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Biocontrol activities of plant growth promoting rhizobacteria (PGPR) against Phytophthora blight and growth promotion of sweet pepper

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ABSTRACT

Fifteen bacterial isolates of plant growth promoting rhizobacteria (PGPR) were isolated from rhizosphere and roots of healthy pepper plants grown in reclaimed soil at Ismailia governorate. The isolates were evaluated for their antagonistic activities against *Phytophthora capsici* Leonian, which the causal agent of root-rot and blight of pepper *in vitro* and *in vivo*. Five isolates showed strong inhibition of the pathogen and increased vigor index of pepper in addition to, had high production of indole acetic acid (IAA) and phosphorous solubilization capability. According to *in vitro* and *in vivo* evaluation, three potent selected isolates were identified based on 16srRNA gene sequence as: *Pseudomonas geniculate* ATCC 19374, *Brevibacillus brevis* NBRC 100599 and *Bacillus cereus* NBRC 15305, that were further tested for their ability to induce resistance, production of defense-related enzymes and phenols in sweet pepper against *Pytophthora capsici*. The maximum activities of peroxidase (PO) and polyphenol oxidase (PPO) were observed in *P. geniculate* pretreated pepper plants challenged with *P. capsici*. Moreover, highest accumulation of phenolic content was observed in *P. geniculate* and *B. brevis* pretreated plants and challenge inoculated with *P. capsici*, and the higher amounts of enzymes and phenolics were noticed even on 10th day after the pathogens challenge inoculation. Under the field conditions, formula of the mixture was superior compared with individual three strains in suppressing root rot disease development and promoting plant growth.

Key words: *Brevibacillus brevis- Bacillus cereus- Pseudomonas geniculate* - biological control- *Phytophthora capsici.*

Introduction

Pepper (*Capsicum annuum* L.) is one of the most important cash crops especially in desert lands in the world, including in Egypt; however, its production and quality have been mainly limited by soil borne diseases such as Phytophthora blight caused by *Phytophthora capsici* . This disease can affect plants at any growth stage, and the damping-off syndrome can kill seedlings within 5 days of infection. The pathogen can also cause crown, leaf and fruit blight, wilting of the whole plant and dark purplish discoloration of the stem (Erwin and Ribeiro, 1996). Although fungicides have shown promising results in controlling the damping-off disease, phytotoxicity as a result of fungicide residues causes environmental pollution leading to health hazards of both human and animals (Ramamoorthy *et al*., 2002). Additionally, that *Pytophthora* sp. develops resistance to fungicide, and this further discourages the use of chemicals for the control of plant diseases (Chakrabrty *et al.,* 2016). Also, global interest has been shifted towards the use of eco-friendly methods for protecting crops against pests and diseases. This simply encourages the application of biological control method which involves the use of antagonists or bio-control agents to improve plant health. Suppression of diseases by bio-control agents is as a result of sustainable balance in interactions among the plant, bio-control agent and the microbial community on and around the plant (Chakrabrty *et al*., 2016).

Among the group of bio-control agents, plant growth promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. PGPR have been widely used for the bio-suppression of various soilborne diseases (Prashar *et al.,* 2013). They offer an excellent combination of traits useful in disease control and plant growth promotion (Harish *et al*., 2009; Sang *et al*., 2013 and Shahid *et al*., 2016). PGPR strains can display disease suppressive effects against various crown, root and foliar diseases through direct inhibition of target pathogens or indirectly via the induction of systemic resistance (ISR) which is

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active throughout the entire plant (Ramamoorthy *et al*., 2002).These PGPR which mostly belong to *Pseudomonas* (Chakraborty *et al*., 2016), *Bacillus* spp. (Xu *et al*., 2016) and *Breveibacillus* sp.(Omar and Elhadidy, 2012 and Omar and Ahmed 2014) are antagonists of recognized root pathogens. PGPR-treated plants showed enhanced emergence potential and increased vegetative and root growth (Chakraborty *et al.,* 2016).

In the current study, aimed to isolate the potent antagonistic rhizobacteria isolates from rhizosphere of pepper and will be assessed for their efficacy as biocontrol agents in sustainable agricultural to manage Phytophthora blight disease and to enhance growth of sweet pepper.

Materials and Methods

Pathogens and inoculum:

Pepper seedlings and mature plants that showed symptoms of root rot and crown rot, were collected from different fields in Ismailia governorate. *Pytophthora capsici* was isolated on Masago, s Phytophthora selective medium (Masago *et al*., 1977) and incubated at 25°c for 7 days. Developed colonies were transferred to V-8 agar plates and were identified according to Stamps *et al*., (1990). Purified isolate was maintained on V-8 agar at 15°c till use. Pathogenicity test of the Pytophthora isolate was performed and the pathogenic potentiality was proven. Mycelial propagules inoculum of the fungal isolate was prepared using 500cm3 of vermiculite and 250 ml of V-8 broth and was added to soil (10cm3/kg soil) as described by Singleton *et al*., (1992).

Isolation and characteristics of rhizobacteria isolates:

Several bacterial isolates were isolated from rhizosphere of healthy pepper grown in reclaimed soil at Ismailia governorate. Bacterial isolates were isolated by the dilution method according to Aysan *et al*. (2003), using on two different media: tryptic soy agar (TSA) for isolation of heterotrophic bacteria according to Gould *et al*., (1985) and King's media B (KB) for isolation of fluorescent Pseudomonads (King *et al*., 1954). Plats were incubated at 28°C for 2-4 days when individual colonies were picked up, purified and stored at 4°C on the appropriate medium. The most effective isolates were purified and identified according to Bergey's manual of Systematic Bacteriology (Holt *et al.,* 1994) at Microbiology Lab., Desert Research Center. Most potent three antagonistic isolates were identified using 16S rRNA sequence by Sigma Scientific Services Co.. Isolation of cellular DNA was performed as described by Ausubell *et al*., (1987) and amplification of 16S rDNA according to (Lane, 1991) using the universal 16S primers (F1 5' AGAGTTT(G/C)ATCCTGGCTCAG 3' R1 5' ACGG/C) TACCTTGTTACGACTT 3').

In vitro assay:

a) Antagonistic effect on Pytophthora capsici :

The antimicrobial activity of the 15 rhizobacterial isolates against *P. capsici* was screened *in vitro*. The bacterial isolates (48-hr-old) were streaked on one side of a Petri dish (1cm from the edge of the plate) with PDA medium. Eight mm diameter mycelial disc of *P. capsici* seven-days-old culture was placed on the opposite side of the Petri dish perpendicular to the bacterial streak (Vidhyasekaran *et al*., 1997). The plates were incubated at room temperature (28°C) for 4 days and the zone of inhibition was measured.

b) Indole acetic acid production:

Selected five isolates rhizobacteria were investigated for their ability to produce indole acetic acid (IAA). Each isolate was grown on NAM (nutrient agar) broth medium containing trptophan (1.0 mg/L) and incubated in shaker with 30ºc and 160 rpm for 48h. Next, bacterial culture was centrifuged at 10000 rpm for 15 min, and 1 ml of culture filtrate was mixed with 1ml of salkowskis reagent (1.5 ml of $Fecl_3$. $6H_2O$ 0.5M solution, in 80 ml of 60% H_2S0_4) and the mixture incubated at room

temperature for 30 min, presence of pink color indicates that isolate can produce indolacetic acid (IAA). Meanwhile (IAA) concentration for each tested strain was quantified colorimetrically in 550 nm by spectrophotometer comparing with IAA standard curve (Gordon and Weler, 1951).

c) Phosphate Solubilization:

Capacity of five selected isolates to solubilize phosphate in form of calcium phosphate was checked qualitatively by using glucose yeast extract agar (GYA) medium containing per1 L distilled water; 10g glucose; 2g yeast extract and 15g agar. In addition, two other solutions were prepared separately; first 5g K_2HpO_4 was dissolved in 50 ml distilled water and second 10g $CaCl_2$ in 100 ml distilled water. These two solutions were added to 1L CYA just before pouring medium to plates (Benduzi *et al*., 2008). Each isolate was grown in GY broth for 24h., and then 10ml of bacterial culture were dropped in each plate and inculcated for 7 days at 28°C. The isolates which showed clear halos around their colonies were considered as phosphate solubilization`.

***In vivo* assay:**

a) Efficacy of rhizobacteria isolates as bio-control agents:

The selected bacterial isolates were tested as bio-control agents *in vivo*, against *P. capsici* using the soil-dishes technique as described by Mosa *et al.* (1997). The Pathogen was grown for five days on a thin layer of potato dextrose agar media, in 9 cm diameter Petri dishes. Then, the fungal colony was covered by autoclaved mixture of peat moss and vermiculite (1:1 v/v). Treated cucumber seeds with rhizobacteria were sown over soil in each Petri dish using sterile tweezers to prevent cross contamination through handling. Set of dishes contained non infested soil served as control. Treatment with the fungicide rizolex-T (2g/kg seeds) was carried out for comparison .Thereafter, seeds covered by soil mixture, watered daily by sterilized distilled water. Percentages of survived seedlings were recorded after twenty five days from sowing date. Seedlings dry weights were also determined.

b) Efficacy of rhizobacteria isolates as plant growth-promoters:

The selected bacterial isolates were grown in flasks (250ml) containing 100ml of King's medium broth (KMB) for fluorescent Pseudomonads and tryptic soyagar (TSA) broth for bacilli, for 48h on a rotary shaker at 28±2°C. Cells were removed by centrifugation at 800g for 10 min at 4°C and washed in sterile water. The pellet was transferred in a small amount of sterile distilled water and then diluted with an adequate amount of sterile distilled water to obtain a bacterial suspension of 10^8 cfu ml^{-1} (Thompson, 1996). For bacterization of seeds of pepper (*Capsicum annum* L.) were surface sterilized with 2% sodium hypochlorite for 30s and rinsed in sterile distilled water and dried overnight under a sterile air steam. Ten milliliter of bacterial inoculum (10^8 cfu ml^{-1}) was put in a Petri dish. To this, 100mg of carboxymethylcellulose was added as adhesive material. One gram of seeds was soaked in 10ml of bacterial suspension for 12h and dried overnight in sterile Petri dish. Plant growth-promoting activity of bacterial isolates was assessed based on the seedling vigor index by the standard roll towel method (ISTA, 1993). The vigor index was calculated by using the formula as described by Abdul Baki and Anderson (1973):

$$\text{Vigor index} = (\text{Mean root length} + \text{Mean shoot length}) \times \text{Germination (\%)}.$$

Potential of bacterial isolates to induce systemic resistance against phytophthora blight in pot experiment:

Three potent antagonistic bacterial strains were used for induction of defense reaction in pepper plants. Treatments were arranged in a randomized complete block design, the following treatments were included in the experiment: (1) seeds treated with bacterial strains; (2) seeds treated with bacterial strains and challenge inoculated *P. capsici* 15 days after planting (50 g sand-corn medium containing 10^3 cfu g^{-1} medium in each pot); (3) plants inoculated with the pathogens 15 days after

sowing; and (4) non-treated plants. The seeds were sown in pots filled with sterilized potting soil at 25 seeds per pot. Three replications were maintained in each treatment: each replicate consisted of five pots. Plants were carefully uprooted without causing any damage to root tissues at different time intervals (0, 5 and 10 days after pathogen inoculation). Four plants were sampled from each replication of the treatment separately for biochemical analysis. Fresh roots were washed in running tap water and homogenized with liquid nitrogen in a pre- chilled mortar and pestle. The homogenized root tissues were stored at -70°C.

Phenols determination:

Root samples (1g) of pepper plants grown in pot experiment were obtained from different treatments after (20 days from inoculation. These samples were homogenized in 10ml of 80% methanol and agitated for 15 min at 70°C (Zieslin and Ben-Zaken, 1993). One milliliter of the metabolic extract was added to 5ml of distilled water and 250µl of Folin-Ciocalteau reagent (1N). The solution was kept at 25°C.The absorbance of the developed blue color was measured using a spectrophotometer at 725nm. Catechol was used as the standard. The amount of phenolics was expressed as µg catechol mg protein^{-1}

Enzymes assay:

1-Peroxidase:

Root samples (1g) of pepper plants grown in pot experiment were obtained from different treatments after (20 days from inoculation were homogenized in 2ml of 0.1M phosphate buffer, pH 7.0 at 4C for 15min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5ml of 0.05M pyrogallol, 0.5ml of enzyme extract and 0.5ml of 1% H_2O_2. The reaction mixture was incubated at room temperature (28 ± 2°C). The changes in absorbance at 425 nm were recorded at 30s intervals for 3min, using spectrophotometer. The enzyme activity was expressed as changes in the absorbance min^{-1} mg protein^{-1} (Allam and Holis, 1972).

2- Polyphenol oxidase:

Root samples (1g) of Pepper plants grown in pot experiment were obtained from different treatments after (20 days from inoculation. These samples were homogenized with 0.2 M Tris HCl buffer, pH 7.8 containing 14 mM B-mercaptoethanol at a rate of 1/3 (w/v). The homogenate was centrifuged at 3000 rpm for 15 min. Polyphenol oxidase (PPO) activity was determined following standard method colorimetric method suggested by (Mukherjee and Ghosh, 1975) and is expressed as increase in absorbance at 420 nm min^{-1} mg protein^{-1}.

Efficacy of peat moss bacterial formulas against Phytophthora blight under field condition:

Solid peat moss formula of plant growth promoting rhizobacteria was prepared to control Phytophthora blight disease of pepper. The peat moss as the carrier material was milled to pass through 200 µm mesh sieves, then they was pasteurized at 70°C/30 min. pH of the peat moss was naturalized by adding calcium carbonate, the material was packed using polyethylene bags containing 1 Kg of it . Each bag containing 1 Kg of peat moss was mixed separately with 100 ml liquid culture of tested bacteria individual as well as the mixture of them, the bags were thoroughly mixed to ensure even distribution and imbibition of the liquid culture into the carrier. The resulting mixture were spread in foil pans and dried for 24 hour at room temperature, the bags were incubated at 25° C for 75 days. Pepper seeds were sown 15 days before transplanting in preformed trays which contain previous preparation the bacterial peat moss formula as seed bed treatment.

The experiment was carried out at Ismailia governorate in the season 2014, to study the efficacy of four peatmos bacterial formulas to control Phytophthora blight disease under natural infested field. Soil was sandy loam pH 7.2 and drip irrigation system was applied in a complete randomized block design. In field was dug in the soil to prepare 5 rows, the dimensions of each row was 60m in

length,50 cm in height and 50cm in width. Pepper transplants which contained a bacterial peat moss formula as well as nonbacterized control were sown at 10 seedlings within each row. Disease incidence and disease severity were recorded after 4 weeks from transplantation. Severity of browning of internal tissue was recorded and conducted with scale proposed by Haware and Nene (1980) based on 0 – 4 scale according to percentage of foliage yellowing or necrosis (0 = 0%, 1 = 1-33%, 2= 34- 66 %, 67-100%, 4= dead plant). Meanwhile, plant height, dry weight and fruit yield were also determined.

Statistical analysis:

Data were subjected to analysis of variance (ANOVA) and were calculated for mean separation analyzed and subjected to Duncan's multiple range tests and comparison after analysis of variance (Duncan, 1955).

Results

In vitro, Screening of rhizobacteria isolates:

a) Antagonistic potential:

Fig. (1) Summarized the results of the *in vitro* assay by 15 rhizobacterial isolates, were isolated from healthy pepper plants and tested for their efficacy to inhabit the mycelial growth of *P.capsici*. Only five isolates B-3, S-8, T-3, T-6 and B-7 showed moderate to strong inhibition to *P. capsici* on plates. The results indicate that, these five isolates recorded higher inhibition zone which measured by 24.5, 24.8, 23.8, 22.9 and 20.6 mm respectively, when compared with other tested isolates.

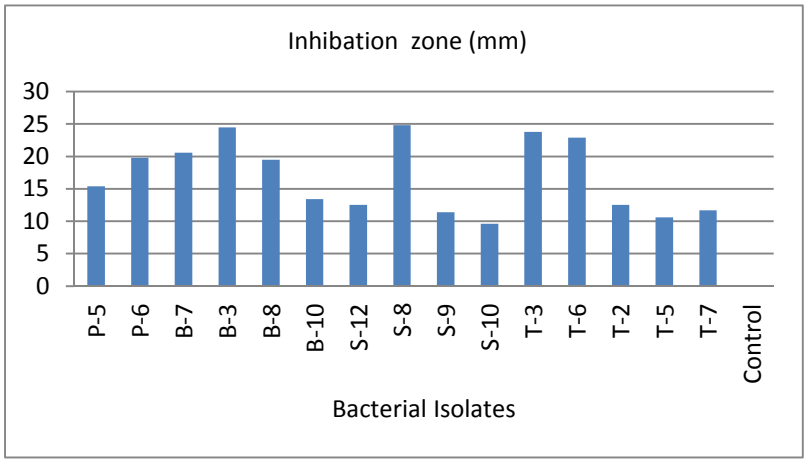

Fig. 1: Antagonistic potential of selected rhizobacteria isolates against the growth of *Pytophthora capsici, in vitro*

b) IAA production and phosphate solubilization:

The selected five isolates B-3, S-8, T-3, T-6 and B-7 showed highly production of indol acetic acid (IAA) that ranged from 18.5 to 35.2 µg/ml (Table 1). The isolates S-8 and T-3 produced highest concentration of IAA that was 35.2 and 32.4 µg/ml. Also, three out of five bacterial isolates B-3, S-8 and T-3 showed phosphate solubilizating ability.

Table 1: Determination of indol acetic acid (IAA) and phosphate for solubilization rhizobacteria isolates

Rhizobacterial Isolates	IAA (µ/mg)	Phosphate solubilization ability
B-3	25.4	+
S-8	35.2	+
T-3	32.4	+
T-6	19.6	-
B-7	18.5	-

***In vivo*, screening of selected antagonistic isolates:**

a) As biocontrol agents

Data in (Table 2) indicated that three bacterial isolates *i.e.* B-3, S-8 and T-3 were reduced damping- off of pepper seedlings significantly. The degree of reduction of damping-off varied according to rhizobacterial isolate and the pathogen. Data in Table (2) also indicated that, there were varied effects of the tested bacterial isolates on seedling survival in pathogen-non infested soil B-3 increased the seedling dry weight by 17mg).

Table 2: *In vivo* screening of selected rhizobacteria isolates against phytophthora blight disease and their effect on seedling dry weight of pepper

Bacterial Isolates	Infested soil		Non infested soil	
	Survived seedlings (%)	Dry weight (mg)	Survived seedlings (%)	Dry weight (mg)
B-3	81 a	15 a	87 a	17 a
S-8	76 a	14 a	83 a	16 a
T-3	78 a	13 a	85 a	15 a
T-6	58 b	11 b	67 b	13 b
B-7	46 c	10 b	62 b	11 b
Rizolex-T (a)	85 a	13 a	63 b	11 b
Untreated (b)	38 d	9 b	46 c	10 b

** Significant at 0.01 level of probability.*
a) Seeds were treated with rhizolex-T at rate of 2 g/kg seeds
b) Seeds were treated with 0.01% MC only.

a) As plant growth promoters

The isolates B-3, S-8 and T-3 showed significant increase vigor index of pepper in the roll towel assay (Fig. 2). Results also illustrated that, the pepper seed germination test in Petri-plates could be used for evaluation the promotion of plant growth.

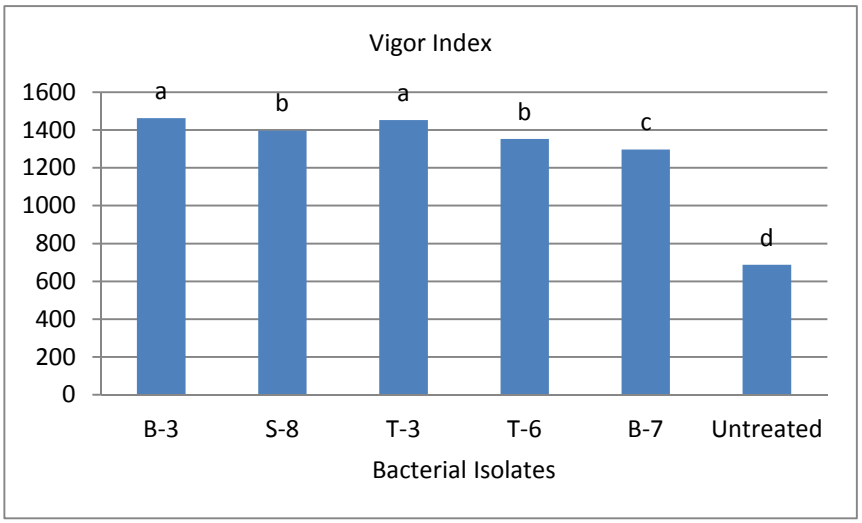

Fig. 2: Plant growth promotion potential of selected rhizobacteria isolates

Identification of the bacterial isolates:

Three most potent antagonistic rizobacterial isolates as well as had IAA production and phosphate solubilization B-3, S-8, T-3 were identified by amplifying and sequencing the 16sr DNA to *Pseudomonas geniculate* ATCC 19374, *Bacillus cereus* NBRC 15305 and *Brevibacillus brevis* NBRC 100599, respectively.

Potential of rhizobacterial isolates to elicit systemic resistance in pot experiment:

Data in (Table 3) indicated that, three selected rhizobacerial strains induced significant disease protection compared with the non-induced disease control to varying degrees against *P. capsici*. The frequency with which various bacterial strains induced significant protection varied with pathogen used. Seeds treated with bacterial strains and challenge inoculated with *P. capsici* significantly reduced root rot compared with untreated treatment (Table 3).

Table 3: Potentiality of bacterial isolates to elicit systemic resistance in sweet pepper against Pytophthora blight disease

Bacterial Isolate	(%) Disease incidence [a]
Brevibacillus brevis	45 c
Pseudomonas geniculata	41 c
Bacillus cereus	64 b
Untreated	86 a

a) Plants inoculated with Pytophthora capsici
** Means within the same column followed by the same later are not significantly different according to Duncan's multiple range test(P≥0.05)*

Biological changes associated with rhizobacterial isolates in pepper plants:

Plants challenge inoculated with the pathogen and the higher amounts of phenolics were noticed the maximum phenolic content was observed in *Brevibacillus brevis* and *P. geniculata* pretreated even on 10th day after the pathogen challenge. In plants inoculated with the pathogen alone the phenolic content decline to the initial level on the 10th day after inoculation. Plants treated with bacterial isolates alone also had increased content of phenolics compared to untreated plants (Fig.3).

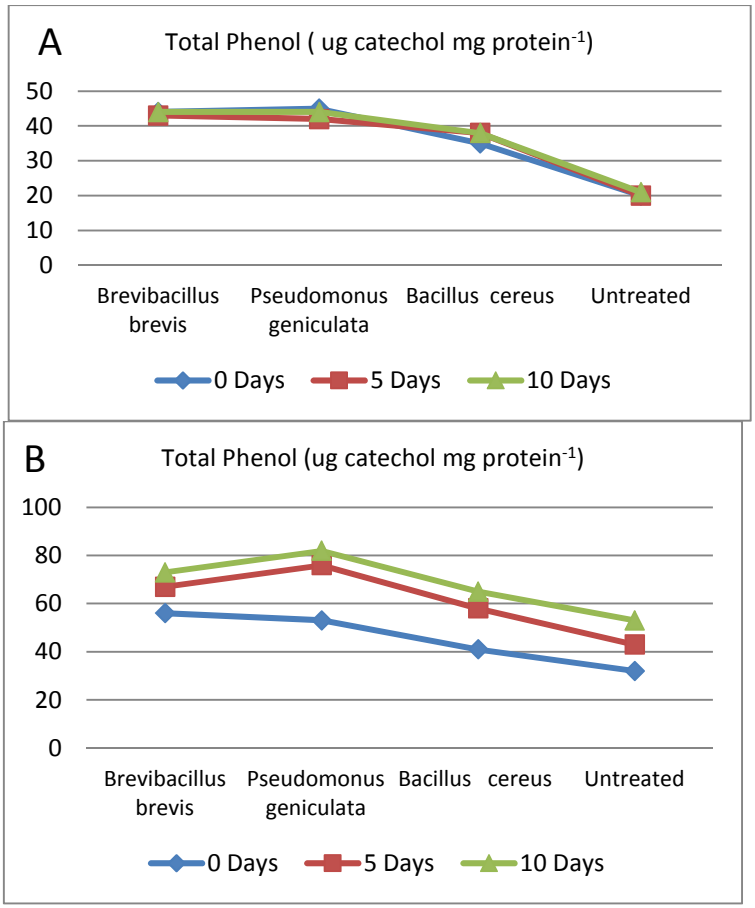

Fig. 3: Total phenol potential in pepper plants inoculated with bacterial isolates only (A), and challenged with *Phytophthora capsici* (B).

Seeds treated with bacterial isolates induced plants to synthesize defense-related enzymes peroxidase (PO) and polyphenol oxidase (PPO) in response to infection by the pathogen. There was more increase in the synthesis was observed in *P. geniculata* and *B. brevis* than *B. cereus* pretreated plants challenge inoculated with *P. capsici*. The activity reached the maximum level on the third day after pathogen challenge and thereafter the activity remained at higher levels throughout the experimental period of 10 days. In plants treated with the pathogens alone, increased activity of peroxidase and polyphenol oxidase was observed for period of 5 days and thereafter declined drastically in plant (Fig. 4 & 5).

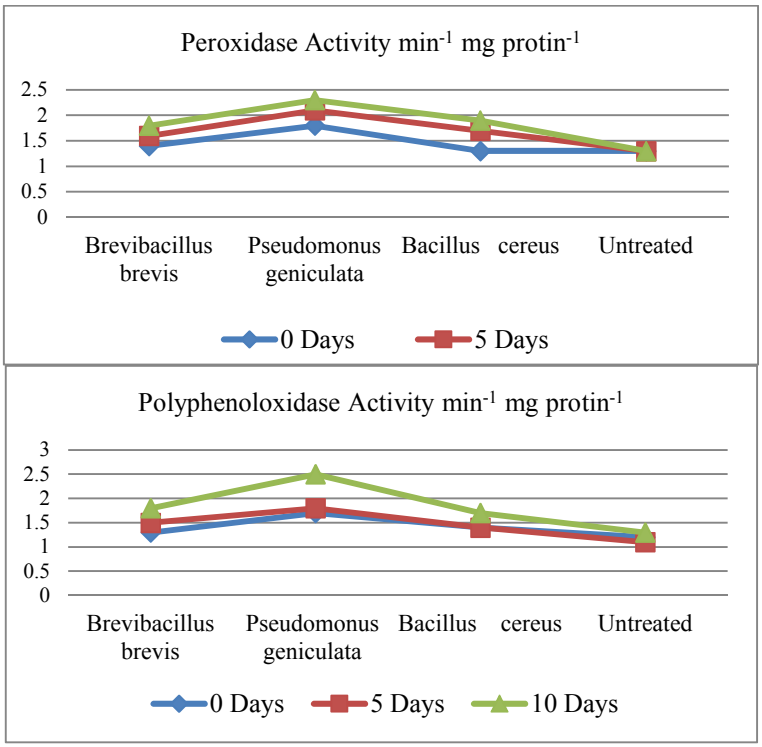

Fig. 4: Enzyme potential in pepper plants inoculated with bacterial isolates only

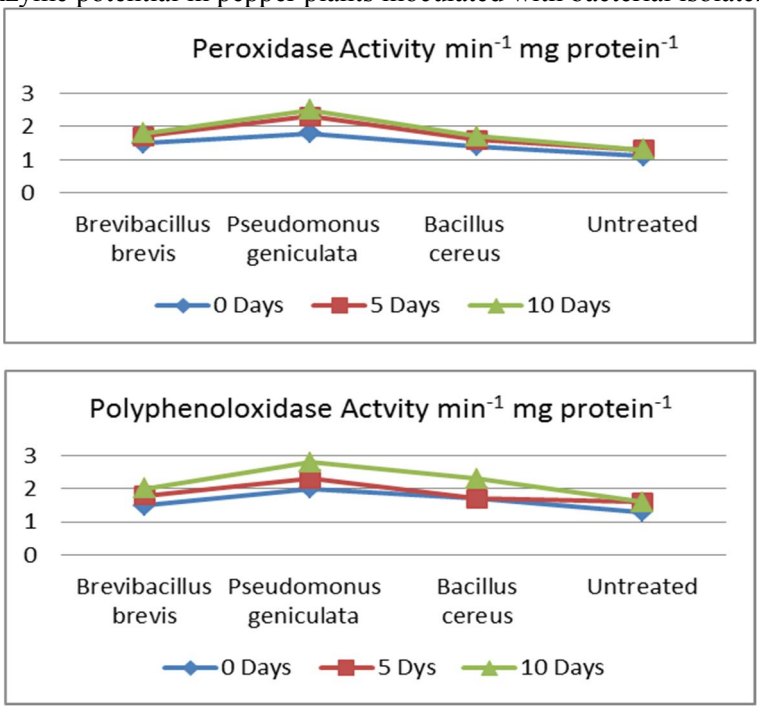

Fig. 5: Enzyme potential in pepper plants inoculated with bacterial isolates and challenged with *Phytophthora capsici*

Based on the *in vitro* and *in vivo* screening of rhizobacterial isolates against *P. capsici*, three potent antagonistic strains were selected for coming studies of assessing their efficacy as biocontrol agents in sustainable agricultural against Pytophthora blight under field conditions.

Efficacy of bacterial isolates against phytophthora blight disease under field conditions*:*

Results in Table (4) indicated that, the peat moss rhizobacterial isolates formula: formula1 (*Brevibacillus brevis* carried on peatmoss), formula2 (*Pseudomonas geniculata* carried on peatmoss), formula3 (*Bacillus cereus* carried on peatmoss), and formula4 (Mixture of three bacterial strains carried on peatmoss) were effective for controlling root-rot and Phytophthora blight in pepper under field condition. Formula4 which contain mixture of three strains was the most effective to decrease percentage of root-rot incidence compared with other formula treatments and untreated treatment. Also, data in (Table 4) illustrate that, all formula treatments led to significant increase in plant growth as measured by the plant height and dry weight, as well as significantly increase effect on yield for the combination of bacterial strains in formula4 (Table 4).

Table 4: Effect of peat moss rhizobacteria isolates formula as seedbed treatment to control phytophthora disease of pepper plants, under field conditions

Bacterial formula	Disease incidence (%)	Disease Severity	Plant growth		Yield (Kg)
			Plant height(cm)	Dry weight(g)	
Formula 1	31.4 c	1.6 c	48.9 c	0.69 b	14.7 b
Formula 2	36.2 bc	1.4 cd	54.1 b	0.65 b	15.2 b
Formula 3	42.3.6 b	1.9 b	46.6 c	0.56 c	13.5 b
Formula 4	21.5 d	1.0 d	64.9 a	0.74 a	18.5 a
Rizolexa-T (a)	11.7 e	0.7 e	41.9 d	0.45 d	21.0 a
Untreated (b)	61.5 a	3.6 a	19.6 e	0.17 e	8.6 c

** Means within the same column followed by the same letter are not significantly different according to Duncan's multiple range test (P≥0.05)*
a) Seeds were treated with rhizolex-T were 800ml suspensions (500 µg active ingredient /ml) were sprayed directly into each seedling tray
b) Seeds were treated with 0.01% MC only.
Formula1 (Peatmos with Brevibacillus brevi), Formula2(Peatmos with Pseudomons geniculata),Formula3(Peatmoss with Bacillus cereus), Formula4 (Peatmos with mixture of three strains)

Discussion

Bacteria that colonize plant roots and promote plant growth are referred to as plant growth-promoting rhizobacteria (PGPR). PGPR are highly diverse and use as biocontrol agents for suppression soil- borne diseases. Their effects can occur via local antagonism to soil-borne pathogens or by induction of systemic resistance against pathogens throughout the entire plant (Beneduzi *et al*., 2012, Prashar *et al*., 2013, Chauham *et al*., 2015 and Meena *et al*., 2016). In present study, fifteen plant growth promoting rhizobacteria were isolated and evaluated for their antifungal potential or induction systemic resistance for the management of root rot and Phytophthora blight in sweet pepper (Zohara Fatematuz *et al.,* 2016). *In vitro* screening, only five isolates B-3, S-8, T-3, T-6 and B-7 showed moderate to strong inhibition to *P. capsici* on plates due to antifungal substances such as: HCN, siderphore and lytic enzymes released by the bacteria into the culture medium (Kumar *et al*., 2012, Kavamura *et al*., 2013 and Chakraborty *et al*., 2016), as well as its showed significant production of IAA and phosphate souliblization ability (Chakraborty *et al*., 2016). Out of five isolates, three bacterial isolates i.e. B-3, S-8 and T-3 were effective in reducing damping- off of pepper seedlings as well as increasing vigor index of pepper in the roll towel assay *in vivo* (Yang *et al*., 2015 and Zohara Fatematuz *et al*., 2016). The PGPR promote plant growth through more than one mechanism that includes secretion of variety of growth stimulating hormones such as IAA and solubilization phosphate has also been reported by Shahab and Khan (2009) and suppression of plant growth retarding agents that are pathogens (Labuschagne *et al*., 2011). In the present study, pepper seeds treated with *Brevibacillus brevi, Pseudomons geniculate* and *Bacillus cereus* and challenge inoculated with *P. capsici* significantly reduced root rot compared with untreated treatment. Several strains of PGPR applied to seeds or roots of field crops have been used as elicitors of induce systemic resistance (ISR), leading to reductions in disease severity in roots (Harish *et al*.,2009 and Chakraborty

et al., 2016).The maximum phenolic content as well as synthesize defense-related enzymes peroxidase (PO) and polyphenol oxidase (PPO) were observed in *Brevibacillus brevis* and *P. geniculate* pretreated plants challenge inoculated with the pathogen and the higher amounts of phenolics were noticed even on 10th day after the pathogen challenge. In the current study, it has been observed that seeds treated with *P. geniculate* and *Brevibacillus brevis* increased the activities of various defense-related enzymes which lead to the synthesis of defense chemicals in the plants (Harish *et al*., 2009). Phenolic compounds may be fungitoxic in nature and may increase the mechanical strength of the host cell wall (Ramamoorthy *et al*., 2002).

In present study under field conditions, the peat moss formula of the three selected bacterial isolates individual or mixture did result in significant protection. Application of formula4 which contain mixture of three isolates *Brevibacillus brevis, Pseudomonas geniculate* and *Bacillus cereus* had resulted in much more intensive plant growth promotion and disease reduction when compared to formula which contain isolates tested singly. The use of fluorescent pseudomonads and bacilli for increasing yield and crop protection are attractive approaches in the modern system of sustainable agriculture (Khabbaz *et al*., 2015). The levels of disease suppression and growth promotion were greater with mixtures than with individual PGPR strains. This might be due to different mode of action for rizobacteria and related to sufficient root colonization and efficiency of biocontrol. Mixtures of plant-growth-promoting rhizobacteria (PGPR) could enhance biological control activity for multiple plant diseases through the mechanisms of induced systemic resistance or antagonism, as well as promote plant growth through more than one mechanism that includes secretion of variety of growth stimulating hormones (auxins, gibberellins and cytokinins) and solubilization phosphate in the soil under field condition (Prashar *et al*., 2013). Therefore, these mechanisms by applying a mixture of the isolates lead to more effective or at least more reliable biocontrol of root-rot and Phytophthora blight of sweet pepper (Sanga and Kim 2012, Yang *et al*., 2015 and Chakraborty *et al*., 2016).

Conclusion

The most common, devastating problem in pepper cultivation is phytophthora blight disease which has a profound effect on growth and yield of the pepper resulting in heavy losses. In order to prevent losses, different chemicals are used indiscriminately, which in turn lead to environmental pollution due to their persistence and toxicity yet employed to meet consumer demand. To fight ever increasing demand and indiscriminate use of chemical agents along with their devastating after effects in agriculture, we need less invasive, eco-friendly and most importantly sustainable practices. Plant growth promoting rhizobacteria (PGPR) influence different physiological activities of the plant through various mechanisms (metabolites, antibiotics, Induced Systemic Resistance and enzymes) and impart protection from pathogens. In the current study, determining the various plant growth rhizobacteria involved in disease inhibition provide a great awareness for their application and possibly commercializing these bacteria as a part of biocontrol strategy under field conditions that it contribute in sustainable agriculture.

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