab	1.54 ab	1.54 ab
P. polymyxa	1.43 a	1.32 a	2.43 a	2.54 a

Effect of different biocontrol agents on Chitinase activity in sugar beet plants:

As shown in Table (6), the tested biocontrol agents increased Chitinase activity compared with untreated control in the two tested cultivars. In Beta max13 cultivar, all tested biocontrol agents elevated Chitinase activity over the control. The highest total Chitinase activity was observed in *P. polymyxa* treatment 0.98 unit/g, and the lowest Chitinase activity was recorded in *Streptomyces sp.* treatment 0.665 unit/g as compared with untreated control 0.21 unit/g. In Diamond cultivar, all tested biocontrol agents elevated Chitinase activity over untreated control. The highest total Chitinase activity was recorded in *P. polymyxa* treatment 0.932 unit/g, and the lowest total Chitinase activity was recorded in *Streptomyces sp.* treatment 0.77 unit/g as compared with untreated control 0.23 unit/g.

Table (6) Chitinase activity of two sugar beet cultivars treated with biocontrol agents before planting in *R. solani* infested soil under greenhouse conditions

Treatment	Chitinase activity	
	Beta max13	Diamond
Control	0.21 d	0.23 b
T. harzianum	0.76 bc	0.87 a
P. fluorescens	0.87 ab	0.889 a
P. putida	0.914 ab	0.889 a
B. brevis	0.901 ab	0.925 a
Streptomyces sp.	0.665 c	0.77 a
B. megaterium	0.854 ab	0.88 a
P. polymyxa	0.98 a	0.932 a

Growth characters as affected by biocontrol microorganisms in *R. solani* infested soil:

Plant growth characters were studies after 120 days and 150 days. Results indicated that all biocontrol treatments had a significant effect on morphological characters. Data showed that the different bioagents treatments improved growth parameters as compared with the control (Table 7). Soil infested with *R. solani* alone, caused severe reduction in growth parameters, however, there were significant increases in growth parameters when seeds were treated with the bioagents. Seed treatment with bioagents led to increase in the plant root length (cm), root fresh weight (g/plant), leaves fresh weight (g/plant), dry root weight (g/plant), leaves dry weight (g/plant) and number of leave/plant. Data in Table (7) showed that different bioagents treatments were effective in increasing the growth parameter (leaves/plant) in both 120 days samples and 150 days samples. Data indicated that *B.brevis* was the most effective bioagent in increasing the number of leaves per plant (14.6) followed by *T. harzianum* (14.3) and *P. putida* were (14.3), *P.polymyxa* (14) and *B. megaterium* (13.6). *P.fluorescens* showed a moderate effect in number of leaves per plant (12.6) and *Streptomyces sp.* had the minimum effect of number of leaves per plant (9.3) when compared with un-inoculated control (4.6) in the samples that were collected in the 120 days sugar beet pants grown in *R. solani* infested soil. Data obtained after 150 days showed that, the bioagent *P.polymyxa* was the most effective bioagents in increasing the number of leaves per plant (17.6), followed by *B. brevis* and *Tharzianum* (17.3), the bioagents *P. putida* and *P.fluorescens* showed a moderate effect in number of leave per plant, *P.putida* (16), *P.fluorescens* (15.6) when compared with un-inoculated control (5.3) of sugar beet plants grown in infested soil. The data indicated that different isolates affected root length with different degrees of efficacy as shown in Table (7). Significant improvement in root length of sugar beet plants were recorded upon inoculation with four isolates whereas three isolates gave non-significant improvement compared with un-inoculated control in 120 days samples. Maximum improvement in root length was obtained by inoculation with the bioagent *B. megaterium* (14.3 cm) followed by *T harzianum* (13.2 cm), *B. brevis* (12.6 cm) and *P. polymyxa* (12.3 cm). The bioagent *P.fluorescens* showed a moderate effect in root length of sugar beet (12 cm), and the bioagents, *P.putida* (10.5 cm) and *Streptomyces sp,* (10.6 cm) had the lowest effect of root length when compared with un-inoculated control (11.7 cm).Data obtained after 150 days, showed that the bioagent *B. megaterium* was the most effective bioagent in increasing of root length of sugar beet plants (19.8 cm), followed by, *P. putida* (17.5 cm), *T.harzianum* (17.2 cm) and *P.fluorescens* (16.4 cm). The bioagents *P. polymyxa* (16 cm) showed a moderate effect on root length of sugar beet plants followed by *B. brevis* (14.3 cm). All antagonistic bioagents stimulated root dry weight of sugar beet plants grown in pots infested with *R. solani* in comparison with un-inoculated control. Data showed that the different bioagents

treatments were effective in increasing dry weight after both 120 days and 150 days. Data indicated that *P. polymyxa* and *T. harzianum* were the most effective bioagents in increasing the root dry weight. The plant root dry weight in case of *P. polymyxa* was (40.3gm), *T. harzianum* (40.29gm), followed by *B. megaterium* (38.94gm), the bioagents *B. brevis* (37.41gm), *P. putida* (37.28gm) showed a moderated effect in root dry weight, followed by *P. fluorescens* (36.93gm) when compared with un-inoculated control (25.25gm) in the samples that were collected after 120 days from sugar beet plants grown in *R. solani* infested soil .Data for 150 days samples showed that, the bioagent *T.harzianum* was the most effective bioagent in increasing the root dry weight (52.36gm), followed by *B. brevis* (50.62gm), the bioagents *P. polymyxa* (46.79gm), *P.fluorescens* (46gm). *P. putida* (45.24gm) showed a moderated effect in root dry weight in sugar beet plants, followed by *B. megaterium* (43.5gm) when compared with un-inoculated control (28.66gm) of sugar beet plants grown in *R. solani* infested soil.All antagonistic bioagents stimulated leaves dry weight of sugar beet plants grown in *R. solani* infested pots as compared with un-inoculated control. Data recorded in table (7), indicated that leaves dry weight was significantly affected by bioagents after 120 and 150 days from planting, the data indicated that different isolates affected dry weight of leaves with different degrees of efficacy. Maximum improvement in leaves dry weight was obtained by inoculation with the bioagent *T.harzianum* (28.34gm), followed by *P. fluorescens* (24.58gm), and *P. putida* (23.81gm), the bioagents *B. megaterium* (23.23gm), *P. polymyxa* (22.84gm) and *B. brevis* (22.78gm) showed a moderate effect on leaves dry weight of sugar beet plants, and the bioagent *Streptomyces sp.* (21.68gm) had the minimal effect of leaves dry weight when compared with un-inoculated control (9.13gm) from sugar beet plants grown in *R. solani* infested soil after 120 days. Data collected after 150 days, showed that the bioagent *T. harzianum* was the most effective bioagent in increasing dry weight of leaves of sugar beet plants (31.48gm), followed by *P.fluorescens* (28.3gm), the bioagent *P. polymyxa* showed a moderate effect in leaves dry weight (27.89gm), followed by *B.megaterium* (26.21gm).

Table (7) Effect of seed treatments with antagonistic bioagents on Growth characters for sugar beet plants after 120 days and 150 days in *R. solani* infested soil.

Treatment	Number of leaves/plant		Root length (cm)		Root dry weight (gm/ plant)		Leaves dry weight (gm/plant)	
	120 Day	150 Day	120 Day	150 Day	120 Day	150 Day	120 days	150 days
Control	4.6 d	5.3 e	7.6 c	8.3 e	25.25 e	28.66 f	9.13 d	11.05 f
T. harzianum	14.3 ab	17.3 ab	12.6 ab	17 a	40.29 a	52.36 a	28.34 a	31.48 a
P. fluorescens	12.6 b	15.6 bc	11.3 b	15.4 ab	36.93 c	46 c	24.58 b	28.3 b
P. putida	14.3 ab	16 bc	8.6 c	15.1 bc	37.28 bc	45.24 c	23.81 b	25.44 d
B. brevis	14.6 a	17.3 ab	9 c	13.6 c	37.41 bc	50.62 b	22.78 bc	24.75 de
Streptomyces sp.	9.3 c	11.6 d	8 c	10.6 d	32.42 d	36.55 e	21.68 c	23.45 e
B. megaterium	13.6 ab	15.3 c	13.3 a	15.6 ab	38.94 ab	43.5 d	23.23 bc	26.21 cd
P. polymyxa	14 ab	17.6 a	11.6 ab	14.6 bc	40.3 a	46.79 c	22.84 bc	27.89 bc

Discussion

Sugar beet (*Beta vulgaris* L.), is grown for sugar production and is considered the second important sugar crop in Egypt. Damping-off and root-rot diseases of sugar beet are the most important diseases that attack both seedlings and adult plants causing serious losses in crop productivity and quality. *R. solani* is the main causal organism for pre, post emergence damping-off and root-rot diseases in Egypt (27). Due to the pollution to the human food by agrochemicals, especially pesticides, therefore there is a growing need to develop alternative approaches for controlling plants diseases other than chemical pesticides. The use of antagonistic microorganisms against plant pathogenic fungi may offer an opportunity for a friendly control method that could minimized the environmental hazards. Biological control of *R. solani* in various crops has much potential for disease management, though there are several problems with practical implementation (46). Several studies have reported the antagonistic effects of *Trichoderma* spp. (27), *Pseudomonas* spp. (30) and *Streptomyces* spp. (47) against causal agents of sugar beet damping-off.

Numerous organisms have demonstrated biocontrol capability against *R. solani* in the present study. The effect of several antagonistic organisms: *T.harzianum*, *P.fluorescens*, *P. putida*, *B. brevis*, *B. megaterium*, *P.polymyxa* and *Streptomyces sp.* on the linear growth of *R. solani* was tested under laboratory conditions. It was

clear that all tested biocontrol agents reduced the mycelial growth of the tested fungi as compared with the control. *T. harzianum* was the most effective bioagent against the tested fungus followed by *P. fluorescens*, the bioagents *P. putida* and *B.megaterium* showed a moderate effect against *R. solani*, followed by *P. polymyxa, B. brevis* and *Streptomyces sp.* The antagonistic reaction between the biocontrol microorganisms and the pathogenic fungus as zone of inhibition showed that, *T. harzianum* isolate had the highest antagonistic effect as it overgrew the pathogen and covered the entire medium surface, the largest zone of inhibition was found by the bioagent *P. fluorescens*, followed by *P.polymyxa*. *B. brevis* showed a moderate zone of inhibition reaction for the pathogenic fungal. *Pseudomonas* sp. inhibited the pathogen due to production of antibiotic and/or siderophores. High production of siderophores antibiotics was also reported by Laha *et al* (48). Wölk and Sarkar (49) found that *P. fluorescens* strongly inhibited *R. solani*. Freitas and Germida (50) found that *P.putida* showed antagonistic effect towards *R. solani in vitro*,. Nielsen and Sørensen (34) found that *P. polymyxa* inhibited *R. solani*. Also (47; 51) found that *Streptomyces* sp. inhibited mycelium growth of *R. solani*.

Data showed production of high levels of protein, the bioagent *B. brevis* showed highest soluble protein content, followed by *P. polymyxa, P. putida* and *T. harzianum.* The bioagent *P. fluorescens* showed a moderate soluble protein content followed by *Streptomyces sp.* The lowest soluble protein content were recorded in *B. megaterium* bioagent. Data showed that *Streptomyces sp.* showed the highest chitinase production followed by *T. harzianum, B. megaterium* and *P. polymyxa*. The bioagent *B. brevis* showed a moderate chitinase production followed by *P. putida* and *P. fluorescens* showed a lowest chitinase production. Data showed that the bioagent *P. polymyxa* showed the highest β-1,3 glucanase production followed by *Streptomyces sp., T. harzianum* and *B. megaterium. B. brevis* showed midrates β-1,3 glucanase production followed by *P. putida* and the bioagent *P. fluorescens* showed the lowest β-1,3 glucanase production. Resistance mechanisms in plants include changes in cell wall composition *de novo* production of pathogenesis-related-proteins such as Chitinase and glucanases, and synthesis of phytoalexins, (52; 53). However, further defensive compounds are likely to exist, but remain to be identified. Indeed, Walters *et al* (54) stated that such resistance can be induced in plants by application of variety of biotic and abiotic agents. Biological control methods gave promising results in reducing fungal diseases and encouraging plant growth and yield. The efficacy of the tested bioagents control against *R. solani* was studied under greenhouse conditions using two sugar beet cultivars. The obtained results revealed that the tested biocontrol agents reduced the percentages of damping-off in soil artificially infested with *R. solani* as compared with the check under greenhouse conditions with subsequent increase in number of survival plants. Moussa (55) found that *B. megaterium* inhibited growth of fungi because antibiotic inhibition of *B. megaterium*. Brewer and Larkin (20) found that *P. polymyxa* inhibited *R. solani* in vitro and produced cell wall-degrading enzymes that reduce stem canker on potato caused by *R. solani*. Berg *et al* (56) found that the most prominent species of all tested microenvironments was *P. putida*.

Analysis of variance for soluble protein content in biocontrol assay showed that there were significant differences between biocontrol treatments compared with untreated control. Data indicated that soaking sugar beet seeds in the solutions of tested biocontrol agents *T. harzianum, P. fluorescens, P. putida, B. brevis, B. megaterium, P. polymyxa* and *Streptomyces sp*. significantly elevated the protein content as compared to untreated control. In Beta max13 cultivar, soaking sugar beet seeds in the biocontrol agents elevated the soluble protein content over the control. The highest total protein content was observed in *P. putida* treatment, whereas the lowest was recorded for *Streptomyces sp.* as compared to the control. Significant differences found among all tested biocontrol agents and control treatments. In Diamond cultivar, the level of protein content in all tested biocontrol agents and control treatment was significant differences. The highest protein content was observed in *P. polymyxa* treatment, whereas the lowest was recorded for *Streptomyces sp.* as compared to the control.

Soaking sugar beet seeds in the solutions of tested biocontrol agents *T. harzianum, P. fluorescens, P. putida, B. brevis, B. megaterium, P. polymyxa* and *Streptomyces sp*. significantly elevated the phenolic compounds as compared to untreated control. In Beta max13 cultivar, soaking sugar beet seeds in the biocontrol agents elevated the phenolic compounds over the control. The highest total phenols was observed in *P. polymyxa* treatment, whereas the lowest was recorded for *Streptomyces sp.* as compared to the control. In Diamond cultivar, the level of phenol compounds in all tested biocontrol agents and control treatment was significant differences. The highest total phenols was observed in *P. polymyxa* treatment, whereas the lowest was recorded for *Streptomyces sp.* as compared to the control. Phenolic compounds are well-known as antifungal and antibacterial compounds that exist naturally in plants (57).

Polyphenol-oxidase activity was higher in the Beta max 13 cultivar than Diamond cultivar. All tested biocontrol agents elevated polyphenol-oxidase activity over the control treatment. The highest polyphenol-oxidase activity in Beta max13 cultivar was recorded in *P. polymyxa*, and the lowest polyphenol-oxidase activity was recorded in *B. megaterium* as compared with control treatment. In Diamond cultivar, the highest polyphenol-oxidase activity was recorded in *P. polymyxa*, and the lowest polyphenol-oxidase activity was recorded in *B. megaterium* as compared with control treatment.

In Beta max13 cultivar, all tested biocontrol agents elevated peroxidase activity over the control. The highest total peroxidase activity was observed in *P. polymyxa*, and the lowest peroxidase activity was recorded in *Streptomyces sp.* as compared with untreated control. In Diamond cultivar, all tested biocontrol agents elevated peroxidase activity over untreated control. The highest total peroxidase activity was recorded in *P. polymyxa*, and the lowest total peroxidase activity was recorded in *Streptomyces sp.* as compared with untreated control.

The tested biocontrol agents increased Chitinase activity compared with untreated control in the two cultivars. In Beta max13 cultivar, all tested biocontrol agents elevated Chitinase activity over the control. The highest total Chitinase activity was observed in *P. polymyxa*, and the lowest Chitinase activity was recorded in *Streptomyces sp.* as compared with untreated control. In Diamond cultivar, the highest total Chitinase activity was recorded in *P. polymyxa*, and the lowest total Chitinase activity was recorded in *Streptomyces sp.* as compared with untreated control.

Enzyme activity plays an important role in plant disease resistance through increasing plant defense mechanisms that are considered the main tool of variety resistance (58). These findings are in agreement with those reported earlier by Mohamed (59) who found that the activates of Polyphenol-oxidase and peroxidase were higher in antagonistic treatment than non-treated plants and in infected plants than in non-infected plants. One possible explanation for its inhibition is the action of chitinases and b-glucanases on chitin or glucan present in these fungal cell walls, acting as protective agents (60).

Plant growth response to various treatments was determined by measuring plant root length, root fresh weight, leaves fresh weight, dry root weight, leaves dry weight and number of leaves per plant. Plant growth characters were studies 120 days and 150 days. Results indicated that all biocontrol treatments significantly reduced root-rot symptoms. Data showed that the different bioagents treatments improved growth parameters as compared with the control. This may be due to the large populations of antagonists that colonized roots in soil and caused protection from infection. Podile and Kishore (61) found that *Pseudomonas* spp. reduced the populations of root pathogens and other deleterious microorganisms in the rhizosphere, thus benefiting the plant growth. Prashar *et al* (62) found that, competition becomes the most important tool of action in cases where the biocontrol agent and target pathogen share high degree of relative proximity because of the similar requirements in terms of nutrients and growth factors, role of siderphores is also irreplaceable as they contribute in many ways ranging from indirect disease control by promoting plant growth, inducing competition among rhizobacteria and pathogens to inducing systemic resistance in plants.

The effect of certain biocontrol agents on seed germination of sugar beet plants grown in sterilized soil under greenhouse conditions was studied using two sugar beet cultivars. Results revealed that the most effective biocontrol agent was *Streptomyces sp.* as determined with percentage of survival plants, followed by *P. fluorescens* and *P. putida*. The bioagent *B. megaterium* showed a moderate effect on percentage of survival plants followed by *T. harzianum* and *B. brevis*. *P. polymyxa* had the least effect on survival plants percentage. Seed Inoculation with plant growth-promoting rhizobacteria (PGPR) significantly enhanced seed germination of maize, leaf and shoot dry weight and also leaf surface area significantly were increased by bacterial inoculation in both sterile and non-sterile soil (63).

Plant growth characters of sugar beet plants grown in uninfested soil were studies 120 days and 150 days. Results indicated that significant differences were observed between most of biocontrol treatments compared with the untreated control. The improved plant growth may be due to the growth regulators produced by antagonists together with their continuous supply to the developing plants as a result of the intimate contact between the seedling and the antagonist (55).The promoting effect of biocontrol agents was also studied by many workers: Kleifeld and Chet (64) found that, The fungus *T. harzianum* which was applied to pathogen-free soil, induced an increase in emergence of seedlings, plant height, leaf area and dry weight. Ryu *et al* (65) found that, *P.polymyxa* when inoculated onto seeds as vegetative cells or as endospores was associated with a consistent increase in foliar growth of cucumber in the greenhouse.

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