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Evaluation of the PGPR Potential of *Bacillus* sp. Isolated from Sandy Loam Soil on the Growth of *Brassica juncea* L.

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ABSTRACT

In this study, three bacterial strains–*Bacillus cereus* (C1, Accession no-ON787613), *Bacillus subtilis* (C2, Accession no-ON787623) and *Brevibacillus borstelensis* (C3, Accession no-ON720969) were isolated from distinct agricultural soils and characterized through molecular techniques. These strains were tested for their potential as plant growth-promoting rhizobacteria (PGPR) in a pot experiment with *Brassica juncea* to evaluate their impact on soil properties and plant growth. The experiment aimed at assessing changes in soil parameters, such as total organic carbon (TOC), pH, water-holding capacity (WHC) and nutrient availability after bacterial inoculation. The results demonstrated that all three strains significantly improved soil health, as indicated by increased TOC, elevated pH, enhanced WHC and improved macronutrient and micronutrient availability. Furthermore, the inoculated plants exhibited higher growth as reflected by increased plant height and dry biomass. When combined with NPK fertilizers, the bacterial isolates further boosted soil and plant performance. These findings underscore the potential of *Bacillus cereus*, *Bacillus subtilis* and *Brevibacillus borstelensis* as effective bio-inoculants for sustainable agriculture.

Key words: *Bacillus, Brassica juncea,* mustard, phosphate solubilization, rhizobacteria

INTRODUCTION

Plant host diverse microbial communities play crucial role in plant growth and health during different biotic and abiotic stresses. The bacterial species present at the plant rhizosphere modulates the plant growth referred as plant growth promoting bacteria (PGPR; Kumar *et al*., 2016). Rhizosphere, a thin zone of plant root which adheres with the soil surface, is considered as hot spot of the plant microbe interaction due to secretion of the root exudates (Wang *et al*., 2023; Solanki *et al*., 2024). Root exudates are nutrient reach photosynthetic or carbon rich compounds which act as energy source or the signalling molecules. Root exudates are constituted of different biochemical compounds such as carbohydrates, lipids, amino acids, flavonoids, etc. These molecules act as signalling molecules for the microbial species and each of the microbial species act have some specific signalling compounds (Lareen *et al*., 2016; Kumar *et al*., 2024).

To promote plant development, PGPR employs both direct and indirect processes. These processes are engaged during growth. Ammonia generation, phosphate solubilization, nitrogen fixation, nutrient absorption improvement, and phytohormone production are some plant growth promoting attributes that affect or modulate the plant growth and plant development (Kumar, 2022). However, to identify plant growth-promoting rhizobacteria and to investigate plant growth promoting activities there are variety of activities aimed at stimulating plant development, while also demonstrating bioremediation potentials by detoxifying contaminants such as heavy metals and pesticides and acting as biopesticides against a variety of phytopathogens. Although the productive efficiency of a certain PGPR may be improved even further by optimizing and

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acclimating it to the local soil conditions (Oleñska *et al*., 2020; Devi *et al*., 2023). In laboratory, greenhouse and field studies, the impact of PGPR on crop production varies. While soil is an unpredictable environment, achieving the desired outcome might be challenging at times. The study's requirement to identify climatic fluctuations has a significant influence on PGPR efficiency yet growing adverse circumstances in the environment are to be anticipated as part of agriculture's usual operation (Etesami and Maheshwari, 2018). However, to evaluate the efficacy of bacterial species various plant growth promoting traits like phosphate solubilization, dinitrogen fixation, ACC deaminase activity, antimicrobial properties, siderophore and IAA biosynthesis have been studied.

Mustard is a significant Indian oilseed widely grown in the various states of India such as Rajasthan, Punjab, Uttar Pradesh, West Bengal and Madhya Pradesh (Singh and Bansal, 2020). Mustard is the second most important oilseed crop in India, with a production of 7.6 million tonnes and the most common mustard type cultivated in India is *Brassica juncea*, often known as Indian or Oriental mustard. In the previous studies, various authors had used different plant growth promoting bacteria to enhance the growth of *Brassica juncea* and for the mitigation of environmental contaminants. For example Meena *et al*. (2020) evaluated the potential of *Pseudomonas* sp. and *Bacillus megaterium* strains for the enhancement of *B. juncea* growth and remediation of Ni. Similarly, Khatoon *et al*. (2024) reported strains like *Variovorax paradoxus*, *Rhodococcus* sp. and *Flavobacterium* sp. and their impact on the growth of mustard plant.

In this study, the plant growth promoting potential of three different bacterial strains have been evaluated and also studied their inoculation impact on the different soil properties.

MATERIALS AND METHODS

The soil samples were collected from transgangatic agroclimatic zone of Rajasthan and Haryana, India's dry regions (Table 1). The bacterial strains were isolated on soil extract agar media and standard biochemical techniques were used to characterize the bacterial strains as per standard protocols (Lareen *et al*., 2016). However, the molecular characterization of the bacterial strains was carried out by 16S rDNA partial gene sequencing analysis. Bacterial cultures were stored at 4°C on the appropriate slants medium until needed.

Table 1. Soil samples used for isolation of *Bacillus* depicting crop grown at sites (locations)

S. No.	Location	District	Crop
1.	Experimental farm CZRI	Bikaner	Guar
2.	Badopal	Fatehabad	Wheat
3.	Raipur	Fatehabad	Mustard

Bacillus cultures were grown on their respective medium for 48 h at 30°C with 100 and 200 mg/ml L-tryptophan. Fully grown cultures were centrifuged at 8000 rpm for 10 min. Four ml of the Salkowski reagent and two drops of orthophosphoric acid were added to the supernatant (2 ml). One ml of 0.5 M $FeCl_3$ in 50 ml of 35% perchloric acid was added and incubated in the dark for one hour. The development of the pink colour indicated IAA production (Rana *et al*., 2020).

Siderophore production by the bacterial strains was addressed using universal chrome azurol S (CAS) agar plate method. *Bacillus* isolates were cultivated on soil extract agar plates, with the help of a cork borer. A CAS agar plate was punched to create a hole and then the collected supernatant (10/µl) from each culture was taken onto the hole. After five days, a yellow halo ring produced surrounding the punched surface area indicated the excretion of siderophore in the taken supernatant. The size of the discoloured halo zones was recorded for each of the isolates (Sultana *et al*., 2021).

For phosphate solubilization by bacteria, *Bacillus* isolates were cultivated on soil extract agar plates, and sterile tooth pricks were used to make spots on the Pikovskaya medium (Sanchez-Gonzalez *et al*., 2022). At 30°C, the plates were incubated. After seven days, the colonies were examined for clear zones. The clear zone and colony diameters were also measured.

The experiment was conducted at Guru Jambheshwar University's Department of Bio and Nano Technology, Hisar, Haryana. Lat Lon = 29.1691991°N 75.7390324°E. The experiment utilized mustard seeds cultivar RH 749 variety taken from Directorate of Research, CCS HAU,

Hisar, Haryana sown on earthen pots filled with sandy soils. The treatments were split into five groups (Table 2). There were four baked clay pots in each category. Each pot was filled with 9 kg of soil. At the bottom of each pot, holes were drilled. Culture 1 was *Bacillus cereus*NS2014B3/4 (c1 isolated from soil sample collected from Experimental Farm CZRI, Bikaner, guar crop), Culture 2 was *Bacillus subtilis*NS2014F8/6 (c2 isolated from soil sample collected from wheat field of Badopal, District Fatehabad), and Culture 3 was *Brevibacillus borstelensis*NS2014F12/8 (c3 isolated from soil sample collected from mustard field of Raipur, District Fatehabad) strains were cultured on soil extract agar medium and kept on soil extract agar slants to make the inoculum for the pots. The seeds were treated with three different isolates as well as added fertilizer. In each category, one pot was used as a control, with no bacterial strain applied. The seeds were planted at a depth of 5 cm. Group 1 received each isolate, group 2 received all isolates and urea (50 ppm solution), group 3 received all isolates and phosphorus (50 ppm solution), group 4 received isolates and potassium (50 ppm solution) and group 5 received isolates, urea and phosphorus and potassium fertilizer. The pots were kept in the plant development room until all of the seeds germinated. Each pot's soil properties were examined. Seven ml phosphorus, 10 ml potassium and 10 ml urea were added to the pots at the start of the experiment.

The soil was collected after 30 and 90 days of cultivation in two seasons and evaluated for the following properties.

For water holding capacity of soil: A clean funnel was used to compute it. It was put in a measuring glass tube with its mouth closed with a cotton stopper. 100 g of soil was weighed and mixed with 100 ml of distilled water. The quantity of water collected in the tube was measured after 10 min to determine the amount of water absorbed by the soil. The water holding capacity (WHC) was calculated using the formula below.

WHC = Total amount of water – Amount of water retained in the glass tube

To evaluate total organic carbon standard protocol of Wet Digestion Method was followed (Benbi, 2018). In a 500 ml flask, 2 g soil was taken. Twenty ml of concentrated H_2SO_4 along with 10 rml of 1 Nr potassiumrdichromate were added. After that, the solution was swirled two to three times and allowed to stand for 30 min. Two hundred ml distilled water was used to dilute the suspension. 0.5 g NaF was added with 1 ml of diphenylamine indicator. Ardeep violet colour appeared. Titration was done with 0.5 N ferrous ammonium sulphate till the colour turned from violet to blue and finally to light green. The volume of ferrous ammonium sulphate used during titration was noted. Blank titration (without soil) was also done as above. A known amount of soil was combined with an

Table 2. Various treatments in five experimental groups

S. No.	Experimental group	Treatment
1.	Group 1	Control
		Soil + c1
		Soil + c2
		Soil + c3
2.	Group 2	Control set for nitrogen (50 ppm)
		Soil + urea + c1
		Soil + urea + c2
		Soil + urea + c3
3.	Group 3	Control set for phosphorus (50 ppm)
		Soil + P + c1
		Soil + P + c2
		Soil + P + c3
4.	Group 4	Control set for potassium (50 ppm)
		Soil + K + c1
		Soil + K + c2
		Soil + K + c3
5.	Group 5	Control with nitrogen, phosphorus and potassium (50 ppm)
		Soil + N + P + K + c1
		Soil + N+ P + K + c2
		Soil + N+ P + K+ c3

excess of alkaline $KMnO_4$ solution and then distilled. In the presence of NaOH, $KMnO_4$ releases nascent oxygen, which causes organic matter condensation and oxidation of released ammonia, which was absorbed in standard acid. The excess content was measured with standard alkali and methyl red as an indicator.

To evaluate phosphorus availability, soil was extracted with 0.5M $NaHCO_3$ (pH 8.3) (Khan *et al*., 2018). In 100 ml wide-mouth plastic bottle or glass bottle, 2 g of soil was taken and a pinch of Dacro G-60 along with 40 ml of 0.5 Ma $NaHCO_3$ solution was added. Flask was shaked for 30 min on shaker. Suspension was filtered through Whatman No.1 filter paper. Ten ml of the filtrate was added to a 25 ml volumetric flask and 5 ml of ammonium-molybdate solution. CO_2 evolved was removed by shaking. When frothing ceased, distilled water was added, washing downsides, and the net volume was brought to about 20 ml. One ml of freshly diluted $SnCl_2$ solution was added. The final volume was made using distilled water. The flask was mixed. Colour intensity (blue) was measured at a wavelength of 660 rnm using a spectrophotometer. Blank was run with all reagents except soil.

Potassium was extracted with neutral ammonium acetate. The solution's potassium content was calculated using a flame photometer (Ramamoorthi and Meena, 2018). One hundred ml flask containing 5 g soil was taken. Twenty-five ml of $NaNH_4OAC$ was added to it. The solution was properly mixed for 5 min and filtered. Potassium concentration was measured in filtrate with a flame photometer. The available micronutrient level was measured following Datta *et al*. (2018). The extractant had a pH of 7.3 and contained 0.1 M triethanolamine, 0.005 M DTPA (diethylenetri-aminepentaacetic acid), and 0.01 M $CaCl_2$. Shaking 10 g of air-dry soil with 20 ml of extractant for 2 h was the soil test. The leachate was filtered, and the filtrate was tested for Fe, Zn, Cu and Mn with the help of Atomic Absorption Spectrophotometer (AAS).

The serial dilution technique was used to count and isolate bacterial cells. Soil samples (stock) were taken, and dilutions were made in test tubes to bring down a dense culture of cells to a more utilizable concentration. The number of bacteria was counted in a diluted sample based on the number of colonies developed on soil extract medium plates. However, for the enumeration of microbial load, standard plate count method was used. One g of triplet soil samples was weighed and mixed properly in a Petri plate. One g of soil from this mixture (3 g) was taken and mixed in test tubes containing 9 ml blanks under aseptic conditions. The test tube (10^{-1} dilution) was kept in a water bath for 10 min at 80°C. Then 1 ml of this 10^{-1} dilution was transferred to the 2^{nd} tube containing 9 ml blank as 10^{-2} dilution. One ml from 10^{-2} dilution was taken and transferred to the 3^{rd} tube with 9 ml blank as 10^{-3} dilution. Similarly, dilutions for other soil samples were prepared. 0.1 ml of all the three dilutions was poured on soil extract Petri plates. Petri plates were incubated for 24-48 h at 33°C. All the steps were performed in a laminar airflow chamber. The number of bacterial colonies was counted using a colony counter. The pH of soil and water was determined by potential measurement using a glass electrode (Bargrizan *et al*., 2018). In brief, saturated soil paste was made by mixing 100 g of soil with 100 ml double distilled water. The mixture was allowed to rest for half an hour, and the pH was measured with the help of pH meter.

All the experiments were carried out in replicates and analysis of variance (ANOVA) was used to analyse the significant differences among the different parameters. The means were compared through LSD test at 5% significance level using SPSS version 26.0. The data were mentioned as Mean ± S.D.

RESULTS AND DISCUSSION

During the isolation process, three bacterial strains were isolated and confirmed for their identity on the basis of molecular characterization. Further the gene sequence to NCBI database was submitted and got the accession number as *Bacillus cereus* (c1), Accession no-ON787613; *Bacillus subtilis* (c2) Accession no-ON787623 and *Brevibacillus borstelensis* (c3) Accession no-ON720969. Culture 1 was *Bacillus cereus* NS2014B3/4 (c1 isolated from soil sample collected from Experimental Farm CZRI, Bikaner, Guar crop), Culture 2 was *Bacillus subtilis* (c2 isolated from soil sample collected from wheat field of Badopal, District Fatehabad) and Culture 3 was *Brevibacillus borstelensis* (c3 isolated from soil sample collected from mustard field, Raipur,

District Fatehabad). After characterization, these bacterial strains were inoculated in the *Brassica juncea* and evaluated for their efficacy in changing soil parameters such as total organic carbon, pH, water holding capacity, macronutrient availability, micronutrient availability in different groups1 to 5.

Table 3 shows the nutritional viability of each soil group which was treated using four different procedures. The mixture of (Seed + NPK + c3) had the maximum nutritional value, whereas group 2 Seed + Nitrogen treatment had the lowest nutrient value (0.135). During the 30 days observation, the highest nitrogen nutrient availability was seen in the fifth group (Seed + NPK + c3), and the lowest was found in the fourth group (Seed + K). Phosphorus availabilities were highest (87.7) in the third group (Seed + P + cl) and lowest in the fourth group (Seed + K), whereas the potassium availability was highest in the fifth group (Seed + NPK + c3) and minimum in the first group (Seed + c1). The zinc availability was minimum in the third group (Seed + P + cl) and maximum in the fourth group (Seed + K + c2). Copper availabilities were higher in the first group (Seed + c3) and lower in the second group (Seed + N). The maximum manganese nutrient content was found in the fifth group (seed + NPK + c3) and the minimum in second group (Seed + N + c3). The presence of iron was highest in the fifth group (Seed + NPK + c3) and lowest in the third group (Seed + P).

The highest nutrient value (486.5) was observed in group 4, the combination of Seed + K + *Bacillus subtilis* (c2), whereas the lowest nutrient value was recorded from group 1 control treated with seed and *Bacillus cereus* (c1). On observation of 90 days, the nutrient values were found to be different as compared to 30 days (Table 4). The nitrogen availability (131.015) was highest in the group 5 the combination of Seed +NPK + *Brevibacillus borstelensis* (c3) and lowest in the group 5 control treated with Seed + NPK. The availabilities of phosphorus were highest (42.175) in the group 3, the combination of Seed + P + *Bacillus subtilis* (c2) and lowest in the group 1, Seed + *Bacillus cereus* (c1) and group 4, seed + K + *Bacillus cereus* (c1). The potassium availability was maximum (486.5) in the group 4, Seed + K + *Bacillus subtilis* (c2) and minimum in the group 5, Seed + NPK + *Bacillus subtilis* c2). The zinc availability was lowest in the group 3, Seed + P + *Brevibacillus borstelensis* (c3) and maximum (0.6389) in the group 1 Seed + *Brevibacillus borstelensis* (c3). Copper availabilities were higher (0.2125) in the group 2, Seed + N + *Brevibacillus borstelensis* (c3) and lower in the group 1, Seed + *Bacillus cereus* (cl). The maximum manganese nutrient content (5.217) was found in the group 1, Seed + *Brevibacillus borstelensis* (c3) and the minimum in the group 3, Seed + P. The presence of iron was highest (1.0775) in the group 5, Seed + NPK + *Brevibacillus borstelensis* (c3) and lowest in the group 3, Seed + P (the same as at 30 days).

Each treatment group showed different levels of total organic carbon content after 30 and 90 days (Fig. 1). After 30 days of evaluation, the combination of Seed + N+ *Bacillus cereus* (c1) and *Bacillus subtilis* (c2) almost showed a similar level of total organic carbon in group 2. In group 5, the maximum level of TOC was observed in the pot treated with Seed+ NPK + *Bacillus cereus* (c1). On comparing all the five groups, the highest level of TOC was found in group 5, which was treated with Seed + NPK and *Bacillus cereus* (c1) isolate.

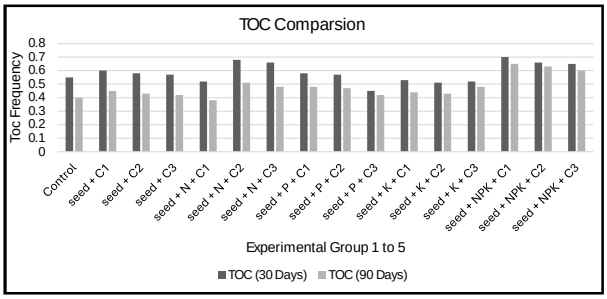


Fig. 1. Total organic carbon (TOC %) in the soil after 30 and 90 days.

After 90 days evaluation, the combination of Seed + N + *Bacillus subtilis* (c2) and *Brevibacillus borstelensis* (c3) almost showed a similar level of total organic compound in group 2. In group 5, the maximum level of TOC was observed in the pot treated with Seed + NPK + *Bacillus subtilis* (c2). On comparing all the five groups, the highest level of TOC was found in group 5, which was treated with NPK and *Bacillus subtilis* (c2) isolate.

After 30 days, the pH level was found to be highest in group 1 compared to other groups (Fig. 2), which meant that it made the soil more alkaline. In the control group, the maximum pH level was found in the combination of seed + *Brevibacillus borstelensis* (c3). In group 5, the

Table 3. Soil nutrient content in different treatments at 30 days (kg/ha)

S. No.	Experimental group	Treatment	Nitrogen	Phosphorus	Potassium	Zinc	Copper	Manganese	Iron
1.	Group 1	Control	84.00±6.30^{b}	18.65±3.75^{e}	213.00±16.58^{b}	0.40±0.02^{d}	0.18±0.007^{bc}	8.39±0.04^{c}	2.51±0.18^{d}
		Seed + c1	97.98±6.27^{b}	65.20±2.84^{c}	202.00±25.52^{b}	0.65±0.01^{ab}	0.19±0.006^{abc}	9.56±0.17^{a}	3.21±0.13^{c}
		Seed + c2	91.00±6.29^{b}	81.70±4.72^{b}	297.00±46.96^{b}	0.60±0.007^{b}	0.20±0.006^{ab}	9.10±0.10^{b}	4.64±0.13^{a}
		Seed + c3	143.45±4.82^{a}	76.20±3.61^{b}	312.99±61.26^{ab}	312.99±61.26^{ab}	0.21±0.007^{a}	9.58±0.20^{a}	4.83±0.15^{a}
2.	Group 2	Control set for nitrogen	94.5 ±4.72^{e}	15.60±2.51^{c}	249.00±32.44^{a}	0.42±0.020^{b}	0.13±0.003^{ab}	5.90±0.03^{b}	2.18±0.10^{c}
		Seed + N + c1	112±6.30^{de}	33.20±1.08^{c}	225.00±33.99^{a}	0.46±0.01^{b}	0.14±0.007^{a}	6.33±0.10^{bc}	2.99±0.16^{a}
		Seed + N + c2	126.00±6.33^{cd}	56.40±4.53^{a}	314.50±58.36^{a}	0.60±0.02^{a}	0.15±0.009^{a}	6.76±0.08^{a}	2.57±0.14^{bc}
		Seed + N + c3	227.50±7.90^{a}	42.40±1.26^{bc}	285.50±40.92^{a}	0.56±0.03^{a}	0.13±0.004^{ab}	6.17±0.05^{cd}	2.97±0.13^{ab}
3.	Group 3	Control set for phosphorus	70.00 ±6.29^{e}	27.20±3.32^{e}	249.83±37.19^{b}	0.42±0.02^{d}	0.16±0.009^{ab}	7.18±0.08^{e}	2.05±0.12^{c}
		Seed + P + c1	87.48±7.86^{de}	87.66±9.98^{b}	261.16±45.99^{ab}	0.50±0.01^{ab}	0.17±0.006^{ab}	7.84±0.13^{b}	2.38±0.11^{ab}
		Seed + P + c2	104.95±3.24^{cd}	84.68±6.60^{bc}	362.50±75.35^{ab}	0.52±0.01^{a}	0.16±0.003^{ab}	7.70±0.07^{bc}	2.31±0.13^{abc}
		Seed + P + c3	111.95±6.28^{c}	71.40±1.60^{cd}	314.50±58.37^{ab}	0.48±0.01^{abc}	0.17±0.005^{a}	7.41±0.06^{de}	2.56±0.11^{a}
4.	Group 4	Control set for potassium	66.50 ±1.64^{e}	13.00±1.13^{c}	259.00±36.22^{a}	0.48±0.001^{d}	0.14±0.001^{d}	6.65±0.66^{c}	2.32±0.14^{d}
		Seed + K + c1	84.00±3.20^{d}	50.50±4.90^{a}	359.50±85.19^{a}	0.53±0.008^{c}	0.15±0.001^{cd}	7.54±0.07^{ab}	2.46±0.10^{cd}
		Seed + K + c2	98.00±3.23^{c}	49.70±7.97^{ab}	401.00±90.78^{a}	0.59±0.008^{b}	0.16±0.001^{bc}	7.24±0.12^{bc}	2.73±0.16^{abc}
		Seed + K + c3	181.95±6.30^{a}	38.00±4.32^{b}	335.50±64.17^{a}	0.67±0.01^{a}	0.17±0.004^{ab}	7.65±0.07^{ab}	2.46±0.07^{cd}
5.	Group 5	Control set with NPK	87.50 ±2.35^{e}	24.70±1.23^{e}	266.00±91.68^{b}	0.54±0.02^{d}	0.19±0.006^{c}	8.96±0.05^{c}	2.92±0.02^{d}
		Seed + NPK + c1	101.50±4.97^{e}	43.50±2.05^{bc}	362.00±79.16^{ab}	0.62±0.01^{ab}	0.21±0.004^{abc}	9.33±0.14^{bc}	3.34±0.08^{c}
		Seed + NPK + c2	122.50±7.90^{d}	58.70±4.07^{ab}	458.00±122.54^{ab}	0.60±0.01^{abc}	0.21±0.003^{ab}	9.48±0.10^{ab}	4.96±0.12^{a}
		Seed + NPK + c3	231.00±3.23^{a}	61.00±9.69^{ab}	479.00±78.26^{a}	0.64±0.01^{a}	0.20±0.005^{bc}	9.72±0.06^{a}	4.98±0.11^{a}

Table 4. Soil nutrient content in different treatments at 90 days (kg/ha)

S. No.	Experimental group	Control	Nitrogen	Phosphorus	Potassium	Zinc	Copper	Manganese	Iron
1.	Group 1	Seed + c1	54.50±2.48	10.5±1.16	198.5±26.17	0.3274±0.005	0.0845±0.007	3.818±0.01	0.7548±0.05
		Seed + c2	68±5.37	30.3±1.02	223.5±36.44	0.5848±0.01	0.09±0.003	5.129±0.08	1.012±0.01
		Seed + c3	54.5±2.51	38.4±1.92	282.075±38.44	0.5689±0.01	0.094±0.006	4.985±0.07	1.0405±0.02
2.	Group 2	Control set for nitrogen	78.335±7.04	41.17±1.94	321±68.42	0.6389±0.01	0.0905±0.008	5.217±0.10	1.0685±0.03
		Seed + N + c1	64.5±1.19	13.4±1.28	262.5±53.00	0.3217±0.01	0.082±0.008	1.991±0.02	0.725±0.04
		Seed + N + c2	85.35±1.05	24.7±1.23	225.665±28.48	0.3685±0.01	0.094±0.005	2.051±0.01	0.9665±0.02
		Seed + N + c3	60.97±1.27	41±0.65	274.5±40.92	0.5102±0.01	0.0865±0.006	3.544±0.10	0.897±0.04
3.	Group 3	Control set for phosphorus	82±5.40	28.5±1.65	336±67.97	0.4376±0.01	0.2125±0.11	2.032±0.02	0.76±0.1
		Seed + P + c1	57.5±3.86	14.6±0.82	242.5±42.71	0.4134±0.01	0.0805±0.006	1.809±0.01	0.64±0.03
		Seed + P + c2	75±2.32	34.265±2.56	255.5±46.73	0.4399±0.006	0.086±0.006	2.568±0.07	0.8875±0.05
		Seed + P + c3	75±8.52	42.175±2.53	400.83±94.28	0.4545±0.01	0.083±0.007	2.307±0.04	0.7895±0.06
4.	Group 4	Control set for potassium	75±5.70	33.3±2.88	334±70.21	0.491±0.005	0.097±0.005	1.8845±0.03	0.9235±0.02
		Seed + K + c1	61±2.35	10.5±1.23	347±91.68	0.4305±0.02	0.069±0.006	1.993±0.05	0.671±0.02
		Seed + K + c2	64.5±1.14	39.7±1.68	363.5±85.91	0.4737±0.01	0.0885±0.005	2.218±0.05	0.9305±0.05
		Seed + K + c3	68±8.50	27.965±0.52	486.5±128.57	0.5321±0.02	0.0765±0.005	2.126±0.07	0.791±0.05
5.	Group 5	Control set with NPK	103±14.76	43.7±10.61	370±83.18	0.6137±0.01	0.085±0.007	3.41±0.05	0.894±0.05
		Seed + NPK + c1	71.5±3.86	17.7±2.21	354.665±88.40	0.5226±0.01	0.088±0.005	4.047±0.12	1.0075±0.04
		Seed + NPK + c2	81.945±2.28	38.5±3.10	374.835±84.89	0.5743±0.01	0.096±0.008	4.092±0.12	0.9065±0.04
		Seed + NPK + c3	67.85±5.31	55.20±4.59	460.00±121.19	0.5234±0.01	0.089±0.008	4.514±0.13	1.043±0.03
		Control	131.015±21.02	39.3±4.83	461.00±124.77	0.5845±0.01	0.0985±0.007	4.191±0.06	1.0775±0.03

maximum level of pH was observed in the pot treated with seed + NPK + *Brevibacillus borstelensis* (c3). After 90 days of assessment, the level of pH was found to be highest in group 1 as compared to other groups. In the control group the maximum level of pH was found in the combination of seed + *Brevibacillus borstelensis* (c3). In the second group, the combination of seed + l N + *Brevibacillus borstelensis* (c3) revealed the maximum level of pH (8.61) and the combination of seed + phosphorus + *Bacillus subtilis* (c2), and *Brevibacillus borstelensis* (c3) showed a similar level of pH in the third group (8.5). In Group 4, the maximum pH level was seen in a pot treated with seed + soil + potassium + *Bacillus cereus* (c1). Total pH levels in each treatment showed differences. The addition of nutrients seed + NPK and *Brevibacillus borstelensis* (c3) strain in group 5 showed the highest levels of total pH compared to that of other treatments. In all the pots, the pH of the soil was found to be alkaline.

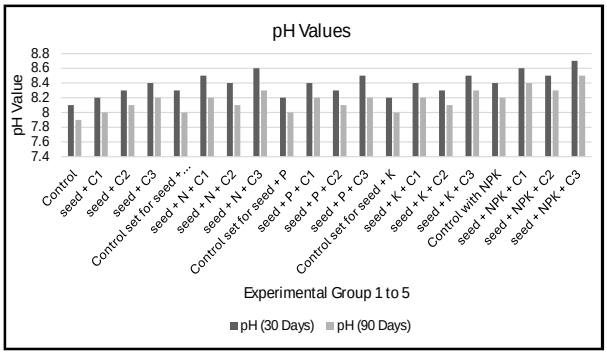


Fig. 2. Soil pH after 30 and 90 days.

The highest water holding capacity was found in pots treated with seed + N + *Bacillus cereus* (c1), which belonged to group 2, whereas the control group showed the lowest water holding capacity (Fig. 3). While after 90 days, the highest water holding capacity was found in pots treated with seed + potassium + *Brevibacillus borstelensis* (c3), which belonged to group 4, whereas the control group showed the lowest water holding capacity.

Fig. 4 shows the microbial load in all the pots treated with five groups after 30 and 90 days. After 30 days, the maximum microbial load was present in pot treated with seed + N + *Bacillus cereus* (c1) of group 2. The control group showed the lowest microbial load.

While after 90 days, the maximum microbial load was present in pot treated with seed + phosphorus + c2 of group 3, similarly, was in

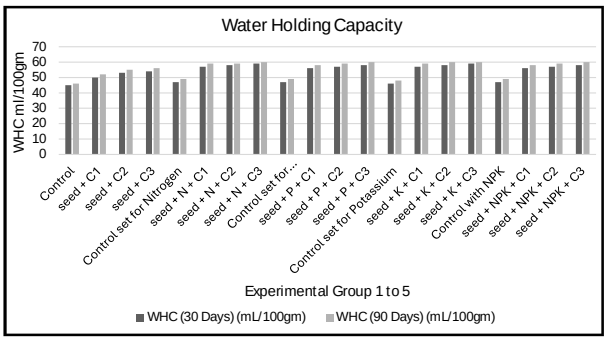


Fig. 3. The water holding capacity (ml/100 g) of soil after 30 and 90 days.

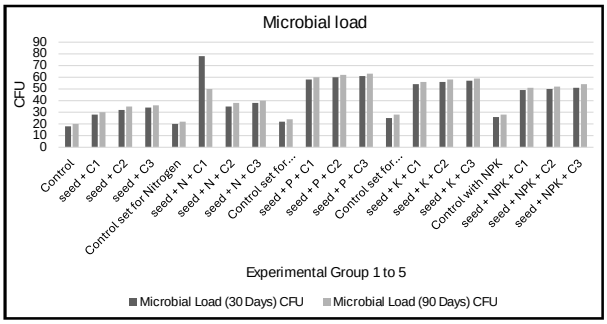


Fig. 4. Microbial load in the soil after 30 and 90 days (1 CFU = 1000 microbial load).

group 5 in the combination of seed + NPK + *Brevibacillus borstelensis* (c3). The control group seed + *Brevibacillus borstelensis* (c3) showed the lowest microbial load.

The dry weight and height of the root and shoot of the plant were checked and noted after 30 and 90 days. In group 5, the height of root and shoot was greater than in the other 4 groups. The dry weight of the plant was maximum in the case of group 5 compared to other groups (Table 5).

Table 6 reveals a statistically significant difference between the 5 groups tested after 30 and 90 days. For nitrogen, pairs 1 and 2 showed a significant difference in the nitrogen availability of the soil after 30 and 90 days. For phosphorus, pairs 1, 2 and 3 showed a significant difference, while for potassium, there was no significant difference in any pair i.e. there was no significant change in potassium availability in the soil after 30 and 90 days.

All the pairs showed a significant difference for zinc availability in the soil except pair 3; similarly, for copper availability, all pairs showed a significant difference except pair 2. The highly statistically significant differences were observed in the magnesium and iron availability in the soil.

Table 5. Shoot height (cm), root length (cm) and plant dry weight (mg/plant) in different seed groups

S. No.	Experimental group	Treatment	Shoot height (cm)		Root length (cm)		Plant dry weight (mg/plant)	
			30 days	90 days	30 days	90 days	30 days	90 days
1.	Group 1	Control (g/kg)	1.157^{fg}±0.026	1.900^{kl}±0.021	0.147^{l}±0.020	0.240^{ij}±0.015	88.537^{qr}±0.026	96.337^{q}±0.030
		Soil + c1	1.333^{ef}±0.023	2.010^{ij}±0.012	0.267^{hi}±0.012	0.290^{gh}±0.012	94.300^{op}±0.153	101.287^{no}±0.032
		Soil + c2	1.300^{f}±0.042	1.960^{jk}±0.017	0.160^{l}±0.017	0.247^{hij}±0.009	99.630^{no}±0.025	112.167^{lo}±0.030
		Soil + c3	1.227^{fg}±0.020	1.950jk±0.017	0.253^{hi}±0.012	0.297^{hij}±0.009	96.037^{op}±0.027	110.833^{lp}±0.027
2.	Group 2	Control set for nitrogen	1.343^{ef}±0.020	2.077^{ij}±0.015	0.410^{fg}±0.015	0.477^{f}±0.019	110.067^{m}±0.026	124.687^{m}±0.058
		Soil + urea + c1	1.440^{d}±0.020	2.157^{hi}±0.015	0.363^{gh}±0.012	0.547^{fg}±0.022	113.240^{lk}±0.026	128.743^{k}±0.101
		Soil + urea + c2	1.373^{de}±0.015	2.233^{gh}±0.020	0.427^{f}±0.015	0.540^{fg}±0.021	111.593^{lo}±0.032	125.667^{m}±0.064
		Soil + urea + c3	1.433^{d}±0.022	2.227^{gh}±0.035	0.467^{ef}±0.015	0.540^{fg}±0.017	118.390^{kl}±0.046	131.653^{k}±0.128
3.	Group 3	Control set for phosphorus	1.557^{c}±0.017	2.270^{fg}±0.021	0.520^{cde}±0.015	0.573^{de}±0.020	129.267^{ij}±0.196	140.327^{ij}±0.035
		Soil + P + c1	1.587^{c}±0.017	2.280^{fg}±0.021	0.580^{abc}±0.047	0.647^{bc}±0.012	119.227^{kl}±0.130	131.667^{k}±0.130
		Soil + P + c2	1.560^{c}±0.015	2.347^{f}±0.020	0.510^{de}±0.026	0.603^{cd}±0.009	124.373^{kl}±0.267	135.613^{k}±0.090
		Soil + P + c3	1.623^{c}±0.026	2.427^{de}±0.019	0.517^{cde}±0.007	0.603^{cd}±0.019	123.523^{kl}±0.266	133.623^{k}±0.162
4.	Group 4	Control set for potassium	1.627^{c}±0.027	2.377^{ef}±0.026	0.543^{cd}±0.033	0.650^{bc}±0.031	134.210^{ij}±0.124	142.237^{ij}±0.241
		Soil + K + c1	1.613^{c}±0.023	2.450^{ef}±0.017	0.543^{cd}±0.020	0.653^{bc}±0.018	133.447^{ij}±0.236	144.210^{ij}±0.029
		Soil + K + c2	1.560^{c}±0.015	2.537^{f}±0.015	0.560^{cd}±0.026	0.660^{bc}±0.015	131.513^{ij}±0.266	143.290^{ij}±0.352
		Soil + K + c3	1.613^{c}±0.009	2.547^{ef}±0.038	0.557^{cd}±0.009	0.677^{bc}±0.015	134.250^{ij}±0.202	145.727^{ij}±0.039
5.	Group 5	Control set with NPK	1.750^{b}±0.017	2.753^{b}±0.015	0.637^{ab}±0.018	0.740^{ab}±0.015	156.280^{gh}±0.147	166.753^{gh}±0.043
		Soil + NPK+ c1	1.827^{a}±0.020	2.773^{b}±0.015	0.573^{ad}±0.022	0.743^{ab}±0.015	160.767^{gh}±0.433	171.197^{gh}±0.026
		Soil + NPK+ c2	1.753^{b}±0.026	2.850^{ab}±0.017	0.550^{cd}±0.023	0.683^{bc}±0.020	161.183^{gh}±0.093	173.197^{gh}±0.050
		Soil + NPK+ c3	1.790^{ab}±0.017	2.960^{a}±0.015	0.637^{ab}±0.018	0.740^{ab}±0.015	165.093^{gh}±0.070	177.110^{gh}±0.064

Table 7 reveals a statistically significant difference based on different parameters between the 5 groups tested after 30 and 90 days. Total Organic Carbon (TOC) showed a significant difference only in pair 2, on the other hand, in the water holding capacity (WHC) only pair 3 showed a significant difference. Pairs 3 and 5 showed significant differences in pH change, while for microbial load, pairs 3 and 4 showed significant differences.

The diversity in PGPR performance may be attributed to various environmental variables that influence their development and have an impact on the plant. Climate, meteorological conditions, soil properties, and the makeup of soil's activity indigenous microbial flora are examples of environmental variables.

Exploring soil microbial diversity for PGPR with a mix of PGP activities and well-adapted to a specific soil environment is one potential method (Alsharif *et al*., 2020). *Bacillus* is the most common species in the rhizosphere, and the PGPR activity of some of these strains has been known for a long time resulting in a comprehensive understanding of the processes involved (Hashem *et al*., 2019). *Bacillus* has also been shown to have the ability to enhance raspberry plant production, growth and nutrition under organic growing circumstances. *Bacillus megaterium* consistently improves root characteristics in mint (root length, root dry matter content and rooting performance (Ilyas *et al*., 2022).

This research aimed at identifying rhizobacterial strains with favourable properties that may be utilized as mustard bio inoculants. Strains of *Bacillus* isolates of rhizobacterial isolates were chosen and tested for the formation of IAA and phosphate solubilization. Under pot house circumstances, three rhizobacterial isolates were evaluated for their ability to alter the soil characteristics. Five groups were treated with different combinations of nutrients and isolate created and evaluated for efficacy.

In this study, the plant's dry weight and composition were assessed and observed the different pH levels, WHC, TOC, micronutrient availability and microbial load of soil. The maximum plant dry weight was seen in group 5, and the height of root and shoot also greater than in other groups (Table 5). pH plays a very important role in a soil sample to check the

Table 6. Comparisons at 30 – 90 days in nutrient values in five different groups

Group	Pairs (days)	Nitrogen	Phosphorus	Potassium	Zinc	Copper	Manganese	Iron
Group 1	30-90	.017*	.029*	.998	.008*	.000*	.000*	.011*
Group 2	30-90	.094	.044*	.767	.001*	.508	.000*	.002*
Group 3	30-90	.034*	.024*	.381	.146	.000*	.000*	.000*
Group 4	30-90	.127	.300	.081	.000*	.001*	.000*	.000*
Group 5	30-90	.087	.052	.592	.027*	.000*	.000*	.010*

*Denotes significant changes.

Table 7. Comparison of soil properties in different groups (p-values)

Group	Pairs (days)	TOC	WHC	pH	CFU
Group 1	30-90	.050	.173	.095	.696
Group 2	30-90	.009*	.405	.137	.663
Group 3	30-90	.311	.009*	.013*	.003*
Group 4	30-90	.807	.554	.118	.011*
Group 5	30-90	.186	.122	.043*	.873

*Denotes significant changes.

availability of acidity and alkalinity of the soil. In the present study, the pH level after 30 and 90 days of cultivation was compared and the highest level was found of pH in group 1 (seed + *Brevibacillus borstelensis*) and group 5 (seed + NPK + *Brevibacillus borstelensis*), which were (8.98), as shown in graph 2. On the other hand, a slight increase in the pH (9) was seen after 90 days (graph 6). A statistically significant change in pH was seen in pairs 3 and 5 (Table 7).

Water holding capacity showed a similar outcome in pot treated with seed + potassium + *Brevibacillus borstelensis* (c3), which belonged to group 4, compared to capacity in pot treated with seed + N + *Bacillus cereus* (c1), which belonged to group 2. It was seen that only group 3 showed a statistically significant difference in the water holding capacity after 30 and 90 days. Total organic carbon measurement provides data on aggregate stability, soil fertility and CO_2 exchange with the atmosphere. According to this experiment, the highest TOC was found after 30 days of cultivation. A statistically significant difference was seen only in group 2.

Graph 4 showed the microbial load in all the pots treated with five groups, and the highest microbial load was present in the pot treated with seed + N + *Bacillus cereus* (c1) of group 2, which was approx. 80 as compared to graph 4 where the microbial load in all the pots treated with five groups and the maximum microbial load was present in pot treated with seed + soil + phosphorus + *Bacillus subtilis* (c2), of group 3. Similar was in group 5 in the combination of seed + NPK + *Brevibacillus borstelensis* (c3). The control group showed the lowest microbial load in both the seasons. A statistically significant change in microbial load of soil was seen in groups 3 and 5.

Azospirillum, *Pseudomonas*, *Klebsiella*, *Azotobacter*, *Alcaligenes*, *Enterobacter*, *Arthrobacter*, *Bacillus*, *Burkholderia* and *Serratia* are only a few of the bacteria that have been identified as PGPR. Direct plant growth promotion by PGPR involves supplying the bacteria with plant growth-boosting chemicals or enabling the absorption of specific plant nutrients from the environment (Pallavi *et al*., 2017). The PGPR promotes indirect plant development by avoiding the harmful impacts of phytopathogenic bacteria. The exact mechanisms by which PGPR promotes plant growth are unknown, but they are thought to include the ability to produce or change the concentration of plant growth regulators such as gibberellic acid, indoleacetic acid, ethylene and cytokinin. The PGPR bacterial strains must also be rhizosphere competent, meaning they must be able to survive and colonize in rhizosphere soil (Santoyo *et al*., 2021).

According to Radhakrishnan *et al*. (2017) the *Bacillus* members can live as endospores for extended periods under severe environmental conditions. They may also produce a range of secondary metabolites that help plants grow faster and fight illness. The capacity to regulate the rhizosphere to improve the competitiveness and survival of these beneficial microbes will be critical to the success of these goods. Rhizosphere management will need consideration of soil and crop cultural techniques and the formulation and administration of inoculants. PGPR is an ecologically friendly solution for increasing health and crop yield (Radhakrishnan *et al*., 2017). The use of molecular techniques improves capacity to understand and control

the rhizosphere, which will lead to new, more effective products.

The phosphate-solubilizing bacterium *Bacillus* sp. is found in the rhizosphere of *B. juncea*, (Sinha and Jee, 2018). IAA production was also discovered in these isolates. IAA is an auxin phytohormone that promotes root growth, cell division and expansion (Sinha and Jee, 2018). Siderophore enhances plant growth directly and indirectly by plant growth promoting rhizobacteria. Soil bacteria isolates including *Azotobacter vinelandii Mac259* and *Bacillus cereus UW85* produce siderophores and they can be used as efficient PGPR to increase the growth and yield of crops (Mushtaq *et al*., 2024). *Bacillus megaterium* from tea rhizosphere is able to produce rhizosphere and help in plant growth promotion and reduction of disease intensity (Chen *et al*., 2023). In this study, *Bacillus cereus* (c1), *Bacillus subtilis* (c2) and *Brevibacillus borstelensis* (c3) were all shown to be acceptable PGPR for *Brassica juncea* L. growth promotion. It is feasible to produce bio inoculants using these three isolates because of their ability to promote plant growth.

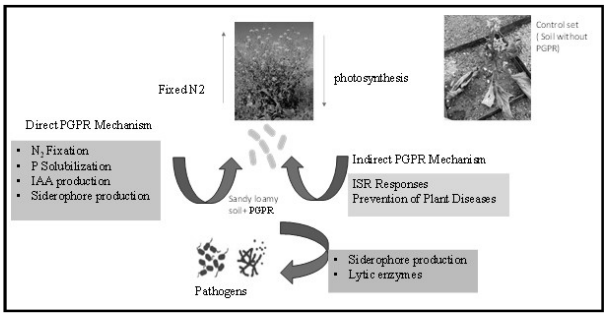
The main aim of this work was to observe the plant's growth after one month of sowing seeds (30 days) and at the end month of crop (90 days), because the crop cycle of *Brassica juncea* L. is 90 days. By combining both the data, the nutrient value in soil was found highest after 30 days of observation, however, the plant could not absorb the nutrient properly. Contrarily by 90 days, plant absorbed the nutrients from soil and appropriately held all of them. Further the nutrients values decreased in the soil after 90 days.

CONCLUSION

Bacteria from the plant rhizosphere positively affected roots. Plant growth-promoting rhizobacteria were strongly linked with plant roots, and they showed beneficial indirect and direct impacts on plant development, including a decrease in biotic stress. This research demonstrated the practical advantages of PGPR for a sustainable agricultural system, with the three cultures: *Bacillus cereus* (c1), *Bacillus subtilis* (c2) and *Brevibacillus borstelensis* (c3). The isolates showed greatest plant growth-promoting capacity in terms of soil influence. The improved soil nutrient management process that leads to soil fertility status was ascribed to the nutrients enrichment of rhizosphere soil inoculated with these microbial inoculants. Furthermore, these isolates have beneficial effects on soil properties and health, which are required for higher plant biomass development.

SUPPLEMENTARY MATERIALS

Graphical Abstract



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