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Plant Growth Promotion and Suppression of Web Blight Disease of Cowpea by Native Rhizobacterial Strains of *Bacillus subtilis* singly or in Combination with *Pseudomonas aeruginosa*

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ABSTRACT: The exploitation of antagonistic microbial populations could be the answer for the eco-friendly and successful management of plant diseases. In the present study, the efficacy of native rhizobacterial strains of *Bacillus subtilis* (PR-19 and B-11) solely or in consortia with *Pseudomonas aeruginosa* (strain GP8) was evaluated for the improvement of plant health and management of web blight disease in cowpea. The application of microbial consortia *Bacillus subtilis* (B-11) with *Pseudomonas aeruginosa* (GP8) through talc-based bioformulation showed 41.72% and 45.29% percentage disease control in 2020 and 2021, respectively, suggesting the potential of utilizing location-specific inoculation strains to obtain an optimum symbiotic benefit. The plant growth and yield parameters of cowpea plants were significantly increased in the treatments comprising PGPR-based bioformulations as compared to the chemical fungicide (Carbendazim 50% WP) and control treatments. The combination of seed treatment followed by soil application of consortia (B-11 and GP-8) performed very well at the field level for enhancing plant growth as well as suppressing web blight disease and increasing the yield of cowpea crops. Further research should be carried out on the shelf-life and cost-effectiveness of the prepared bioformulation, as well as their feasibility to be used in integrated disease management programs.

Keywords: Web blight, biocontrol, *Bacillus*, *Pseudomonas*, PGPR, sustainable agriculture.

INTRODUCTION

With the rise of the population, the demand for food is increasing, whereas the modern-day intensive agricultural system, along with the application of chemical pesticides, results in considerable environmental pollution and unavoidable impacts on soil, water, and animals, as well as on human health (Sharma *et al.*, 2020; Raffa and Chiampo 2021). However, the need for pest and disease management is unavoidable to secure our food requirements. Therefore, the development of biopesticide-based pest management strategies is preferred and encouraged globally to ensure food safety as well as food security (Samada and Tambunan 2020). There are different types of biopesticides, like bio-derived chemicals (pyrethrum, rotenone, neem oil, etc.), various essential oils, entomopathogenic fungi, viruses, microbial pesticides, plant growth-promoting rhizobacteria (PGPR), etc. (Boye and Arcand 2013).

PGPRs are remarkable biopesticides, as they not only control several plant diseases but also promote plant health (Gupta *et al.*, 2021). They colonize the plant root rhizosphere and can multiply and maintain their population. PGPR can promote plant growth and development by releasing plant growth regulators or other active compounds and uptake of nutrients through fixation and mobilization. They can minimize the pathogen population by means of competition, antibiosis, or inducing systemic resistance in plants (Riaz *et al*., 2021). They also play an important role in soil fertility. Some examples of PGPR include *Rhizobium*, *Acetobacter*, *Azospirillum*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Micrococcus*, etc. (Mashabela *et al.*, 2022). Among different PGPRs, *Bacillus* sp. has been found effective against several fungal and bacterial pathogens as well as inducing resistance against several abiotic stresses (Egamberdieva, 2016). Bacillus is a gram-positive aerobic endospore-forming genera that is capable of antibiotic production, the production of various enzymes like pectinase, cellulase, and various proteolytic enzymes, nitrogen fixation, and good plant growth-promoting activities, along with biological control through antibiosis and lysis (Kumar *et al*., 2012). The gram-positive bacterial genus *Bacillus* sp. is the dominant component of the soil microflora and is found in widely varying habitats. The successful application of *Bacillus* sp. as bioinoculants depends to a great extent on their capability to colonize roots and compete with the indigenous microbiome in the rhizosphere (Babalola *et al.*, 2021). It is characterized as important because of its biocontrol ability against several disease-causing plant pathogens. *Bacillus* sp. can control a wide range of soil-borne fungi through antibiotics, enzymes, volatile and non-volatile compounds, or by triggering systemic resistance in the plant (Garcia-Gutierrez *et al.*, 2013). The glittering promise of *Bacillus* sp. in biological control is exemplified by many research works, yet very few studies are made on region-specific effective *Bacillus* sp. On the other hand, *Pseudomonas* species are widely distributed in nature and possess numerous qualities that make them the ideal PGPR (Singh *et al.*, 2019). The fluorescent *Pseudomonas* is gram-negative, aerobic, rod-shaped, motile, and capable of producing a water-soluble yellow-green pigment. They can thrive in both rhizospheres and rhizoplanes. They show great compatibility with Bacillus and efficiently suppress plant diseases (Singh *et al*., 2021). Plant growth-promoting rhizobacteria are subjected to variations in climatic and soil factors, climatic factors like rainfall pattern, temperature, and relative humidity, and soil factors like soil temperature, pH, soil moisture, and organic matter, affecting the number of viable microorganisms available in or near the rhizosphere (Yuliatin *et al.*, 2019). All these factors restrict the application of specific strains to specific agro-climatic regions. The application of location-specific strains needs to be emphasized to obtain an optimum symbiotic benefit. Uncountable specificity of different isolates of Bacillus is present due to variation in different agro-climatic zones, which makes it difficult to enumerate successful and effective Bacillus and its application procedures. Enormous research has been conducted on *Bacillus* spp. as a bio-control agent, but the exploration of native agro-climatic region-specific *Bacillus* spp. in eastern India is rare. Therefore, the objective of the present study is to evaluate the efficacy of native rhizobacterial strains of Bacillus subtilis solely or in consortia with *Pseudomonas aeruginosa* (strain GP8) for the improvement of plant health and management of web blight disease in cowpea (*Vigna unguiculata* (L.) Walp.).

MATERIALS AND METHODS

Rhizobacterial strains

Potential strains of native rhizobacterial *Bacillus subtilis* (B-11and PR-19) and *Pseudomonas aeruginosa* (GP-8) were collected from The Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India, and used in the present investigation.

Maintenance of PGPR

Actively grown PGPR bacteria (*Bacillus* and *Pseudomonas*) cultures were transferred to nutrient-agar Petri plates using a sterilized inoculating wire loop following standard bacteriological procedure. The *Pseudomonas* strain was collected from the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, India. Subsequently, it was then incubated at 28 ± 2°C for 72 h to obtain active growth. Thereafter, they were kept in a refrigerator (4 ±1 °C) for future use. Sub-culturing was performed at 15-day intervals to maintain an active bacterial population for future use.

Preparation of bacterial broth: The nutrient broth was prepared for the mass multiplication of the bacteria. The broth was inoculated with the native rhizobacterial strains *Bacillus subtilis* (B11 and PR19) and *Pseudomonas aeruginosa* (GP-8) and incubated in shaker fitted BOD incubator at 28±2°C for 48 h. These homogenized bacterial cultures were utilized further in the experiments.

Bio formulation of PGPR. Talc is widely used as an inorganic carrier for bioformulation of different PGPR/Bio agents. PGPR formulation was prepared using1kg of talc powder mixed uniformly with 150 mL of homogenized broth of bacterial culture manually with proper safety measures. After reaching optimum moisture conditions, the bio-inoculated talc powder was stored in moisture-proof packaging (aluminum bags) and kept in a cool place for future use.

Field experiments. The field experiments were conducted for two years (2020 and 2021) at Instructional Farm, BCKV, Mohanpur, Nadia, West Bengal, India (located at 22.56"52" (N) latitude and 88.32"20"(E) longitude with an elevation of 7.6 m above mean sea level) during Kharif season. The date of sowing for the first year was 19th June 2020 and for the second year, 16th June 2021 using cowpea as the host crop following a Randomized Block Design (RBD)having three replications for each treatment with a 3.0m × 4.0 m plot size. In each replication, the number of plots was seven. Therefore, each field experiment consisted of 21 plots, with row-to-row and plant-to-plant spacing of 30 cm and 15 cm, respectively.

The two most potential strains of *Bacillus* (B-11 and PR-19) against *Rhizoctonia* were applied solely or in combination with *Pseudomonas* (GP-8) to check whether their bipartite interaction may improve the bioefficacy or not. Seeds were treated with *Bacillus* subtilis based formulation alone (B-11 or PR-19) or in combination (B-11+PR-19, B-11+GP-8, PR-19+GP-8) @ 5g/kg of cowpea seeds before sowing. Seeds were placed in a capped plastic container, prevailing minimum moisture condition, followed by a rotary motion for homogenous distribution of bacterial cells surrounding the seed coat. The bacterial-mixed seed was kept for 5-6 hours before sowing. Sowing of the treated seeds was done followed by soil drenching by the prepared formulations @ 5 g/L of water at 10 days after the sowing of seeds. The treatments of the field experiments were (i) *Bacillus* sp. –based bioformulation-I (PR-19), (ii) *Bacillus* sp. –based bioformulation-II (B-11), (iii) Two *Bacillus* sp.–based bioformulation (B-11+PR-19), (iv) *Bacillus* sp. and *Pseudomonas* sp.–based bioformulation-I (B-11+GP-8), (v) *Bacillus* sp. and *Pseudomonas* sp.–based bioformulation-II (PR-19+GP-8), (vi) Carbendazim 50% WP, and (vii) Control.

Standard agronomic practices like manure and fertilizer application, hand weeding, and need-based irrigation were followed as and when required. Two prophylactic sprays with Imidacloprid (17.8% SL) @ 1 mL/ 3 L of water were done uniformly at 30 and 60 days after sowing (DAS)in all the plots to protect the crop against different sucking insect pests of cowpea, while Chloropyriphos 20% EC @2.5 mL/L was used against different leaf-cutting insects as and when required. The disease severity of web blight of cowpea (*R. solani*) was recorded at 50 DAS.

Statistical analysis. SPSS statistical software was used to analyze all the components of the present work. Randomized Block Design (RBD) with three replications per treatment was used in the case of the field experiments. For comparing the treatment means, the least significant difference (LSD) test was used at the p<0.05 level.

RESULTS

Efficacy of prepared PGPR-based bioformulations on the plant growth characteristics of cowpea (cv. Kashi Kanchan)

Effect on plant height, root length, and shoot-root ratio of cowpea

Plant height. Results obtained from the field trial conducted in 2020 (Table 1) revealed that the maximum plant height was recorded in the combination of bacterial bioagents B-11 + GP-8 (*Bacillus* sp.+*Pseudomonas* sp.) (21.20 cm), followed by PR-19 + GP-8 (*Bacillus* sp. + *Pseudomonas* sp.) (20.37 cm) in comparison to the control (15.27cm) when the observation was recorded at 30 DAS. Other bacterial isolates, like B-11 (20.12 cm), B-11+PR-19 (19.10 cm), and PR-19 (18.62 cm), also exhibited significant plant height increases over control. When plant heights were recorded at 60 DAS, a similar trend was recorded in different treatments, and these were B-11 + GP-8 (31.23 cm), PR-19+GP-8 (29.87 cm), B-11 (28.42 cm), B-11 + PR-19 (27.77cm), PR-19 (26.63 cm), and carbendazim (23.50 cm) over the control treatment (18.03 cm) (Table 1).

Experimental results obtained from the field trial conducted in 2021 showed a similar effect on the plant height of cowpeas obtained at 30 DAS and 60 DAS (Table 1). At 30 DAS, maximum plant height (22.90 cm) was recorded in treatment B-11 + GP-8 (*Bacillus* sp. + *Pseudomonas* sp.), followed by 21.10 cm in PR-19 + GP-8 (*Bacillus* sp. + *Pseudomonas* sp.), and 20.20 cm in B-11(*Bacillus* sp.) in comparison to control (15.03 cm) and standard fungicidal check (16.23 cm). *Bacillus* sp. (PR-19) alone and in combination with another *Bacillus* sp. (B-11) also showed significant plant heights of cowpea (18.30 cm and 18.77 cm) in contrast to the control and standard fungicidal checks. Data recorded for cowpea plant height at 60 DAS also exhibited a similar trend. These were 34.27 cm in B-11 + GP-8 (*Bacillus* sp. +*Pseudomonas* sp.), 32.23 cm in PR-19 + GP-8 (*Bacillus* sp. +*Pseudomonas* sp.), 29.57 cm in B-11 (*Bacillus* sp.), 28.53cm in B-11+PR-19 (*Bacillus* sp. + *Bacillus* sp.), and 26.90 cm in PR-19 (*Bacillus* sp.), which were significantly higher than control (21.23 cm) and standard fungicidal check (23.37 cm), respectively.

Root length. The root length of cowpea was recorded at 30 and 60 DAS from both the experimental trials conducted in the years 2020 and 2021. The field trial conducted in 2020 showed maximum root length at 60 DAS and was recorded from the treatment B-11 + GP-8 (*Bacillus* sp.+ *Pseudomonas* sp.) (20.41 cm), followed by PR-19 + GP-8 (*Bacillus* sp. + *Pseudomonas* sp.) (19.54 cm), and in B-11 (*Bacillus* sp.) (18.22 cm) in comparison to control (13.97 cm) and standard fungicidal check (15.33 cm). *Bacillus* sp. (PR-19) alone (16.77 cm) and in combination with another *Bacillus* sp. (B-11+PR-19) (17.51 cm) also showed significantly higher root length in contrast to the control and standard fungicidal check (Table 1).

A similar trend of effect by the bacterial isolates on the root growth of cowpeas was recorded in the field trial conducted in 2021. Results (Table 1) revealed that treatment (B-11 + GP-8) showed maximum root length, followed by PR-19 + GP-8, B-11, B-11+PR-19, and PR-19 in comparison to the control and standard fungicidal check at both 30 and 60 DAS.

Shoot: Root ratio. The shoot-root ratio of the cowpea plant was recorded at 30 and 60 DAS from both the experimental trials conducted in 2020 and 2021, which showed no significant difference among the treatments (Table 1). The shoot-root ratio varied from 1.76 to 2.48 at 30 DAS and 1.30 to 1.67 at 60 DAS in the field trial conducted in 2020, while it was recorded to be 2.03-2.30 at 30 DAS and 1.52-1.77 in 2021.

Effect of prepared PGPR-based bioformulations on the number of branches, leaves, and nodules per plant of cowpea (cv. Kashi Kanchan)

Number of branches per plant. A significant difference among the different treatments was observed in the number of branches, leaves, and nodules of the cowpea crop at different time intervals when crops were imposed with bacterial bioagents and standard fungicide at two locations. Results from the field trial conducted in 2020 (Fig 2) recorded the highest number of branches per plant in B-11+GP-8 (5.88 & 6.73), followed by PR-19 + GP-8 (5.11 & 6.00), and by B-11(4.78 & 5.74) in comparison to fungicide (3.85 & 4.96), and control treatment (3.22 & 4.66) at 30 and 60 DAS, respectively.

Results obtained from the field trial conducted in 2021 (Fig. 3) also showed a similar trend in the number of branches per plant. The number of branches per plant was highest in theB-11+GP-8 treatment (5.78 and 6.70), followed by PR-19+ GP-8 (5.29 and 6.00), and B-11 (4.64 and 5.66) in comparison to the fungicide check (3.78 and 4.92) and control treatments (3.04 and 4.74) at 30 and 60 DAS, respectively.

Number of leaves per plant. Results obtained from the field trial conducted in 2020 (Fig. 2) showed the highest number of leaves per plant by the combination of inoculation with B-11+GP-8 (20.33 and 25.33), followed by PR-19+ GP-8 (19.33 and 23.67), and by B-11(18.33 and 22.33) in comparison to fungicide check (15.33 and 18.33) and control treatment (13.33 and 16.33) at 30 and 60 DAS, respectively.

Results from the field trial conducted in 2021 (Fig. 3) also revealed a significant increase in leaf number per plant in different treatments, with the highest nominal value in B-11+GP-8 (21.00 and 26.00), followed by PR-19+ GP-8 (20.67 and 24.00), and B-11 (19.00 and 23.00) in comparison to the fungicide check (16.00 and 19.00) and control (14.00 and 17.00) treatments at 30 and 60 DAS, respectively.

Number of nodules per plant. The effect of different rhizobacteria on nodulation in cowpeas was recorded from both experimental fields at 50 DAS, and results obtained from the field trial conducted in 2020 (Fig. 2) showed a significant increase in nodule number in different treatments over control. Maximum nodules per plant were obtained in plants treated with bacteria B-11+GP-8 (31.33), followed by PR-19 + GP-8(30.33), and B-11 (29.00) in comparison to fungicide check (20.00) and control treatment (19.67). A combination of bacteria B-11 + PR-19 (27.33) and PR-19 (26.67) also showed increased nodule numbers per plant over fungicide check and control.

Results from the field trial conducted in 2021, presented in (Fig. 3) showed a similar trend in the nodulation of cowpea. The nodule numbers per plant as recorded were B-11+GP-8 (32.67) followed by PR-19+GP-8(31.33), B-11 (29.67), PR-19 (28.33), and B-11+PR-19 (26.33) in comparison to fungicide check (21.67) and control (24.33).

Effect of prepared PGPR-based bioformulations on pod number and yield of cowpea (cv. Kashi Kanchan)

Results obtained from the field trial conducted in 2020 (Table 2) revealed that maximum pods per plant (26.33), yield (10.22 t/ha), and percent increase of yield over control (45.17%) were recorded in the bioagent consortia of B-11 + GP-8, which were followed by consortia of PR-19 + GP-8 (number of pods/plant: 25.67; yield: 10.04 t/ha; percent increased of yield over control: 42.61%) and by B-11 (number of pods per plant: 23.67; yield: 9.63 t/ha; percent increased of yield over control: 36.89%), significantly higher than the test chemical fungicide treatment (number of pods per plant: 19.33;yield: 8.33 t/ha; percent increase of yield over control: 18.32 %). The lowest number of pods/plant (16.67) and yield (7.04 t/ha) were recorded in the control treatment.

Results from the field trial conducted in 2021 (Table 2) showed a similar trend in pod number per plant, total yield, and percent increment of yield over the control treatment. Among the different bioagents and their consortia, maximum pods per plant (21.52), yield (9.25t/ha), and percent increase of yield (48.95 %) over control were recorded in the bioagents consortia of B-11+GP-8, followed by consortia of PR-19+ GP-8 (number of pods/plant: 20.26; yield: 8.78 t/ha; percent increase of yield over control: 41.38 %), and by B-11 (number of pods/plant: 19.04; yield: 8.32 t/ha; percent increase of yield over control: 33.98 %), which were significantly higher than the test chemical fungicide treatment (number of pods/plant: 17.15; yield: 7.99 t/ha; percent increase of yield over control: 28.66 %). The lowest pods/plant (15.67) and yield (6.21 t/ha) were recorded in the control treatment.

Effect of prepared PGPR-based bioformulations on web blight disease incidence in cowpea (cv. Kashi Kanchan) under field conditions. Results obtained from the field trial conducted in 2020 (Table 3) showed that inoculation with *Bacillus* spp. (B-11 and PR-19) alone or in combination with *Pseudomonas* sp. (GP-8) significantly reduced the web blight disease severity in comparison to the control. The data revealed that minimum disease severity (16.66%) was recorded in fungicide (Carbendazim 50% WP)-treated plots and gave maximum disease control (52.84%). Among the treatments with bioagents and their combinations, B-11+GP-8 exhibited the lowest disease severity (20.59%) and highest disease reduction (41.72%) of web blight, followed by PR-19+ GP-8 (21.07% & 40.36%), B-11+PR-19 (23.03% & 34.81%), B-11 (24.04% & 31.95%), and PR-19 (26.07% & 26.21%) with comparison to the disease severity of control treatment.

A similar trend of disease severity was recorded for web blight disease conducted in 2021. Results (Table 3) showed that all the treatments significantly reduced the disease severity of web blight over untreated control. Treatment with the test fungicide Carbendazim-50% WP exhibited minimum severity (20.11%), while treatments with bacterial formulations (B-11+GP-8), (B-11+PR-19), (PR-19 + GP-8), B-11, and PR-19 showed significantly reduced disease severity, *viz*., 26.18%, 26.67%, 27.82%, 28.89%, and 30.07%, respectively, over the disease severity of the untreated control (47.85%). The percent disease control of web blight was also recorded as highest when treatment was imposed with carbendazim (57.97%). This was followed by treatments with other bacterial formulations like B-11+GP-8 (45.29%), B-11+PR-19 (44.26%), PR-19+ GP-8 (41.86%), B-11 (39.62%), and PR-19 (37.16%), respectively.

DISCUSSION

In the present experiment, three selected PGPR-based formulations *viz*., (PR-19- *Bacillus* sp.); B-11- *Bacillus* sp.; GP-8-*Pseudomonas* sp.) were explored alone or in combination as seed treatments and soil drenching on cowpea. Overall, B-11- *Bacillus* sp. + GP-8-*Pseudomonas* sp. showed promising results on different plant growth characteristics like plant height, root length, noodle number, pod yield, and total yield of cowpea. Other formulations also showed good potential in comparison to the control treatment. The efficacy of different bacterial formulations alone or in combinations showed also promising results in reducing disease severity against web blight of cowpea, but results were not reached to the level performed by the test chemical fungicide, carbendazim 50% WP.

Cowpea web blight disease severity was found in plots treated with *Bacillus* sp. alone or in combinations as compared to the control in the present field studies, which indicated that seed treatment followed by soil drenching with bacterial antagonists leads to an increase in their population with time in the surrounding area of the seed, which protects them from attacks by different plant pathogens (Mitra *et al.,* 2021).

Bacterial bioagents normally require more time for the initial establishment to exert their efficacy against pathogens through the elicitation of ISR (Induced Systemic Resistance) mechanisms, especially by the *B. subtilis* strains for controlling plant diseases. Moreover, jasmonic acid (JA), ethylene, and the regulatory gene NPR1 are thought to be essential for the signal transduction pathways that *B. subtilis* activates in plants (Garcia-Gutierrez *et al.,* 2013). These findings indicated that in the present study, the increased total yield of cowpea in the bacterial bioagent-treated plot over fungicide treatment might be due to the extended activity of the bioagent that protected the crop later in the crop phase and led to an enhancement of crop yield.

Several works on the management of soil-borne diseases through the application of bacterial antagonists have supported the present investigation of cowpea web blight management. For instance, the ability of *B. subtilis* IMP 215 to prevent the growth of carrot root infections while in storage was very promising. In conjunction with appropriate storage techniques, processing carrots with *B. subtilis* - IMP 215 was reported to be a helpful tool for minimizing microbial damage and minimizing losses (Pershakova *et al*., 2018). In another study, it was reported that the application of an indigenous *B. subtilis* strain showed promising natural biopesticide agents in cowpea crops, suppressing several soil-borne pathogens like *F. verticilloides*, *F. equiseti*, *F. solani*, *F. oxysporum*, and *R. solani* (Abaidoo *et al.,* 2011).

The enhancement of plant growth and productivity of crops in cowpeas in the present experiments might also be due to the secretion of some extracellular metabolites called siderophores by different bacterial formulations used. The presence of side rophore-producing PGPR in the rhizosphere increases the rate of Fe^{3+} supply to plants and therefore enhances the plant growth and productivity of crops (Mustafa *et al*., 2019). According to the current research, the growth promotion of crops using *Bacillus* sp. may also be a result of other factors, such as the production of phytohormone precursors like indole acetic acid (IAA-auxin), phosphate solubilization, siderophore production, and biocompatible species like nitrogen-fixing species like *Azospirillum* and *Azotobacter* in soils that increase the soil's fertility (Kashyap *et al.*, 2019). *Bacillus* sp. alone or in combination exhibited improved plant growth, possibly due to more nutrient uptake in plants and a synergistic interaction of *Bacillus* with other microbes in the plant root that promotes plant growth, mineral nutrition, and stress tolerance (Egamberdieva, 2016).

In the crop field, a variety of *Bacillus* and *Paenibacillus* species can support crop health in various ways. They can stimulate plant development by improving nutrient uptake through soil mineral mobilization, as well as by activating the host plant's defense mechanisms through ISR before infection (Kumar *et al*., 2011). In our study, *Bacillus* sp. (B-11) and *Pseudomonas* sp. (GP-8) and their consortia exhibited significantly taller plants, suggesting that direct length promotion might be due to cell elongation as an activity of ACC deaminase reduced ethylene levels in the plants and maximizing plant growth (Ji *et al*., 2020).

The production of various phytohormones and enzymes, such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, the mineralization of nutrients, such as nitrogen, phosphate, potassium, and zinc solubilization, nitrogen fixation, increased root absorption capacity, and the production of siderophores by *Bacillus* and *Pseudomonas*, may all contribute to the growth promotion and increased yield of cowpea in the current investigation (Chowdappa *et al.,* 2013).

Enhanced pod number and increased yield of cowpeas as found in the present study are also evident by an experiment done earlier by Ahmad *et al.* (2019) that treatments by PGPR *B. subtilis* B4 gave highly dry weight and number of healthy pods and a higher increased yield percentage compared to control both in green house and open field environmental conditions.

Table 1: Efficacy of prepared PGPR-based bio formulations on plant height, root length, and shoot-root ratio of cowpea (cv. Kashi Kanchan) at field trial conducted in the year 2020 and 2021.

	2020						2021						Average (2020 & 2021)		Average (2020 & 2021)		Average (2020 & 2021)	
Treatments	Plant height(cm)		Root length (cm)		Shoot: Root ratio		Plant height(cm)		Root length (cm)		Shoot: Root ratio		Average Plant height (cm)		Average Root length (cm)		Shoot: Root ratio	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
PR-19	18.62	26.63	10.32	16.77	1.82	1.59	18.3	26.9	8.1	16.73	2.3	1.63	18.46	26.765	9.21	16.75	2.00	1.60
B-11	20.12	28.42	10.66	18.22	1.89	1.57	20.2	29.57	9.39	18.08	2.16	1.64	20.16	28.995	10.025	18.15	2.01	1.60
B-11+PR-19	19.1	27.77	10.69	17.51	1.8	1.67	18.77	28.53	8.49	17.62	2.21	1.63	18.935	28.15	9.59	17.565	1.97	1.60
B-11+GP-8	21.2	31.23	12.13	20.41	1.76	1.65	22.9	34.27	11.37	19.47	2.03	1.77	22.05	32.75	11.75	19.94	1.88	1.64
PR-19+ GP-8	20.37	29.87	11.22	19.54	1.83	1.61	21.1	32.23	10.1	19	2.1	1.7	20.735	31.05	10.66	19.27	1.95	1.61
Carbendazim 50%WP @1g/kg	17.42	23.5	8.11	15.33	2.18	1.54	16.23	23.37	7.37	15.2	2.21	1.54	16.825	23.435	7.74	15.265	2.17	1.54
Control	15.27	18.03	6.2	13.97	2.48	1.3	15.03	21.23	6.93	14	2.18	1.52	15.15	19.63	6.565	13.985	2.31	1.40
LSD(p<0.05)	2.617	4.131	1.81	2.299	NS	NS	1.773	2.303	1.52	1.52	NS	NS						

(PR-19= *Bacillus* sp.; B-11- *Bacillus* sp.; GP-8= *Pseudomonas* sp., DAS=Days after sowing)

Table 2: Efficacy of prepared PGPR-based bio formulations on pod number and yield of cowpea (cv. Kashi Kanchan) at field trial conducted in the year 2020 and 2021.

Treatments	No. of pods/plant	Yield (t/ha)	% increase in yield over control	No. of pods/plant	Yield (t/ha)	% increase in yield over control	Average no. of pods/plant	Average Yield (t/ha)	Average% increase in yield over control
	2020			2021					
PR-19	20.67	8.7	23.58	16.37	7.31	17.71	18.52	8.005	20.645
B-11	23.67	9.63	36.89	19.04	8.32	33.98	21.355	8.975	35.435
B-11+PR-19	22	9.37	33.09	18.74	8.45	36.07	20.37	8.91	34.58
B-11+GP-8	26.33	10.22	45.17	21.52	9.25	48.95	23.925	9.735	47.06
PR-19+ GP-8	25.67	10.04	42.61	20.26	8.78	41.38	22.965	9.41	41.995
Carbendazim 50%WP@1g/kg	19.33	8.33	18.32	17.15	7.99	28.66	18.24	8.16	23.49
Control	16.67	7.04	-	15.67	6.21	-	16.17	6.625	-
LSD(p<0.05)	4.372	0.765	-	3.369	1.227	-			-

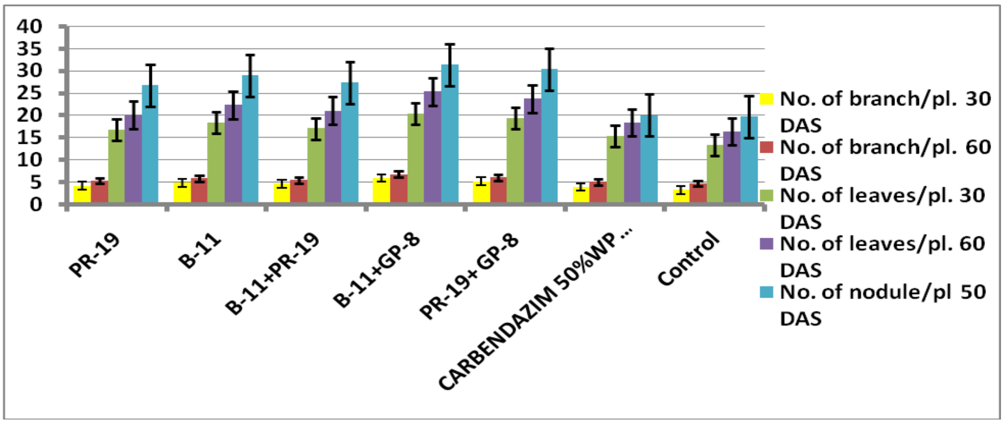
(PR-19= *Bacillus* sp.; B-11- *Bacillus* sp.; GP-8= *Pseudomonas* sp., DAS=Days after sowing)

Table 3: Efficacy of prepared PGPR-based bioformulations against Web blight disease incidence of cowpea (cv. Kashi Kanchan) at field trial conducted in the year 2020 and 2021.

Treatments	%. disease severity	% disease control	%. disease severity	% disease control	Average (2020 & 2021) % disease control
	2020		2021		
PR-19	26.07(30.96)	26.21	30.07(33.55)	37.16	31.685
B-11	24.04(29.67)	31.95	28.89(32.80)	39.62	35.785
B-11+PR-19	23.03(28.97)	34.81	26.67(31.38)	44.26	39.535
B-11+GP-8	20.59(27.33)	41.72	26.18(31.07)	45.29	43.505
PR-19+ GP-8	21.07(27.66)	40.36	27.82(32.14)	41.86	41.11
Carbendazim 50%WP @ 1g/kg	16.66(24.45)	52.84	20.11(26.98)	57.97	55.405
Control	35.33(36.76)	-	47.85(44.06)**	-	
LSD(p<0.05)	2.3481	-	3.487	-	

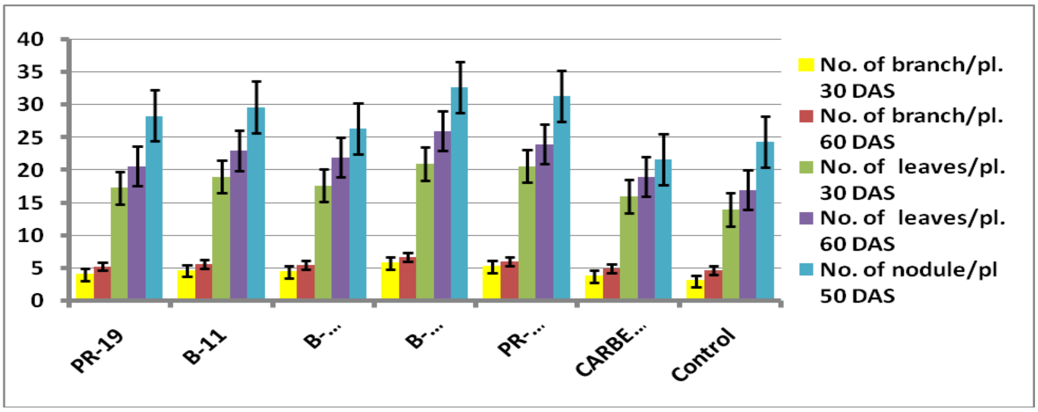
(PR-19= *Bacillus* sp.; B-11- *Bacillus* sp.; GP-8= *Pseudomonas* sp, ** Parenthesis within the brackets arc sine transformed value)

Fig. 1. Web blight (*R. solani*) disease infected cowpea plant.



[PR-19= *Bacillus* sp.; B-11- *Bacillus* sp.; GP-8= *Pseudomonas* sp., DAS =Days after sowing
*Average of 3 replications]

Fig. 2. Efficacy of prepared PGPR-based bio formulations on number of branches, leaves, and nodules per plant of cowpea (cv. Kashi Kanchan) at Field study-I (2020).



[PR-19= *Bacillus* sp.; B-11- *Bacillus* sp.; GP-8= *Pseudomonas* sp., DAS =Days after sowing *Average of 3 replications]

Fig. 3. Efficacy of prepared PGPR-based bio formulations on number of branches, leaves, and nodules per plant of cowpea (cv. Kashi Kanchan) at Field study-II (2021).

(a) (b)

Fig. 4. Pods (a) and nodules (b) of a plant treated by B-11+GP-8 at 50 DAS.

CONCLUSIONS

In the present study, different strains of *Bacillus subtilis* and *Pseudomonas aeruginosa* based bio formulations were used alone or in combination. The field trials were conducted to evaluate the effect of the prepared bioformulations on the plant growth parameters and Web Blight disease of cowpea caused by *R. solani* Wested & Wallays. The result showed that better disease control occurred when combinations of the different PGPR-based bioformulations were used. The plant growth attributes like plant height, root length, number of branches per plant, and number of leaves per plant after 30 DAS and 60 DAS were also significantly improved under consortial application of *Bacillus* as compared to sole application of *Bacillus*, chemical application, and control. The Highest nodulation per plant after 50 DAS was registered in treatment B-11+GP-8, i.e., *Bacillus* sp. with fluorescent *Pseudomonas*, than all other treatments. Lower web blight disease incidence and higher vegetative pod yield of cowpea were recorded by the plot treated with consortia of B-11+GP-8, whereas the lowest yield was recorded in the control plot. Therefore, the combined application of *Bacillus* sp. and fluorescent *Pseudomonas* both as seed treatment and soil application may be used in the development of integrated disease management strategies for different crops in the near future as a step towards environmental as well as agricultural sustainability.

FUTURE SCOPE

Isolation and deployment of zone specific bioformulation for promotion of higher yield and suppression of disease have a potential for the sustainable development with low cost input, further study of plant and microbe's metabolites can reveal the synergistic interaction between the host and PGPR's and can give us a insight about the unknown interacting partners.

Authors' contribution. S. Paul & S. K. Ray: experiment designing, experiment execution (field and lab experiments), data compilation, analysis, manuscript writing; S. Dutta: Conceived and designed the experiment, experiment execution, and manuscript editing, fund management; S. Pati, A. Patwari, R. Mandal, A. Roy Barman, and E. Venu: experiment execution, data compilation, analysis, manuscript writing

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Conflict of Interest. None.

REFERENCES

Abaidoo, R. C., Killani, A. S., Akintokun, A. K., & Abiala, M. A. (2011). Antagonistic effect of indigenous *Bacillus subtilis* on root-/soil-borne fungal pathogens of cowpea.

Ahmad, A. G. M., Attia, A. Z. G., Mohamed, M. S., & Elsayed, H. E. (2019). Fermentation, formulation and evaluation of PGPR *Bacillus subtilis* isolate as a bioagent for reducing occurrence of peanut soil-borne diseases. *Journal of Integrative Agriculture*, *18*(9), 2080-2092.

Babalola, O. O., Emmanuel, O. C., Adeleke, B. S., Odelade, K. A., Nwachukwu, B. C., Ayiti, O. E., & Igiehon, N. O. (2021). Rhizosphere microbiome cooperations: strategies for sustainable crop production. *Current Microbiology*, *78*, 1069-1085.

Boye, J. I., & Arcand, Y. (2013). Current trends in green technologies in food production and processing. *Food Engineering Reviews*, *5*, 1-17.

Chowdappa, P., Kumar, S. M., Lakshmi, M. J., & Upreti, K. K. (2013). Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. *Biological control*, *65*(1), 109-117.

Egamberdieva, D. (2016). *Bacillus* spp. a potential plant growth stimulator and biocontrol agent under hostile environmental conditions. *Bacilli and Agrobiotechnology*, 91-111.

Garcia-Gutiérrez, L., Zeriouh, H., Romero, D., Cubero, J., de Vicente, A., & Pérez-García, A. (2013). The antagonistic strain *Bacillus subtilis* UMAF 6639 also confers protection to melon plants against cucurbit powdery mildew by activation of jasmonate-and salicylic acid-dependent defence responses. *Microbial Biotechnology*, *6*(3), 264-274.

Gupta, A., Bano, A., Rai, S., Dubey, P., Khan, F., Pathak, N., & Sharma, S. (2021). Plant Growth Promoting Rhizobacteria (PGPR): A sustainable agriculture to rescue the vegetation from the effect of biotic stress: A Review. *Lett. Appl. NanoBiosci*, *10*, 2459-2465.

Ji, J., Yuan, D., Jin, C., Wang, G., Li, X., & Guan, C. (2020). Enhancement of growth and salt tolerance of rice seedlings (*Oryza sativa* L.) by regulating ethylene production with a novel halotolerant PGPR strain *Glutamicibacter* sp. YD01 containing ACC deaminase activity. *Acta Physiologiae Plantarum*, *42*, 1-17.

Kashyap, S., Mahajan, S., & Lal, M. (2019). Effects of topical tranexamic acid during open reduction and internal fixation of acetabular fractures: A retrospective study. *Acta Orthopaedica et Traumatologica Turcica*, *53*(3), 175-179.

Kumar, A., Prakash, A., & Johri, B. N. (2011). Bacillus as PGPR in crop ecosystem. In *Bacteria in agrobiology: crop ecosystems* (pp. 37-59). Berlin, Heidelberg: Springer Berlin Heidelberg.

Kumar, P., Khare, S., & Dubey, R. C. (2012). Diversity of bacilli from disease suppressive soil and their role in plant growth promotion and yield enhancement. *New York Sci. J.*, *5*(1), 90-111.

Mashabela, M. D., Piater, L. A., Dubery, I. A., Tugizimana, F., & Mhlongo, M. I. (2022). Rhizosphere tripartite interactions and PGPR-mediated metabolic reprogramming towards ISR and plant priming: A metabolomics review. *Biology*, *11*(3), 346.

Mitra, D., Mondal, R., Khoshru, B., Shadangi, S., Mohapatra, P. K. D., & Panneerselvam, P. (2021). Rhizobacteria mediated seed bio-priming triggers the resistance and plant growth for sustainable crop production. *Current Research in Microbial Sciences*, *2*, 100071.

Mustafa, S., Kabir, S., Shabbir, U., & Batool, R. (2019). Plant growth promoting rhizobacteria in sustainable agriculture: from theoretical to pragmatic approach. *Symbiosis*, *78*, 115-123.

Pershakova, T. V., Kupin, G. A., Mihaylyuta, L. V., Babakina, M. V., Panasenko, E. Y., & Viktorova, E. P. (2018). Investigation of antagonistic properties of bacteria *Bacillus subtilis* against carrot phytopathogenes *in vitro* and *in vivo* experiments. *Journal of pharmaceutical sciences and research*, *10*(6), 1619-1622.

Raffa, C. M., & Chiampo, F. (2021). Bioremediation of agricultural soils polluted with pesticides: A review. *Bioengineering*, *8*(7), 92.

Riaz, U., Murtaza, G., Anum, W., Samreen, T., Sarfraz, M., & Nazir, M. Z. (2021). Plant Growth-Promoting Rhizobacteria (PGPR) as biofertilizers and biopesticides. *Microbiota and biofertilizers: a sustainable continuum for plant and soil health*, 181-196.

Samada, L. H. & Tambunan, U. S. F. (2020). Biopesticides as promising alternatives to chemical pesticides: A review of their current and future status. *Online J. Biol. Sci*, *20*(2), 66-76.

Sharma, A., Shukla, A., Attri, K., Kumar, M., Kumar, P., Suttee, A., & Singla, N. (2020). Global trends in pesticides: A looming threat and viable alternatives. *Ecotoxicology and Environmental Safety*, *201*, 110812.

Singh, S. K., Singh, P. P., Gupta, A., Singh, A. K., & Keshri, J. (2019). Tolerance of heavy metal toxicity using PGPR strains of *Pseudomonas* species. In *PGPR amelioration in sustainable agriculture* (pp. 239-252). Woodhead Publishing.

Singh, S., Balodi, R., Meena, P. N., & Singhal, S. (2021). Biocontrol activity of *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* against *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*. *Indian Phytopathology*, *74*(3), 703-714.

Yuliatin, E., Ardyati, T., & Suharjono, S. (2019). Effect of Soil Physicochemical Properties on PGPR Density at A Coffee Plantation in Malang, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 391, No. 1, p. 012071). IOP Publishing.

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