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Development of Microbial consortium for plant growth promotion, nutrient and disease management in rice-horticulture based cropping system in Sikkim

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FOREWORD

Sikkim is endowed with rich floral & faunal diversity and it has become the first organic state with the adoption of 100% organic farming. However, farmers are facing challenges to ensure higher farm production and profitability. With the emerging challenges of climate change, the situation is expected to aggravate further. This demands due attention to develop a comprehensive microbial package required to manage insect and disease problem apart from enhancing productivity under organic cultivation.

The bulletin on "Development of microbial consortium for plant growth promotion, nutrient and disease management in rice-horticulture based cropping system in Sikkim", is an attempt to provide holistic information about role of native microbes in enhancing yield of local rice and different vegetable crops under Sikkim condition. This will help the stakeholders in maximizing the farm output with increased farm family income.

I congratulate the authors for their efforts in bringing out this useful publication that would serve as a reference material for different stakeholders.

(Dr. Dipankar Maiti)

Honorable Shri L N Sharma
Minister
Government of Sikkim
Department of Agriculture, Horticulture,
Animal Husbandry and Veterinary Services,
Information and Public Relations, Printing and Stationery

MESSAGE

It gives me immense pleasure to learn that ICAR-National Rice Research Institute, Cuttack and Sikkim University is publishing a research bulletin highlighting the major research outcomes of the DBT sponsored twining mode project on “Developing Microbial consortium for horticulture crops in rice based cropping system to promote growth, nutrient uptake and diseases management in organic farming in Sikkim”.

Sikkim with a great emphasis on green economy, adopting organic farming in the entire state, have many technical challenges including the nutrient and pest management. I am happy to know that the project undertaken by ICAR-NRRI and Sikkim University have come out with the valuable research bulletin entitled “Development of microbial consortium for plant growth promotion, nutrient and disease management in rice-horticulture based cropping system in Sikkim” and hope this will be much useful for the farming community of Sikkim.

I convey my best wishes to the implementing institute and particularly the project team.

(Shri L N Sharma)

MINISTER
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The Vice-Chancellor

Date : 05/10/2020

Prof. Avinash Khare
Vice Chancellor
Sikkim University
Sikkim, Gangtok

MESSAGE

I am delighted to know that the ICAR- National Rice Research Institute (NRRI), Cuttack and Department of Horticulture, Sikkim University is publishing the research bulletin highlighting the major research outcomes of the DBT- Twinning Project, "Developing microbial consortium for horticulture crops in rice based cropping system to promote growth, nutrient uptake and diseases management in organic farming in Sikkim"

It is learnt that the research was concluded with very good results for nutrient and pest management in organic farming system. I hope the research bulletin entitled "Development of microbial consortium for plant growth promotion, nutrient and disease management in rice-horticulture based cropping system in Sikkim" would be much useful for the farming community and policy makers of the state. I commend the endeavor of the project investigators and team for their efforts and looking forward to have more collaboration with ICAR- NRRI, Cuttack in future.

Date: 5/10/2020
Place: Gangtok

(Prof. Avinash Khare)

कुलपति
Vice-Chancellor
सिक्किम विश्वविद्यालय
Sikkim University

PREFACE

Sikkim has been declared the first fully organic state by Hon'ble Prime Minister of India on 18th January 2016. Presently farming in Sikkim is carried out without the use of synthetic fertilizers and pesticides, providing access to safer food choices and making agriculture a more environment-friendly activity. Sikkim is located in northeast India and blessed by a fertile land that largely supports agriculture. Prominent cropping system in the state is rice and maize based, wherein rice is cultivated in the well-designed terraces up to an altitude of 1700 m from MSL, followed by cultivation of Rabi vegetables like potato, tomato, cabbage, cauliflower and greens etc. Due to prevalence of favorable climatic conditions for pest and disease occurences like continuous heavy rainfall, low temperature and high humidity in the Sikkim Himalayan region causes enormous yield losses. Currently, several management practices are available for improving plant growth and pest management, especially in Sikkim's organic farming. However, the development of a holistic and ecologically sustainable strategy using native microbial strains having insecticidal and fungicidal properties with plant growth promotion abilities, could be a viable option for sustainable agricultural production under Sikkim condition.

To address the above issue, ICAR - National Rice Research Institute, Cuttack, Odisha and Sikkim University, Sikkim has jointly operated DBT sponsored twining mode project entitled “Developing microbial consortium for horticulture crops in rice based cropping system to promote growth, nutrient uptake and diseases management in organic farming in Sikkim”. This platform provided immense opportunity to develop microbial consortium for sustainable organic agriculture production under Sikkim condition. The microbial technologies developed under this project have been popularized among Sikkim famers through participatory field demonstration and trainings. For the benefit of farming community, researchers and policy makers, we have compiled the research findings of the project in the form of a research bulletin titled “Development of microbial consortium for plant growth promotion, nutrient and disease management in rice-horticulture based cropping system in Sikkim”. This is expected to be highly useful for the farming community of Sikkim and also other stakeholders who are involved in organic farming as well as upliftment of the economic status of the organic farmers without degenerative environmental footprint.

Authors

CONTENTS

Sl. No.	Contents	Page No.
1.0	Introduction	**08**
2.0	Geographic location, climate and agricultural production in Sikkim	**10**
3.0	Screening and characterization of PGP bacteria from Sikkim soil	**11**
4.0	Assessing multifunctional PGP traits of bacterial strains isolated from Sikkim	**12**
5.0	Bio-control potential of PGP bacterial strains isolated from Sikkim	**15**
6.0	Development of bacterial consortium	**20**
7.0	Evaluation of microbial and bio-fertilizers consortium for different vegetable and rice crops under field condition	**21**
8.0	Imparting trainings to farmers in Sikkim	**29**
9.0	Conclusion	**32**
10.0	Way forward	**32**
11.0	Annexure	**33**
12.0	References	**35**

1. Introduction

Scientific research has clearly established the role of microbes in crop growth and development. Microbes play important role in the management of plant stress arising mostly due to soil nutrient depletion and pest infestations (Jacoby et al., 2017). By altering soil microbes in rhizosphere, plant-microbe interaction has been found to be influenced, thereby increasing plant yield (Adhya and Annapurna, 2018). Different beneficial bacteria are broadly categorized into many groups in soil *viz*., nutrient mobilizers, nitrogen fixers, phytohormone producers and biocontrol agents, which are commonly grouped as plant growth promoting bacteria (PGPB) (Panneerselvam et al., 2019). Microbial formulations can be developed using single strain of bacterial isolate, but due to their inconsistency in performance under different field conditions, emphasis has been given towards developing microbial consortium *i.e.* combination of several PGPB in single carrier. Now a days, farmers want one stop solution for nutrient and pest management. Therefore, use of microbial inoculants particularly microbial consortium formulation, has to be developed for accruing multifaceted benefits to the crop plants. With the introduction of 'green revolution', modern agriculture is getting more and more dependent upon the steady supply of synthetic inputs (mainly fertilizers and chemical pesticides). Adverse effects are being noticed due to excess and imbalanced use of these synthetic inputs. In agricultural production, overuse of chemical fertilizers, pesticides and inadequate soil management practices, can significantly affect the soil quality by changing their physical, chemical, and biological properties (Prashar and Shah, 2016). This situation has led to identification of harmless inputs like beneficial microbes. Use of such natural products like bio-fertilizers in agricultural production will help in safeguarding the soil health and also the quality of crop products.

At present, most of the farmers are not evincing interest for applying bio-fertilizers for crop production due to various reasons such as slow effect, poor performance, and non-availability of the inoculants at the farm gate in a timely manner and finally lack of awareness. To overcome these problems, we need to develop a suitable delivery system and retention of beneficial microbes in the rhizosphere of crop plants. For most of the strains, when seed inoculated, they only colonize the tap root, whereas lateral roots are colonized by native ineffective microbes that make the introduced bioinoculant incompetent. Development of bioinoculant for better colonization and their survival in crop rhizosphere is essential. There is immediate need to develop compatible and effective microbial consortium for agricultural crop production. The major problem in bio-inoculant technology is poor quality microbial cultures and inability of those inoculants to compete with the native microflora (O'Callaghan, 2016). The major reason for non-survival of introduced microbes in the rhizosphere of the crop plants is due to less numbers and initial difficulty in adapting to newer rhizospheric environment. Hence the fate of introduced microbes in crop rhizosphere and their competence with native microorganisms has to be studied in detail to design rhizosphere competent strains for future microbial management strategies. Keeping these aspects in mind, the present technology has been developed for sustainable agricultural production under Sikkim conditions.

In bio-fertilizers application, co-inoculation of *Azospirillum*, *Azotobacter*, P-solubilizing bacteria and fluorescent pseudomonads has been found to be significantly better than their single inoculation (Mącik et al., 2020; Bargaz et al., 2018). Scientific evidence clearly showed that combined application of nitrogen fixing, and phosphate solubilizing and mobilizing microbes, on vegetables had positive effect on crop growth and yield (Mitra et al., 2020; Bargaz et al., 2018). ICAR-NRRI in collaboration with Sikkim University under DBT sponsored twining mode project has developed microbial consortium for management of nutrients and pest problems. This technology apart from reducing cost of application will also harness the synergistic effects of microbial combinations.

2. Geographic location, climate and agricultural production in Sikkim

India is a major agrarian country comprising 29 states, of which Sikkim has become the first organic state with the adoption of 100% organic farming. Sikkim situated in the North eastern part of India, is known for its rich biodiversity endowed with rich floral and faunal diversity. Farming system practiced in the state is unique and diverse. Prominent cropping system in the state is rice- and maize-based, wherein rice is cultivated in the well-designed terraces up to an altitude of 1700 m from MSL, followed by cultivation of rabi vegetables like potato, tomato, cabbage, cauliflower and greens etc. Rice occupies an area of about 12000 hectares with average productivity of 18 q ha^{-1} (Anonymous, 2014). Sikkim officially announced adoption of organic farming in the year 2003 to cover entire state under organic farming practice through a declaration in legislative assembly (Yadav, 2017). Now, it is a fully certified state of India as per the guidelines laid down by the National Programme on Organic Farming. The state was officially declared organic on 18th January 2016 by Hon'ble Prime Minister of India while inaugurating the National Conference on Sustainable Agriculture and Farmers Welfare, at Gangtok in Sikkim. At present time, Sikkim is well known as an organic state not only in the country but at International level also. Currently, various management practices are available for improving plant growth and disease management, especially in Sikkim's organic farming. However, the development of an effective microbial strategy using native microbial strains with insecticidal and/or fungicidal properties with plant growth promotion abilities could be a viable option for sustainable agriculture production under Sikkim condition. Keeping this in mind, attempts were made under DBT sponsored twining mode project (**Developing Microbial Consortium for horticulture crops in rice-based cropping system to promote growth, nutrient uptake and diseases management in organic farming in Sikkim**) to develop microbial consortium for sustainable agriculture under Sikkim condition.

In the eastern Himalayas, Sikkim is located at latitudes of 27°5' N to 20°9' N and longitudes of 87° 59' E to 88°56' E. Positioned in north-east, Sikkim is the second smallest state of India sandwiched between Nepal in the west and Bhutan in the east, China in the north and West Bengal in the south, covering an area of 7,096 km^2. Sikkim gets water from rivers like Teesta and Rangeet and receives plenty of monsoon showers (both south-west and north-east monsoon combined) round the year. With the elevation ranging from 280 m (920 feet) to 8,585 m (28,000 feet), Sikkim has diverse climate ranging from tropical to tundra. The geographical diversity of Sikkim is probably the reason for the rich collection of flora and fauna in this small state. Being rocky, however, less area is available for farming and agriculture. The average annual temperature for the major part of Sikkim is recorded 18°C (64°F) approx. During the months of March - May, the sun shines at its best in the state of Sikkim. The weather remains wintry and humid, since it rains most of the time.

Agriculture has been the mainstay of the rural population of Sikkim. Due to adverse geographical location and difficult terrain, the expanse of area under cultivation is limited. The challenge is two-fold, very low per-capita availability of land and decreasing number of farmers working on farms. Being the first organic state of India, Department of Science and Technology has been involved propagating and advocating farmers to adopt new and modern technologies of organic farming system to sustain productivity and production. The policies and programmes on organic farming in tune with our natural endowments envisage to make Sikkim a model organic State. The march towards organic farming has led to substantial departmental interventions. The agriculture department has formulated a programme to curtail the percentage of food grain deficit. Maximum thrust is being given in increasing the productivity of various crops through proper crop management under organic system of farming, extension of irrigation facilities, farm mechanization, value addition and post-harvest processing etc. The rice-based cropping systems followed in Sikkim are rice-potato or rice-vegetables (cabbage, cauliflower, radish, carrot, spinach or local vegetables). It is highly imperative to support the winter vegetable production in order to sustain the rice cultivation. In many cases, there has been tremendous crop diversification owing to the less remunerative rice cultivation. For making the cropping system more remunerative or increasing the vegetable productivity in winter, nutrient management has been seen as one of the most important initiative to be followed. Similarly, proper crop protection measures are equally important. Among the available strategies for improving plant resistance to insect pest and diseases, microbe induced defense strategy may offer a promising alternative to chemical control.

3. Screening and characterization of plant growth promoting (PGP) bacteria from Sikkim soil

Survey was conducted on 30 different farmer's field in east and west Sikkim and soil samples were collected from rice based horticulture cropping system. In total, 436 bacterial isolates were isolated, of which 16 bacteria (Table 1) were selected based on their multiple plant growth promoting attributes. All the isolates were tested by adopting standard methods for phyto-hormone production (Restu et al., 2019; Panneerselvam et al., 2019), nitrogen fixation (Tang et al., 2020; Jiménez et al., 2011), phosphate (Tang et al., 2020), potash solubilization (Etesami et al., 2017) and antagonistic potential (Panneerselvam et al., 2018; El-Sayed et al., 2014) against soil born fungal pathogens. Efficient selected PGPR isolates (*B. subtilis* BioC-WB (NAIMCC-B-02285), *B. luciferensis* K2 (NAIMCC-B-02286) *B. amyloliquefaciens* K12 (NAIMCC-B-02288) and *B. megaterium* (NAIMCC-B-02287) were deposited at ICAR-NBAIM, Mau, UP.

Table 1: Molecular identification of potent plant growth promoting bacterial species isolated from Sikkim soil

Sl No.	Strain No.	16SrRNA Identification	Accession No.
1	PANS13	*Pseudomonas putida*	MT020300
2	PANSK10	*P. fluorescens*	MT020294
3	PANA13	*Bacillus subtilis*	MT020289
4	PanAz3	*Azotobacter chroococcum*	MT186700
5	PanPs4	*P. putida*	MT186701
6	PanAzb5	*A. beijerinckii*	MT186701
7	USR4	*Burkholderia ambifaria*	MK359019
8	K18	*Bacillus amyloliquefaciens*	MK359016
9	K12	*B. amyloliquefaciens*	MG490140
10	R32/R3	*B. megaterium*	MG490139
11	LS3	*Variovorax paradoxus*	MG490138
12	AR1	*Burkholderia* sp.	MG490137
13	SSR2	*B. thuringiensis*	MG490136
14	K13	*B. subtilis*	MG490135
15	K2	*B. luciferensis*	MG490134
16	BioC-WB	*B. subtilis*	MG490133

4. Assessing multifunctional PGP traits of bacterial strains isolated from Sikkim

Based on PGP traits, the following three bacterial strains *viz.*, nitrogen fixer (*A. chroococcum* (NCBI: MT186700), phosphate (*B. megaterium* (NAIMCC-B-02287) and potassium solubilizers (*P. putida* NCBI: MT020300) were selected and their PGP traits are presented in Table 2. *A. chroococcum* recorded 119.7 µg/mL of IAA, 5.7 µg/mL of GA_3 production and 11.3 mg of nitrogen fixed per gram of sucrose utilized. The phosphate solubilizing bacterium *B. megaterium* could produce IAA (53.1 µg/mL) and GA_3 (4.0 µg/mL) and had ability to solubilize 2646.3 µg/mL of tricalcium phosphate in broth culture. *P. putida* (NCBI: MT020300) could solubilize potash and produced 18.5 mm clear zone in KSB medium, this strain also having ability to produce IAA, GA_3 and phosphate solubilization.

The PGP traits were also assessed (Table 3) in biocontrol bacterial strains used for microbial consortium preparation viz., *B. luciferensis* K2, *B. amyloliquefaciens* K12 and

B. subtilis BioCWB. Among the three strains, B*. luciferensis* K2 significantly produced higher GA_3 and IAA phytohormones (GA_3: 10.6 µg mL^{-1} and IAA: 97.1 µg mL^{-1}) compared to other two bacterial isolates. In phosphate solubilizing ability, *B. subtilis* BioCWB showed higher tricalcium phosphate solubilization (570 ppm) followed by *B. luciferensis* K2 (417.3 ppm), the BioCWB also possess nitrogen fixing ability (Figure 2). In addition, the selected bacterial strains potential for rhizosphere competence were tested in chilli plants under glass house condition. All the three strains having good abilities to colonize rhizosphere of chilli (1.8-2.1x10^6 cfu g^{-1}) plants compared to other strains. In addition, the efficiency of nitrogen fixing ability by the diazotropic bacteria was found to be higher in *Azotobacter* species followed by *B. subtilis*.

Table 2: Characterization of plant growth promotion and biocontrol traits in bacterial strains used for preparation of bio-fertilizers consortium

Parameters	***A. chroococcum***	***P. putida***	***B. megaterium***
Indole 3-acetic acid (IAA)(µg/mL)	119.7	512.4	53.1
Gibberlic acid (GA_3) (µg/mL)	5.7	5.8	4
Phosphate solubilization (µg/mL)	0	2354.9	2646.3
Potash solubilization zone (mm)	Nd	18.5	14.3
Nitrogen fixation (mg/g sucrose)	11.3	nd	nd

nd: not deducted

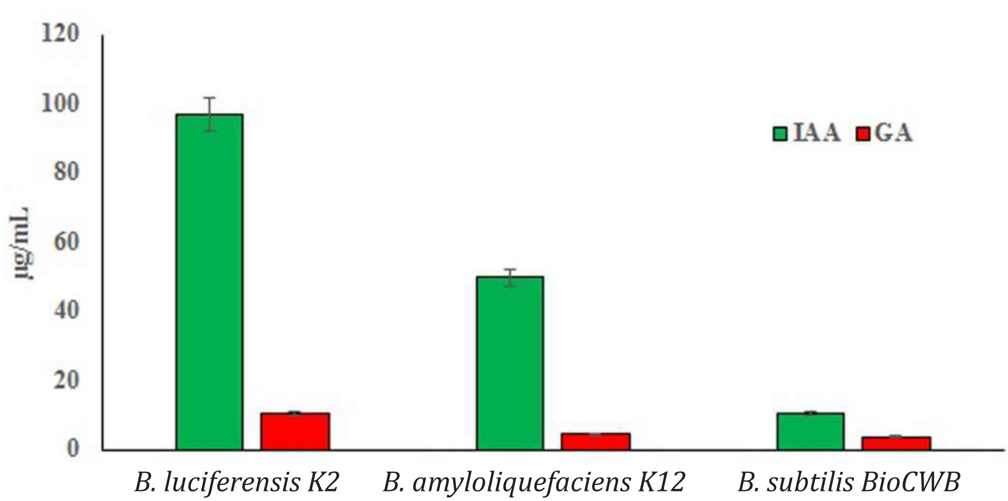


Figure 1: IAA and GA_3 production by plant growth promoting bacteria

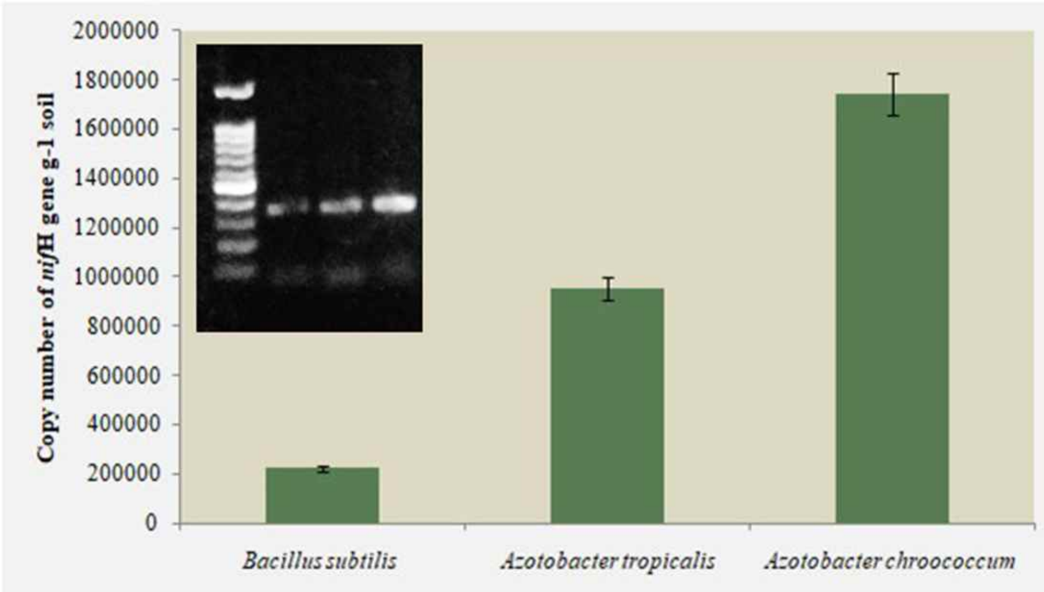


Figure 2: N_2 fixing efficiency of PGP isolates through *nif*H gene abundance using RT-PCR

Figure 3: Phosphate solubilization of *B. subtilis* BioCWB strain

5. Bio-control potential of PGP bacterial strains isolated from Sikkim soil

The following three efficient strains *viz*., *B. subtilis* BioC-WB (NCBI: MG490133), *B. luciferensis* K2 (NCBI: MG490134) and *B. amyloliquefaciens* K12 (NCBI: MG490140) were assessed for their antagonistic potential against some important plant pathogenic fungi of rice *viz*., *Sclerotium rolfsii* (seedling blight), *Fusarium proliferatum* (sheath rot) and *Rhizoctonia solani* (sheath blight). The *in-vitro* assay studies indicated that all the three strains have good antagonistic potential against above said plant pathogens (Table 3 and Fig 4). In pot experiment, the liquid-based bioformulation of above said three bacterial strains were evaluated for their potential in controlling rice sheath blight. Thirty-five days old rice plants were inoculated with *R. solani* by using standard methods and periodic observations were recorded. Results of the studies revealed that application of *B. subtilis* suppressed rice sheath blight incidence by 71.1%, followed by *B. luciferensis* (53.4%) and *B. amyloliquefaciens* (27.35%). The chemical treatment showed 83.07% suppression and the maximum disease incidence was recorded in un-inoculated control. These finding indicates that the above-mentioned *Bacillus* spp. exhibits significantly higher bio-control potential against *R. solani* which is the causal organism for rice sheath blight.

Table 3: Characterization of plant growth promotion and biocontrol traits in bacterial strains used for preparation of microbial consortium for pest and disease management

Parameters	*B. luciferensis*	*B. amyloliqufaciens*	*B. subtilis*
IAA (µg/mL)	97.1	49.9	10.4
GA_3 (µg/mL)	10.6	4.6	3.9
Phosphate solubilization (µg/mL)	417.3	364.1	570
Nitrogen fixation (mg/g sucrose)	0	0	1.8
Siderophore production	+	+	+
Hydrogen cyanide production	+	+	+
Ammonia production	+	+	+
Protease activity	+	+	+
Chitinase activity	+	+	+
LC50 (CFU x10^4 ml^{-1})	2.0	2.9	2.1
Suppression sheath blight (%)	53.4	27.3	71.1
Plate assay			
R. solani (%)	75.0	22.9	79.6
F. proliferatum (%)	83.3	72.5	86.0
S. rolflsii (%)	86.3	82.9	86.7
Broth assay			
R. solani (%)	80.8	25.3	84.5
F. proliferatum (%)	79.5	65.9	76.6
S. roflsii (%)	78.0	65.1	87.8

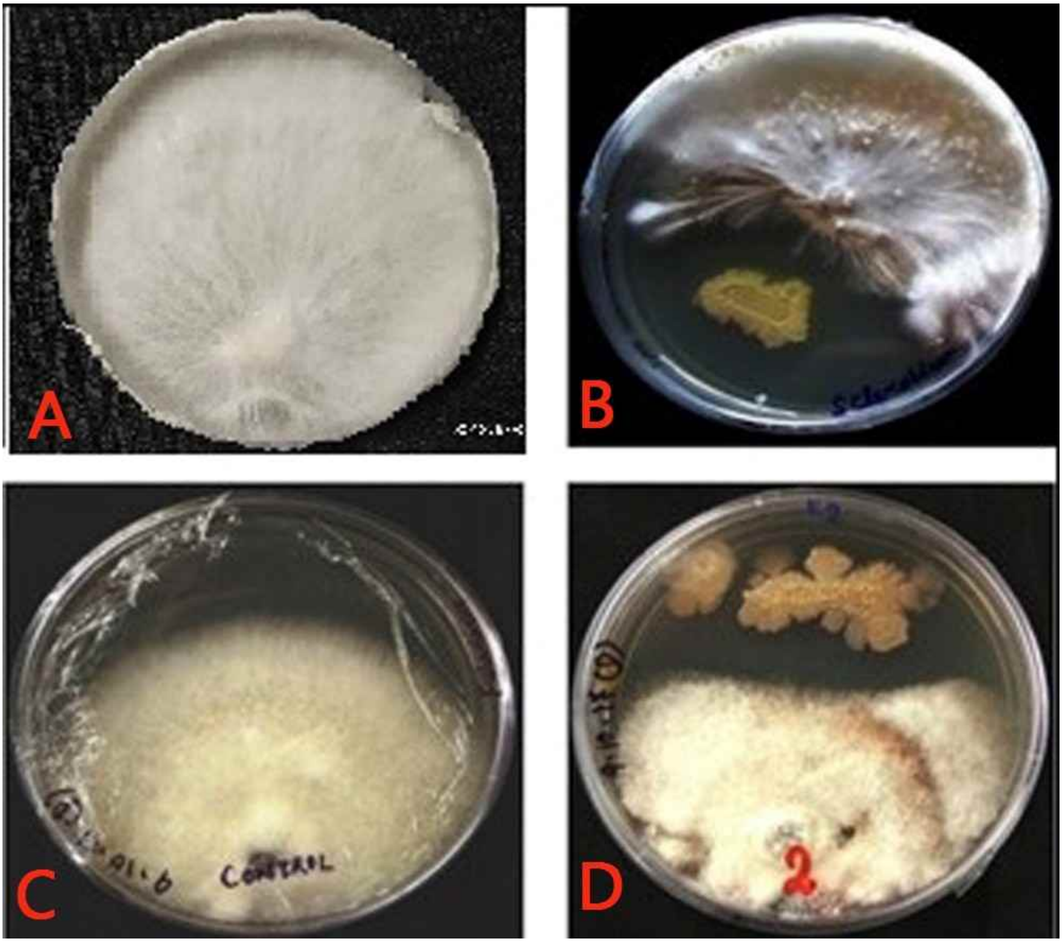


Figure 4: Antagonistic activity of *B. subtilis* BioC-WB against *S. rolfsii* and *F. proliferatum* **(**A: control – only *S. rolfsii;* B: *S. rolfsii + B. subtilis* BioC-WB*;* C: Control – only *F. proliferatum*; D: *F. proliferatum + B. subtilis* BioC-WB)

5.1. Larvicidal potential of PGP bacterial strains used for microbial consortium

The larvicidal potential of three efficient bacterial strains *viz*., *B. subtilis* BioC-WB (NCBI: MG490133), *B. luciferensis* K2 (NCBI: MG490134) and *B. amyloliquefaciens* K12 (NCBI: MG490140) were evaluated in potted rice plants (variety TN1), among the three strains, *B. luciferensis* treated leaves showed 50% (LC_{50}) leaf folder larval mortality with the cell load of 2.0 x 10^4 CFU ml^{-1} whereas *B. subtilis* treated leaves showed 50% (LC_{50}) leaf folder larval mortality with 2.10 x 10^4 CFU ml^{-1} and *B. amyloliquefaciens* 2.90 x 10^4 CFU ml^{-1} respectively (Table 3 and Figs. 5 & 6), which indicates that all the three strains have good larvicidal potential against rice leaf folder with minimum cell load in the range of 2.0 to 2.97 x 10^4 CFU ml^{-1}.

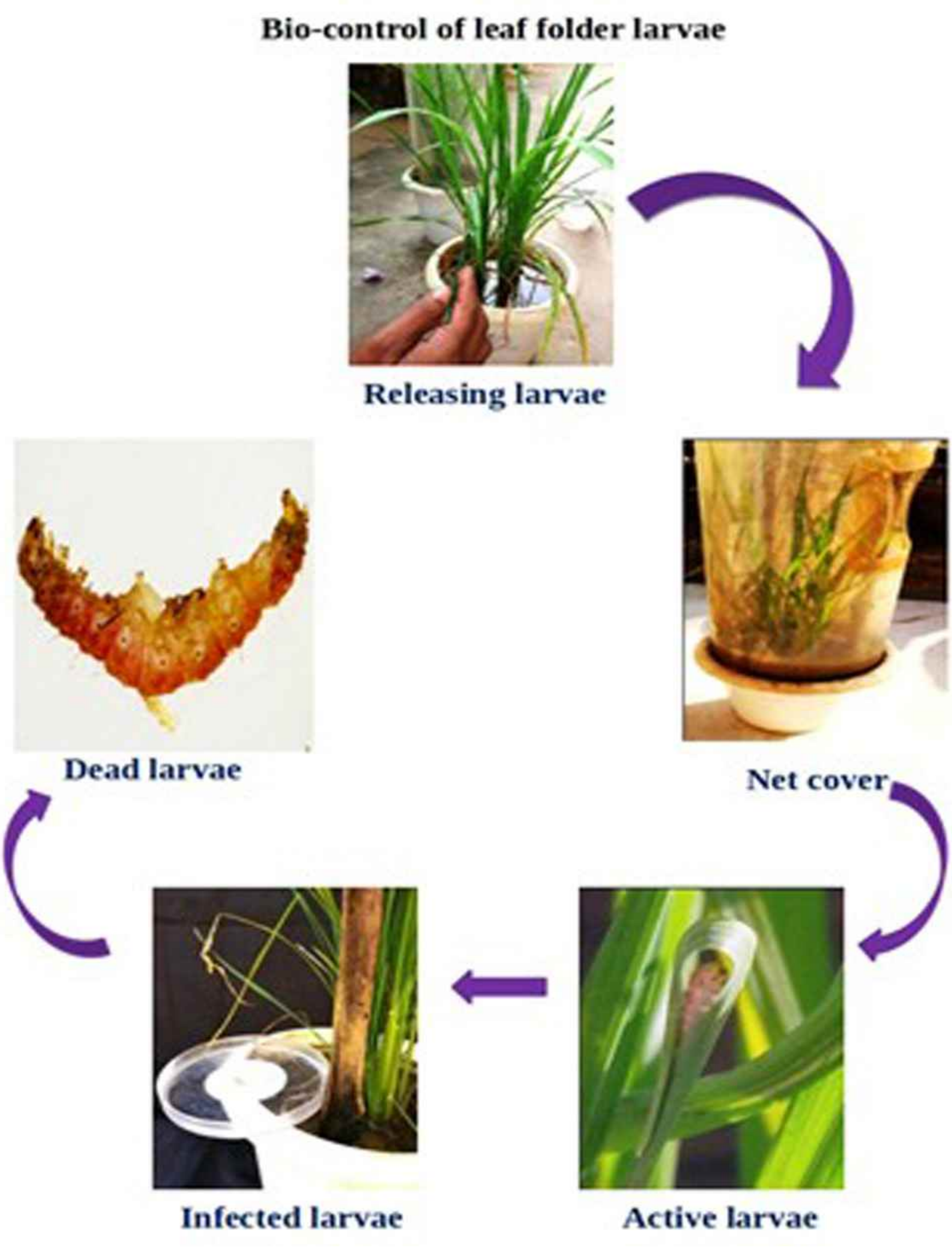


Figure 5: Larvicidal activity of PGP isolates against rice leaf folder larvae under glass house conditions

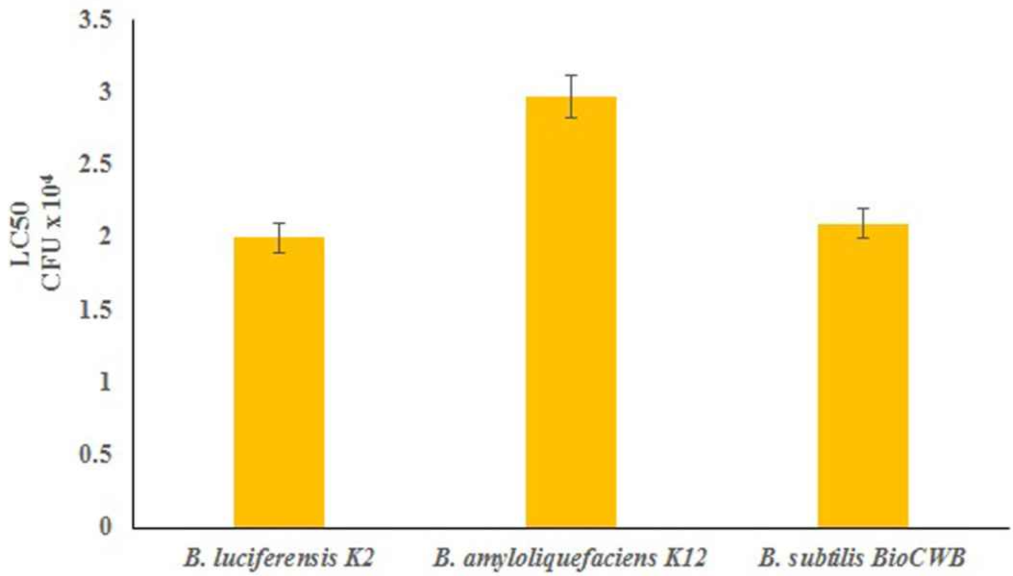


Figure 6: Toxicity of *Bacillus* spp against third instars larva of rice leaf folder (*C. medinalis* Guenee)

5.2. Assessment of volatiles and metabolites produced by PGP bacterial strains

To understand the possible antagonistic mechanisms of *Bacillus* spp., the volatiles and soluble metabolites released by them were analyzed. The results (Table 4 and Fig 7) showed that *B. luciferensis* K2 contained 29 different types of volatiles which included the most abundant furan, 2-methyl- (17.56%), acetic acid, anhydride with formic acid (14.944%) and disulfide, dimethyl (8.189%). Whereas *B. subtilis* BiocWB recorded 23 volatiles with the maximum production of 1, 3-pentadiene, (Z)- (17.984%), 3-hexyn-2-ol (15.435%) and disulfide, dimethyl (10.248%). *B. amyloliquefaciens* K12 possessed 22 volatiles where the most abundant were Disulfide, dimethyl (15.795%) and furan, 3-methyl- (11.242%). Among the soluble metabolites, several aromatic, alkane, aldehyde, alkene, esters, ethers and amides were recorded. *B. luciferensis* and *B. subtilis* showed the maximum number of soluble metabolites with 9-octadecenamide, (Z)- (23.256%) being the most abundant. *B. luciferensis* strain K2 recorded twenty-six metabolites, of which E-15-heptadecenal (9.89%) was found to be maximum followed by 3-Eicosene, (E)- (9.31%). All the three bacterial strains had multiple antimicrobial peptide genes (Fig 8), which includes mycosubtilin, mersacidin, ericin, subtilin, surfactin, iturin, bacilysin and subtilosin (Panneerselvam et al., 2019). The above information clearly indicated that the combined effect of antimicrobial peptides, volatiles, soluble metabolites, siderophore, ammonia, protease and chitinase production might have played major role in pest and disease suppression.

Table 4: Mass spectroscopy analysis of metabolites released by multipotent plant growth promoting *B. subtilis* strain BiocWB

RT	Metabolite Name	Area%
11.89	Phenol, 2,6-bis(1,1-dimethylethyl)-	4.607
13.73	Hexadecen-1-ol, trans-9-	4.691
17.94	5-Octadecene, (E)-	6.074
20.43	L-Proline, N-valeryl-, undecyl ester	5.23
21.91	3-Eicosene, (E)	5.735
25.55	1-Docosanol, methyl ether	4.329
28.29	9-Octadecenamide, (Z)-	23.256
28.86	Cyclotetracosane	3.029
33.54	Trans-13-docosenamide	4.198

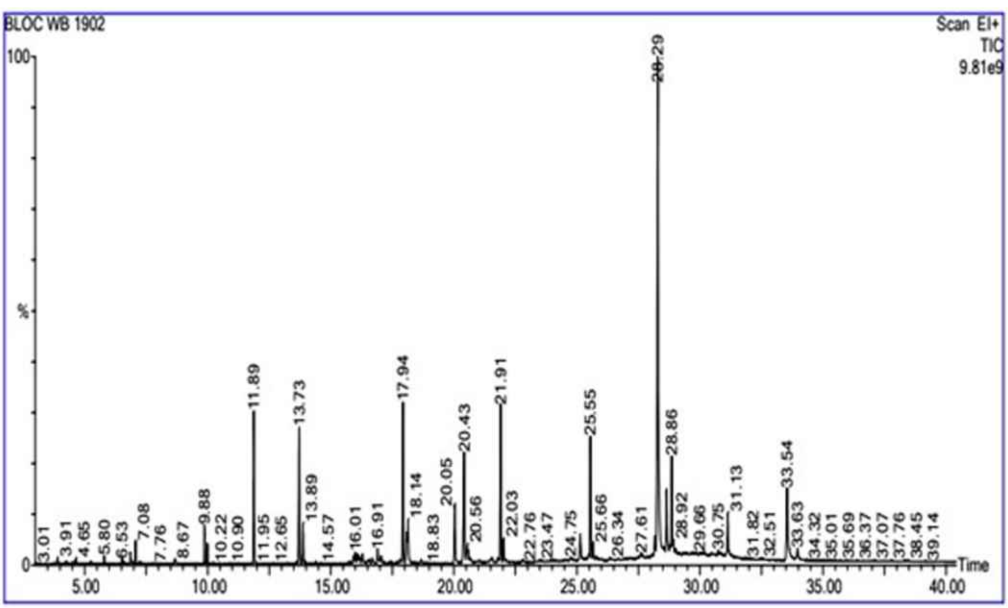


Figure 7: GC/MS profiles of volatiles emitted by *Bacillus* spp.

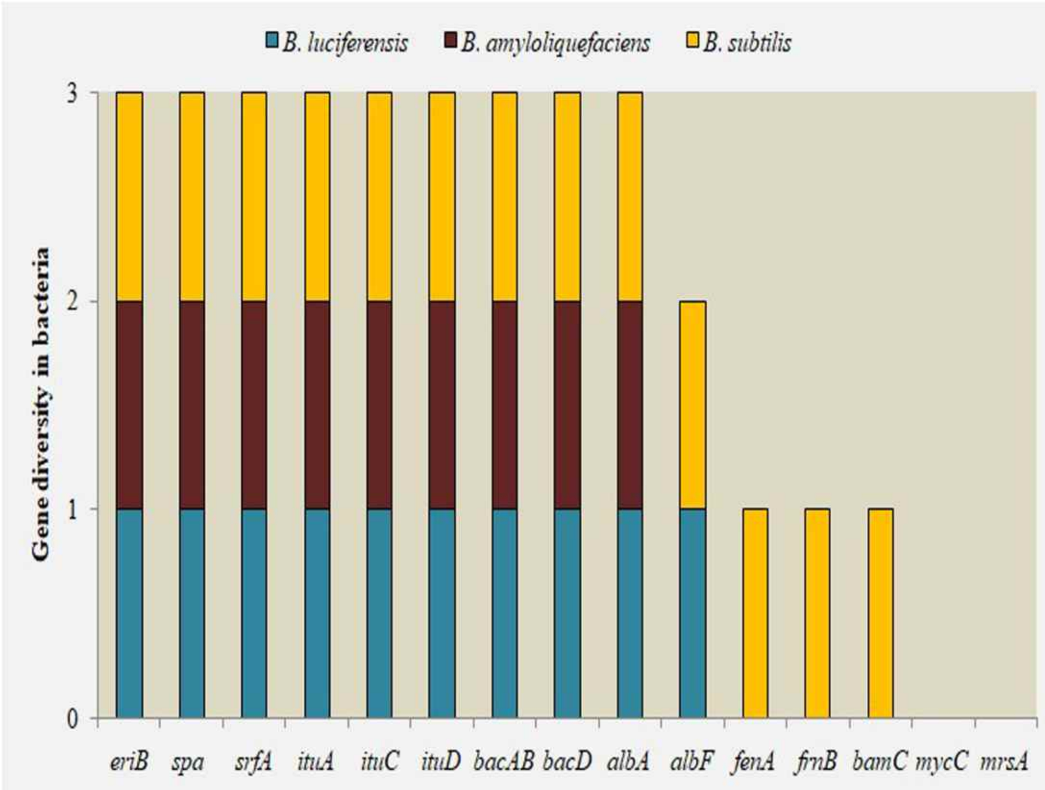


Figure 8: Antimicrobial peptide genes of different *Bacillus* spp.

6. Development of bacterial consortium

After characterization and compatibility assessment, the selected isolates were multiplied in large quantities (4.0 L) in respective broth (all *Bacillus* spp. and *Pseudomonas* multiplied in NA broth, *Azotobacter* in Jenson broth) and incubated till they attain log phase (*i.e*. *Bacillus* spp for 36- 48 h, *Azotobacter* for 72 h) with a cell load of 10^9 -10^{10} cells ml^{-1} and, then used for inoculant preparation. The liquid microbial consortium was prepared by mixing the log phase cultures (10^9 -10^{10}cells ml^{-1}) of BioCWB, K2 and K12 with equal proportion. After preparation, osmo-protectants (glycerol and polyvinyl pyrolidone) were added in different proportion and mixed gently. The prepared microbial consortium were stored in plastic bottles. Similarly, bio-fertilizer consortium was prepared using following microbial strains *viz*., *A. chroococcum*, *B. megaterium* and *P. putida* (Fig 9)

Figure 9: Formulations of bio-fertilizers and microbial consortium

7.0. Evaluation of microbial and bio-fertilizers consortium for different vegetable and rice crops under field condition

The following two microbial formulations *viz*., i) Bio-fertilizer consortium (BC) for nutrient management and ii) Microbial consortium (MC) for pest and disease management developed under the DBT project were evaluated in different vegetable crops *viz*., broccoli, brinjal, cabbage, capsicum, okra, tomato and rice under greenhouse and field condition (Fig10). Microbial and bio-fertilizers consortium were applied separately (5-liter ha^{-1}) and in combination (2.5-liter microbial consortium + 2.5-liter bio-fertilizer consortium ha^{-1}) by soil drenching.

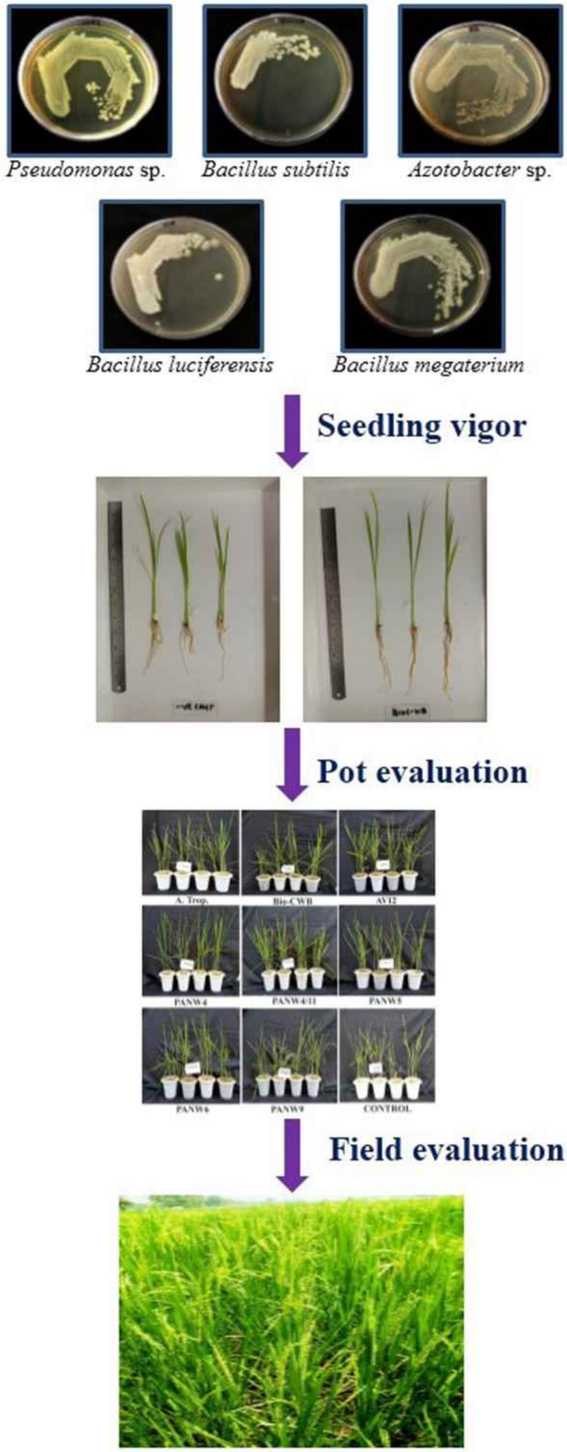


Figure 10: Evaluation of microbial consortia for plant growth improvement

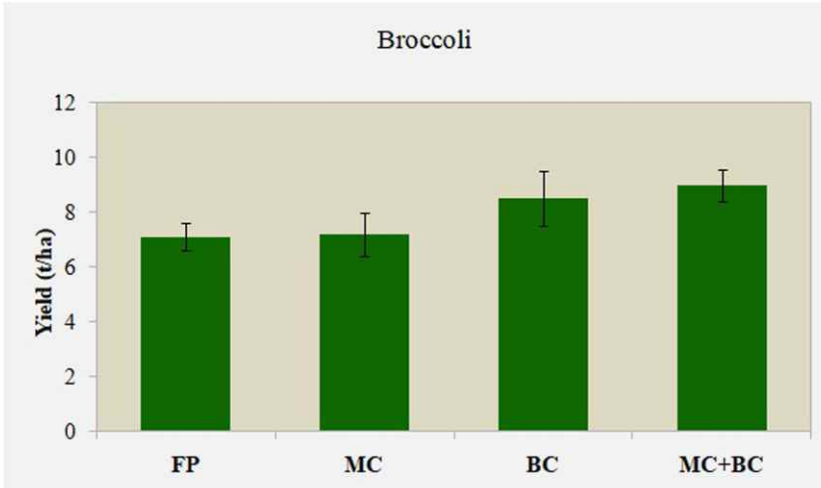
7.1. Evaluation of microbial technology in broccoli under field conditions

Broccoli (*Brassica oleracea* L.) variety Marathon (F1 hybrid) with medium to late (90-100 DAT) duration was used in field trials. The following four different treatments were imposed *viz*., (1) FP: farmer practice by applying 20 t/ha FYM, (2) MC: microbial consortium, (3) BC: bio-fertilizer consortium and (4) combined application of MC and BC. The results of the field trials indicated that combined application of MC and BC significantly increased yield (8.36 t/ha) as compared to farmer's practices. Individual application of BC and MC recorded 8.10 t/ha and 7.80 t/ha yield, respectively. These observations clearly indicated that the combined application of BC and MC could increase broccoli yield under Sikkim condition as compared to existing farmer's practices (Fig 11-13).

Figure 11: Evaluation of bio-fertilizers and microbial consortium in broccoli seedlings

FP - Farmers practice
MC- microbial consortium
BF - biofertilizer consortium,
M+B- microbial
+ biofertilizer consortium

Figure 12: Evaluation of bio-fertilizers and microbial consortium in broccoli seedlings under field condition

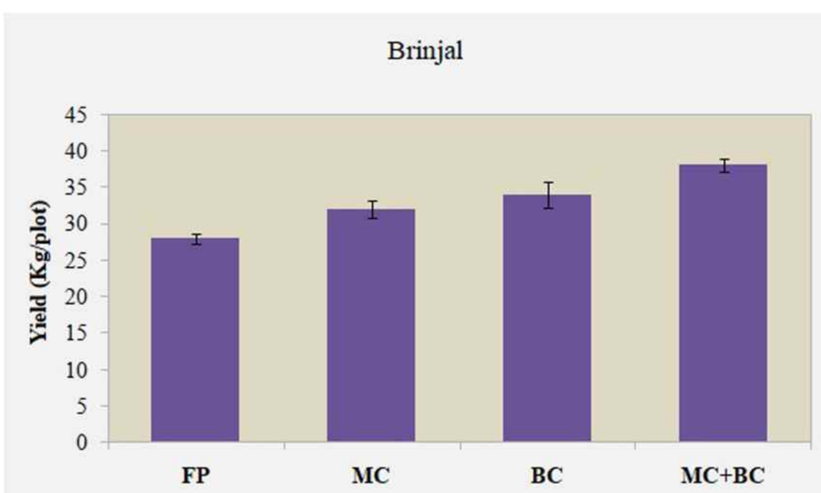


FP: Farmer practice MC: Microbial consortium BC: Bio-fertilizer consortium

Figure 13: Effect of bio-fertilizers and microbial consortium on yield of broccoli.

7.2. Evaluation of microbial technology in brinjal under field conditions

The following two microbial technologies *viz*., i) bio-fertilizer consortium ii) microbial consortium were evaluated in brinjal under field condition. Liquid microbial consortium was applied at rate of 5 liter ha^{-1} by soil drenching after 30DAT. Both the products significantly increased brinjal yield. Application of BC plus MC recorded significantly higher brinjal yield (34.0 kg per 12 m^2) than only organic manure application (26.5 kg per 12 m^2) (Fig 14).



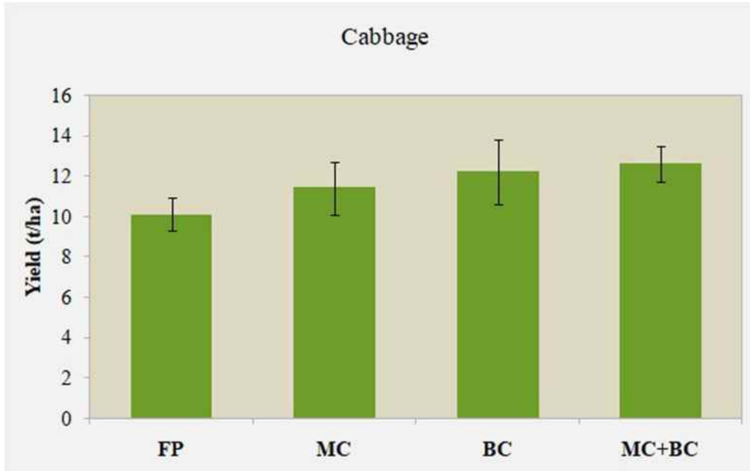
FP: Farmer practice MC: Microbial consortium BC: Bio-fertilizer consortium

Figure 14: Effect of bio-fertilizers and microbial consortium on brinjal yield

7.3. Evaluation of microbial technology in cabbage (variety Rareball) under field conditions

In this field trial there were four treatments *i.e.* (a) FP (farmers practice) (b) application of MC (microbial consortium), (c) application of BC (bio-fertilizer consortium) and (d) combination of microbial and bio-fertilizer consortium. Results indicated combined application of MC and BC recorded significantly higher yield (12.2 t/ha) as compared to farmer practices (10.0 t yield /ha). The present evaluation revealed that application of BC+MC increased yield by 22.0 % in cabbage as compared to FP, which provides evidence that microbial intervention improves cabbage yield under Sikkim conditions (Fig 15 -16).

Figure 15: Evaluation of bio-fertilizers and microbial consortium in cabbage (variety Rareball) under field condition



FP: Farmer practice MC: Microbial consortium BC: Bio-fertilizer consortium

Figure 16: Effect of bio-fertilizers and microbial consortium on cabbage yield

7.4. Evaluation of microbial technology in capsicum under field conditions

In this evaluation, individual and combination of bacterial inoculants were evaluated in capsicum under field condition. The following treatments were imposed T1: uninoculated control, T2: *B. subtilis* BioCWB, T3: *B. amyloliquefaciens* K12, T4: *B. luciferensis* K2, T5: *B. subtilis* BioCWB+ *B. amyloliquefaciens* K12+ *B. luciferensis* K2. The results showed that *B. luciferensis* K2 application significantly increased root dry weight, plant dry biomass and fruit number as compared to *B. amyloliquefaciens* K12 and *B. subtilis* BioCWB. However, combined application of three strains was found superior in enhancing plant height (48.3 cm), shoot weight (10.0 g), root weight (0.7 g), plant biomass (10.7 g) and fruit number (14.3) as compared to individual strains (Fig 17).

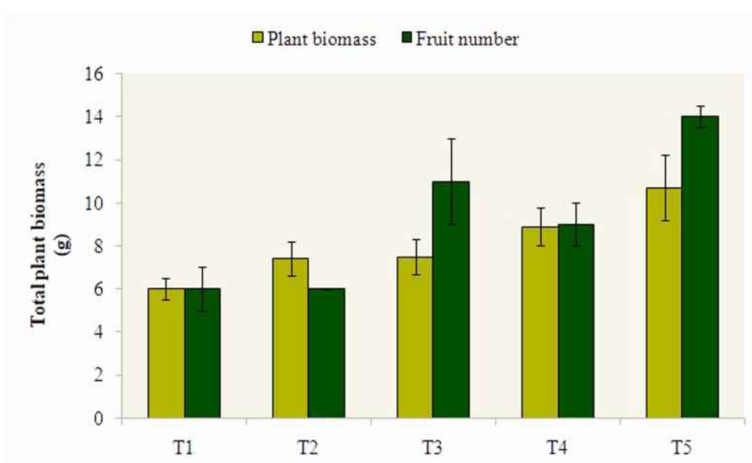
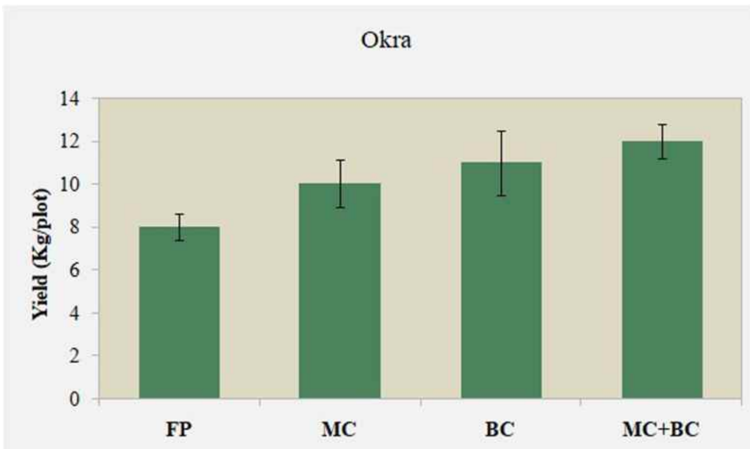


Figure 17: Effect of bio-fertilizers and microbial consortium on capsicum yield

7.5. Evaluation of microbial technology in okra under field conditions

Bio-fertilizer consortium and microbial consortium were evaluated in okra under field condition. Liquid microbial consortium was applied at rate of 5 liter ha^{-1} by soil drenching after 30 DAT. Both the products showed significant improvement in yield, wherein the bio-fertilizers consortium application recorded yield of 11.0 kg/12 m^2 area plot and microbial consortium recorded 10.0 kg yield /plot as compared to organic manure application (8.0 kg/plot) (Fig 18).



FP: Farmer practice MC: Microbial consortium BC: Bio-fertilizer consortium

Figure 18: Effect of bio-fertilizers and microbial consortium on okra yield

7.6. Evaluation of microbial technology in tomato under field conditions

Solanum aethiopicum, a local crop germplasm has high demand as vegetables by local community of Sikkim Himalayas. The crop is cultivated in every household of Sikkim state and abundantly grown in North Eastern part of India. The cultivation of *S. aethiopicum* in Sikkim hills has gain more importance after the declaration of Sikkim as Organic state owing to its wide adaptability to biotic and abiotic factors. In this study, the growth of *S. aethiopicum* was enhanced in the fields inoculated with consortium along with recommended dose of FYM and vermicompost. Highest yield of 5546 kg/ha was obtained with application of microbial consortium which was 40 % higher than uninoculated control.

7.7. Evaluation in rice plants under field condition

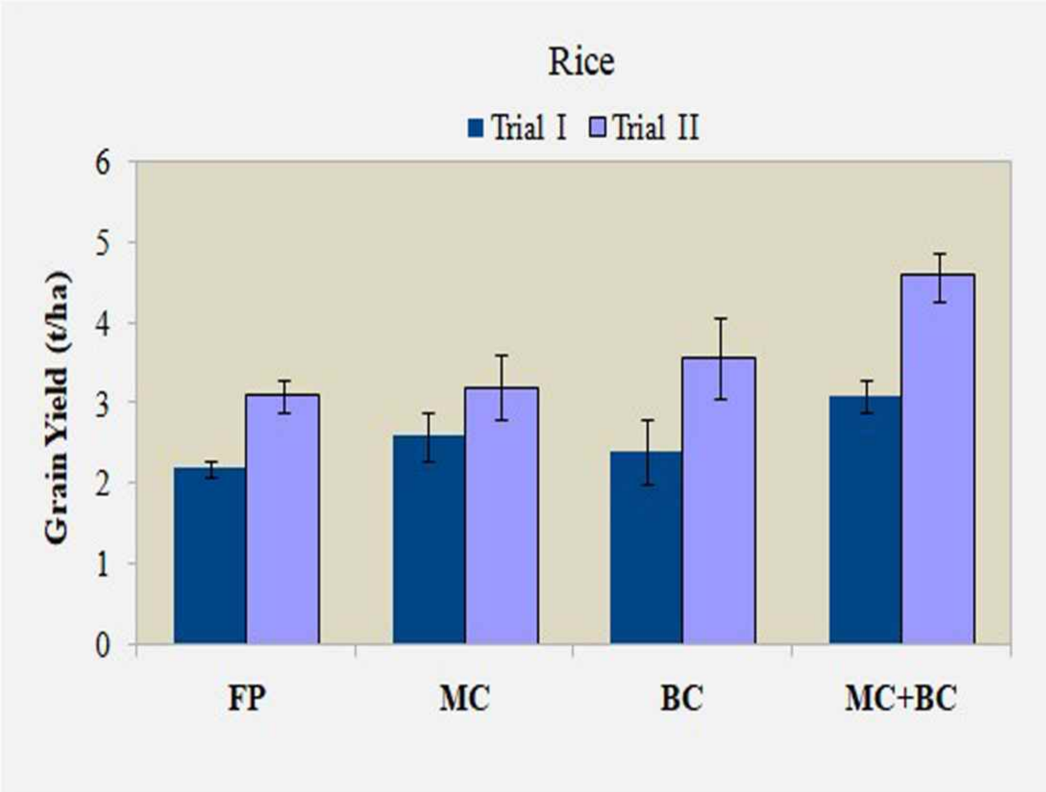
Bio-fertilizer and microbial consortium were evaluated in local rice variety (*cv.* Attey) under field condition. The results showed that the combined application of these two products significantly increased rice grain yield (2.6-4.5 t/ha) as compared to un inoculated control (1.9 - 3.1 t/ha). Application of either microbial consortium or bio-fertilizer consortium recorded higher yield as compared to farmer's conventional practices (Figs 19-21). The present study indicated that application of MC plus BC increased yield by 26.9 -31.0 % as compared to FP.

FP: Farmer practice   MC: Microbial consortium   BC: Bio-fertilizer consortium

Figure 19: Experimental field view of rice field at Rabitar village, Sikkim

FP: Farmer practice   MC: Microbial consortium   BC: Bio-fertilizer consortium

Figure 20: Harvesting stage of rice (Attey variety) at Rabitar village Sikkim



FP: Farmer practice MC: Microbial consortium BC: Bio-fertilizer consortium

Figure 21: Effect of bio-fertilizers and microbial consortium on rice (variety-Attey) grain yield

8. Imparting trainings to farmers in Sikkim

The two microbial formulations *i.e.* microbial and bio-fertilizers consortium developed under DBT twining mode project in collaboration with Sikkim University were demonstrated in the farmers' field at Sikkim. Also, both men and women farmers were given hands-on training on method of application of bio-fertilizers at field level. In this demonstration programme, more than 50 farmers including 20 female farmers participated. Different mode of delivery of microbial inoculants at field level *i.e.* seed and seedlings treatment, soil drenching, basal application and composts/ FYM enrichment was demonstrated. This programme was successfully conducted by ICAR-National Rice Research Institute in collaboration with Department of Horticulture, Sikkim University (Figs 22-25)

Figure 22: Demonstration cum training on method of bio-inoculants application at farmer's field at Pachey village, Sikkim

Figure 23: Demonstration cum training on application methods of bio-inoculants at farmer's field at Gairi Gaon village, Sikkim

Figure 24: Demonstration of method of application for bio-inoculants at farmer's field at Pachey village, Sikkim

Figure 25: Training conducted on microbial technology at Sikkim for agricultural professionals

9. Conclusion

Several combinations of bacterial strains were tested both under *in-vitro* and *in-vivo* conditions among which the combinations of the following native bacterial isolates from Sikkim *viz*. *B. subtilis* BioC-WB (NAIMCC-B-02285), *B. luciferensis* K2 (NAIMCC-B-02286) and *B. amyloliquefaciens* K12 (NAIMCC-B-02288) were found suitable for preparation of "Microbial formulation" for plant health improvement. Similarly, the following three efficient bacterial strains *viz*., nitrogen fixer (*A. chroococcum* (NCBI: MT186700), phosphate (*B. megaterium* (NAIMCC-B-02287) and potassium solubilizers (*P. putida* NCBI: MT020300) were selected for bio-fertilizer consortium preparation. Both the formulations had good compatibility with each other. Assessment of shelf life under room condition indicated that this product can be stored without any deterioration up to six months. Both the microbial products were evaluated for different crop plants including broccoli, brinjal, capsicum, cabbage, okra, tomato and rice under Sikkim condition. The combined application of microbial and bio-fertilizer consortium significantly increased the yield in different vegetable crops and rice as compared to farmer's practice in Sikkim condition.

10. Way forward

Microbial consortium prepared by using indigenous microbes isolated from Sikkim soils for plant growth promotion and disease management have shown better performance for yield improvement in important different vegetable and rice crop. These microbial products should be popularized among farmers and up scaled by Government of Sikkim to harness the benefits of these products.

Annexure I
Method of Application

Crop	Mode of application	Dosage	Description of method of application
Vegeta-bles	Seed treat-ment	10-20 ml inoculums per 100 g seeds	This is most common method used for most of the crop plants and this method is more effective and economic. Around 10-20 ml inoculums are sufficient to treat 100 g vegetable seeds. Take the required seed materials in small clean polythene bag and add sufficient inoculum along with 0.1 % carboxy methyl cellulose (sticking agent), then bag should be closed and squeezed for 3-4 minutes until all the seed are uniformly wetted. After through coating, the bag is opened and the seed is dried under the shade for 30 minutes. If we want to treat for large amount of seeds, big containers like plastic bucket can be used. The microbial treated seeds should be sown immediately after shade drying.
	Soil Drench-ing	2.0 lit inocu-lums per acre	Microbial consortium should be mixed with water @ 25 ml/ litre and then applied near the root zone on 5-10 days after transplanting.
	Root dipping	500 ml inoculums per acre seedlings	The required quantity of inoculums should be mixed with 50 litres of water and the roots of seedlings should be immersed for half-an-hour before transplanting.
	Main field application	2.0 lit inocu-lums per 500 kg FYM enrichment	For the main field application for one acre of land, 2-liter microbial consortium should be mixed with 500 kg of FYM and applied near the root zone of standing crop. Alternatively, it can be applied basally at the time of land preparation. Spread the compost in shaded place and uniformly sprinkle the diluted inoculums (one lit inoculums mixed in 20 L water) over the compost. Mix the inoculums thoroughly, after mixing, heap the compost and allow for 1-2 days for further multiplication. The enriched compost should be broadcasted in the field or it should be applied near the root zone of standing crop.

Rice	Seed treat-ment	500 ml inoculums per one acre	Around 500 ml inoculums are sufficient to treat the rice seeds for one-acre field. Take the required seed materials in small clean polythene bag and add sufficient inoculum along with 0.1 % carboxy methyl cellulose (sticking agent), then bag should be closed and squeezed for 3-4 minutes until all the seed are uniformly wetted. After through coating, the bag is opened and the seed is dried under the shade for 30 minutes. If we want to treat for large amount of seeds, big containers like plastic bucket can be used. The microbial treated seeds should be sown immediately after shade drying.
	Root dipping	500 ml inoculums per acre seedlings	The required quantity of inoculum (500 ml) should be mixed with 50 litre of water and the roots of seedlings should be immersed for half-an-hour before transplanting.
	Main field application	2.0 litre inoculum per 500 kg FYM enrichment	For the main field application of one acre of land, 2-litre microbial consortium can be mixed with 500 kg of FYM and it can be applied basally at the time of land preparation. Spread the compost in shaded place and uniformly sprinkle the diluted inoculum (one litre inoculum mixed in 20 litre water) over the compost. Mix the inoculum thoroughly, after mixing, heap the compost and allow for 1-2 days for further multiplication. The enriched compost can be broadcasted in field.

Important points to be followed during the time of applications

- Always wear protective gear (goggles, masks, gloves).
- Keep the product beyond the reach of children.
- Avoid inhalation and skin contact during the mixing
- Don't eat / drink / smoke during the mixing process
- In case of contact with eyes: Flush with water liberally for 20 minutes
- After the work, wash hands with soap water
- Microbial consortium should not be mixed with insecticide, fungicide, herbicide and fertilizers

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