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Plant growth promotion and antifungal activities of the mango phyllosphere bacterial consortium for the management of *Fusarium* wilt disease in pea (*Pisum sativum* L.)

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Abstract

Root rot caused by the pathogen *Fusarium oxysporum* L. is the number one cause of pea plant (*Pisum sativum* L.) death. There are many potential advantages to using rhizobacteria, endophytic bacteria and phyllospheric bacteria for managing plant diseases and promoting plant growth. This study investigated the potentiality of consortium species of bacteria to suppress root rot disease and their ability to promote the growth of pea plants compared with their individual and control plants. A total of 55 phyllospheric bacteria were isolated from mango flower and *Bacillus* sp. LBF- 02, *Bacillus* sp. LBF- 03 and *Bacillus* sp. LBF- 05 showed the most potent antimicrobial activity against root rot pathogens in a dual culture assay. Identification of phyllobacterial strain LBF- 01, LBF- 03 and LBF-05 were done by 16S rDNA sequence analysis using 704f forward primer (5'-AGATTTTCCGACGGCAGGTT-3') and 907r reverse primer (5'-CCGTCAATTCMTTTRAGTTT-3') with the PCR conditions. Their ability to solubilize phosphate, produce ammonia, siderophore and indole acetic acid, as well as produce extracellular enzymes *in vitro* was excellent. The results of a greenhouse study found that pea seed treated with consortium isolate significantly increased high germination rates and vigour indexes, as well as shoot and root length, fresh and dry weights, as compared with seed treated with single isolate and control. The defense enzyme activities in consortium treated pots were higher than those in individual and control pots. The plants treated with consortium exhibited higher levels of chlorophyll and carotenoids content in their leaves compared to the untreated control and single treated plants. A significant variation in the chemical profile of pea plants was found ($F_{7,16} \geq 2.598$; $P \leq 0.048$) resulting from different treatments (T1-T8). After evaluating a variety of growth and microbiological parameters, it was concluded that inoculation with the microbial consortium contributed to raising healthy and vigorously growing pea seedlings in greenhouse conditions, which is applicable in the field in future for sustainable farming.

Keywords

Antifungal, consortium, root rot, Phylobacteria, sustainable farm

Introduction

Agricultural crops have always been plagued by numerous pests such as fungi, weeds and insects, causing a dramatic drop in the yield of the plants (1). The use

of chemicals is highly effective for the control of these pests, but their adverse effects not only affect a wide variety of hosts, but also negatively impact the environment and farming systems. In particular, pests and pathogens have developed resistance to pesticides, making the problem even more serious. Therefore, synthetic pesticide use has declined by 2% per year in commercial agriculture as a result of regulatory restrictions, while biopesticide use has increased by 10% as an alternative agrochemical (2). Biopesticides are derived from animals, plants and other natural materials such as fungi, bacteria, algae, viruses, nematodes and protozoa. Microbial biopesticides are one of the most widely known types of biopesticide and they command 5% of the global pesticide market, with microbial biopesticide taking first place (3). Although biopesticides are promising, their widespread adoption is hampered by a lack of available products to meet farmer needs, the high cost of refined products, and their slow action levels (4). However, these drawbacks must be weighed against a tolerable level of toxicity, if any, that biopesticides manifest. In addition, they are biodegradable, specific in action (harmless to non-target organisms) and provide an alternative method of dealing with pest resistance issues caused by synthetic pesticides (5). As science waits to address the drawbacks of this approach, raw extracts of pesticidal plants can be used in the meantime, especially by local farmers and countries in developing regions. Sustainability through biopesticide-driven agriculture is socially acceptable, stimulates economic productivity and encourages environmental responsibility.

The pea, *Pisum sativum*, is a popular vegetable crop from western Asia and North Africa, belonging to the Leguminosae, a family of legumes. There are a number of pest and diseases that affect pea plants, including bacteria, viruses and fungi, which can significantly affect the quality and yield of the crop. There are numerous soil-borne fungal diseases that threaten agriculture across the globe, resulting in yield losses of up to 50%. It is estimated that at least 1200 species of fungal pathogen are responsible for soil-borne plant diseases and crop failures (6, 7). Root rot disease in different regions of the world may be caused by several fungal pathogens and fungal-like organisms including *Fusarium solani*, *Rhizoctonia solani*, *Pythium* spp. and *Fusarium oxysporum* (8). Plant infections caused by *Fusarium*, an ascomycete, include head blights, vascular wilts, patch diseases, root rots and yellowing. Biocontrol of *Fusarium* is performed by *Pseudomonas* sp. and *Bacillus* species that utilize multiple strategies including siderophore-mediated iron competition, induced systemic resistance and antibiotic production (9). A secondary metabolite synthesized by *Aspergillus flavus* controls insect pathogens by producing alphatoxins (B1, B2, G1 and G2) (10). In various larval stages of *Spodoptera litura*, *Aspergillus flavus* is highly toxic to agriculture pests (11). Insecticidal activities of *Bacillus subtilis* chitinase that can be identified by matrix-assisted laser desorption/ionization time-of-flight/time of flight mass spectrometry has been demonstrated against *Spodoptera litura* Fab (12). Plant defense against *Xanthomonas oryzae* pv. *Oryzae* rice infection by methyl salicylate treatment and foliar application (13).

In conventional farming, farmers use large amounts of chemical fungicides to control fungi like *Fusarium oxysporum* which are damaging the environment (14). There are many ways that microbes can be used as biocontrol agents to suppress pathogens in plants without affecting any surrounding environment factors. Among the microorganisms used to control *Fusarium* wilt, *Bacillus* spp., *Pseudomonas* spp. and *Trichoderma* spp. are most attractive. *Bacillus* sp. produce antagonistic secondary metabolites, such as fengycin, bacillomycin, iturin and surfactin (15). They are also able to produce cell wall degrading enzymes and induce systemic acquired resistance in the targeted hosts (16). A variety of *Bacillus* sp. have been identified as plant-growth-promoting bacteria (17) and biocontrol agents (BCAs) (18). *B. subtilis, B. licheniformis* and *B. amyloliquefaciens* are some of the most commonly studied PGPB/BCA (19). The PGPB uses both direct and indirect strategies for enhancing plant growth and survival (20). Phytohormone production, the acquisition of nutrients such as phosphorous and nitrogen and pathogen control are the most common direct mechanisms, for example, by the production of hydrolytic enzymes, antifungal compounds, lipopeptides or antibiotics. Protection against abiotic stress caused by drought, salinity etc. stimulation of defense-related pathways, particularly the induction of systemic resistance (SSR) against pathogens and pests (21) and the release of volatile organic compounds (VOCs), called bacterial volatile compounds (BVCs) (22). In recent years, numerous articles and reviews have published about how PGPB promotes plant growth (23). This study aimed to determine the inhibitory effect of strains LBF-01, LBF -03 and LBF-05 of *Bacillus* sp. against *F. oxysporum* in pea plants, both *in vitro* and *in vivo*, through disease protection, growth enhancement and *in vivo* defense enzyme production.

Materials and Methods

Microorganisms and Culture Conditions

The strains *Bacillus* sp. LBF- 01, LBF- 03 and LBF- 05 (GenBank Ac. no. KX656669, KX665548 and KX665549) were isolated from mango flowers (24). To perform different experiments, bacterial isolates were sub-cultured and maintained on nutrient agar slant media at 4 °C as stock cultures. The root rot pathogen was isolated from the rhizosphere of pea plants. The infected roots of the pea were collected in the field and taken to the laboratory. Pea root samples were surface sterilized using 5% sodium hypochlorite solution for 1 min (24), followed by 3 washes with ethanol and sterile distilled water. The root was longitudinally cut with the help of sterile blade aseptically and placed on sterile Potato Dextrose Agar (PDA) medium amended with streptomycin sulphate (300 mg/L), (w/v) for 1 week at 25 °C (25). The pathogens were identified by the morphological characteristics of their hyphae and spores.

Antifungal screening of Bacterial strains

The dual culture plate method was used to determine the antifungal activity of *Bacillus* sp. LBF- 01, LBF- 03 and LBF-05 *in vitro* against the pea root rot fungus as reported earlier (24). A comparison of the fungal mycelia diameter on the control plate and the test plate was used to

determine the antagonistic activity and inhibition was calculated using [(C - T)/C] × 100, where C and T are the fungal mycelia diameters of the control plate and the test plate respectively (26).

In vitro screening for multiple plant growth promoting traits

The multiple PGP traits of *Bacillus* sp. LBF- 01, LBF- 03 and LBF- 05 were tested *in vitro*. Indole acetic acid (IAA) production was tested using Salkowski's reagent as described (27). Phosphate solubilisation was determined using the protocol previously described (28). The Schwyn and Neiland, method was used to assess the siderophore production ability (29). Ammonia production was tested using protocol (30). Formation of clear zones around the colony in each plate was considered as positive response.

Extra cellular enzyme activities of *Bacillus* sp.

Chitinase productions of 3 *Bacillus* sp. strains were tested on chitinase detection agar (CDA) plates according to standard procedure (31). The formation of halo zone around the colony after adding iodine solution indicates chitinase enzyme production. The protease activity of the strains LBF- 01, LBF- 03 and LBF- 05 was assessed in sterile skim milk agar according to the method (32). The strain LBF- 01, LBF- 03, LBF- 05 were spot inoculated on starch agar plates (33). Cellulase activity was tested on cellulose granules agar plate (cellulose granules 1g and agar powder 2% dissolved 100 mL water). Measurement of net reaction zone was done by the formula 'Active unit (AU) = Diameter of reaction zone ÷ diameter of colony.

In vitro physiological parameters of seed germination and vigour index of Pea plant

Preparation of bacterial inocula for pea seed treatment

Bacterial strain was cultured in 250 mL conical flasks containing 100 mL of Nutrient broth in an orbital shaker at 120 rpm for 72 h at 37 ± 0.2 °C. Bacterial cells were collected by centrifugation at 8000 rpm for 5 min at 4 °C and pellet was washed twice with sterile distilled water. The bacterial pellets were suspended in 1 mL sterile distilled water (approximately 10^6 CFU/seed), vortex mixed and used for seed treatment. Approximately 10-15 pea seeds were surface sterilized with 5% sodium hypochlorite (NaOCl) for 1 min and washed 3 times in sterile distilled water (24). Dry seeds were immersed in bacterial suspension and the preparation was stirred frequently for 5 min. The treated seeds were spread on a petri dish and air dried overnight at room temperature. The number of bacterial cells per seed was determined via serial dilutions and was set to approximately 10^6 CFU/seed.

Effect of bacterial seed treatment on germination and vigour index of Pea seeds.

To assess the effect of the strain LBF- 01, LBF- 03 and LBF- 05 on germination and seedling vigour in treated with different combination parameter such as control, *Bacillus* sp. LBF- 01, *Bacillus* sp. LBF- 03, *Bacillus* sp. LBF- 05, *Bacillus* sp. LBF- 01 + LBF- 03, *Bacillus* sp. LBF- 01 + LBF- 05, *Bacillus* sp. LBF- 03 +LBF- 05 and *Bacillus* sp. LBF- 01 + LBF- 03 + LBF- 05. Total 15 bacterized seeds placed in two 9 cm petri dishes with 2 layers of moistened filter paper (Whatman No. 1) and incubated at 28 °C ± 2 °C in a light incubator. As a control treatment, seeds treated with distilled water instead of bacterial suspensions were also established. In order to maintain sufficient moisture for germination, 5 mL distilled water was added to the petri dishes in every alternate day. Germination was considered to have occurred when the radicals were half of the seed length. The germination % was recorded every 24 h for 10 days. Root and shoot length were measured after the 10 days. The experiment was repeated thrice. The germination rate and vigour index were calculated using following formula (24):

Germination rate (%) = (number of seeds germinated/ total number of seeds) × 100

Vigour index = % germination × total plant length

In vivo physiological assessment of pea plant in pot conditions

Preparation of pot soil and application of *Fusarium oxysporum* in pot soil

The experimental soil was collected from 0-15 cm soil depth at the University campus. After air-drying and grinding, the soil was sieved (2 mm mesh) to remove the small stones and bricks. The soil was autoclaved at 121 °C for 20 min and again air dried. About 2 kg of dry soil was taken in each soil pot (9 cm diameter; 12 cm deep). The mass inoculum of *Fusarium* sp. was prepared by inoculating mycelia block in pre-sterilized sand maize medium and the inoculum was mixed thoroughly in double autoclaved earthen pot and allowed for mycelia growth of *Fusarium* sp. for 7 days.

Application of PGP bacterial strain and pea seed treatment

Surface sterilized pea seeds were then soaked in a 48 hr old suspension of the bacterial strain at 10^6 CFU/ml for 24 h. The 8 treatments imposed were: (i) Negative control, sterile soil (SS) infested with *Fusarium* sp. inoculum; (ii) SS + *Fusarium* sp.+ *Bacillus* sp LBF- 01; (iii) SS + *Fusarium* sp.+ *Bacillus* sp LBF- 03; (iv) SS + *Fusarium* sp.+ *Bacillus* sp LBF- 05; (v) SS + *Fusarium* sp.+ *Bacillus* sp. LBF- 01+ LBF- 03. (vi) SS *Fusarium* sp.+ *Bacillus* sp. LBF- 01 + LBF-05; (vii) SS + *Fusarium* sp.+ *Bacillus* sp. LBF- 01 + LBF- 03 + LBF- 05; (viii) SS+ Superphosphate fertilizer (Positive control). The seeds of 6 peas of similar size and shape were sown in every pot and properly labeled. For each of the 8 sets, 22 pots were selected and all the experiments were carried out in the spring (month of March-April). To maintain sterility, all inoculated and control pots were maintained in a sunny location and sprayed with sterile water regularly. In each pot, the rate of seed germination was observed and the % of seed germination was determined. In each pot, data on different growth parameters was collected for 20-35 days. In each pot, shoot length, root length, shoot dry weight and root dry weight were measured. To determine the dry weight of the shoots and roots, the plant fresh materials were incubated at 65 °C for 72 h.

Effect of *Bacillus* sp. on seed treatment against root

rots disease of pea plant caused by *Fusarium* sp.

The strain LBF- 01, LBF- 03 and LBF- 05 were inoculated into pea seeds and grown in a sterilized petridish for 15 days. In each petri dish, seedlings were inoculated with 500 or 1000 µl spores' suspension as described (34) and kept inside humid chambers for 48 h. A total of 15 seedlings were used per treatment in 3 replications for each experiment. The number of alive plants was counted after 7 days of inoculation. A control group was treated with non-inoculated seeds. % of disease incidence (PDI) and % of disease protection (PDP) by the strain were calculated using the following formula (24):

$$PDI = \text{Number of infected plants/Total number of inoculated plants} \times 100$$

$$PDP \text{ by PGP bacteria (\% protection)} = [(A-B)/A] \times 100$$

Where, A = PDI in non-inoculated control plants; B = PDI in PGPR-treated plants.

Biochemical Analyses of Defense Related Enzymes

Estimation of Phenylalanine Ammonia Lyase (PAL)

The leaf samples were collected, crushed and extracted on ice using 5 mL of a sodium borate buffer (pH - 8.8) containing 2 mM β-mercaptoethanol. A centrifuge is then used at 15000 rpm at 4 °C for 20 min and the collected supernatant is used for testing and analysis. For the assay, 500 mL crude enzyme was mixed with 300 mL of 0.3 M borate buffer (pH 8.0), 300 mL of 2% L-phenylalanine and 1.9 mL distilled water. The mixture was incubated for 1 h at 40 °C and then the absorbance was measured at 290 nm. In this study, the amount of cinnamic acid produced by an enzyme from 1 g tissue was determined by using a standard curve and the enzyme activity was expressed as the amount of cinnamic acid produced by the enzyme from 1 g tissue per min (35).

Estimation of β-1, 3-Glucanase activity

The assay of β 1, 3-glucanase was performed following standard procedure (35). To conduct the assay, leaves were collected and crushed in chilled 5 mL of sodium acetate buffer (pH 5.0) for 5 min. The extract was then centrifuged for 15 min at 4 °C at 10000 rpm and the supernatant collected was used to make crude enzyme. The assay was performed by adding 62.5 mL crude enzyme to 62.5 mL of 4% laminarin and incubating the mixture for 10 min at 40 °C, followed by adding 375 mL DNSA (dinitro salicylic acid) and incubating for 5 min on a boiling water bath. Finally, to determine the amount of glucose liberated from the solution, it was diluted with water and the standard curve was drawn. The enzyme activity was expressed as mg glucose released/min/g tissue.

Estimation of chitinase activity

To assay chitinase, leaves were collected and enzyme extracted in 5 mL of chilled 0.1 (M) sodium acetate buffer (pH 5.0), followed by centrifugation to obtain crude enzyme (35). The assay mixture consists of 10 mL of 1 M Na -acetate buffer (pH 4), 0.4 mL crude enzyme solution and 0.1 mL colloidal chitin. The mixture was incubated at 37 °C for 2 h and centrifuged for 3 min at 10000 rpm. After that, 0.3 mL of supernatant was added to 30 mL of 1 M K-PO4 buffer (pH 7.1), and 3% Helicase (20 mL) was mixed and incubated at 37 °C for 1 h. The incubation period was concluded by adding 70 mL of 1 M Na-borate buffer (pH 9.8). The reaction was then stopped by incubation in a boiling water bath for 3 min and then rapidly cooled in ice water. Finaly, 2 mL of DMAB (2% dimethyl amino benzaldehyde) was added and incubated for 20 min at 37 ° C. The amount of GlcNAc released was measured and enzyme activity was expressed as mg GlcNAc released/ min/g fresh wt. tissue.

Analysis of photosynthetic pigments

The terminal opened tender leaves of the fungus treated and control plants were cut into small pieces and treated with 7 mL of dimethyl sulphoxide (DMSO) in test tubes for 3 h at 65 °C.The volume was diluted with DMSO up to 10 mL and the absorbance of the clear extract was measured on a UV-vis spectrophotometer (UV-Vis1800, Shimadzu, Japan) at 645 and 663 nm with pure DMSO as a blank (36). Pea leaves ground with 80% acetone were used to extract photosynthetic pigments. A method of Lichtenthaler was used to estimate the chlorophylls and carotenoid content (37). Samples from 3 different plants were used for the estimation in triplicate and 3 times. Total chlorophyll, chlorophyll a, chlorophyll b and carotenoids were expressed as mg/g of fresh leaf tissue. The absorbance for chlorophylls-a and b and carotenoid were recorded at 663, 645 and 470 nm respectively.

Phytochemical analysis:

Freshly collected pea, *Pisum sativum* L. plants under eight (T1-T8) treatment conditions with *Bacillus* sp. (LBF-01, LBF -03 and LBF-05) were separately rinsed with distilled water and dried by paper toweling for phytochemical analysis. They were extracted in different solvents for extraction of different primary (PM) and secondary (SM) metabolites as well as they were estimated by various standard protocols (38-40). Determination of each biochemical analysis was repeated for three times during 2021-2022 and was expressed in dry weight basis.

Statistical analyses

The data on growth and defense enzyme activity in pea plants under different treatment conditions were in normal distribution (Kruskal-Wallis test) and analyzed by one-way ANOVA followed by Tukey's HSD test (41). Similarly experimental data of different phytoconstituents under 8 (T1-T8) treatment conditions with *Bacillus* sp. (LBF - 01, LBF- 03 and LBF- 05) were subjected to one-way analysis of variance (ANOVA) and correlation analysis (41). Means of different phytochemicals were also compared by Tukey's test (HSD) when significant values were obtained (41). The data obtained for different treatments and or analyses were also analyzed by using correlation analysis and paired t-test accordingly (41). All the statistical analysis was performed by using SPSS, version 16.0 (40).

Results

Characterization of the phyllobacterial strains

The strains LBF- 01, LBF- 03 and LBF- 05 were rod-shaped

Table 1. *In vitro* evaluation of PGP traits of phyllobacterial strains LBF-01, LBF-03 and LBF-05

PGP traits	Strains No.		
	LBF-01	LBF-03	LBF-95
Direct PGP traits	**Response units**		
Indole acetic acid production	+++	+++	+++
Phosphate solubilization	3.800 ± 0.058	4.067 ± 0.088	3.700 ± 0.058
Ammonia production	+++	+++	+++
Indirect PGP traits	**Response units**		
Siderophore production	6.233 ± 0.088	4.033 ± 0.088	3.500 ± 0.058
Protease	7.300 ± 0.058	7.000 ± 0.058	6.433 ± 0.120
Amylase activity	7.400 ± 0.058	7.700 ± 0.058	7.967 ± 0.088
Cellulase activity	9.467 ± 0.088	9.800 ± 0.058	9.833 ± 0.088
Xylanase activity	8.533 ± 0.088	8.233 ±0.033	8.467 ±0.088
Chitinase activity	6.700 ± 0.058	6.733 ± 0.120	6.700 ± 0.115

Gram-positive, fast-growing, round to irregular colonies with elevations and smooth surfaces. Based on the sequence similarity and phylogenetic relations, the isolate LBF- 01, LBF- 03 and LBF- 05 were identified as *Bacillus* sp. (Fig. 1) and assigned as *Bacillus* sp. LBF- 01 (GenBank Accession No. KX656669), *Bacillus* sp. LBF-03 (GenBank Accession No. KX665548) and *Bacillus* sp. LBF- 05 (GenBank Accession No. KX665549). The of antifungal activity result from assay plate of the strains LBF- 01, LBF- 03 and LBF- 05 against *Fusarium* sp. showed that LBF- 01 form 9 mm, LBF- 03 form 8 mm and LBF- 05 form 7 mm clear inhibition zone as shown in Fig. 2.

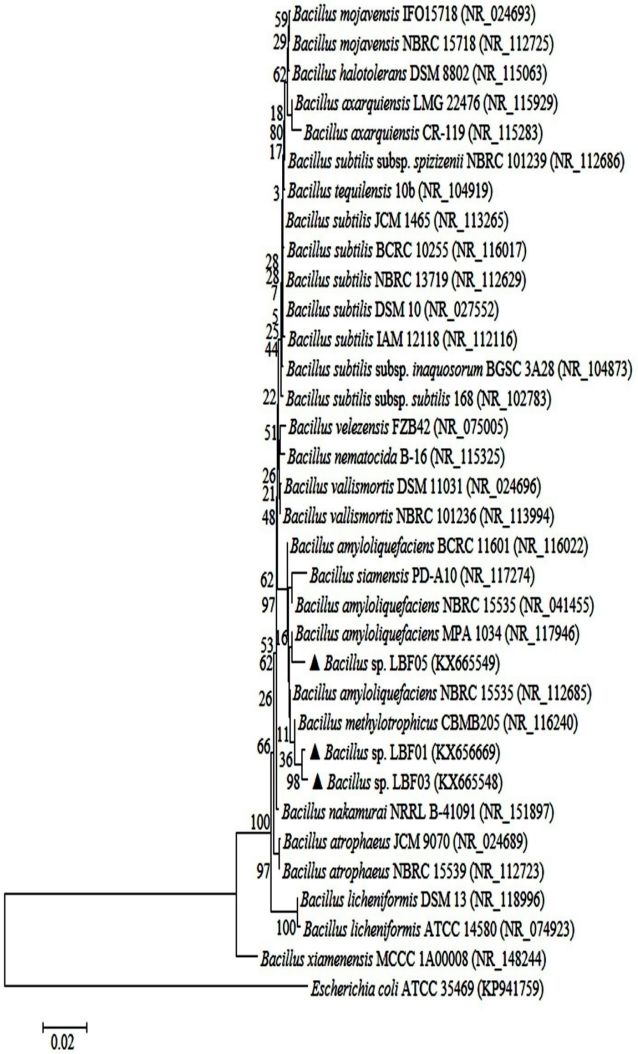

Fig. 1. Phylogenetic relation of the strains LBF-1, LBF-03 and LBF-05 is using the neighbour-joining method. The number in the bracket is the GenBank accession number given for each organism and the numbers at the clades are bootstrap values based on 1000 replications

Characterization of the plant growth promoting traits and extracellular hydrolytic enzymes production

The strainsLBF-1, LBF- 03 and LBF- 05 showed significant plant growth promoting activity in *in vitro* through the phosphate solubilization, IAA production, siderophore production, ammonia production and extracellular cell wall degrading enzymes production. The strains LBF-1, LBF- 03 and LBF- 05 were produced clear halo zone around colony in NBIRP agar medium indicating as phosphate solubilizer. The solubilizing efficiency (SE) of strain LBF-03 was strong, i.e., 4.067 ± 0.088, followed by LBF-01 was 3.8 ± 0.058 and LBF-05 was 3.7 ± 0.058. The strain could also produce IAA. The trainsLBF-1, LBF- 03 and LBF- 05 produced siderophore in term of produced clear halo zone around colony in CAS media. The solubilizing efficiency (SE) of strain LBF-01 was strong i.e., 6.233 ± 0.088, followed by LBF-05 was 4.033 ± 0.058 and LBF-03 was 3.5 ± 0.058. The strain could also produce IAA. The strains LBF-1, LBF-03 and LBF- 05 produced moderate amount of ammonia and hydrogen cyanide.

All three strains produced α-amylase with an AU of 7.400 ± 0.058 by LBF-01 followed by 7.700 ± 0.058 by LBF-03 and 7.967 ± 0.088 by LBF-04. Extracellular protease activity of the strains LBF-01 was7.300 ± 0.058 AU followed by LBF- 03 was 7.00 ± 0.058 AU and LBF- 05 was 6.433 ± 0.120 AU.

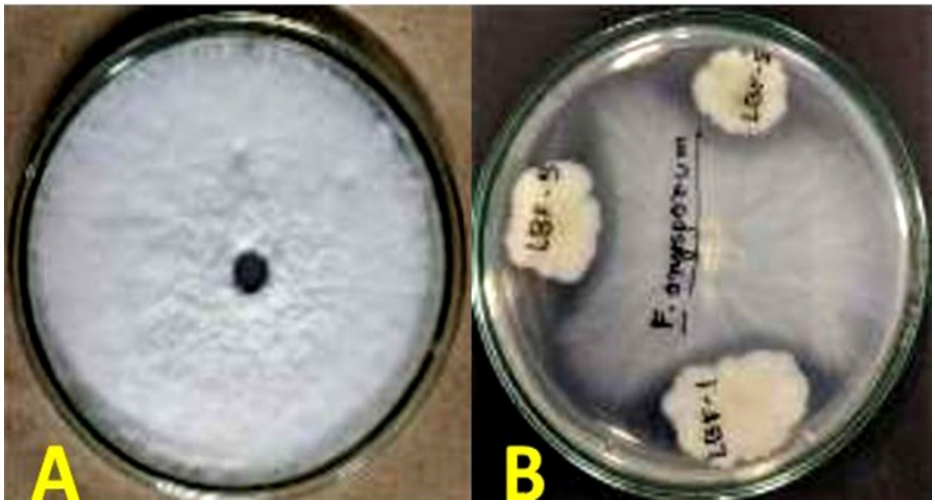

Fig. 2. Antifungal assay of LBF-1, LBF-03 and LBF-05 against root rot pathogen (*Fusarium oxysporum*). (A) Control plate; (B) Treated plate.

Cellulase production was 9.467 ± 0.088 AU by LBF-01, 9.800 ± 0.058 AU by LBF-03and 9.833± 0.088 AU by LBF-05. The chitinase detection agar (CDA) plates of the strains LBF-1, LBF - 03 and LBF- 05 indicated their capability of secretion of extracellular chitinase with an AU of 6.700 ± 0.058,6.733 ± 0.120 and 6,700 ± 0.115 respectively. Xylanase production by the strain LBF-01 was 8.533 ± 0.088, LBF-03 was 8.233 ± 0.033 and LBF-05 was 8.467 ± 0.088. All the three strains produced enzymes with significant differences ($P \leq 0.05$). Solubilization effciency (SE) = Halo zone ÷ Diameter of colonies; Activity unit (AU) = Diameter of reaction zone ÷ Diameter of colony. '+' = positive result. Values are means of three replicates ± standard error (SE).

In in vitro seed germination and vigour index of Pea seedling

The results of seed germination rate and vigour index of pea plants treated with *Bacillus* sp. LBF-01, LBF- 03 and LBF- 05 as individual and consortium treatments are shown in Fig. 3. One application of *Bacillus* sp. LBF- 01, *Bacillus* sp. LBF- 03 and *Bacillus* sp. LBF- 05 significantly increased the rate of seed germination by 33.33%, 26.66% and 26.66% respectively. Comparative to control plants, the consortium application of 2 strains LBF- 01+ LBF- 03 resulted in a 33.33%, 40% and 40% increase of LBF- 01+ LBF- 05 and LBF- 03+ LBF- 05. The 3-strain consortium (LBF-01+ LBF-3+ LBF-05) significantly improved seed germination by 46.77% as compared to controls. Similarly, the excess vigour index for each single strain is 596.38%, 429.57%, 509.97%, while 2 consortium strains are increased by 683.05%, 869.62%, 962.95% and 3% consortium strains by 1009.61% as compared to control.

All the data related with seed germination and vigour index differed significantly within the treatments (one-way ANOVA, $F_{7,16} \geq 47.321$, $P<0.001$).

In vivo Plant Growth Promotion in pea plant

The development of different growth parameters revealed that bacterial application increased growth in pea plants up to 30 days, as depicted in Fig. 4. The shoot and root lengths of the plants treated with *Bacillus* sp. LBF-1 increased by 7.5 ± 0.20 cm and 3.23 ± 0.088 cm respectively, whereas they were 4.9 ± 0.058 cm and 2.5 ± 0.115 cm respectively, for untreated plants. The application of *Bacillus* sp. LBF- 01 increased the shoot fresh and dry weights of a pea plant (6.73 ± 0.120 g and 2.8 ± 0.058 g), as well as root fresh and dry weights (2.13 ± 0.088 g and 1.10 ± 0.058 g), compared to untreated sets for shoot fresh and dry weights (4.86 ± 0.120 g and 2.40 ± 0.058 g) and root fresh and dry weights (1.73 ± 0.033 g and 0.50 ± 0.034 g). A comparison of the shoot and root lengths of the pea plants treated with *Bacillus* sp. LBF- 03 with those of the controls showed a significant increase of 7.7 ± 0.115 cm and 3.66 ± 0.088 cm. In comparison with untreated control pot, *Bacillus* sp. LBF- 03 increased fresh and dry shoot (6.7 ± 0.088 g, 3.06 ± 0.088 g) and root weights (2.5 ± 0.058 g, 1.56 ± 0.033 g) of pea plants. The shoot and root lengths of the pea plants treated with *Bacillus* sp. LBF- 05 were significantly longer than those of the controls, by 8.10 ± 0.088 cm and 3.80 ± 0.088 cm respectively. *Bacillus* sp. LBF- 05 increased pea plant fresh and dry shoot weights (7.26 ± 0.088 g and 3.60 ± 0.058 g) and root weights (2.90 ± 0.058 g and 1.80 ± 0.058 g) in comparison to untreated control pots. All the data related with the effect of *Bacillus* sp. LBF- 01, *Bacillus* sp. LBF- 03 and *Bacillus* sp. LBF - 05 on various plant growth parameters of Pea seedlings were differed significantly within the treatments along with controls (one-way ANOVA, $F_{7,16} \geq 211.058$, $P<0.001$).

Plants treated with a consortium application of *Bacillus* sp. LBF- 01 and LBF- 03 increased in shoot and root length by 9.63 ± 0.088 cm and 4.33 ± 0.088 cm respectively, as opposed to 2 individual applications. A two-species consortium application of *Bacillus* species (LBF- 01 + LBF- 03) significantly increased shoot fresh and dry weight (8.53 ± 0.088 g and 4.16 ± 0.145 g) and root fresh

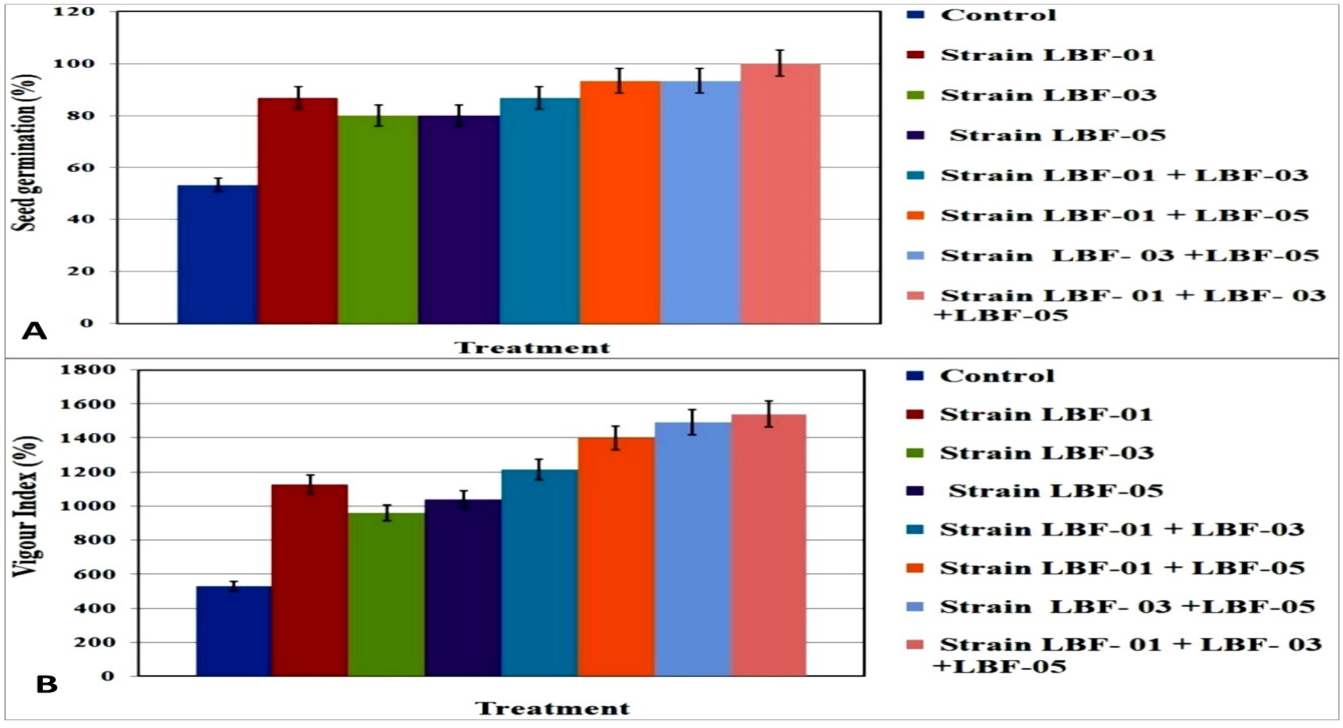

Fig. 3. Seed germination and vigour index of pea seed. (A) % of seed germination; (B) % Vigour index.

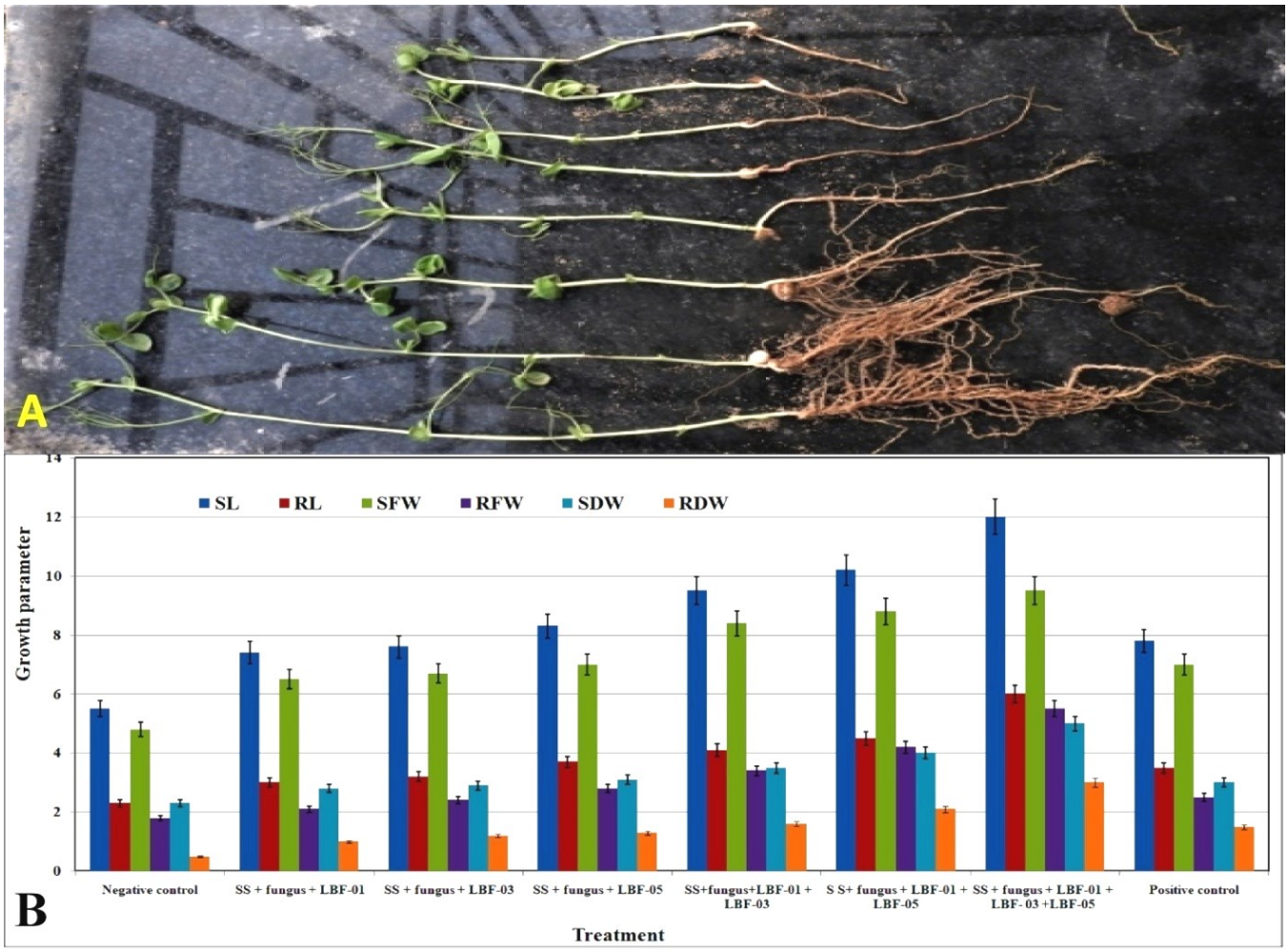

Fig. 4. Consortium and individual effect of *Bacillus* sp. LBF-1, *Bacillus* sp. LBF-03 and *Bacillus* sp. LBF-05 on various plant growth parameters of pea seedlings up to 30 days. (A) Shoot length and length of different treatments and control (B) Graphical representation of different growth parameters of pea seedling. Here, the data are displayed as mean ± standard error; of Shoot length, Root length.

and dry weight (3.56 ± 0.088 g and 2.00 ± 0.058 g) of pea plants compared with 2 individual applications of *Bacillus* sp. LBF- 01 and *Bacillus* sp. LBF- 03. When combined application of *Bacillus* sp. LBF- 01 and LBF- 05 strains increased the length of the shoots by 10.50 ± 0.115 cm and the root length by 4.60 ± 0.058 cm, the results were better than 2 individual applications. *Bacillus* sp. consortium applications (LBF- 01 + LBF- 05) significantly increased shoot fresh and dry weight (9.06 ± 0.120 g and 5.26 ± 0.088 g) and root fresh and dry weight (4.53 ± 0.120 g and 2.40 ± 0.058 g) of pea plants than single applications of *Bacillus* sp. LBF- 01 and *Bacillus* sp. LBF- 03. Comparatively with 2 individual applications, the shoot and root lengths of the pea plants treated with *Bacillus* sp. LBF- 01 and LBF- 03 increased by 12.40 ± 0.173 cm and 5.66 ± 0.088 cm respectively, when the consortium treatment was applied. Considering 2 *Bacillus* sp. consortium applications (LBF- 05 + LBF- 03), shoot fresh and dry weight was significantly higher (9.90 ± 0.115 g and 3.10 ± 0.058 g) as well as root fresh and dry weight (5.66 ± 0.088 g and 3.10 ± 0.058 g) of pea plants compared with 2 individual applications of *Bacillus* sp. LBF- 01 and *Bacillus* sp. LBF- 03. Similarly, multi-consortium application of strains LBF- 01, LBF- 03 and LBF- 05 increase the growth parameter such shoot length, root length, shoot fresh and dry weight and root fresh and dry weight in respect to all treatment. The application of superphosphate as a positive control, the value of growth parameter approximately matched with *Bacillus* sp. LBF- 01. All the data related with pea plant growth parameters were also differed significantly within the treatments including control (one-way ANOVA, $F_{7,16} \geq 93.702$, $P < 0.001$).

Effect of bacterized seed for protection against root rots disease of pea seedling

As shown in Fig. 5, we have determined both the % of disease incidence and the % of protection after 15 days of inoculation with 500 µL and 1000 µL. According to the results of inoculating seedlings with 500 µL and 1000 µL fungal spores and the individual strains, PDI is highest (53.33%, 66.66%) for LBF- 03 strain in both cases and lowest (40%, 46.66%) for LBF-05 strain in comparison with control (100%). Based on the 2-strain consortium application, 33.33% by 500 µL and 40% by 1000 µL spores' inoculation was observed, which is highest by LBF-01+ LBF - 03 followed by 26.66% by 500 µL and 33.33% by 1000 µL by LBF- 01+ LBF- 05. Furthermore, strains LBF- 01 + LBF- 03 + LBF- 05 showed 20% and 26.66% PDI with 500 µL and 1000 µL. In the application of individual strains in 500 µL and 1000 µL inoculated seedlings of pea, there was the highest level of protection in case of LBF- 03 (60% and 53.34%) and the lowest level in case of LBF- 05 (46.67% and 33.34%) as compared to control. The 2 consortium applications show the highest score of 73.34% and 66.67% for LBF- 01 + LBF- 03 and the lowest score of 66.67% and

60% for LBF- 01 + LBF- 05. Based on all treatment parameters, the 3 consortium application results showed the greatest degree of protection, at 80% and 73.34% respectively with significant (P<0.05) variations.

Defense enzymes activity of leaf and root of pea plants

The defense enzyme activity of leaf and root of pea plants under different treatment conditions is shown in Fig. 5. *Bacillus* sp. LBF- 05 was found to have the highest levels of β -1,3 glucanase and PAL enzyme activity compared with *Bacillus* sp. LBF- 01 and *Bacillus* sp. LBF- 03 as compared with control. Comparing all individual and control plants, *Bacillus* sp. LBF- 01 + LBF- 05 consortium application showed the highest activity of β -1,3 glucanase and PAL, followed by *Bacillus* sp. LBF- 01 + LBF- 03. In all treatment conditions, 3 consortium applications showed the highest performance in terms of β -1,3 glucanase and PAL activities. It is found that the root of the pea plant contains more -1, 3 glucanase and PAL than the leaves. In leaf and root treated with *Bacillus* sp. LBF- 03, Chitinase activity was higher than LBF- 05 and LBF- 01 strains as compared with controls. Based on comparison with a control, *Bacillus* sp. LBF- 01 + LBF- 05 demonstrated higher chitinase activity than *Bacillus* sp. LBF- 01 + LBF- 03. The results of 3 consortium application studies indicated the highest levels of activity for β -1, 3 glucanase, PAL and chitinase regardless of treatment conditions. The enzyme activities of positive control plants are lower than those of combined application, but higher than those of individual and control plants. All the data related with enzyme activity were differed significantly within the treatments for both leaf (one-way ANOVA, $F_{7,16} \geq 234.923$, $P<0.001$; Table 4) and root system (one-way ANOVA, $F_{7,16} \geq 49.890$, $P<0.001$; Table 3).

Photosynthetic pigment content of pea plant

According to the chlorophyll content study for *Bacillus* sp. LBF- 05 treated plants, chlorophyll (a and b) contents were 0.36953 mg/g for *Bacillus* sp. LBF-05, followed by 0.3571 mg/g for *Bacillus* sp. LBF- 03 and 0.3571 mg/g for *Bacillus* sp. LBF - 01 among the individual strains compared to control plants. The highest level of chlorophyll-a (0.19554 mg/g) and chlorophyll-b (0.31842 mg/g) was found in *Bacillus* sp. LBF- 01 + LBF- 05 treated healthy plants, followed by the standard control and LBF- 01 +LBF- 03. According to the results from the 3 consortium applications, 0.28842 mg/g chl-a and 0.38220 mg/g chl-b are more than any other treatment parameter. From positive control, the chl-a is 0.16523 mg/g and the chl-b is 0.17522 mg/g, which is higher than both individual and control. Additionally, *Bacillus* sp. LBF- 01 had the highest amount of carotenoid estimated (0.00257 µg/mL), followed by *Bacillus* sp. LBF- 03 (0.00189 µg /mL) and *Bacillus* sp. LBF- 01 (0.00125 µg /mL). The presence of carotenoid was significantly enhanced by the application of *Bacillus* sp. strains LBF- 01+ LBF- 05 to the 2 strains. The result was 0.00778 µg /mL in the case of LBF- 01 + LBF- 03 and 0.00698 µg /mL for LBF- 01 + LBF- 05. The carotenoid content in the 3 consortium applications is 0.00895 µg/mL, which is the highest among the treatment conditions.

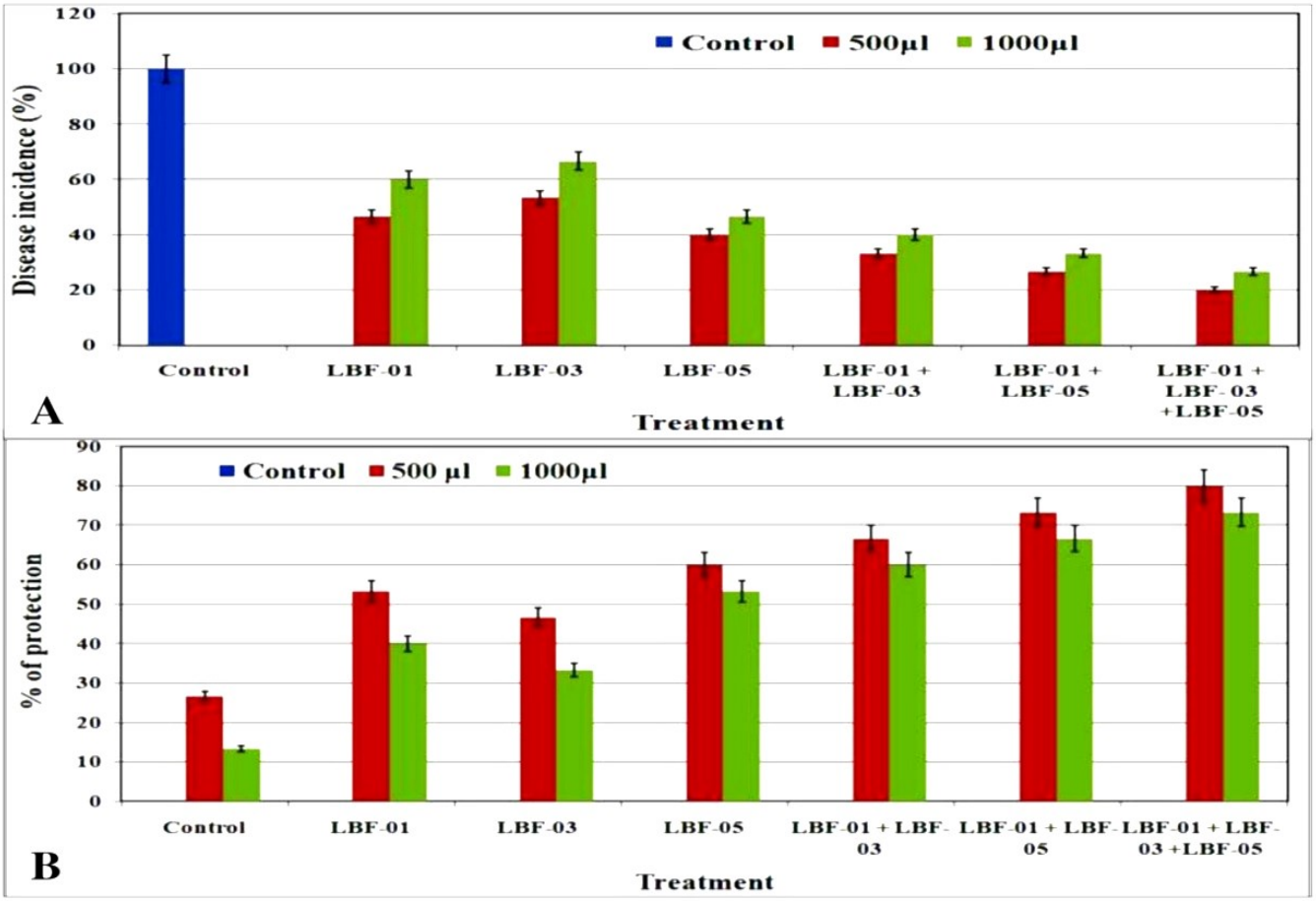

Fig. 5. Disease incidence by *Fusarium oxysporum* and protection by *Bacillus* sp. LBF-1 on pea plant. (A) Disease incidence of *Fusarium oxysporum*; (B) Protection of disease by *Bacillus* sp. LBF-1 against *Fusarium oxysporum*.

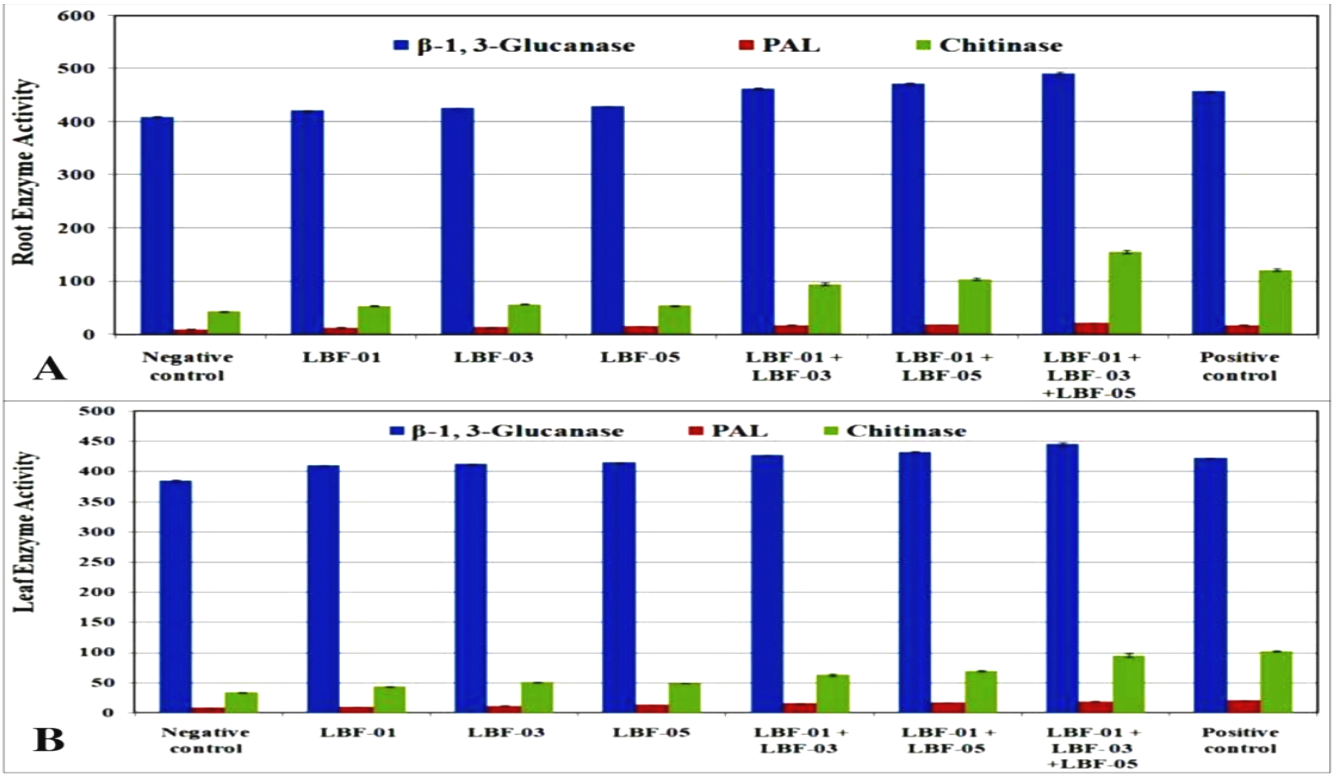

Fig. 6. Defense enzyme activity (A) Root and (B) Leaf. The data are displayed as mean ± standard error. Bar with the same letters indicate no significant differences according to DMRT (p = 0.05).

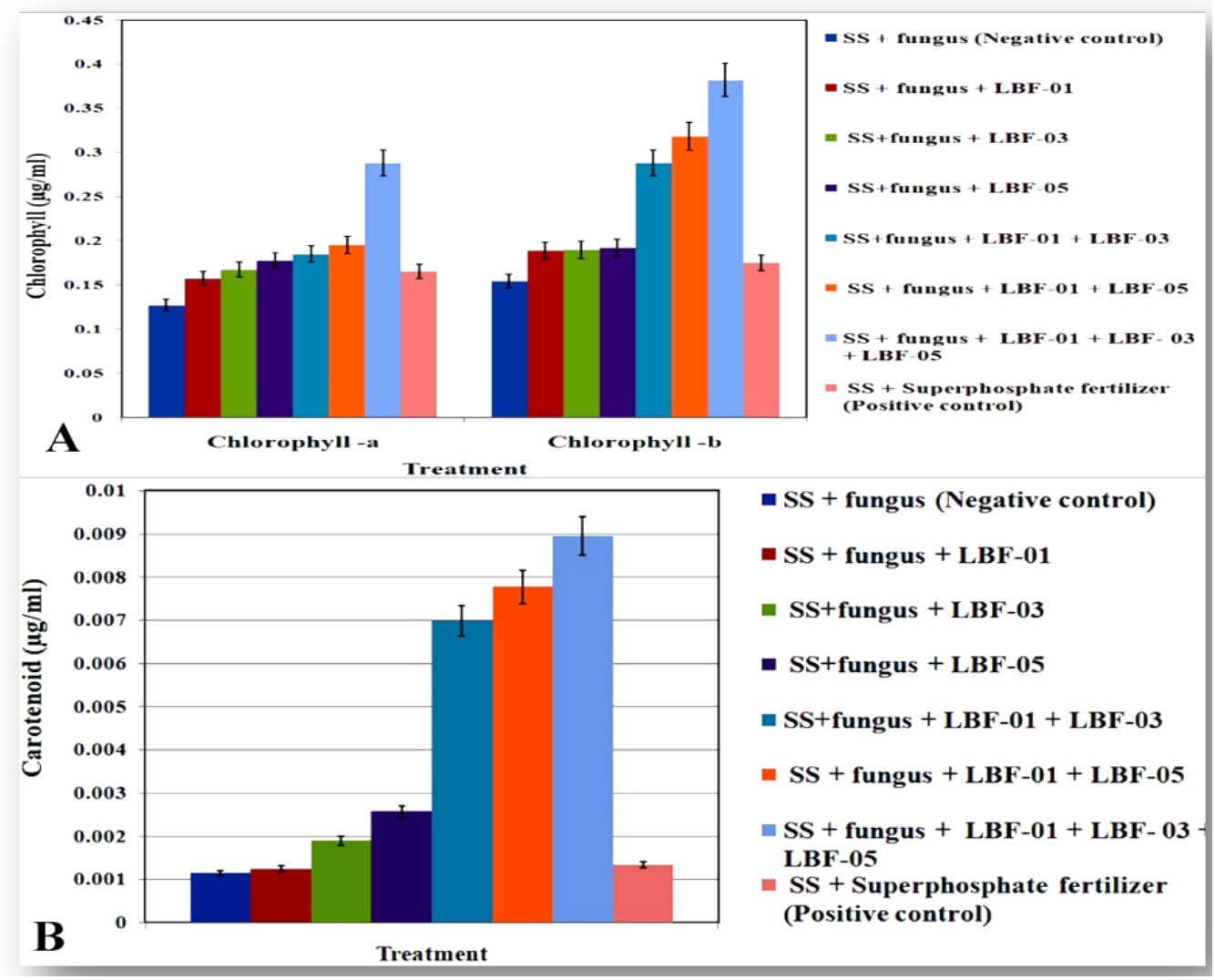

Fig. 7. Photosynthetic pigment (A) Chlorophyll and (B) Carotenoids pigments of tomato plants.

Host Phytochemicals

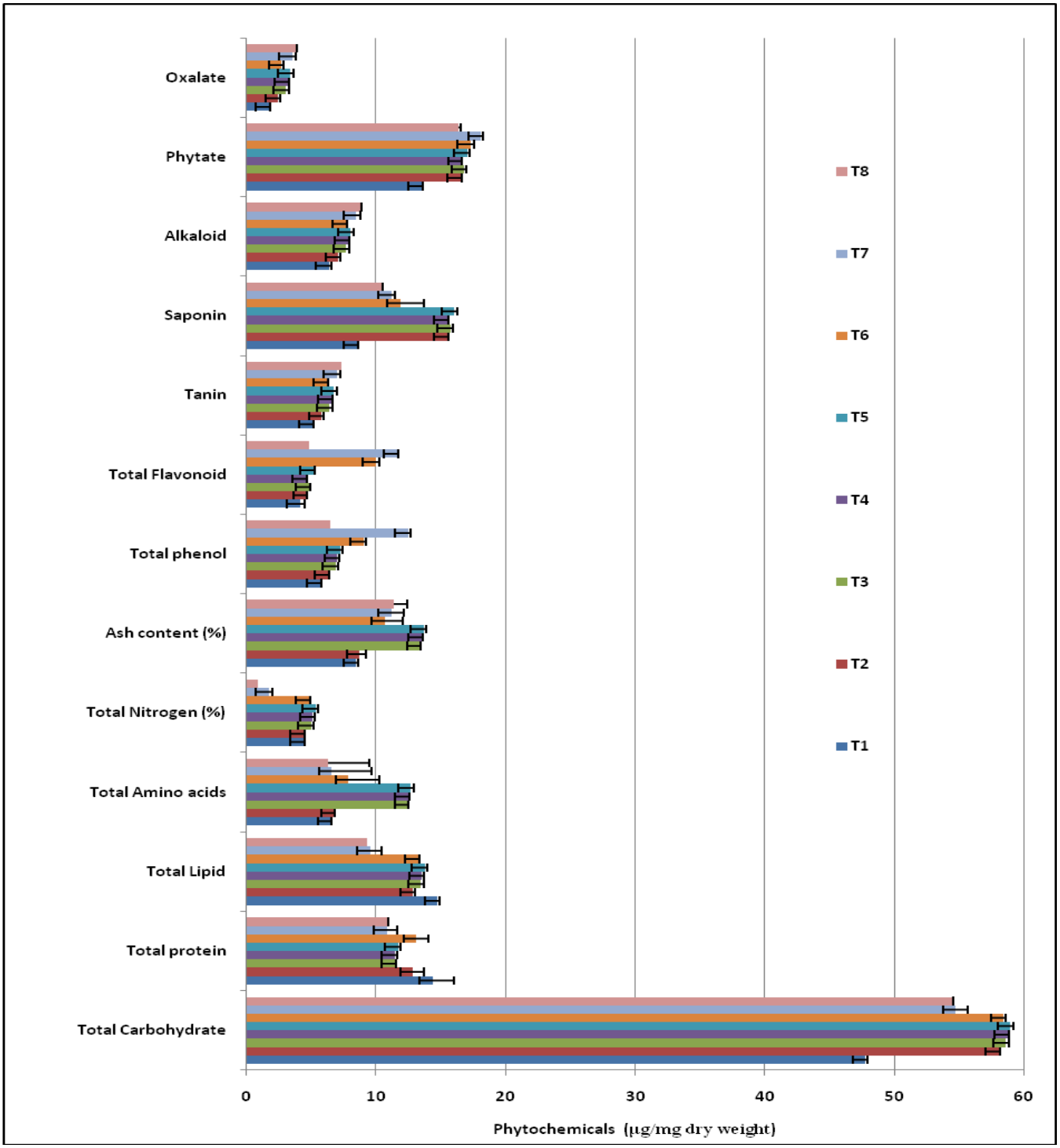

Fig. 8. Phytochemicals of the Pea plants under eight (T1-T8) treatment conditions with *Bacillus* sp. LBF-01, LBF-03 and LBF-05

The variations in chemical regime in pea, *P. sativum* plants under 8 (T1-T8) treatment conditions with *Bacillus sp*. (LBF-01, LBF- 03 and LBF- 05) were presented in Fig. 1. The chemical profile of pea plants was varied significantly ($F_{7,16}$ ≥2.598; P ≤ 0.048) due to different treatments (T1-T8). Total carbohydrate and lipid content were varied with high significant value ($F_{7,16}$≥ 33.787; P < 0.001) whereas, protein and amino acid contents were with lower significant value ($F_{7,16}$≥ 2.598; P ≤ 0.048) in the treatments (Fig. 1). Among the SMs, total phenol, flavonoidand phytate content in the treatments were varied with higher significant ($F_{7,16}$≥ 71.248; P<0.001) values in the treatments (T1-T8) (Fig. 1). The other SMs (tannin, saponin, alkaloid and oxalate) were also varied with lower significant ($F_{7,16}$≥14.690; P<0.001) values in the treatments (T1-T8) (Fig. 1). The overall photochemical changes due to different treatments can be arranged in the order of T7> T6> T5> T4> T3>T2 > T1 and T8 varied with in T1 to T3 range (Fig. 1). Among the PMs only carbohydrate contents were positively correlated (r=0.225, P=229) whereas, excluding saponin (r=-0.184, P=389) all SMS were positively correlated with the treatments (Table 2).

Discussion

The present study evaluated 3 phyllobacterial strains isolated from the inflorescence of *Mangifera indica* L. for their biocontrol agents and growth-promoting characteristics *in vitro.* PGPR activity was demonstrated by 3

Table 2. Correlation between the treatments and specific phytochemicals in pair wise comparison along with paired t-test under eight (T1-T8) treatment conditions with Bacillus sp. LBF-01, LBF-03 and LBF-05.

Pair	Comparisons	N	Correlation	Sig.	t	*df*	Sig. (2-tailed)
1	Treatment &carbohydrate	24	0.225	0.290	-64.501	23	<0.001
2	Treatment & protein	24	-0.501	0.013	-10.712	23	<0.001
3	Treatment & lipid	24	-0.771	0.000	-9.693	23	<0.001
4	Treatment & amino acids	24	-0.116	0.590	-4.607	23	<0.001
5	Treatment &nitrogen	24	-0.655	0.001	0.731	23	0.472
6	Treatment & ash content	24	0.300	0.154	-12.415	23	<0.001
7	Treatment & phenolics	24	0.569	0.004	-7.406	23	<0.001
8	Treatment &flavonoid	24	0.561	0.004	-3.439	23	0.002
9	Treatment & tannin	24	0.817	0.000	-5.059	23	<0.001
10	Treatment & saponin	24	-0.184	0.389	-10.262	23	<0.001
11	Treatment & alkaloid	24	0.870	0.000	-9.234	23	<0.001
12	Treatment & phytate	24	0.643	0.001	-32.686	23	<0.001
13	Treatment & oxalate	24	0.779	0.000	4.014	23	0.001

isolates, identified as LBF- 01, LBF- 03 and LBF- 05 and these isolates were selected for further *in vivo* testing based on their positive results in all tests. All 3 bacterial isolates were identified as *Bacillus* sp. by morphological examination and 16S r DNA sequencing. Phylogenetic closeness tree constructed from 16S r DNA sequence analysis using neighbor-joining methods for strain LBF- 01, LBF- 03 and LBF- 05 further confirmed that this isolate is phylogenetically related to other *Bacillus* sp. (Fig. 2). *Bacillus amyloliquefaciens* has been widely studied and used in agriculture as a phytopathogen inhibitor as a result of its inhibitory effects against different phytopathogens (42, 43). It was reported that *B. velazensis* is a potent biocontrol agent that can fight fungi and promote plant growth against *Ralstonia solanacearum* and *F. oxysporum* (44). In this context, a significant result obtained in the present study exhibited that applied to pea root rhizospheres in the field, *Bacillus* sp. LBF- 01, LBF- 03 and LBF- 05 reduced root rot disease of pea by *Fusarium* sp. A key element for plant growth and productivity of agricultural crops is phosphorus, which is also the least available nutrient in soils. PGP microorganisms increase soil phosphorus availability by releasing insoluble and fixed forms of phosphorus (45). The studies established that *Bacillus* sp. LBF- 01, LBF- 03 and LBF - 05 are capable of liberating phosphate from their fixed forms for direct utilization by plants. As a result of its ability to solubilize mineral phosphate, it demonstrated a good solubilizing efficiency, indicating its capability to increase plant growth. We have not yet found any phyllospheric bacteria capable of phosphorus solubilization. *Bacillus* sp. LBF- 01, LBF- 03 and LBF- 05 are phyllospheric bacteria that have phosphorus solubilizing abilities.

In agriculture, microorganisms are one of the most important candidates for producing IAAs, which serves as a good source of fertilizers (46). They are the chief producers and enhancers of IAA, which also increases root surface area through soil-based nutrients (47). There have been numerous reports of *Bacillus* sp. producing significant amounts of IAA *in vitro*. In contrast to the above observation, the production of IAA by *Bacillus* sp. LBF- 01, LBF- 03 and LBF- 05 was similar. PGPR produced IAA in response to culture conditions, growth stage and substrate capability. The presence of siderophores producing bacteria significantly impacts the availability of various metals to plants, including Fe, Zn and Cu as reported earlier (48). The production of siderophores directly influences the biosynthesis of numerous antimicrobial compounds, which suppress the growth of pathogenic microbes. Specifically, *F. oxysporum* and *Rhizoctonia solani* act as stress factors that make the host immune. Our studies revealed that *Bacillus* sp. LBF-1, LBF-03 and LBF-05 produces siderophore in a similar fashion to other isolates of plant growth promoting bacteria, confirming this bacteria's ability to produce antimicrobial compounds and enhance plant growth. Crop plants need nitrogen in higher amounts than any other essential element to perform various cellular functions and increase crop yields. Microorganisms associated with Phyllospheric, Rhizospheric and Endophytic plants are advantageous to plants due to their production of ammonia (NH_3), which is an essential fertilizer for plant growth. Microorganisms that fix nitrogen in crop fields or release NH3 for plants play a crucial role in plant nutrition (44). The rhizospheric and endophytic strains of *Pseudomonas* sp. and *Bacillus* sp. produce NH_3 in different environments, which can promote plant growth (43). A few reports, however, indicate that phyllospheric bacteria produce NH_3. According to our studies, *Bacillus* spp. LBF- 01, LBF- 03 and LBF- 05 is a significant producer of NH_3, suggesting secondary functions as a source of biofertilizer. The production of extracellular enzymes by microorganisms that degrade cell walls is an important mechanism by which microorganisms inhibit phytopathogens. Enzymes disrupt the structure of the target pathogen's walls, indirectly promoting the growth of the host plant. The cell wall matrix is composed of 3 main components: chitin, glucans and proteins. A fungus' cell wall is rigid and morphologically defined by glucans and chitin polymers. Certain proteins play an essential role in the integrity of cell walls, such as

enzymes that catalyze cell wall synthesis and lysis and structural proteins. As a result of this destruction, fungal hyphae usually lose their biological function and change in morphology. It was previously reported that *B. velezensis* TXJ2-6 lysed *Colletotrichum fructicola* hyphae from the suppressed edges of the fungus colony (48). As shown in this study, *Bacillus* sp. LBF- 01, LBF- 03 and LBF- 05 possessed high levels of chitinase, cellulase, amylase and protease. Therefore, *Bacillus* sp. LBF- 01, LBF- 03 and LBF- 05 have the potential to degrade chitin, protein, starch and cellulose of the fungal hyphae. Besides improving the growth of plants, all the trains produced ά-amylase, which plays a crucial role in destroying the cell walls of oomycetes such as *Phytophthora* sp. and *Pythium* sp. *Bacillus* sp. produce proteases and chitinases that degrade fungal cell walls (24). This hydrolytic enzyme production may also be utilized for producing industrial enzymes. Seed bacterization of pea with strain LBF- 01, LBF- 03 and LBF- 05 strains in individual and consortium significantly increased the rate of the seed germination and vigour index in comparison to the untreated control (Fig. 3 A & B). Pea seeds treated with individual strain LBF- 01, LBF- 03 and LBF- 05 significantly increased the rate of seed germination and % of vigour index as compared with the negative control. All the plant growth parameters were positively correlated with each other (Pearson correlation; r≥0.726). The 2 strains consortium application showed that the rate of seed germination and % of vigour index were also enhanced in compare to individual strain treated seeds and negative control seeds. Similarly, 3 strain consortium treated seeds showed the highest rate of seed germination and % of vigour index than all others treated parameters. PGPR indirectly enhanced seed germination and vigour index by reducing the incidence of seed phytopathogens (49). *Bacillus* sp. LBF- 01 also enhanced seed germination of tomato and chili and vigour index by reducing the incidence of against *Fusarium oxysporum* (24).

In the present study, there was significant increase in plant growth parameters in seedlings applied with *Bacillus* sp. LBF- 01, LBF- 03 and LBF- 05 in different treatment conditions with comparison to negative control and positive control. Application of *Bacillus* sp. LBF- 05 showed that shoot and root length increase 65.30% and 52%, shoot fresh weight and root fresh weight increase 49.31% and 67.33 %, shoot biomass and root biomass increase 50% and 39.47% which is highest among the single strains (LBF- 01and LBF-03) and negative control.

Among the application of 2 strain consortium, the highest shoot and root length increase 114.28% and 84%, shoot fresh weight and root fresh weight increase 86.29% and 94.22%, shoot biomass and root biomass increase 61.11% and 33.33% by the strain LBF-01 + LBF-05 consortium as compare to control and other consortium. Application of 3 strain consortium showed that shoot and root length increase 153.06% and 126.68%, shoot fresh weight and root fresh weight increase 103.41% and 227%, shoot biomass and root biomass increase 141.66% and 516.30% which is highest among the treated and negative control. Enhancement of plant growth by root-colonizing *Bacillus* sp. is well documented (24, 35). For example, *Bacillus velezensis* strain FZB42 produces indole-3-acetic acid (IAA) (36). *Bacillus velezensis* and *Bacillus megaterium* also produce cytokinin (36). Some beneficial microorganisms produce gibberellin or jasmonic acid. These growth regulators directly increase plant growth. The earlier study reported that *Bacillus velezensis* strain BAC03 has antimicrobial (28) and biological control activities in greenhouse and field conditions (35). It also displayed potential growth promotion ability during pathogen exposure (24). To utilize this bacterium for enhancing plant growth to get a better result, it is necessary to evaluate LBF-1 for plant growth activity and determine the optimal strategies of LBF1 application for plant growth promotion. A comparison of defense enzyme activity between leaf and root of pea plants under different treatment conditions is shown in Fig. 5. All the data related with enzyme activity were differed significantly within the treatments for both leaf as well as root system (one-way ANOVA, $F_{7,16} \geq 49.890$, $P<0.001$). All the plant growth parameters were positively correlated with different strains (Pearson correlation; r≥0.592) as well as within the treatments (Pearson correlation; r≥0.539) due to different enzyme activities. In present study, pea plant treated with *Bacillu*s sp. LBF- 01, LBF- 03 and LBF- 05 in various combinations suppressed the *Fusarium* sp. and promotes the growth due higher level of defense enzyme production and their distribution in root and leaves with significant differences among the treatments (one-way ANOVA, $F_{7,16} > 47.236$, $P<0.001$). The PAL enzyme also attacked the cell wall polymers components and cause degradation of fungal hyphae. *Bacillus* sp. LBF-01 treated plants significantly enhanced total chlorophyll content of leaves by 77.78% (chlorophyll a by 58.22% and chlorophyll b by 95%) over untreated control plants. Chlorophyll biosynthesis has been considered as an indicator of net physiologically available iron to the plant. Higher absorption of iron is correlated with higher contents of chlorophyll a, chlorophyll b and total chlorophyll (35). The increase in chlorophyll content could be due to the utilization of microbial siderophore by the plants. Similarly, the application of strain LBF-01 increases the amount of carotenoid in treated plant over the control.

Biological control using antifungal antibiotic compound-producing microorganisms against plant diseases offers a powerful alternative to the use of synthetic chemicals that are hazardous to humans and environment (24). *Bacillus* sp. LBF-1 had selected as a most potent antifungal activity against *Fusarium* sp. and other pathogenic fungi. The crude compounds extracted from bacterial isolate inhibit mycelia growth in *in vitro* and also suppress in field conditions. In this study, *Bacillus* sp. LBF-01 had significantly higher antifungal activity due to defensive enzyme production as well as significantly higher plant growth parameters under different treatments. All the plant growth parameters and the treatments were positively correlated with each other due to respective enzyme activities. The qualitative and quantitative alterations of different phytochemicals (PMs and SMs) including elevation of different oxidative enzymes in response to stresses is a general phenomenon (50). The complex mixture of other SMs in many plants may provide

effects in defense against a range of different stresses (40). In the present study, chemical profile of pea plants was varied significantly ($F_{7,16}$≥2.598; P≤0.048) due to different treatments (T1-T8). Mainly, the SMs in different treatments were varied significantly ($F_{7,16}$≥14.690; P<0.001). Thus, overall photochemical changes due to different treatments can be arranged in the order of T7> T6> T5> T4> T3> T2 > T1 and T8 varied with in T1 to T3 range. Among the Pms, only carbohydrate contents were positively correlated (r=0.225, P=229) whereas, excluding saponin (r=-0.184, P=389) all SMS were positively correlated with the treatments. Thus, the treatment T7 will be the best defensive one among all the treatments again such pathogens for their sustainable alternative management in future.

Conclusion

In the present study, 55 plant growth-promoting phyllobacteria were isolated from the mango flowers. Among them, 3 phyllobacterial isolates were found to effectively promote the growth of pea seedlings and consistent PGPR identified. The consortium of these PGPR performed better than when used singly. Moreover, the consortium treatments of these phyllobacterial isolates were found to activate the defense mechanisms in pea seedlings through the induction of Glucanase, chitinase and PAL activities. The increase in chlorophyll content could be due to the utilization of microbial siderophore by the plants. The pea seedlings inoculated with the microbial consortium showed significantly improved growth as compared to uninoculated seedlings. Based on the various growth and microbiological parameters studied including phytochemical regimes, it was concluded that inoculation with microbial consortium is beneficial for raising healthy and vigorously growing pea seedlings under greenhouse condition.

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Authors contributions

SKC designed, carried out the experimental works and wrote the paper. NR conducted experiment, data analysis and editing of the manuscript, MB wrote the paper and data analysis, DB phytochemical analysis and wrote the paper.

Compliance with ethical standards

Conflict of interest : Authors do not have any conflict of interests to other than publication of this MS.

Ethical issues : None

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