Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 4193 - 4208
Received 25 April 2021; Accepted 08 May 2021.

Growth Promotion Activities of Plant Growth Promoting Rhizobacteria (Pgprs) Isolated from Vidarbha Region, Maharashtra, India: Study on Cotton Crop

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Abstract
Plant growth promoting rhizobacteria are the microbes which are found in the rhizospheric region of soil. These promotes the growth of crops and plays an important role as biofertilizer and pesticidal/fungicidal agent for crops. The chemical fertilizers leave harmful impact in the environment releasing toxic residues in the environment, while PGPRs are utilized as the alternate strategies for maintaining sustainable agriculture. From past few years, the awareness amongst the farmers and agricultural commodity has generated the requirement of such bioformulations. In the present investigation, the PGPRs and their bioformulations were studied on cotton crop in order to determine their growth promotion activities. The results determined the significant growth promotional activities of the PGPRs consortia on the cotton crop.

Keywords: PGPR, biofertilizer, agriculture, cotton crop, antifungal, bio-pesticide.

Introduction
In many developing countries agriculture practices provides an important role for national income as well as export earnings. Sustainable agriculture helps an important role for the growth and also to fulfill the need of future agriculture. Now a days there has been a great interest in eco-friendly products because of the great loss of natural product or deterioration of environment. PGPRs already proved themselves as an eco-friendly biofertilizers, insecticides and pesticides as compared to the synthetic products [1]. The term 'rhizobacteria' given by Kloepper and Schroth in 1978. These are the bacteria found in soil that competitively colonized in plant roots and promote the growth rate of plant and control the rate of plant disease. In 1981 Kloepper and Schroth termed these beneficial 'rhizobacteria 'as plant growth Promoting Rhizobacteria (PGPR). These act as the essential part of rhizosphere biota that when grown in corporation with the host plant can induce the growth of the host. Due to their adaptive and flourishing nature and availability of versatile biochemical which metabolize a wide range of natural and xenobiotic compounds, PGPR successfully getting established in soil ecosystem [2]. PGPRs are basically categorized into three categories-arbuscular mycorrhizal fungi (AMF), other plant growth promoting rhizobacteria and nitrogen fixing rhizobia [3-5]. These PGPRs works as "Team-work" in the rhizospheric community and form the niche of the ecosystem in balancing the nutrients and dissolution of complex substances in the soil. The present study was performed to screen PGPRs for enhancing the agricultural productivity of the cotton crops.

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Materials and Methods

Study area

Vidarbha is the eastern region of the Indian state of Maharashtra, comprising Nagpur Division and Amravati Division. Amravati division's former name is Berar (Varhad in Marathi). It occupies 31.6% of the total area and holds 21.3% of the total population of Maharashtra. It borders the state of Madhya Pradesh to the north, Chhattisgarh to the east, Telangana to the south and Marathwada and Khandesh regions of Maharashtra to the west. Situated in central India, Vidarbha has its own rich cultural and historical background distinct from rest of Maharashtra. The largest city in Vidarbha is Nagpur followed by Amravati.

Image 1: Vidarbha region, Maharashtra, INDIA

Sample collection and their preparation

Systematic random soil samples were collected from the fields of Vidarbha region having plantations of different crops during Year 2016- Year 2020. The care was taken, to collect the soil samples from rhizospheric region. A total of 15-20 soil samples were collected. The soil samples weighing about 0.5 kg each were brought to the laboratory in properly sealed polythene bags. The visible plant debris and fauna was removed and stored at 4 °C until they are transferred for residual analysis. In soils, the concentration was calculated on air-dry basis for which samples were air-dried at room temperature. A portion of the samples were air-

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dried, passed through a 2 mm sieve, and used for physicochemical analysis. Such sampling procedure was adopted to study other contaminants in agricultural soils.

Isolation of plant growth promoting rhizobacteria (PGPRs)

Bacteria were isolated from the rhizospheric soil samples by serial dilution technique on nutrient agar (NA) plates and incubated at 28±2 ^{0}C for 72 hours. After incubation period, NA plates were observed for morphological appearances and number of bacterial colonies. Bacterial isolates having different morphological appearance on agar plates were selected and maintained on nutrient agar slants and 50% glycerol at -80°C. All the isolates were morphologically characterized as per method described in Bergey's manual of determinative bacteriology [6-8].

Characterization of bacterial isolate for different plant growth promoting activities

The bacterial isolates were screened for positive PGPR activities. The strains were screened to determine qualitatively and quantitatively the production of phyto-hormones [9-11].

a) IAA production

Indole acetic acid (IAA) production was quantitatively estimated by Salkowski method. Bacterial cultures were grown on Luria broth liquid medium at 36±2 °C. The cultures in the flask showing dense milky white growth were tested for purity. Fifty milliliter of Luria Bertani (LB) broth containing 0.1% DL tryptophan was inoculated with 500 µl of 24 h old bacterial cultures and incubated in refrigerated incubator shaker at 30±0.1°C at 180 rpm for 48 h in dark. Fully grown bacterial cultures were centrifuged at 10,000 rpm for 10 minutes at 4°C. Estimation of IAA production in the supernatants was done using colorimetric assay. One milli liter (1 ml) of supernatant was mixed with 100 ml of 10 mM orthophosphoric acid and 2 ml of the Salkowski reagent (1 ml of 0.5 M FeCl3 in 50 ml of 35% HCIO4) at 28±2 ^{o}C for 30 minutes. Development of pink colour in test tubes at the end of the incubation indicated IAA production.

b) Phosphate solubilization

A loop full of isolated pure fresh bacterial cultures were streaked on the centre of agar plates modified with Pikovskaya agar with insoluble tricalcium phosphate (TCP) and incubated for 120 h at 28±2°C 18. The presence of halo zone around the bacterial colonies indicated positive phosphate solubilization ability.

c) Siderophores and HCN production

Qualitative estimation of siderophore production by the bacterial isolates was determined by adapting the modified method on chrome azurol sulphonate (CAS) assay. Production of siderophore were determined by the development of orange halo zone around bacterial colonies. In addition, all the bacterial isolates were screened for the HCN production. Colour change of the filter paper from deep yellow to reddish-brown colour indicated production of HCN.

d) Catalase activity

Bacterial cultures were grown in nutrient agar medium for 48 h at 28°C. The 48-hour old bacterial colonies were added with 2-3 drops of hydrogen peroxide (3%) on a clean glass

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slide and mixed using a sterile tooth pick. The evolution of oxygen as effervescence indicated Catalase activity.

Antifungal activity of screened PGPR isolates against fungal phyto-pathogens

The antifungal activity of broth culture of PGPRs (Streptomyces isolates) was determined against fungal phytogens by well diffusion method [12].

Determination of compatibility amongst the potent PGPR strains isolated

Bacterial cultures were streaked on nutrient agar plates in such a way that for every single bacterial culture in the centre of the plate, other cultures were streaked radiating from the centre. The plates were incubated at 37°C for 48 h and the inhibition of growth of the specific bacterial culture was recorded. The cultures which showed inhibitory growth against each other were not considered for the study [13].

Preparation of Bio-consortia

The compatible strains were formulated in a synthetic designed media having optimum concentration of carbon and nitrogen sources. The media was having desired nutrients and ingredients responsible for the growth of all individual PGPR strains with respect to their characteristic attributes as determined by above mentioned assays for PGPRs. The 5 days growth of the mixed culture was extracted with solvent (Methanol: Chloroform- 2:1) to collect organic layer which was further dried to obtain the crude fraction insecticidal and pesticidal activities against the pests on cotton crop [14].

Results

As per the studies, 150 soil samples (rhizospheric region) were collected from different field areas of Vidarbha region (Butibori, Nagpur, Wardha, Amravati, Gadchiroli and Akola districts) having the frequent usage of organic and chemical fertilizers. A total diversity of 530 microbes were isolated. Amongst which 220 microbial strains were contributed by 12 dominant genera and 18 species of PGPRs. Different other microbial strains were also isolated but such strains didn't possess positive PGPR traits, thus not considered for the study. The PGPR isolates were isolated on specific agar media and characterized by morphological colonies appearance and staining procedures. The results are shown in **Table 1.1** and **Figure 1.1.** The isolated PGPR cultures are categorized as per the strain name, code and type of organism **(Table 1.2; Figure 2.1; 2.2).** PGPRs were further qualitatively characterized **(Table 1.3).** The antifungal activity of selective PGPR strains was determined **(Table 1.4).** The effect of PGPRs on vegetative growth of cotton crop was determined **(Table 1.5; Figure 3).** determined. The results showed promising activity of the same on vegetative and reproductive growth of cotton crop.

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Table 1.1: Percent diversity of PGPR isolates from rhizospheric region of soil samples of Vidarbha region of Maharashtra, INDIA

Soil samples	Total number of microbes isolated	PGPR isolates	Diversity of PGPR genera	Diversity of PGPR species
150	530	220	12.0	18.0

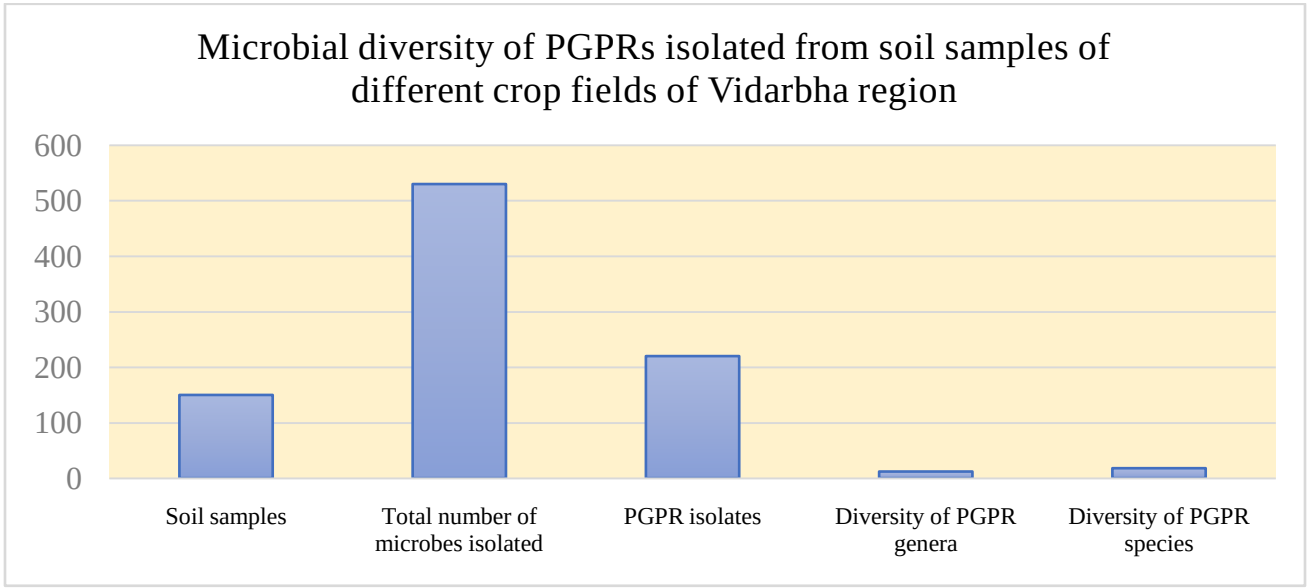

Figure 1.1: Graphical representation of microbial diversity isolated from soil samples of Vidarbha region of Maharashtra, INDIA

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Table 1.2: Isolated Diversity of PGPR cultures (bacterial and fungal cultures)

S.No.	Strain code	PGPR isolates	
		Strain Name	**Type of strain (Bacterial/Fungal)**
1.	MK12	*Bacillus subtilis*	Bacterial strain
2.	MK15	*Bacillus cereus*	Bacterial strain
3.	MK37	*Bacillus mycoides*	Bacterial strain
4.	MK46	*Pseudomonas fluorescens*	Bacterial strain
5.	MK123	*Glomus sp.*	Fungal strain/Mycorrhiza
6.	MK152	*Gigaspora sp.*	Fungal strain/Mycorrhiza
7.	MK167	*Acaulospora sp.*	Fungal strain/Mycorrhiza
8.	MK178	*Streptomyces AM 101*	Fungal strain/Actinomycetes
9.	MK182	*Streptomyces AM 102*	Fungal strain/Actinomycetes
10.	MK189	*Streptomyces AM103*	Fungal strain/Actinomycetes
11.	MK193	*Rhizobium sp.*	Bacterial strain
12.	MK195	*Trichoderma viride*	Fungal strain/Biocontrol
13.	MK199	*Streptomyces AM104*	Fungal strain/Actinomycetes
14.	MK203	*Beauveria bassiana*	Fungal strain/Entomopathogenic
15.	MK207	*Trichoderma harzianum*	Fungal strain/Biocontrol

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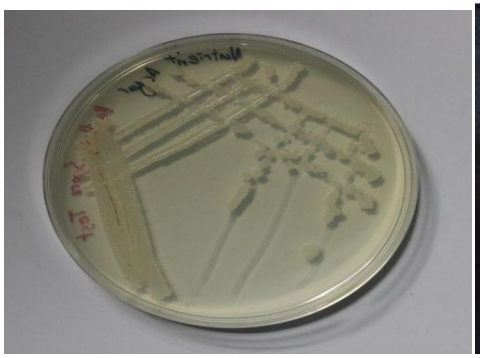
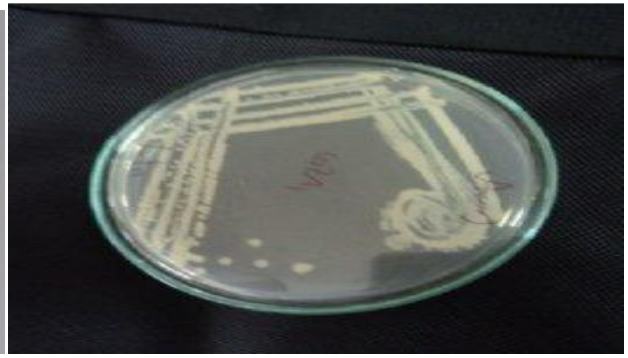
16.	MK209	*Streptomyces AM105*	Fungal strain/Actinomycetes

Bacillus subtilis (MK12)

Bacillus cereus (MK15)



Bacillus mycoides (MK37)



Pseudomonas fluorescens (MK46)

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Streptomyces sp AM 101 (MK178) Streptomyces AM 102 (MK182) Streptomyces AM 103 (MK189)

Rhizobium sp. (MK193) Trichoderma viride (MK195) Streptomyces AM104 (MK199)

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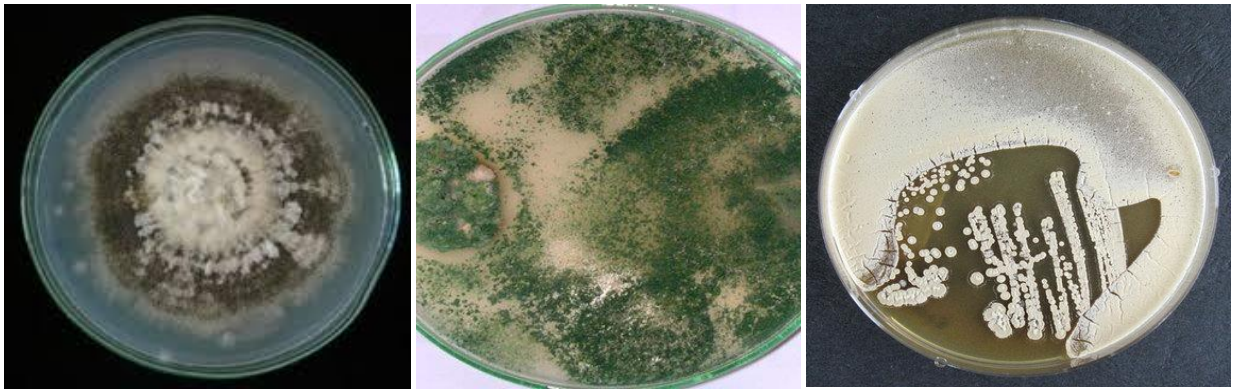
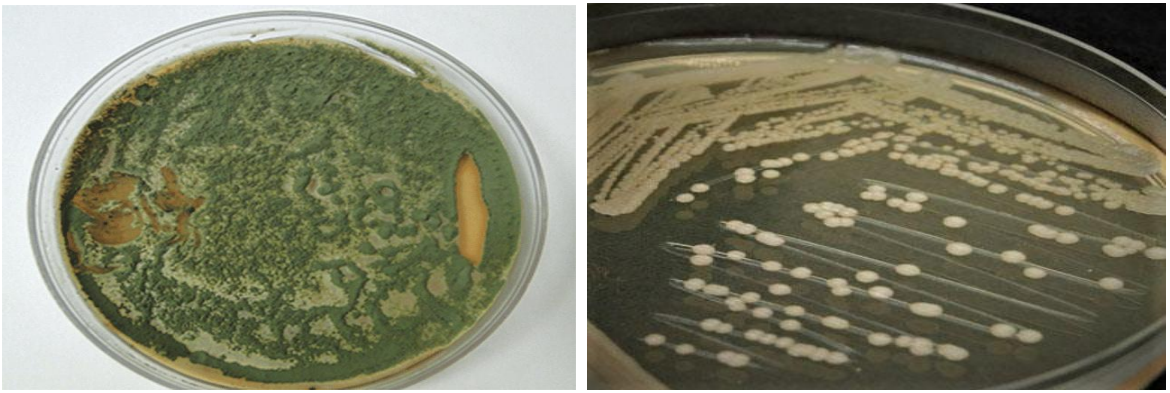


Beauveria bassiana (MK203) Trichodema harzianum (MK207) Streptomyces AM105 (MK209)



***Metarhizum anisopliae (MK213*)** **Bacillus megaterium* (MK214)**

Figure 2.1: Bacterial and Fungal-PGPR isolates

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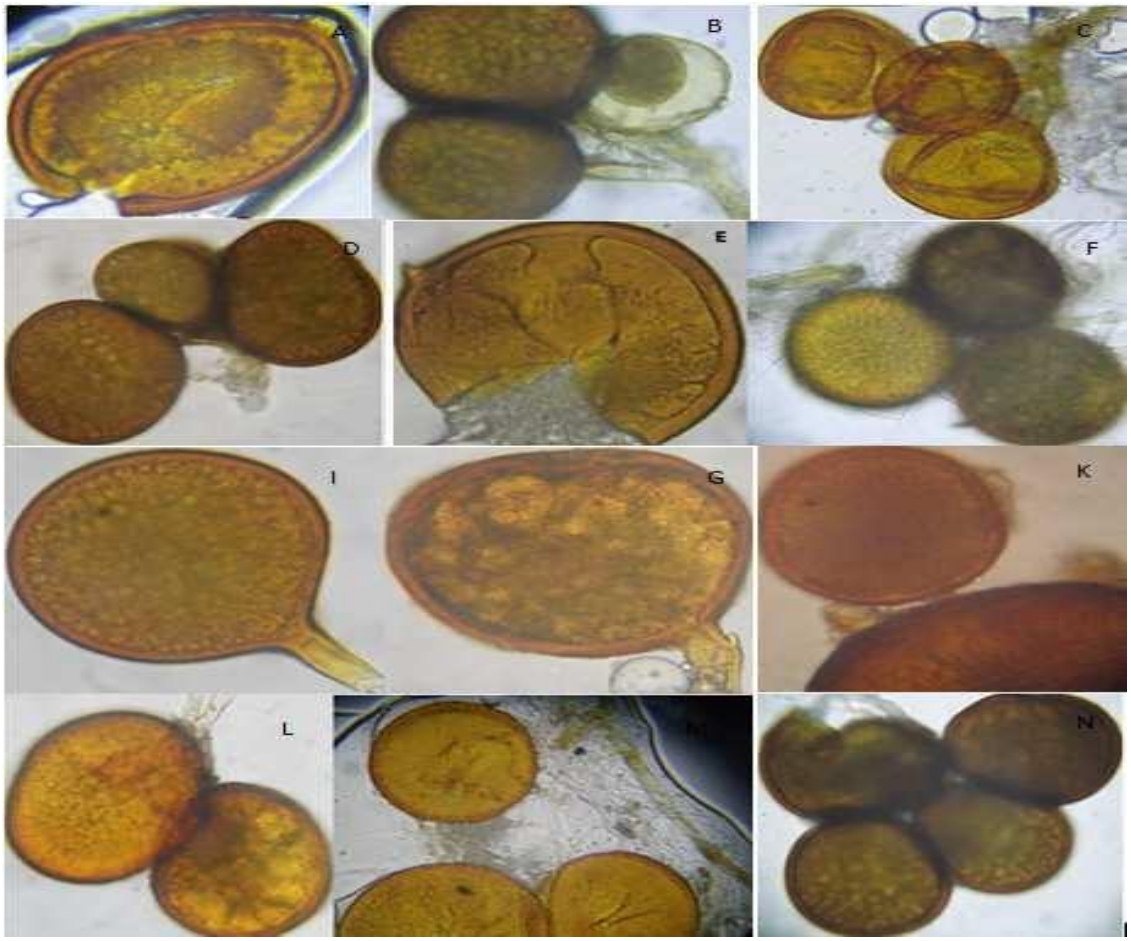

Figure 2.2: Spores (a-n)of Glomus sp. (MK123); Gigaspora sp. (MK152); Acaulspora sp. (MK167)

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Table 1.3: Qualitative screening of PGPR isolates

S.No.	Strain code	Qualitative tests of PGPR				
		Strain Name	**IAA production**	**Phosphate solubilization**	**Siderophores and HCN production**	**Catalase production**
1.	MK12	*Bacillus subtilis*	++++	+++	+++	++++
2.	MK15	*Bacillus cereus*	++++	++++	++++	++++
3.	MK37	*Bacillus mycoides*	++++	++++	++++	++++
4.	MK46	*Pseudomonas fluorescens*	++++	++++	+++	++++
5.	MK123	*Glomus sp.*	++++	++++	++++	++++
6.	MK152	*Gigaspora sp.*	++++	++++	++++	++++
7.	MK167	*Acaulospora sp.*	++++	++++	++++	++++
8.	MK178	*Streptomyces AM 101*	++++	+++	+++	++++
9.	MK182	*Streptomyces AM 102*	++++	++++	++++	++++
10.	MK189	*Streptomyces AM103*	++++	++++	++++	++++
11.	MK193	*Rhizobium sp.*	++++	++++	++++	++++
12.	MK195	*Trichoderma viride*	+++	++++	++	++++
13.	MK199	*Streptomyces AM104*	++++	++++	++++	++++

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14.	MK203	*Beauveria bassiana*	++++	++++	++++	++++
15.	MK207	*Trichoderma harzianum*	++++	++++	++++	++++
16.	MK209	*Streptomyces AM105*	++++	++++	++++	++++
17.	MK213	*Metarhizum anisopliae*	++++	++++	++++	++++
18.	MK214	*Bacillus megaterium*	++++	++++	++++	++++

*++++, potent producer; +++, medium producer; ++, producer

Table 1.4: Antifungal activity of selected PGPRs against fungal phyto-pathogens

Antifungal activity of PGPR extracts via well diffusion method - Diameter of zone of inhibition (mm)						
S.No.	**Strain code**	**Strain Name/Positive Control**	***Fusarium oxysporum***	***Sclerotium rolfsii***	***Colletotrichum sp.***	***Rhizoctonia solani***
1.	MK178	Streptomyces AM 101	35.0±0.020	28.0±0.065	43.0±0.0015	40.23±0.025
2.	MK182	Streptomyces AM 102	38.0±0.054	33.0±0.056	35.0±0.022	37.0±0.047
3.	MK189	Streptomyces AM103	35.0±0.067	38.0±0.048	36.0±0.023	34.0±0.053

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4.	MK199	Streptomyces AM104	38.0±0.020	45.0±0.020	43.0±0.018	40.0±0.035
5.	-	Fluconazole/ Positive Control (1 mg/ml)	42.0±0.025	43.0±0.020	42.0±0.020	45.0±0.018

*P<0.05, level of significance; ±, standard deviation

Table 1.5: Observation sheet for vegetative growth in cotton crops after treatment with PGPRs: (Average mean of 5 plants) after 15 days of sowing

S.No	PGPR strains	Germination %	Vigor Index	Root Length (cm)	Shoot Length (cm)	No of leaves	Root Pattern	Yellow appearance
T1	MK12	80	1440	10	8	4	Straight	NIL
T2	MK15	83.33	1333	9	7	4	Fibrous	NIL
T3	MK37	96.66	1728	9	9	5	Fibrous	NIL
T4	MK46	73.33	1686	16	7	4	Fibrous & long	NIL
T5	MK123	86.66	1290	8	7	4	Fibrous	NIL

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T6	MK152	83.33	1494	12	6	4	Fibrous long	NIL
T7	MK167	66.66	792	6	6	4	Straight	NIL
T8	MK207	86.66	1548	9	9	4	Fibrous	NIL
T9* Consortia	MK178+MK182+MK189+MK199+MK209	80	1280	9	7	4	Fibrous	NIL

Figure 3: Vegetative growth in cotton crops after treatment with PGPRs (Average mean of 5 plants) after 15 days of sowing

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Discussion

Increasing use of chemical fertilizers in agriculture make country self- dependent in food production but it deteriorates environment and cause harmful impacts on living beings. Due to insufficient uptake of these chemical fertilizers by plants, they reach into water bodies through rain water, cause eutrophication in water bodies and affect living beings including growth inhabiting microorganism. The excess uses of chemical fertilizers in agriculture are costly and also have various adverse effects on soils as depletion of water holding capacity, soil fertility and disparity in soil nutrients. It was felt from a long time to develop some low cost effective and ecofriendly fertilizers which work without disturbing nature. Now, certain species of microorganism are widely used which have unique properties to provide natural products, and serve as a good substitute of chemical fertilizers. The results of the study are in corelation with the previous findings [15-20].

Conclusion

The studies thus concluded that, PGPRs are the need for today as these are the effective agents releasing significant molecules and metabolites responsible for growth of crops and enhancing sustainable agricultural productivity. The utilization of such bio-inoculants/PGPRs as products by the farmers can aid in value enhancement with least expenses and sustainable agricultural productivity.

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