Advances in PGPR Technology for Banana Cultivation for Betterment of Agriculture and Environment of *Khandesh* in *Tapati* river basin

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Purpose of the study was to

- reduce the consumption of chemical fertilisers
- enhance the fertiliser use efficiency
- resort to the drip irrigation

Why we studied this problem?

Markets for an Indian bananas are under exploited and there exists a high value product potential in bananas

Our interest was in exploring the possibility of banana production, processing and trade as an aid for sustainable development and how can a banana crop, already part of north maharashtrian agriculture and cultural landscape, be transformed into an engine of regional economic development?

Comparative advantages about the benefits for community, region and country

Food security and the natural resource base

Theme of the study

Khandeshi farmers cultivating bananas in *Tapi*-river basin have no alternative than adopting sustainable production practices (SPPs) including management of natural resources due to

- ongoing deterioration of soils,
- high annual nutrient turn over in banana eco-systems,
- over-exploited water reservoirs and
- overall non-remunerative stagnation of banana production to 52 MTha^{-1}.

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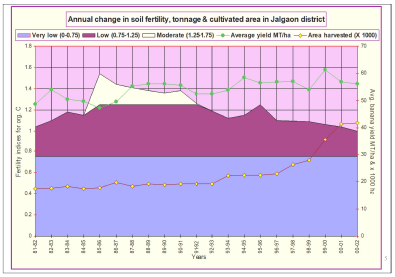

Theme of the article

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Among the probable ways to cope up with the ever-growing demand of qualitative and quantitative increase in banana production are

- fostering the farmer's co-operative movement in the region,
- utilising the tissue-culture derived and/ or naturally and anthropogenically selected high yielding varieties,
- managing the irrigation appropriately,
- preserving the soil health through implementation of eco-friendly technologies and
- increasing the vitality through application of amino-acid based plant growth regulators.

continued...

Probable ways

Theme of the article

...continued.

The individual or combined application of

- soil conditioner after its production from voluminous banana orchard waste;
- consortium of efficient biofertilisers specially dedicated to banana mycorrhizosphere;
- coal-combustion ash as a source of nutrients;
- judiciously applied doses and frequencies of chemical fertilisers and irrigation water

are the proven practices to sustain banana production with preservation of soil and plant health and productivity in the region.

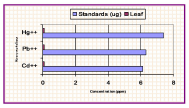
continued...

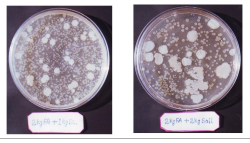
Bioconversion of voluminous trash into fluffy compost

Biofertilisers dedicated to banana mycorrhizosphere

Coal-combustion ash as a source of nutrients

FA contained negligible C & N, little P (< 8000 mg Pkg^{-1}) vis-à-vis soils. FA contains sufficient amounts of all major and minor nutrients essential for plant growth.





Theme of the article

...continued.

Adoption of these SPPs would help *Khandeshi* farmers to

- conserve and recycle the available natural resources,
- protect their environment,
- develop profitable farming system and
- enhance their livelihood.

Overall possibilities for fetching sustainability in banana production in the region were recommended.

Thank You

Any questions? please.

Enhancing the antinematode activity of bacterial based lipopeptide by integrating with plant growth-promoting rhizobacteria

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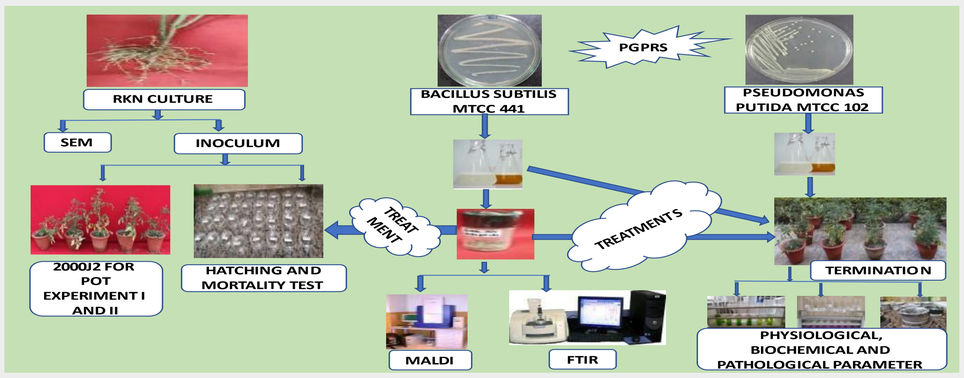
OBJECTIVES

- Keeping in mind the biocontrol potential of PGPR, the current study was planned to evaluate the ovicidal and larvicidal efficacy of *Bacillus subtilis* (MTCC441) derived lipopeptide against *Meloidogyne incognita* under laboratory.
- In addition, nematicidal effect of lipopeptide and cultures of *Bacillus subtilis* (MTCC441) and *Pseudomonas putida* (MTCC102) on *M. incognita* reproductive parameters infecting tomato was examined, and consequently, plant growth and biochemical parameters were investigated.

INTRODUCTION

- Plant parasitic nematodes, especially *Meloidogyne* species are considered to be the most important nematodes affecting tomato production worldwide.
- Globally, estimated annual losses of about US$78 billion were caused by such root-knot nematodes (RKN) (Lima *et al.* 2017).
- Chemical nematicides are toxic and can cause significant environmental damage.
- The application of plant growth-promoting rhizobacteria (PGPR) is an environmentally friendly and host-directed approach for managing plant diseases.
- *Bacillus* spp. and *Pseudomonas* spp. have been investigated as bio-nematicides, as they can generate secondary metabolites, antimicrobial compounds, enzymes and exotoxins that have nematocidal activity (Subedi *et al.* 2020)

METHODS



EXPERIMENTAL DESIGN FLOW CHART

TREATMENTS FOR POT EXPERIMENT I

T1 = *M. incognita* (2000J2s) $\xrightarrow{\text{after 1week}}$ *B. subtilis* (20 mL)

T2 = *M. incognita* (2000J2) $\xrightarrow{\text{after 1week}}$ *P. putida* (20 mL)

T3 = *M. incognita* (2000J2s) $\xrightarrow{\text{after 1week}}$ *B. subtilis* (10 mL) + *P. putida* (10 mL)

T4 = *B. subtilis* (20 mL) $\xrightarrow{\text{after 1week}}$ *M. incognita* (2000J2s)

T5 = *P. putida* (20 mL) $\xrightarrow{\text{after 1week}}$ *M. incognita* (2000J2s)

T6 = *B. subtilis* (10mL) + *P. putida* (10 mL) $\xrightarrow{\text{after 1week}}$ *M. incognita* (2000J2s)

UUC = Untreated Uninoculated control (control),

UIC= Untreated Inoculated control (nematode only)

EXPERIMENT II

F1 = Root dip in crude lipopeptide for 15min $\xrightarrow{\text{after 1week}}$ *M. incognita* (2000J2s),

F2= Root dip in crude lipopeptide for 15min $\xrightarrow{\text{after 1week}}$ *M. incognita* (2000J2) $\xrightarrow{\text{again after 1week}}$ 500µL of crude lipopeptide

UUC = Untreated Uninoculated control (control),

UIC= Untreated Inoculated control (nematode only)

RESULTS

- SEM of perennial pattern of RKN was identified as *M. incognita* (Fig.1)
- The peaks in the samples of *B. subtilis* show a molecular mass of surfactin, suggesting that this extract contains lipopeptide based surfactin molecule m/z 1036. The surfactin molecule was detected in positive mode. The peak at m/z = 1058 [M+Na]+ that resembles surfactin (C13–C15) was clearly visible in the spectrum (Fig. 2)
- The FTIR spectrum implies production of a lipopeptide biosurfactant (Fig 3)

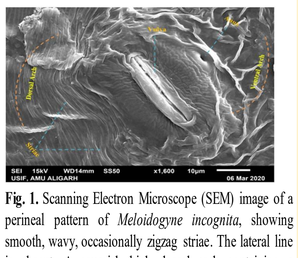


Fig. 1. Scanning Electron Microscope (SEM) image of a perineal pattern of *Meloidogyne incognita*, showing smooth, wavy, occasionally zigzag striae. The lateral line is absent. A squarish high dorsal arch containing a distinct whorl around the tail terminus is the most conspicuous diagnostic character of the perineal pattern of *Meloidogyne incognita*

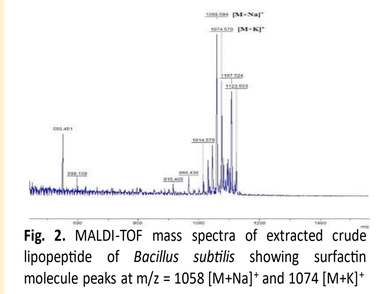


Fig. 2. MALDI-TOF mass spectra of extracted crude lipopeptide of *Bacillus subtilis* showing surfactin molecule peaks at m/z = 1058 $[M+Na]^+$ and 1074 $[M+K]^+$

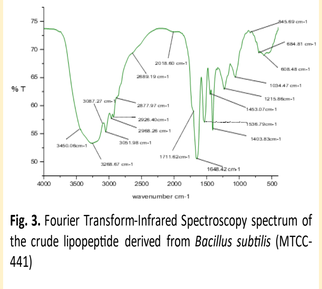


Fig. 3. Fourier Transform-Infrared Spectroscopy spectrum of the crude lipopeptide derived from *Bacillus subtilis* (MTCC-441)

- **MORTALITY TEST-** In an aqueous concentration of 35 ppm lipopeptide, there was 85% nematode mortality after 96 h of exposure, which is the maximum for all treatments.
- **HATCHING TEST-** A significant reduction in egg hatching (83.97%) was observed with 35 ppm lipopeptides after 96 h of exposure.

RESULTS (continued)

Table 1. Effect of *B. subtilis* (MTCC441) and *P. putida* (MTCC102) alone or in combination on growth parameters of J2s inoculated tomato.

Treatment	Length (cm)		Total length (cm)	Fresh weight (g)		Total fresh weight (g)	Dry weight (g)		Total dry weight (g)
	Shoot	Root		Shoot	Root		Shoot	Root	
T1	$32.6^{ef}\pm1.4$	$16.3^{de}\pm0.8$	$49.9^{f}\pm1.2$	$41.90^{f}\pm1.60$	$13.42^{de}\pm0.71$	$55.32^{f}\pm1.44$	$9.37^{f}\pm0.26$	$2.72^{de}\pm0.21$	$12.10^{f}\pm0.17$
T2	$30.0^{f}\pm0.9$	$14.7^{e}\pm0.6$	$44.7^{f}\pm0.4$	$33.17^{g}\pm1.06$	$11.05^{ef}\pm0.66$	$44.22^{g}\pm1.06$	$8.10^{g}\pm0.15$	$2.07^{ef}\pm0.22$	$10.17^{g}\pm0.35$
T3	$35.7^{e}\pm1.1$	$18.9^{d}\pm1.4$	$54.6^{e}\pm1.2$	$47.85^{e}\pm1.08$	$14.20^{cd}\pm0.64$	$62.05^{e}\pm0.81$	$11.05^{e}\pm0.45$	$2.92^{d}\pm0.25$	$13.97^{e}\pm0.35$
T4	$53.6^{c}\pm1.2$	$23.5^{bc}\pm1.1$	$77.1^{c}\pm2.3$	$63.45^{c}\pm1.33$	$16.72^{c}\pm0.51$	$80.17^{c}\pm1.73$	$17.62^{c}\pm0.65$	$4.05^{c}\pm0.22$	$21.67^{c}\pm0.75$
T5	$47.4^{d}\pm1.3$	$19.7^{cd}\pm1.2$	$67.2^{d}\pm1.2$	$58.47^{d}\pm0.81$	$15.27^{cd}\pm1.17$	$73.75^{d}\pm0.91$	$14.65^{d}\pm0.55$	$3.50^{cd}\pm0.42$	$18.15^{d}\pm0.54$
T6	$58.0^{b}\pm0.8$	$27.2^{b}\pm1.3$	$85.2^{b}\pm0.5$	$67.35^{b}\pm0.58$	$20.02^{b}\pm1.19$	$87.37^{b}\pm1.13$	$19.80^{b}\pm0.46$	$5.15^{b}\pm0.30$	$24.95^{b}\pm0.47$
UUC	$63.8^{a}\pm1.4$	$34.4^{a}\pm2.5$	$98.3^{a}\pm3.8$	$75.00^{a}\pm0.79$	$25.22^{a}\pm1.13$	$100.22^{a}\pm1.11$	$22.52^{a}\pm0.28$	$6.50^{a}\pm0.25$	$29.02^{a}\pm0.24$
UIC	$23.9^{g}\pm1.2$	$12.5^{e}\pm0.6$	$36.4^{g}\pm1.6$	$25.22^{h}\pm1.82$	$9.80^{f}\pm0.88$	$35.02^{h}\pm2.22$	$4.80^{h}\pm0.30$	$1.55^{f}\pm0.17$	$6.35^{h}\pm0.29$

Table 2. Effect of *B. subtilis* derived crude lipopeptide, surfactin molecule on growth parameters of J2s inoculated tomato.

Treatment	Length(cm)		Total Length	Fresh Weight (g)		Total Fresh weight (g)	Dry Weight (g)		Total Dry weight (g)
	Shoot	Root		Shoot	Root		Shoot	Root	
F1	$37.9^{c}\pm1.1$	$17.7^{b}\pm0.5$	$55.6^{c}\pm1.5$	$48.10^{c}\pm0.99$	$11.85^{c}\pm0.61$	$62.95^{c}\pm0.71$	$11.52^{c}\pm0.63$	$3.27^{c}\pm0.26$	$14.80^{c}\pm0.53$
F2	$52.7^{b}\pm0.8$	$18.5^{b}\pm0.8$	$71.2^{b}\pm1.5$	$61.85^{b}\pm1.32$	$17.95^{b}\pm0.83$	$79.80^{b}\pm0.75$	$18.55^{b}\pm0.75$	$4.47^{b}\pm0.29$	$23.02^{b}\pm0.53$
UUC	$65.7^{a}\pm1.7$	$37.2^{a}\pm2.7$	$102.9^{a}\pm3.8$	$72.85^{a}\pm1.50$	$25.77^{a}\pm1.28$	$98.62^{a}\pm2.36$	$22.65^{a}\pm0.86$	$6.72^{a}\pm0.42$	$29.37^{a}\pm1.11$
UIC	$24.3^{d}\pm1.5$	$13.0^{c}\pm0.8$	$37.3^{d}\pm1.6$	$25.50^{d}\pm1.63$	$10.35^{d}\pm1.08$	$35.85^{d}\pm1.98$	$4.97^{d}\pm0.47$	$1.85^{d}\pm0.25$	$6.82^{d}\pm0.34$

Table 3. Effect of *B. subtilis* and *P. putida* alone or in combination on the biochemical and pathological parameters in J2s inoculated tomato.

Treatments	Chlorophyll content (mg g^{-1})	Carotenoid content (mg g^{-1})	NRA (µmh^{-1}g^{-1})	Egg masses /root system	Nematode population/250 g soil	Root gall index (RGI)
T1	$1.39^{f}\pm0.00$	$0.33^{e}\pm0.01$	$0.216^{e}\pm0.005$	$105.0^{c}\pm1.2$	$1054.75^{b}\pm83.57$	$2.80^{c}\pm0.10$
T2	$1.15^{g}\pm0.02$	$0.28^{ef}\pm0.00$	$0.186^{f}\pm0.003$	$113.2^{b}\pm1.7$	$1097.25^{b}\pm39.70$	$3.17^{b}\pm0.12$
T3	$1.54^{e}\pm0.01$	$0.36^{e}\pm0.02$	$0.242^{e}\pm0.007$	$97.7^{d}\pm1.2$	$984.50^{bc}\pm38.76$	$2.20^{d}\pm0.10$
T4	$1.89^{c}\pm0.03$	$0.65^{c}\pm0.06$	$0.362^{c}\pm0.005$	$85.0^{f}\pm1.9$	$781.50^{d}\pm30.69$	$1.67^{e}\pm0.12$
T5	$1.76^{d}\pm0.04$	$0.50^{d}\pm0.03$	$0.281^{d}\pm0.014$	$91.7^{e}\pm1.3$	$886.75^{cd}\pm33.56$	$2.0^{de}\pm0.10$
T6	$2.16^{b}\pm0.02$	$0.81^{b}\pm0.03$	$0.415^{b}\pm0.009$	$75.2^{g}\pm1.1$	$566.00^{e}\pm35.59$	$1.2^{f}\pm0.10$
UUC	$2.53^{a}\pm0.04$	$0.96^{a}\pm0.00$	$0.489^{a}\pm0.003$	$0^{h}\pm0$	$0^{f}\pm0$	$0^{g}\pm0$
UIC	$1.04^{h}\pm0.05$	$0.20^{f}\pm0.00$	$0.152^{g}\pm0.017$	$129.0^{a}\pm1.2$	$2075.75^{a}\pm55.63$	$5.0^{a}\pm0$

Table 4. Effect of *B. subtilis* derived crude lipopeptide, surfactin molecule on biochemical and pathological parameters of J2s inoculated tomato.

Treatment	Chlorophyll content (mg g^{-1})	Carotenoid content (mg g^{-1})	NRA (µmh^{-1}g^{-1})	Egg masses / root system	Nematode population/250g soil	Root gall index (RGI)
F1	$1.60^{c}\pm0.03$	$0.36^{c}\pm0.00$	$0.273^{c}\pm0.002$	$89.0^{b}\pm1.2$	$827.25^{b}\pm9.88$	$2.0^{b}\pm0.06$
F2	$2.05^{b}\pm0.03$	$0.78^{b}\pm0.00$	$0.394^{b}\pm0.004$	$81.2^{c}\pm1.1$	$577.25^{c}\pm10.96$	$1.4^{c}\pm0.04$
UUC	$2.64^{a}\pm0.04$	$0.97^{a}\pm0.00$	$0.479^{a}\pm0.003$	$0^{d}\pm0$	$0^{d}\pm0$	$0^{d}\pm0$
UIC	$1.01^{d}\pm0.06$	$0.22^{d}\pm0.00$	$0.159^{d}\pm0.019$	$123.0^{a}\pm1.2$	$1948.25^{a}\pm52.47$	$5.0^{a}\pm0$

Values are the mean ± standard error of four replicates. Means on each column followed by the same letters are not significantly different according to Duncan's multiple range test DMRT at p≥0.05

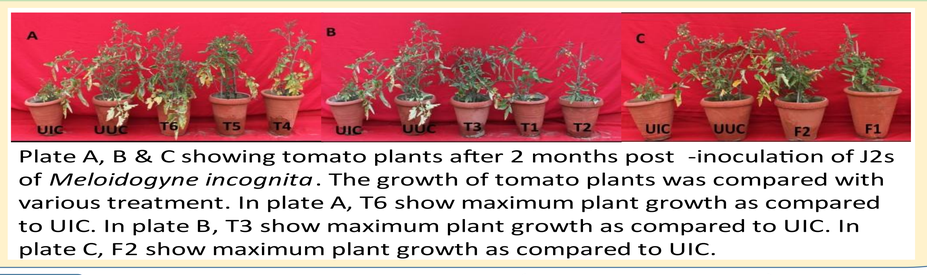


Plate A, B & C showing tomato plants after 2 months post -inoculation of J2s of *Meloidogyne incognita*. The growth of tomato plants was compared with various treatment. In plate A, T6 show maximum plant growth as compared to UIC. In plate B, T3 show maximum plant growth as compared to UIC. In plate C, F2 show maximum plant growth as compared to UIC.

CONCLUSION

- Surfactin, could be an important source for production of an antinematode product with novel nematocidal activity
- Surfactin significantly effect J2 hatching and mortality.
- In the tomato pot experiment, when cultures of both *B. subtilis* and *P. putida* were applied before inoculation of J2, control efficiency was higher compared to when applied after inoculation.
- This bacteria-based lipopeptide, surfactin, also plays a crucial role in improving plant growth.

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Role of Chitinases in the Development of Transgenic Plants and as a Bio-control Agent of Plant Pathogens: Recent Developments

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Abstract

Chitinolytic enzyme (chitinases) is a group of enzyme that degrades chitin into low molecular weight oligomer and N- acetyl-D-glucosamine (NAG) monomer components by breaking the glycosidic bonds. The enzyme chitinase is broadly found in fungi, bacteria, archaea, crustaceans, invertebrates and also in higher plants. Fungal plant diseases are one of the major problems to agricultural production. Plant chitinase have antifungal activity to several fungi containing chitin component in cell wall. Biological control of phytopathogens provides an alternative for management of fungal disease without any negative impact on environment. Manipulations of cloned chitinase gene in transgenic plant play a significant role in plant defense. Chitin and chitinolytic enzymes has an extensive range of applications currently in morphogenesis, biocontrol agent, preparation of single cell protein (SCP), bioconversion of water, mosquito and nematode control, bio-pesticides, pharmaceutical and in medicinal field. Using protein engineering and biochemistry we can produce chitinases with particular features that will make them more useful in the development of transgenic plants and for the biocontrol of phytopathogens.

Introduction

- Chitin is a natural biopolymer of N – acetyl D- glucosamine linked by β-1,4-glycosidic linkage.
- Chitin is a major component of fungal cell wall , exoskeleton of crustacean shells , insects, plants, bacteria and algae etc.
- Chitinase degrade chitin into monomer product.
- Several organism including fungi, bacteria, plants, insects and animal produce chitinases .
- Chitinases have potential for biological control of the plant disease caused by several phytopathogenic fungi and insect pests that can be used as an alternative to chemical pesticide.

Waste management

- The use of chitinase enzyme to control the insects pests and phytopathogens offer a significant approach in agriculture field.
- Several chitinolytic bacteria shows huge potential for plant pathogens including *Paenibacillus sp*. and *Streptomyces sp*. against Fusarium wilt of *Cucumis sativus* caused by *Fusarium oxysporum*.
- Chitinases from Yam also have been used as a bio control agent for powdery mildew in strawberry.
- So far SCP (single cell protein) have been produced from *Saccharomyces serevisiae*.
- Mahadevan et al (1997) reported the antagonistic action of *S. lydicus* WXEC108 against *Pythium ultimum* and *Rhixzoctonia solani*, which caused diseases in pea and cotton.
- Cheng et al (2003) reported the growth inhibiting properties of plant pathogenic fungi from *B. cereus* Y8308 against *Fusarium oxysporum*, *F. solani*, and *P. ultimum*.

Bio control of phytopathogens

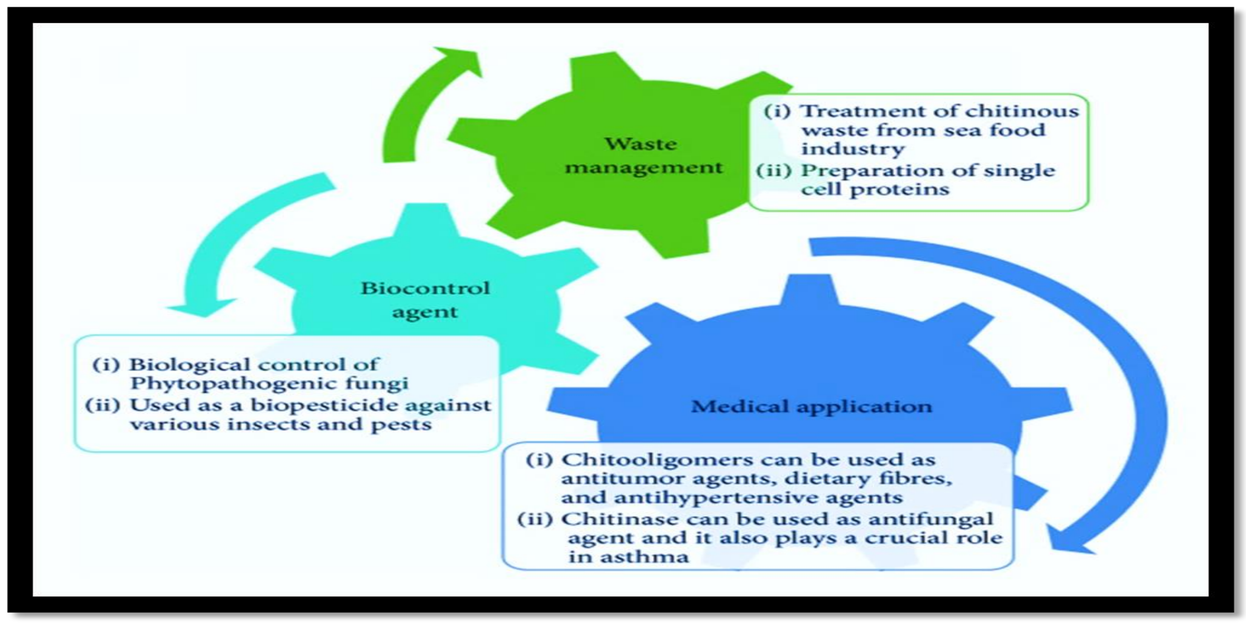
- Chitinases are present in plants besides the various pathogenesis related protein as a plant defense mechanism.
- Since overexpression of a combination of various chitinases in transgenic pants may assists against fungal pathogens.
- Chitinases can also be used directly as a biopesticides against various fungi and insects that may be an alternative to chemical pesticides.
- Commonly chitinases used as a biocontrol agents as chitinases act as a target for biopesticide because chitin play a major role in insect metamorphosis as well as in gut of insects.
- Allosamidin (a pseudotrisaccharide) act as an inhibition of chitinases and potentially used as a biopesticide.

Transgenic plants with chitinase gene

The production of transgenic plants overexpressing chitinase gene had been indicated to get resistance against phytopathogens. Chitinase Chi A from chitinolytic bacteria including *Sanitaria marcens* and *Enterobacter agglomerans* are potential agents for the biological control of plant diseases caused by various pathogens .

Table:1 Expression of chitinase in transgenic crops

Chitinase Gene	Source	Transgenic crops	Application	References
Chitinase RCH 10	Plant Rice	*Lilium oriental Canola*	Resistance against *Botrytis cinerea*	González FF et al., (2015)
Chitinase Chit 33	Fungus (*Trichoderma atroviride)*	*Brassica napus*	Resistance against stem rot disease caused by *Sclerotinia sclerotiorum*	Solgi et al., (2015)
Endochitinase geneIIHR-JBMch	Fungus *(Trichoderma harzianum)*	Guava (*Psidium guajava L.*)	Resistance against wilt disease caused by *Fusarium oxysporum*	Mishra et al., (2014)
chitinase geneBI333-EN4-RCC2 and pBI333-EN4-RCG3	Plant Rice	Banana (*Musa acuminate*)	Confers resistance to Black leaf streak disease caused by the fungus *Mycosphaere lla fijiensis*	Kovacs et al., (2013)
Endochitinase geneBI121-CHI	Lant Bean (*Phaseol us vulgaris*)	Cotton (*Gossypium hirsutum*)	Resistance to wilt caused by *Verticillium dahliae*	Tohidfar et al., (2012)
Chitinase geneRCC11	Lant Rice	litchi (*Litchi chinensis Sonn*)	Transgenic plants showed die-back, leaf spots and blight pathogen (*Phomopsis sp.*)	Das et al., (2012)
Chitinase gene(CaMV-UbiChi1	Plan Rice	Grapevine (*Vitis vinifera L.*)	Resistance against powdery mildew caused by *Erysiphe necator*	Nirala KN et al., (2010)
Endochitinase geneThEn -42	Fungus (*Trichoderma harzianum*)	Broccoli	Resistance to *Alternaria brassicicola*	Mora and Earle. (2009)
Class II chitinase gene AHCBarChit	Lant Barley	Wheat	Enhanced resistance against Fusarium head blight caused by *Fusarium graminearum*	Shin et al., (2008)
Chitinase geneCHIT33 and CHIT42	Fungus (*Trichoderma harzianum*)	Tobacco	Resistance to *R. solani* and abiotic stress	Dana et al., (2006)



Future prospective

- As a food additives to increase shelf life
- Antifungal drug
- Anti-tumor drug
- Therapeutic agent for asthma
- Insecticides
- Remediation of organic contaminates

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I would like to thank to the Principal “Dr. Shashi Sharma” and Head of Department Miss. Kalpna for providing me all the facility that were required.





Synthesis of Green Nanoparticles: Antagonistic Studies for Biocontrol of Plant Pathogens

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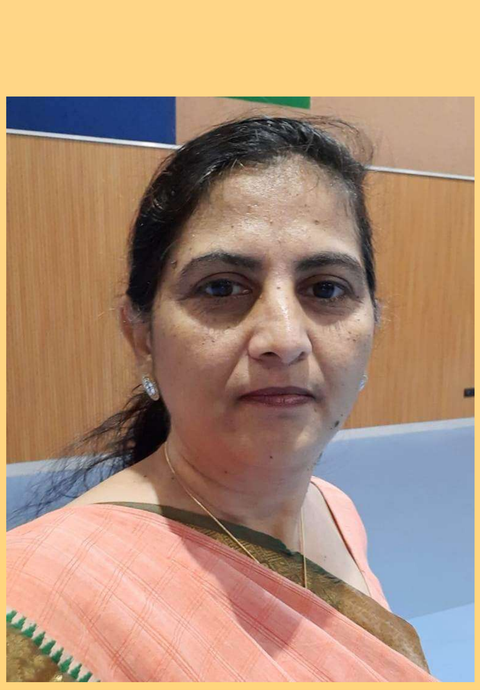

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Abstract: The green synthesis of nanoparticles is an important aspect of current nanotechnology research. In the present study, green synthesis of silver and copper nanoparticles were carried out using bacterial isolates obtained from the agricultural soil of Navsari and Dang district of the South Gujarat region, Gujarat. Total 10 isolates were taken for this extracellular synthesis of Silver and Copper nanoparticles. Characterization were done by visual observation and then by UV-Vis spectrophotometer. The nanoparticles were also evaluated for their Antifungal activity against plant pathogenic fungi *Fusarium oxysporum*, *Rhizoctonia soloani*, *Pythium spp*., and *Sclerotium rolfsii* . Furthermore, Antioxidant activities of these metal nanoparticles were done using Ascorbic acid as a standard.

Introduction

- Nanoparticles can be synthesized by physical and chemical methods, but have many drawbacks such as high energy requirements, are expensive, and also form toxic byproducts. Thus an alternative for the fabrication of nanoparticles is a biological route that is environment friendly and cost effective.

Objectives

- 1.To synthesis metal nanoparticles by microbial routes.
- 2. Characterization of synthesized metal nanoparticles.
- 3. To evaluate antifungal activity of synthesized metal nanoparticles against Plant pathogens.
- 4. To evaluate antioxidant activity of synthesized metal nanoparticles.

Methodology

Various soil samples were collected using standard microbiological protocol from Navsari and Dang district of the South Gujarat region of Gujrat.

Isolation of bacteria were carrying out using different culture media like Nutrient agar, R2A agar, and Actinomycetes agar

Studied their morphological, colonial and biochemical characteristics

Supernatant was collected for the synthesis of metal nanoparticles

Bacteria were subjected to grow in Luria Bertani media for 24 hours

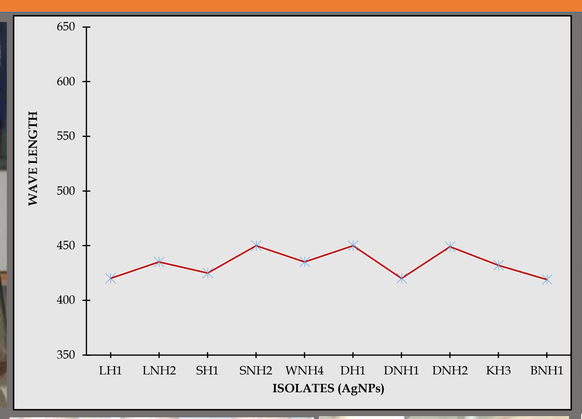
Total 10 isolates were selected for the green synthesis of nanoparticles

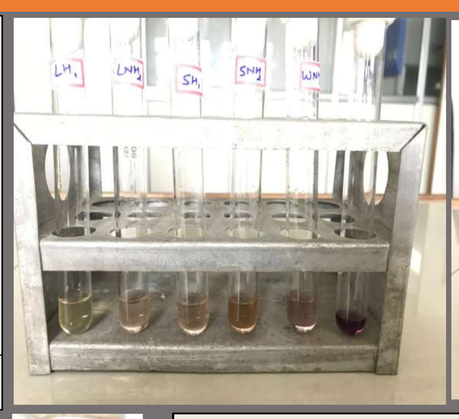
$AgNO_3$/ $CuSO_4$ was mixed with supernatant and incubate in dark for 24 hours at room temperature

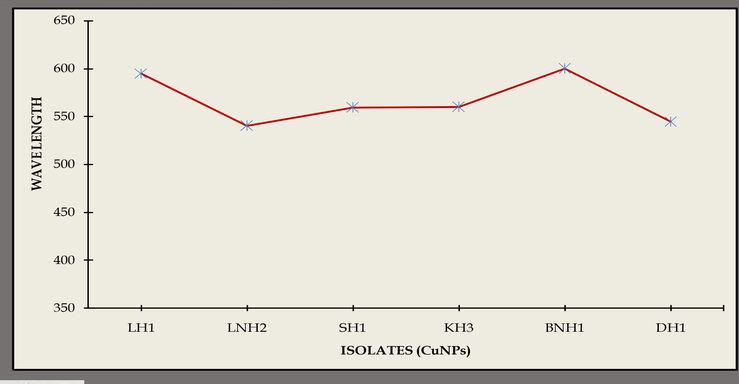
Characterization done by visual observation of color changed and then by Uv Vis spectroscopy

Antifungal activity by Kirby and Bauer method and Antioxidant activity were performed

Results & Discussion







Among the 10 isolates (LH1, LNH2, Sh1, SNH2, WNH4, DH1, DNH1, DNH2, KH3, BNH1) purified from the soil, all have ability to form silver nanoparticles as observed by color change and showed λ max between 420 to 450 nm. Also these all silver nanoparticles have good antioxidant activity were confirmed by color change and by absorbance at 517nm. AgNPs from LH1, LNH2, SNH2 and WNH4 were found to good antifungal activity against *Pythium spp*. AgNPs from LH1 also showed antifungal activity against *Rhizoctonia solani* .

6 isolates out of 10 synthesized copper nanoparticles, confirmed by color changed and UV- Vis spectral analysis. CuNPs from DH1 showed very good antifungal activity against all four plant pathogenic fungi named *Fusarium oxysporum*, *Rhizoctonia soloani*, *Pythium spp*., and *Sclerotium rolfsii*. Another CuNPs from KH3 was found good antifungal activity against *Pythium spp*. and *Rhizoctonia solani*. BNH1 CuNPs showed activity only against *Rhizoctonia solani*. Also all these CuNPs showed good antioxidant activity.

Conclusions: Nanoparticles have many applications due its small size and high surface to volume ratio. In the current study green synthesis of Silver and Copper nanoparticles were successfully done. Silver and Copper nanoparticles had good antifungal activity against plant pathogens. Also all synthesized metal nanoparticles shows good antioxidant activities.

Acknowledgements: I would like to thank my research guide Dr. Arti Raval for continuous support and guidance. I also express my gratitude to Shrimad Rajchandra Vidyapeeth,Dharmpur,for providing practical and instrumental facilities.

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3. Vidhya Lakshmi Das, Roshmi Thomas, Rintu T. Varghese, E. V. Soniya, Jyothis Mathew, E. K. Radhakrishnan,(2013). Extracellular synthesis of silver nanoparticles by the Bacillus strain CS 11 isolated from industrialized area. 3 Biotech (2014) 4:121–126.

6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment
(3-4, September 2021)

Beneficial aspects of lignin in biofuel production by molecular cloning of COMT and CCoAOMT genes in *Sorghum bicolor* for attaining better biofuel yield

(Registration No. 4.6, presenting by A.VinodKumar**)**

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INTRODUCTION

Lignin, a major component of plant secondary cell wall (biomass). The bioenergy produced from cellulose is obstructed by polymer of lignin which crosslinks with cellulose. The biofuels are non-polluting and safe to the environment. Upstream processing of separation of lignin is the major step in the production.

The composition of lignin is modified by molecular cloning of COMT and CCoAOMT genes (These genes expression produces mono lignols thereby lignin molecules), the modification include alteration of lignin biosynthetic pathway at different steps, so that the upstream procedure of biofuel production is easier and less cost effective. Hence the lignin can be easily separated which helps in production of biofuel and the separated lignin is used in formation of activated carbon, binders, motor fuel, sorbents, etc.

OBJECTIVES

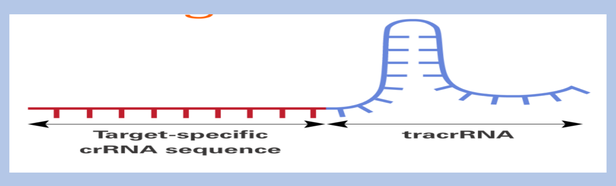
- ❖To clone the full length genes encoding the COMT and CCoAOMT from *Sorghum bicolor.*
- ❖To characterize the sequence data of genes encoding the COMT and CCoAOMT from *Sorghum bicolor* using bioinformatics tools.
- ❖To prepare the CRISPR/Cas9 constructs with COMT and CCoAOMT genes respectively and introduce into *Agrobacterium tumefaciens* strains.
- ❖To genetically transform Sorghum with *Agrobacterium tumefaciens* strains harboring CRISPR/Cas9 constructs of COMT and CCoAOMT genes respectively.
- ❖To characterize (detection of mutation) the transformed plants harboring CRISPR/Cas9 constructs of COMT and CCoAOMT genes respectively.
- ❖To analyze the lignin content and cellulose contents and evaluate the ethanol production in transformed *Sorghum* lines along with controls

METHODOLOGY

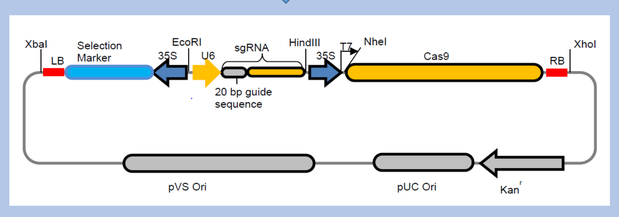
***Sorghum bicolor*, R-16 Cultiver grown in green house**

COMT **CCoAOMT**

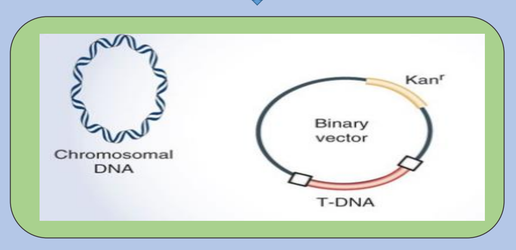
Isolation of desired genes (COMT/CCoAOMT)



gRNA design for (COMT / CCoAOMT)



Schematic representation of CRISPR-Cas9 construct (containing gRNA and cas9 of desired gene (COMT/ CCoAOMT).



A.tumefacience harbouring CRISPR construct of COMT and CCoAOMT

Transgenic *sorghum* bicolor, R-16 Cultiver

Evaluation and analysis of transgenic *S.bicolor* lines with controls

RESULTS

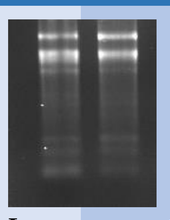


Figure 1: Lanes representing , Total RNA isolated from stem tissues of *S. bicolor*

COMT

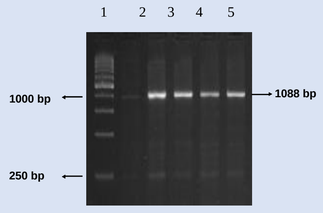


Figure 2: PCR amplicon of full length *SbCOMT* Coding Sequence Lane: +1kb ladder, Lane: 2-5 SbCOMT Gene (1088 bp)

Confirmation of isolated PCR product by Sangers sequencing method

CAAGTGGCTCACCCCTAACGAGG

Target gRNA sequence designed by online tool (chopchop)

CCoAOMT

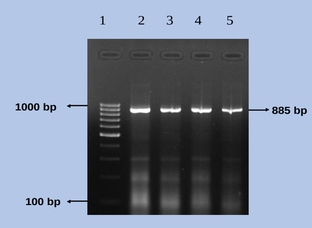
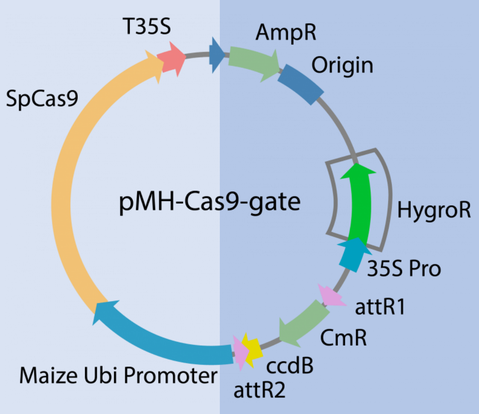


Figure 3: PCR amplicon of full length *SbCcoAOMT* Coding Sequence Lane: 1kb ladder, Lane: 2-5 SbCcoAOMT Gene (885 bp)

Confirmation of isolated PCR product by Sangers sequencing method

GACACGAGCGTGTACCCGCGGG

Target gRNA sequence designed by Sigma-Aldrich



CRISPR-Cas9 construct (containing gRNA and cas9 of desired gene (COMT/ CCoAOMT).

INFERENCE

- ❖ **The amplified product of 1088 bp of COMT gene and 885 bp of CCOAOMT genes were confirmed by PCR (Figure 2 & 3).**
- ❖ **Sequencing of the COMT/ CCoAOMT genes were carried out and analyzed by Sangers sequencing method. The sequencing of the CCoAOMT was shown >90 percent homology with related lignin biosynthetic genes existing in NCBI database.**
- ❖ **Designed target gRNA sequence by online tools (Chopchop and Sigma-Aldrich) and cloned into CRISPR construct.**

FUTURE WORK

In the future, we plan to characterize (detection of mutation) the transformed plants harboring CRISPR/Cas9 constructs of COMT and CCoAOMT genes respectively and to analyze the cellulose, lignin contents followed by evaluating the ethanol production in transformed Sorghum lines along with controls.

REFERENCES

Engler et al., 2008; Bout and Vermerris, 2003; Saballos et al., 2008; Sattler et al., 2012; Dien B, Sarath G, Pedersen J, Sattler S, Chen H, Funnel-Harris D, Nichols N, Cotta M 2009.

ACKNOWLEDGEMENTS

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Bacterial biostimulants: revitalization of plant and soil health

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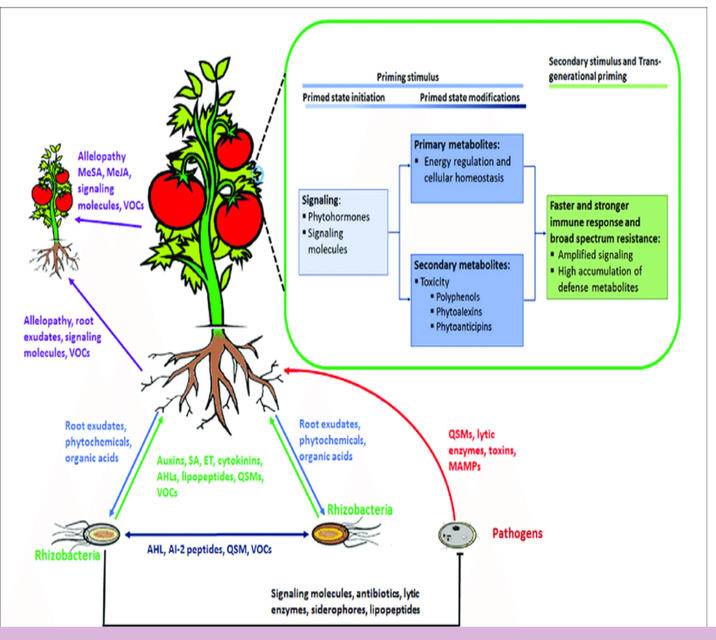
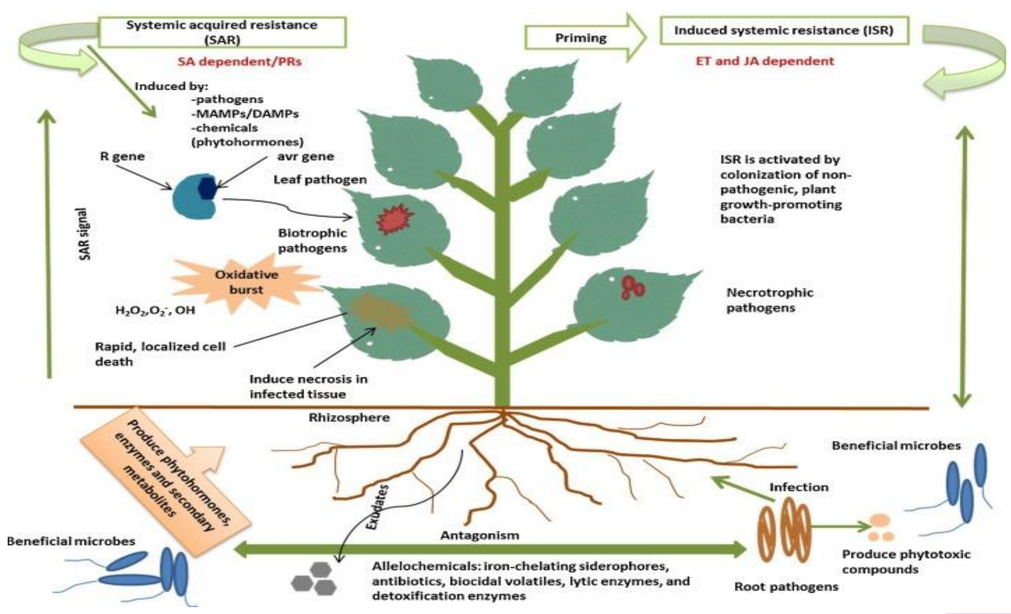
Introduction

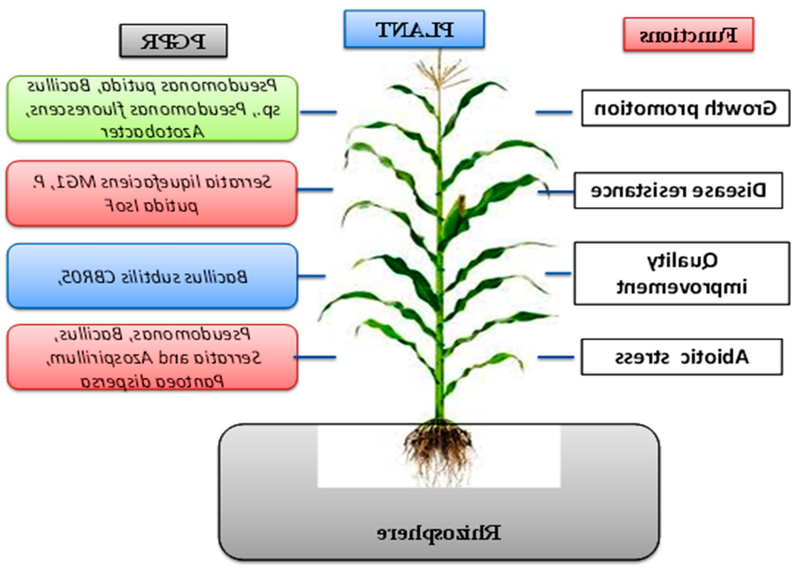
The rhizosphere is the narrow zone of soil specifically influenced by the root system (Dobbelaere *et al.*, 2003). rich in nutrients providing energy and nutrients for bacteria (Gray and Smith, 2005). 10 to 100 times higher than that in the bulk soil (Weller and Thomashow, 1994) rhizosphere is populated by a diverse range of microorganisms and rhizobacteria (Schroth and Hancock, 1982).

❖The direct promotion byPGPR is by providing phytohormones or facilitating the uptake of certain nutrientsGlick, 1995).and indirectly by producing antagonistic substances or by inducing resistance to pathogens (Glick, 1995).

❖ PGPR as biocontrol agents acts through various mechanism by effecting auxin levels (Patten and Glick, 2002), decrease of ethylene levels (Glick *et al.*, 2007) or by biological nitrogen fixation by roots (Döbereiner, 1992).





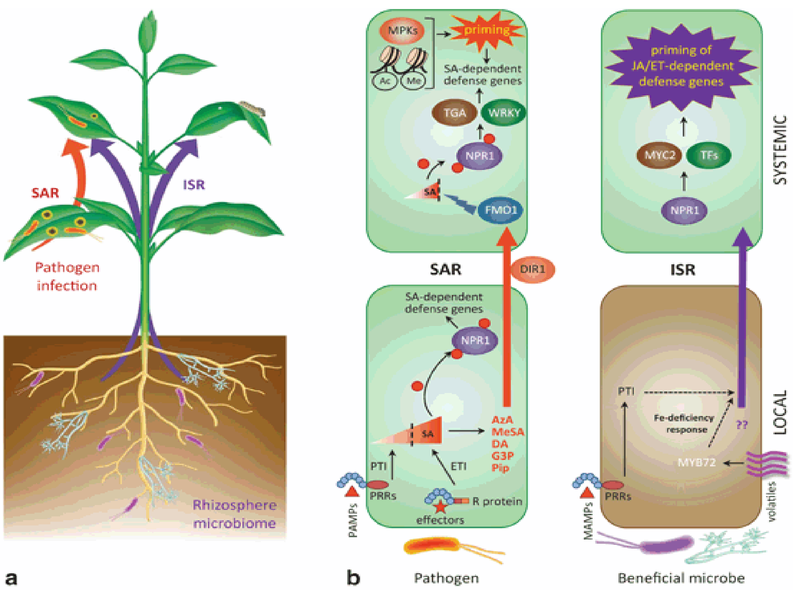


Role of PGPR in revitalization of Plant and soil health

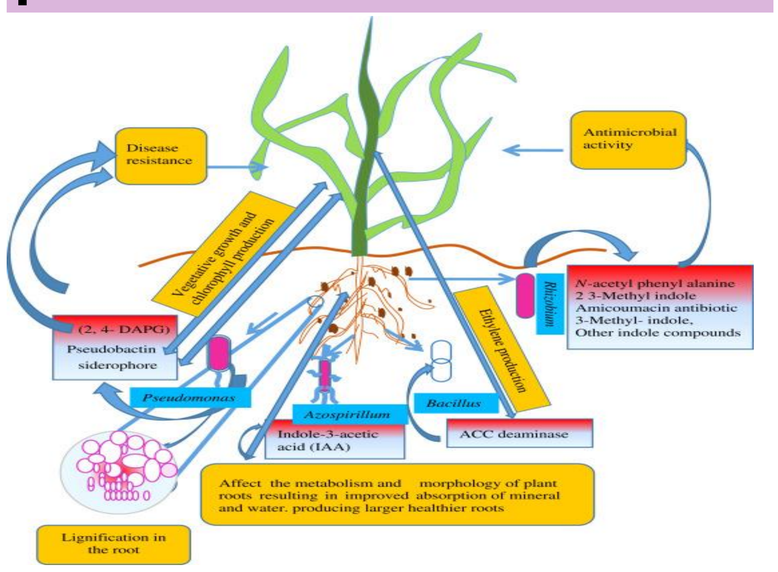
Miicrobial antagonism

Microbial Bioconsortia as bio stimulants

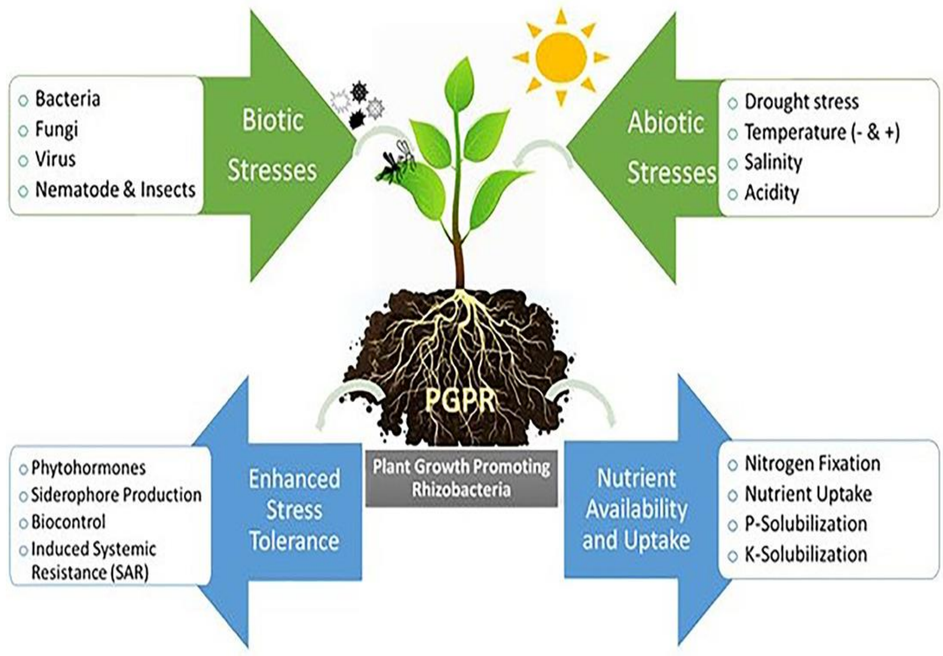
Induced Systemic resistance & systemic acquired resistance



Secondaary metabolites and siderophores production



Probiotics in Agriculture



Conclusion

The ability of microbial consortia in production of bio stimulants (siderophores,) primary metabolites l(enzymes) secondary metabolites(antibiotics) to suppress phyto pathogens could be of significant agronomic importance.

Resistance-inducing and antagonistic rhizo bacteria might be useful in formulating new inoculants, offering an eco-friendly biological control of plant disease , improving the cropping systems .

These new PGPR require a systematic strategy designed to completely utilize all beneficial factors, applying combinations of different mechanisms of action allowing crop yields toincrease while chemical treatments are being reduced.

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EXPLOITING PLANT GROWTH PROMOTING ACTIVITIES OF ACTINOMYCETES FOR SUSTAINABLE AGRICULTURE

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❑INTRODUCTION

Most of the microorganisms play a significant role in agriculture by maintaining fertility and improves the quality of the soil by various processes. Soil microorganisms enrich soil with nutrients like carbon, nitrogen, phosphorous, potassium etc. The plant growth-promoting (PGP) bacteria improve soil and plant health by an adapting attractive method for developing sustainable agricultural systems due to their eco-friendliness, low production cost and minimizing consumption of non-renewable resources. Actinomycetes are one of the best examples of plant growth promoting bacteria which are involved in the process of organic matter recycling. Actinomycetes are predominantly present in various natural habitats such as soil and plant tissues, so their isolation is easy. They grow on fresh substrates more slowly rather than bacteria and fungi, additionally they are also involved in production of hydrolytic enzymes, synthesize valuable bioactive compounds such as antibiotics, neutraceuticals, antitumor agents, plant growth regulators and vitamins. By producing such products they help in plant growth promotion and through their antagonist activity against phytopathogens also control various plant diseases. However, the applications of Actinomycetes as enzyme producers in agriculture field are relatively less explored Rani.K, 2021). The objective of this study was to isolate and screen potassium solubilizing actinomycetes from ceramic industry soils.

❑METHOD

Majority of the ceramic industries are using insoluble source of potassium i.e. feldspar as their raw material so samples were collected from the various ceramic industries. Total 15 samples were collected from different ceramic industries of Gujarat nearby Morbi, Meshana and Kadi region. The samples labelled as S1 to S15.

Isolation and Screening of Potassium Solubilizing Actinomycetes

Enriched samples were inoculated after serial dilution from 10^{-1} to 10^{-6} on Aleksandrov's agar medium constituted 1% glucose, 0.05% $MgSO_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$,0.2% $CaPO_4$ and 0.5% potassium aluminium silicate, agar 3 % pH-6.5 (Sugumaran and Janartham, 2007) and incubated at 28± 2^0 C for 1 week. Colonies exhibiting clear zone of potassium solubilization were selected. Secondary Screening was carried out on the basis of study of zone diameter of the different isolates by using Khandeparkar's selection ratio on same Aleksandrov's agar medium.

Ratio = D/d = Diameter of zone of clearance / Diameter of growth

To study the mechanism of potassium solubilization, selected Actinomycetes isolates were also inoculated
on the same Aleksandrov's medium with pH indicator dye (0.025% Bromothymol blue).(K B Prajapati, 2012)

Macroscopic / Colony morphological Characterization

Colony characteristics of the selected Actinomycetes strains were studied on Glycerol Asparagine Agar (GAA) medium. Cell morphologies of the isolates were observed using a compound microscope
after performing Gram staining.

Nitrogen fixing and Phosphate Solubilizing Ability of Selected Isolates

All promising isolates were grown on Ashby's Mannitol agar for nitrogen fixation and the selected isolates were spot inoculated on the Pikovskaya's agar plates to check their ability to solubilize insoluble inorganic phosphate.

Production of Growth Promoting Substances by Potassium Solubilizers

All promising selected isolates were subjected to qualitative analysis for the production of Indole acetic acid (IAA) and Gibberellic Acid and examined for the production IAA and GA on Luria agar supplemented with SDS (0.06%) and glycerol (1%). (Gordon & Paleg,1957)

❑RESULTS

Colonies exhibiting zone of clearance on Aleksandrov's agar medium were selected as potassium solubilizers. Total 22 Morphologically distinct actinomycetes colonies wereselected and labeled as KSA1 to KSA22 (Table 1). Isolatesshows higher ratio of potassium solubilization by Khandeparkar's selection ratio were selected, i.e. KSA9, KSA10, KSA12, KSA16 and KSA17 (table 2). The isolates which having ability to solubilize inorganic phosphate are exhibiting zone of inorganic phosphate solubilization. The 2 Actinomycetes (KSA09 and KSA16) gave SR value higher than all other isolates.

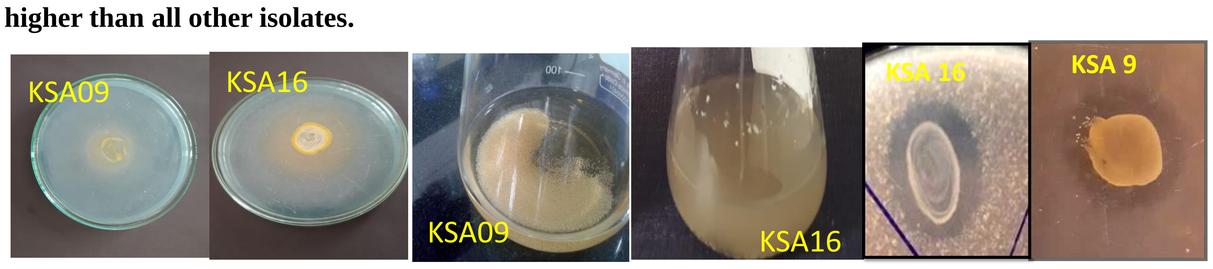


Fig-1 Yellow color formation on Aleksandrov's agar +BTB medium

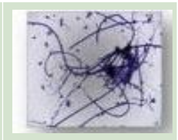
Fig-2 Insoluble potassium i.e. Feldspar solubilization on Liquid medium

Fig-3 Zone of Potassium solubilization on Aleksandrov's agar medium

Table-1. Potassium solubilization values of Actinomycetes isolates by Khandeparkar's selection ratio mm

Isolates	Diameter of Growth and Clearance (D) mm	Diameter of growth (d)	D/d (ratio)
KSA01	09	09	1
KSA02	10	10	1
KSA03	11	10	1.1
KSA04	12	12	1
KSA05	09	08	1.13
KSA06	10	10	1
KSA07	11	11	1
KSA08	09	08	1.13
KSA09	**14**	**09**	**1.56**
KSA10	**11**	**08**	**1.37**
KSA11	10	10	1
KSA14	10	10	1
KSA15	10	09	1.11
KSA16	**13**	**08**	**1.62**
KSA17	**10**	**08**	**1.25**
KSA18	11	11	1
KSA19	10	10	1
KSA20	11	10	1.1
KSA21	12	12	1
KSA 22	09	08	1.13

Table-2. Potassium solubilizing Actinomycetes Isolates Description (colony morphology, microscopic features)

Isolates no.	Description (Colony Morphology &)Microscopic Features	Growth on GAA medium	Microscopic view
KSA09	Aerial mycelium cream, smooth, powdery, circular, colony reverse off white Filaments branched.		
KSA10	Aerial mycelium gray, rough, powdery, circular, colony reverse gray Filaments branched.		
KSA12	Aerial mycelium brown, rough, irregular, colony reverse brownish pigment Filaments branched.		
KSA16	Aerial mycelium dark yellow, smooth, circular, reverse light yellowish pigment producer Filaments branched,		
KSA17	Aerial mycelium creamy, smooth, irregular, colony reverse off white, Filaments branched.		

The strains exhibited clear zone of potassium solubilization and yellow color formation around the growth in Aleksandrov medium + BTB in KSA 09 and KSA 16 indicates potassium solubilization is through acid production (Fig-1). Both isolates were able to solubilize feldspar in liquid medium also(Fig-2)

Both isolates were able to grow on Ashby's Mannitol agar so they were Nitrogen fixer and give zone of Phosphate solubilization on Pikoskavyas agar which indicates phosphate solubilization by both the isolates.

Based on the development of red colour on the filter paper (Qualitative method) and green fluorescence under UV light, both isolates were considered as positive for IAA and GA production.

❑ DISCUSSION

No reports found for Potassium solubilizing Actinomycetes, but the research suggested that microbixal solubilization of mineral phosphate might be either due to the excretion of organic acids causing acidification of the external medium or to the excretion of chelating substances (such as sideropshores) that form stable complexes with phosphate adsorbents (aluminium, iron and calcium) (Whitelaw, 1999,Welch et al. 2002; Hamdali et al. 2008) Five selected Actinomycetes isolates showed low pH associated with yellow color formation on the Aleksandrov's medium supplemented with Bromothymol blue after 72 hrs of incubation indicates that K solubilization was through acidification mechanism. The zone of Potassium solubilization with Bromothymol blue containing medium by KSA09 &KSA16. Actinomycetes are able to thrive in extremely different soils, play important ecological roles in soil nutrient cycling and are recently being regarded as plant growth promoting Rhizobacteria (Pathom-Aree et al., 2006; Franco-Correa et al., 2010, Sun F., et al. 2020)

❑CONCLUSION

➢Total 22 Actinomycetes strains were isolated from various K rich ceramic industries soil samples. All the isolates were characterized for morphological and cultural characters.Among them 5 Actinomycetes isolates were selected for further study due to their higher potassium solubilization capabilities.

➢All the isolates were able to solubilize (feldspar) insoluble potassium mineral under in vitro condition. Further two Actinomycetes strains i.e.KSA 9 and KSA 16 were selected which showed the highest zone of potassium solubilization on Aleksandrov's agar medium.

➢ Selected actinomycetes isolates showed low pH associated with yellow colour formation on the Aleksandrov's Agar medium supplemented with bromothymol blue indicates that potassium solubilization is due to acid production.

➢With this solubilization both the isolates were able to fix Nitrogen and solubilize phosphate as well as they were also producing growth promoting substances i.e.IAA and GA in good quality

➢Based on these potential applications of Actinomycetes as PGPR, their exploration as rhizosphere actinomycetes is being considered as an promising approach in sustainable agricultural field. Overall, the multifunctional property of actinomycetes makes them unique and their potentials are yet to be fully exploited.

❑ACKNOWELDGEMENT

The authors are thankful to the Department of Microbiology, HVHP Institute of Post Graduate Studies &Research, Kadi and Management of Kadi Sarva Vishwavishvidyalaya (KSV) Gandhinagar, for facilitating this research.

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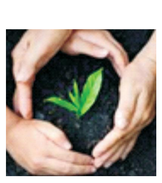
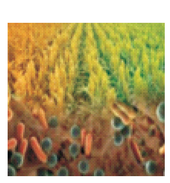


4.10

BACILLUS- NOVEL GENERA OF PGPR BY ITS BIO-PROSPECTING PROPERTIES

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Introduction

➢Research in the area of plant microbe's interaction (PMI) has opened up a fascinating world of remarkable diversity not only in terms of the rhizobacteria but also in terms of the beneficial microbe and their effects involved in agriculture.

➢Numerous species of Bacillus flourish in the rhizosphere of plants and activate or stimulate plant growth by plethora of mechanisms.

➢In recent times, chemical fertilizers and pesticides are indispensable for higher yield of crops, impart hazardous effect on soil-microbe-ecological balance and residual problem.

➢This has diverted the attention of researchers toward alternate methods plant disease control.

Objectives

The present study was carried out to evaluate and characterize of various PGPR properties and induction of defense related enzyme by cellulolytic bacteria *Bacillus amyloliquefaciens* isolated from soil rhizosphere in singly and consortia assortment against *M. Phaseolina* (Tassi.) Goidanich.

Materials and Methods

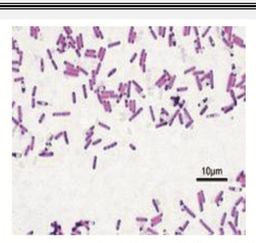
➢ *B. amyloliquefaciens* was isolated from the soil-rhizosphere using NA and purified by TSA (Hi-Media) with endospore stain kit (Fig. 1b).

➢Single colony was taken for selection of the isolates (Fig. 1c).

➢16S r- DNA gene was PCR amplified with Forward (5'-AGAGTTTGATC CTGGCTC-3') and Reverse (5'-GGTTACCTTGTTACGACTT-3') primers in ABI 3730xl sequencer (Fig. 1a).

➢Amplicon electrophoresed in 1% Agarose gel and visualized under UV by BLAST of NCBI.

Fig.1a. Genomic DNA of B. *amyloliquefaciens*



Microphotograph of *B. amyloliquefaciens*

Fig.1b

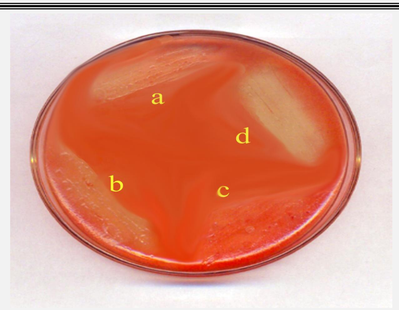


Fig.1c. Pure culture of B. *amyloliquefaciens*

Results and Discussion

Table 1. green house studies of PGPR activities

Treatments/ Std. Error Mean	Germination %	Root length (cm)	Shoot length (cm)	Fresh weight of seedling (gm)	Dry weight of seedling (gm)	Vigour Index (VI)
T1	91.94	3.76	11.93	3.46	0.53	1409.66
SEM (±)	0.91	0.03	0.29	0.11	0.08	8.14
T2	**95.33**	**4.95**	**13.20**	**4.13**	**0.63**	**1730.23**
SEM (±)	**0.85**	**0.02**	**0.20**	**0.22**	**0.06**	**21.10**
T3	89.66	3.92	12.50	3.64	0.40	1472.23
SEM (±)	0.33	0.02	0.15	0.17	0.00	6.89
T4	88.00	3.76	12.20	3.27	0.33	1404.30
SEM (±)	2.08	0.07	0.17	0.16	0.03	18.54
T5	84.50	3.75	11.60	3.01	0.30	1296.56
SEM (±)	1.18	0.02	0.25	0.14	0.00	9.53
T6	83.27	3.76	12.06	2.88	0.30	1317.93
SEM (±)	1.40	0.06	0.39	0.11	0.00	38.74
T7	84.99	3.79	11.50	3.24	0.30	1300.16
SEM (±)	0.33	0.02	0.00	0.13	0.00	7.38
T8	85.08	4.21	12.00	3.76	0.50	1378.63
SEM (±)	2.48	0.03	0.11	0.15	0.00	30.53
T9	90.00	4.50	12.83	4.36	0.40	1560.16
SEM (±)	1.15	0.14	0.16	0.11	0.00	37.60
T10	83.33	3.77	12.40	3.45	0.30	1447.23
SEM (±)	4.40	0.00	0.17	0.08	0.00	73.78
T11 (Diseased Control) SEM (±)	73.16 1.46	3.46 0.03	10.40 0.05	2.97 0.08	0.30 0.00	1014.23 24.18
T12 (Healthy Control) SEM (±)	83.22 1.23	3.80 0.02	11.23 0.03	2.99 0.11	0.33 0.03	1251.16 18.51
Total	86.04	3.95	11.98	3.43	0.38	1381.87
SEM (±)	1.00	0.06	0.13	0.08	0.01	

Acknowledgements

The author is grateful to the Director, ICAR-Central Research Institute for Jute and Allied Fibres (Indian Council of Agricultural Research), Barrackpore, Kolkata 700121 and Head, Microbiology Laboratory, Department of Botany, University of Kalyani-741235, Nadia, India for providing the necessary facilities for the experiments.

Results and Discussion

➢Among isolated strains *Bacillus amyloliquefaciens* (AB909000) showed the best effect in phosphate solubilisation efficiency (73.33%) (Fig 2a), seed germination (96.66%) and seedling growth of jute (Table 1), almost near the highest ability to pathogen inhibition (74.26%) (Fig. 2c), reduction of stem rot disease severity (62.9%) in the green house test.

➢It was unique compared to all parameters (iron chelation by siderophore) (Fig. 2b). and enhanced the activity of defence enzyme peroxidase (PO) (Fig. 4). even after challenge inoculation (Fig. 5), has tremendous potentiality to control notorious pathogen *M. phaseolina* and plays unique role in plant growth promoting activities (IAA, Siderophore, HCN) in jute (Fig. 3). by its novel properties over the others.

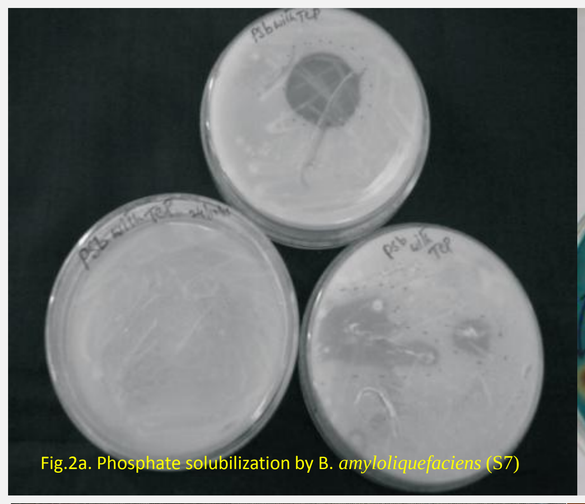


Fig.2a. Phosphate solubilization by B. *amyloliquefaciens* (S7)

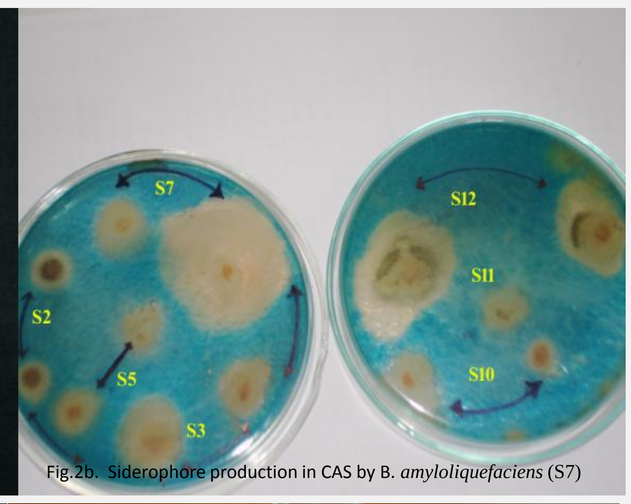


Fig.2b. Siderophore production in CAS by B. *amyloliquefaciens* (S7)

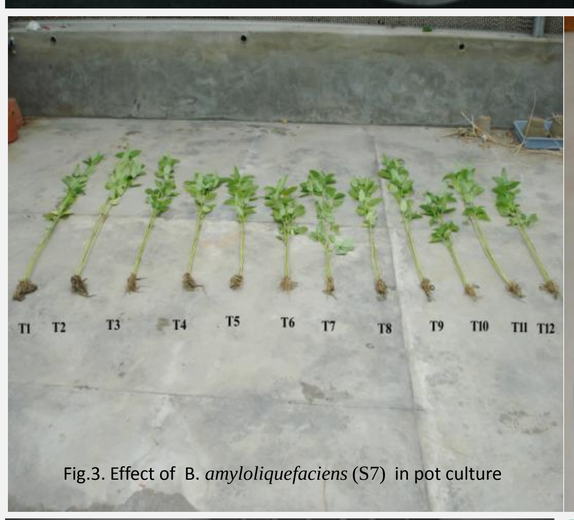


Fig.3. Effect of B. *amyloliquefaciens* (S7) in pot culture

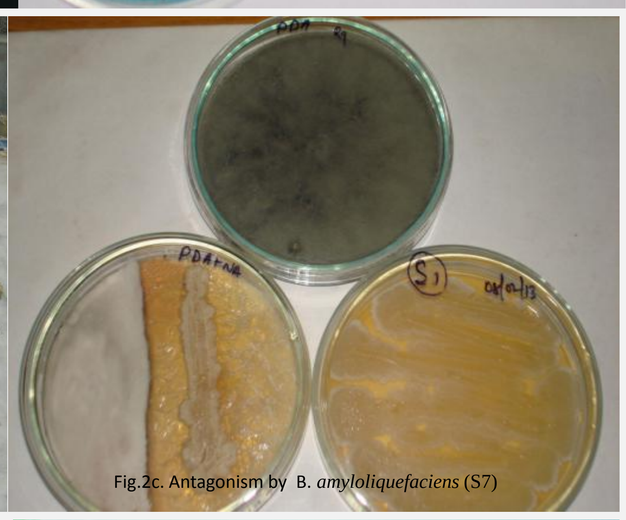


Fig.2c. Antagonism by B. *amyloliquefaciens* (S7)

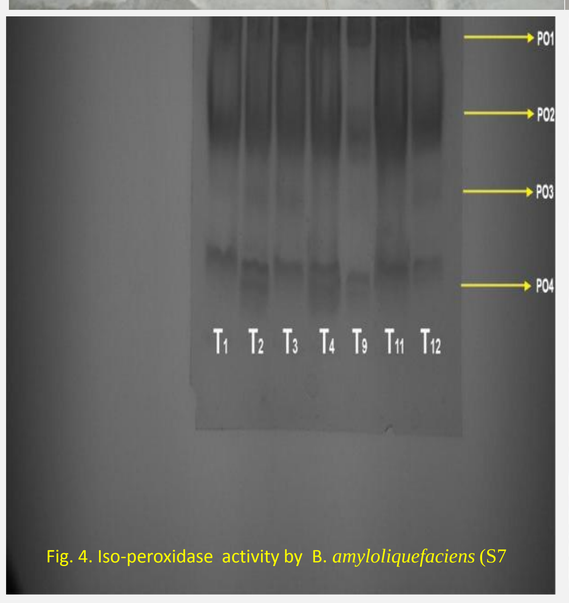


Fig. 4. Iso-peroxidase activity by B. *amyloliquefaciens* (S7

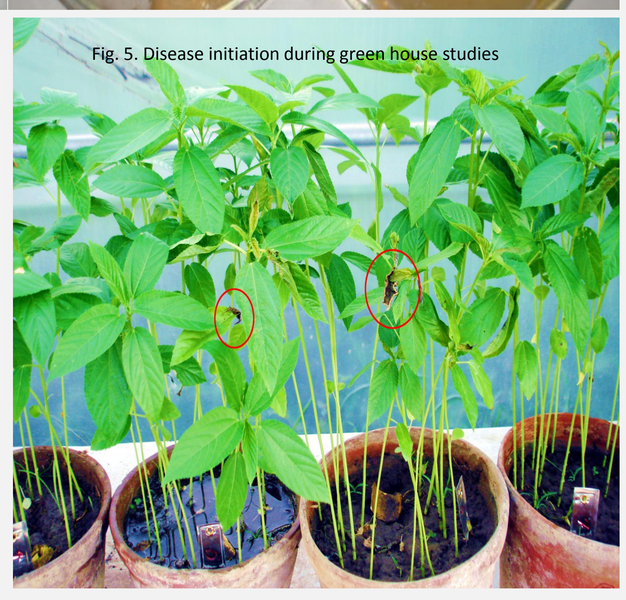


Fig. 5. Disease initiation during green house studies

Conclusion

➢Studies indicated, use of plant growth promoting rhizobacteria (PGPR) and/or fungal strains (PGPF) consortium with leading agrochemicals and growth regulator have nothing detrimental or adverse effects on each other based on their compatibility for multiple benefits of pathogen suppression, plant nutrient supply and growth promotion in jute crops which could be the most important emerging area of research in other crops for future prospects.

➢New and novel isolate of Bacillus amyloliquefaciens which has tremendous potentiality to control notorious pathogen, M. phaseolina, and also plays unique role in plant growth promoting activities in jute.

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❖**S. K. Bhattacharyya, C. Sengupta, N. K. Adhikary and J. Tarafdar. 2014. *Bacillus amyloliquefaciens* - A Novel PGPR Strain Isolated From Jute Based Cropping System. *The Bioscan* 9(3): 1263-1268.**

Post-biotics produced by plant beneficial microbes for sustainable crop productivity and environment

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Introduction

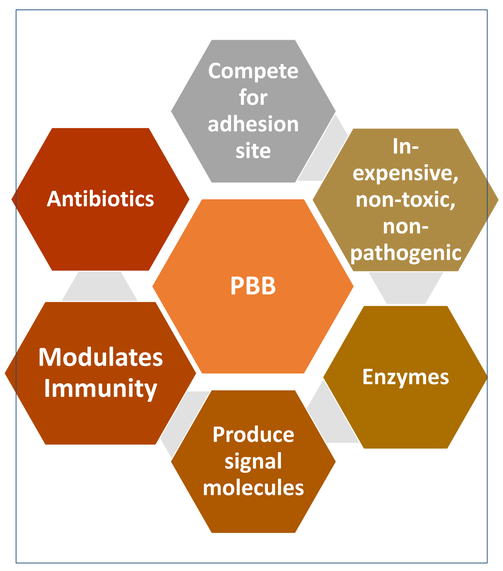
Chemicals → Ecological imbalance → Environment and Human health

Plant Beneficial Microorganisms (PBM / PBB)

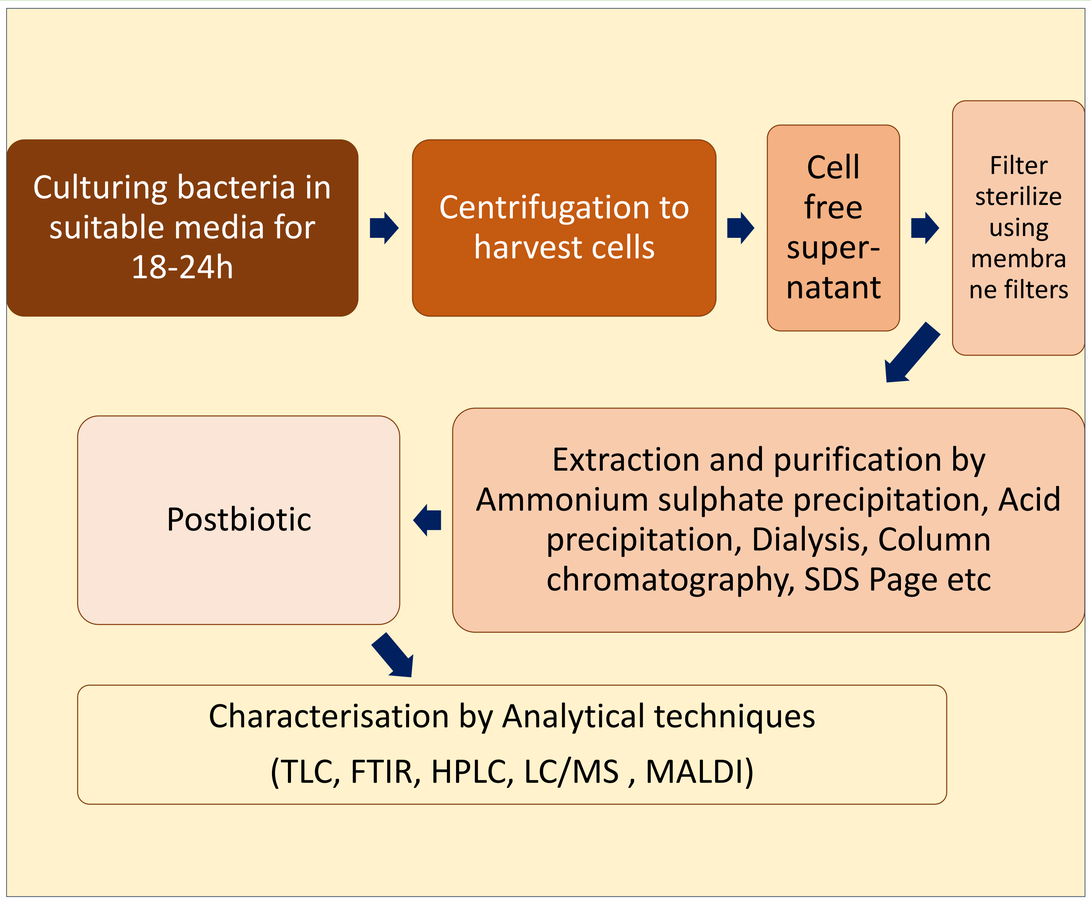
Prebiotics & Synbiotics (Organic Materials	**Probiotics** (Bio-Formulations: Single strain or consortium)	**Postbiotics (Fermented metabolites Raw/Purified)**
• Organic materials • Composts • Biochar • Biostimulants	• PGPB + PGPR • VAM + PGPR • Trichoderma + PGPR	• Extracellular metabolites: • Biosurfactants, VOC, OA, Hormones, Enzymes, Vitamins QS, QQ……

Methodology

Plant growth promotion activities	Hydrolytic enzymes	Biotic & abiotic stress management
• IAA, GA, Cytokinines • Ammonia production • HCN • ACC Deaminase • P solubilisation • Zinc solubilisation • Potassium solubilisation • VOC • ISR	• Chitinase • Pectinase • Cellulase • Xylanase • Amylase • Protease • Lipase	• Drought • Temperature • Metals • Salinity • Pathogens • Insects • Weeds

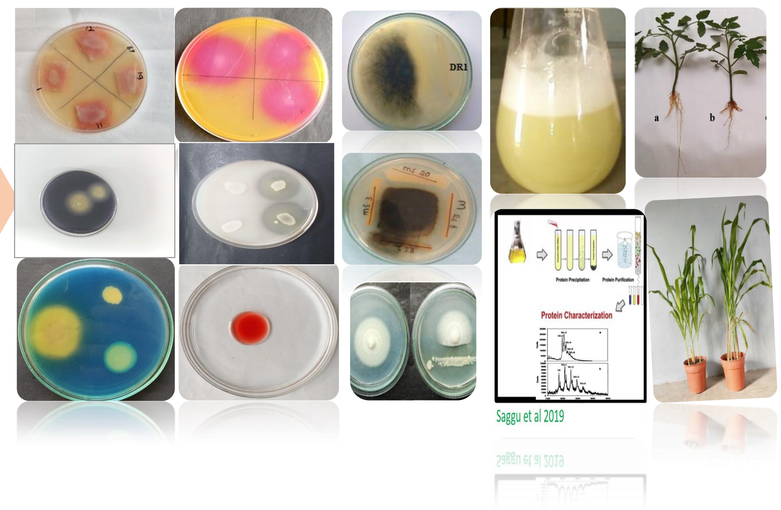


- Plant Growth Promotion
- Antifungal Activity
- Production of Postbiotics: Microbial surfactants, peptides, bacteriocins, QS molecules etc
- Tolerance to PAH and Heavy metals



Results

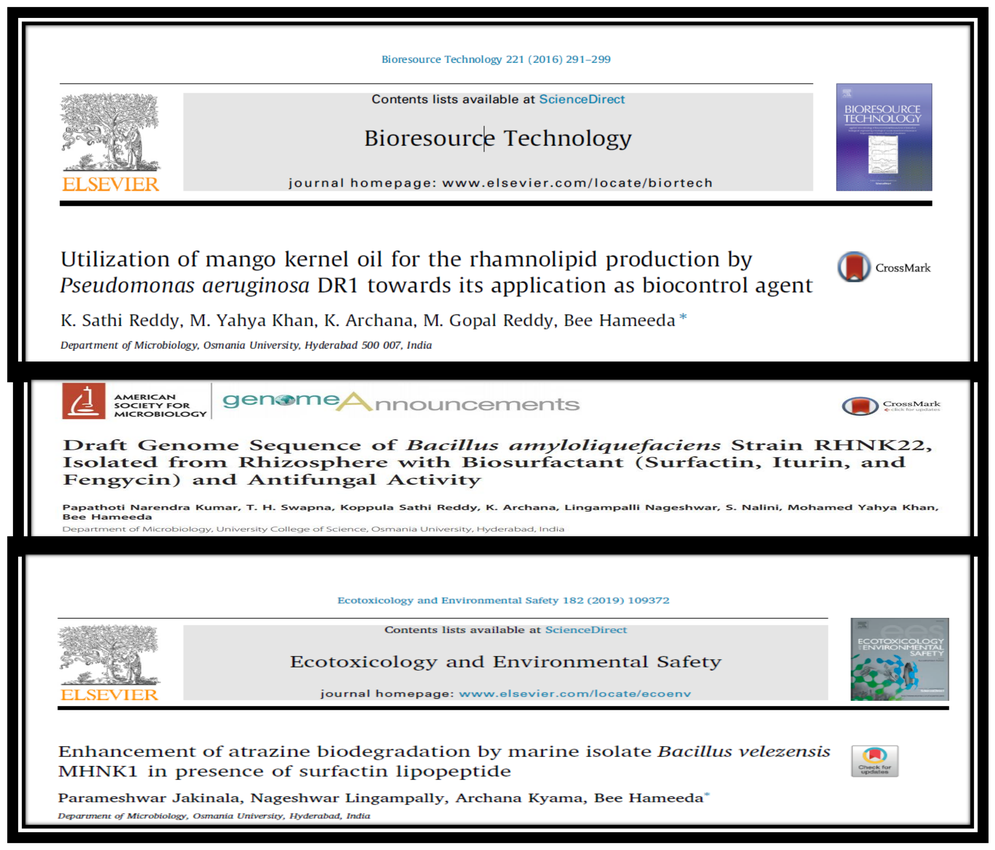
Bacillus spp.
Pseudomonas spp.
Stenotrophomonas maltophila
Rhizobium spp.
Pantoea spp.



Bacteria	PBB/Post biotic molecules	References (Our studies)
Pseudomonas aeruginosa DR 1	Mono, di-RHL	Reddy et al. 2016
Bacillus amyloliquefaciens RHNK 22	Antigungal metabolites LPs, Polyketides	Narendra et al. 2017, Hameeda et al. 2019
Bacillus velezensis MHNK 1	Surfactin	Paramesh et al. 2019
Bacillus velezensis MS 20	Surfactin, Biofilm, ISR	Kavitha et al. 2019
Streptomyces puniceus RHPR9	THL, Melanin, antifungal bioactive molecules	Ravinder et al. (unpublished)
Rhizobium undicola	Glycolipid	Imran et al. unpublished
Bacillus cereus	Antifungal, peptide fractions	Humera et al,, unpublished
Bacillus subtilis MAH84	Peptide	Adeeb et al. unpublished
Bacillus mojavensis RHPR20	Surfactin	Manasa et al. unpublished
Exoguobacterium indicum LS4	Keratinase, aminoacids	Shiva et al. unpublished

Conclusions

-For successful application of PBB, formulations should be inclusive of biostimulants and postbiotics



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Impact of *Acetobacter* isolates in sweet corn (*Zea mays L. saccharata*) in field experiment.

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INTRODUCTION:

Chhattisgarh is regarded as the "rice bowl of India" due to more acreage under rice besides the staple food of the majority of the community. The uncertainty of rice in upland, especially in low rainfall areas lead the farmers to go for other alternative crops which give more remunerative profits. Under such conditions, the scope to grow sweet corn seems to be the best choice for upland farmers. In order to popularize its cultivation among the farming society, it is essential to standardize its biofertilizers technique for it's prospective.

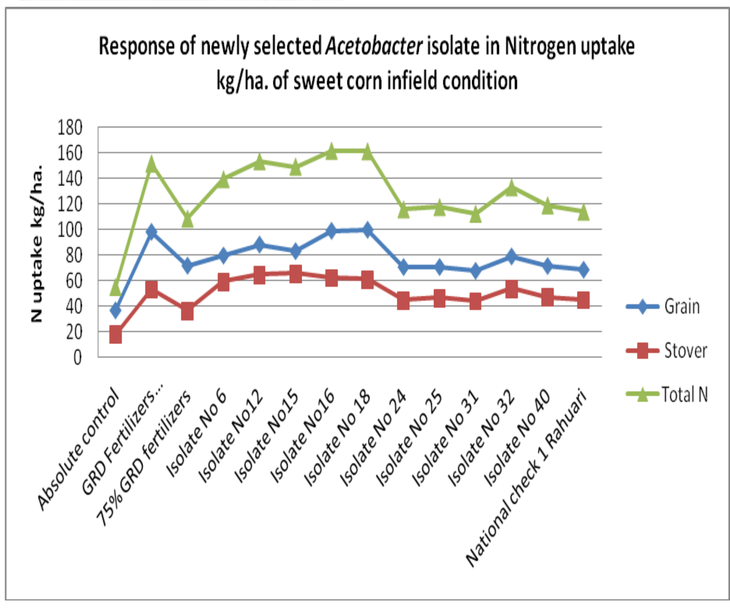
Sweet corn (*Zea mays* L.saccharata) is one of the premier commercialized maize types in poaceae family. It has a sweetened rather than a starchy endosperm and a creamy texture. The low starch level makes the kernel wrinkled relatively than plumy .When the moisture content is higher than 74 per cent the cobs are not fully formed and below 70 per cent they drop the sweetness and develop an unlikable taste and texture. In India, maize is grown over an area of 7.27 million ha with an annual production of 15.86 million tones and an average production of 2181 kg ha-1 (Anonymous, 2011). In Chhattisgarh, maize grown in 102.70 thousand ha with an annual production of 185.80 thousand million tones and an average output of 1809 kg ha-1 (Anonymous, 2010).

Besides, Chhattisgarh soil has a more demand of biological N_2-fixing and P mobilizing microbial population to reduce the use of chemical fertilizers. The low population density of endophytic, diazotrophic bacteria are mainly due to high temperature 48^0C during summer, soil surface temperature away from 60^0C and low moisture up to 3-4% for extended period of summer period resulting to loss of organic matter and population of favorable microbes (Anonymous, 1996). In addition, to the available soil nitrogen is one of the most limiting plant nutrients of are low to medium in soils of Chhattisgarh. In rising demand of chemical fertilizers, depleting soil fertility and increasing prices it is necessitates to develop effective bio-inoculants like *Acetobacter* for sweet corn crop is the need of this area. So an attempt will made to develop suitable *Acetobacter* inoculants for Sweet corn growers of Chhattisgarh with the following objectives. Effect of *Acetobacter isolates* on performance of sweet corn and response of nitrogen fixing ability of newly collected endophytic bacteria *Acetobacter spp.* in field condition.

MATERIAL METHOD

Isolation of *Acetobacter* and preparation of inoculums of *Acetobacter* isolates were isolated from sugarcane, fresh root of sweet corn, barley, maize, sweet potatoes, and surrounding soil in LGIP media. The isolated *Acetobacter* isolates was multiplied in the departmental laboratory. After preliminary glass house study on the basis of growth performance, out of 45 isolates 10 best effective isolates was selected, for field experiment.

The field experiment was laid out as per the layout plan in *Vertisols* The number of, replicated thrice in randomized block design, 14 treatments were as followings Absolute control, GRD Fertilizers 120:60:60::N:P:K,75% GRD fertilizers, isolate No 6, 12, 15, 16, 18, 24, 25, 31, 32 , 40 and one National Checks along with 75% GRD. The field was prepared by two repeated ploughing followed by leveling. Nitrogen, phosphorus and Potassium were applied in all the treatments except absolute control at the rate of 120:60:60 kg/ha through urea, single super phosphate and murate of potash, respectively. Potash and phosphorus was applied as basal and Nitrogen was applied in three split doses, first dose at the time of sowing, second and third doses at 30 and 45 DAS, respectively. Seeds were inoculated with isolates of *Acetobacter* according to treatment. 50 ml amount of individual broth cultures of all ten *Acetobacter* isolates so that each and every seed received at least 100,000 live bacterial cells. Un-inoculated seeds were treated with distilled water.. The Green cobs were harvested after 95 days of growth. The shoots were harvested at maturity at 110 DAS and weight was expressed in g/plant. After the harvesting of the crops soil samples were collected from the experimental pots. Available soil nitrogen was determined by Alkaline potassium permanganate method suggested by Subbiah and Asija (1956). Available soil phosphorus was done by the method given by Olsen (1982) . Available soil potassium was estimated by Hanway and Heidal(1952).



RESULT S:

Table.: Response of various *Acetobacter* isolate on Soil nutrient status after harvest of crop in field study.

Name of Treatment	Dry Root weight (g)/plant	Dry shoot weight g^{-1} plant	Fresh Cob weight (g/plant)	Dry cob weight (g/plant)	Grain weight (g/plant)	Fresh Cob yield kg/ha	Stover yield kg/ha.	Total sugars contents (Brix %)	Available Soil N kg/ha.	Available Soil P kg/ha.	Available Soil K kg/ha.
Control	36.70	27.34	287.00	51.00	32.90	3478.54	3340.20	3	163.07	11.35	381.96
GRD Fertilizers 120:60:60::N:P:K	87.27	49.53	624.33	112.67	73.94	7870.21	7520.44	6	188.16	14.13	401.30
75% GRD fertilizers	67.15	39.45	469.67	94.00	62.94	6239.17	6513.10	5	171.43	12.25	353.00
Isolate No 6 + 75% GRD	64.24	40.24	573.00	98.00	63.15	7450.63	7057.30	5	179.80	14.93	380.61
Isolate No12 + 75% GRD	52.08	40.28	645.67	114.00	73.49	7738.54	7474.38	5	183.98	10.75	346.33
(Isolate No15+75% GRD	69.69	40.40	577.00	98.5	61.42	7245.42	6938.54	6	188.16	11.35	368.85
(Isolate No16+75% GRD	82.93	52.59	706.33	125.67	82.55	7766.04	7519.73	7	213.25	15.32	353.00
Isolate No 18+75% GRD	91.93	56.34	738.67	131.67	85.74	8524.79	8036.82	8	204.89	14.04	380.61
Isolate No 24+75% GRD	79.72	53.42	574.33	99.33	64.13	6868.54	6525.71	7	184.31	12.25	394.69
Isolate No 25+75% GRD	73.51	42.49	509.67	88.67	57.37	6625.00	6401.27	7	209.07	10.45	370.16
Isolate No 31+75% GRD	80.28	49.65	556.00	101.00	66.25	6419.58	6544.87	6	171.43	11.95	360.10
Isolate No 32+75% GRD	74.52	44.21	584.33	98.67	67.62	6669.79	6652.46	5	200.70	13.44	358.66
Isolate No 40+75% GRD	84.81	46.75	595.00	90.00	68.43	7221.25	6770.29	7	200.70	14.63	350.34
National check 1 Rahuari +75% GRD	67.73	43.50	596.00	97.67	67.83	7098.75	6538.69	7	183.98	12.25	348.54
CD (0.05)	**4.85**	**2.23**	**109.46**	**6.62**	**3.26**	**659.96**	**531.38**	**NS**	**17.94**	**1.70**	**NS**
CV	**7.53**	**5.58**	**14.00**	**7.74**	**5.83**	**10.62**	**8.93**		**10.67**	**15.37**	**-**

Plant biomass study in field experiment data revealed that The highest dry shoot was recorded (56.34 gm/plant) due to inoculation of *Acetobacter* isolate no. 18 associated with isolate *Acetobacter* followed by isolate no.24 (53.42 g/plant) and minimum was noticed in absolute control (27.34g/plant. The highest dry root wt. was recorded (91.93 gm/plant) due to inoculation of *Acetobacter* local isolate no. 18 followed by local isolate no. 40 (84.81 g/plant) and minimum was observed in absolute control (36.70g/plant). The highest fresh cob weight was recorded (8424.79 kg/ha), associated with local isolate no.18 of *Acetobacter* followed by local isolates no.16 (7766.04 kg/ha) and minimum observed in absolute control (3478.54kg/ha). The highest Stover weight was recorded (8036.82kg/ha), associated with local isolate no.18 of *Acetobacter* followed by local isolates no.16 (7519.73 kg/ha) and minimum was observed in absolute control (3340.20kg/ha). Total Sugar content not increased significantly by inoculation of newly selected local *Acetobacter* isolates. The highest Brix percentage was noticed in isolates no. 18, (8%) and Lowest was absolute control (3%). Among these isolates all the isolates showed atpar with isolates no. 18.

In case of available soil nitrogen and phosphorus treatments showed significant variation over control. Maximum available soil N 213.23 kg/ha was observed in local isolates no.16 fallowed by isolate no.25 (209.07kg/ha) and minimum in control (163.07kg/ha). In available soil phosphorus maximum available soil P_2O_5 (15.32 kg/ha) was recorded in local isolates no.16 fallowed by local isolates no. 6 (14.96 kg/ha) and minimum in control (11.35kg/ha).

Shoot nitrogen accumulation study indicated that highest shoot N-content was observed in local isolate no.16 (1.96% per plant) followed by local isolate no.32 (1.83 % per plant). Total N uptake, it is observed from the data that maximum amount of N accumulated in sweet corn (161.60kg/ha) due to inoculation of local *Acetobacter* isolate no.16 followed by local isolates no.18 (161.17 kg/ha) and minimum was observed in absolute control uninoculated treatment (55.51kg/ha).

CONCLUSION:

Keeping in view of above mentioned findings, it can be concluded that combination of local isolate no.18 was most efficient nitrogen fixer and followed by local isolate no. 16 among all the combinations tested under the present investigation.

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4.16. Influence of Organic and Biodynamic Manures on Soil Microbial Dynamics and Soil Nutrient Parameters in Chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Thai Chen Queen

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Introduction

Excessive use of inorganic fertilizers in raising of crops is deleterious to soil health and environment. Application of organic and biodynamic manures help to maintain soil fertility, soil microbial population and quality flower production. Keeping in view, an experiment was conducted at Model Floriculture Centre, GBPUAT, Pantnagar, during 2018-19 and 2019-20 to study the response of effects of organic nutrient management practises on the biological properties of soil in chrysanthemum cv. Thai Chen Queen. The experiment consisted of sixteen treatment combinations plotted using a randomised block design, replicated thrice. During chrysanthemum harvest, soil treated with Panchagavya 6 % + common basal dose (T_7) had considerably higher bacterial, fungal, and actinomycetes populations, as well as more N-fixers and P-solubilizers than the other treatment combinations. Additionally, the impact of organic farming practises on soil health in the region was investigated using basic soil parameters. The results indicate that when 6% Panchagavya along with common basal dose is applied, both the microbial population and essential nutrients increased in soil. The pH, E.C and organic carbon concentrations were all close to neutral. ***CBD consists of *Azotobacter* + *Azospirillum* + *PSB* + Potash bacteria + VAM + Vermicompost**

Objective

To study the response of organic and biodynamic manures on Soil Microbial Dynamics and Soil Nutrient Parameters in Chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Thai Chen Queen

Methodology

The experiment was laid out in a randomized block design (RBD) with three replications and 16 treatments consisting of 1–10 % Panchagavya treatments, 10 – 50 % Jivamrita treatments along with control. Each treatment treated with common basal dose of *Azotobacter* + *Azospirillum* + *PSB* + Potash bacteria + VAM + Vermicompost along with FYM. Estimation of soil microbial population was done by the method of soil analysis given by Wollum, 1982, P-solubilizers as well as N-fixers in rhizosphere soil was determined by the method given by Allen, 1959**.**

Results and Discussion

Treatment combination of T_7 (6 % Panchagavya + CBD) resulted in maximum bacterial population (290 x 10^5, 288 x 10^5 CFU g^{-1}), fungal population (142 x 10^5, 140 x 10^5 CFU g^{-1}) and actinomycetes population (147 x 10^5, 146 x 10^5 CFU g^{-1}) as well as more N-fixers (74 x 10^4, 71 x 10^4 CFU g^{-1}) and P-solubilizers (163 x 10^3,160 x 10^3 CFU g^{-1}) in both seasons of 2018-19 and 2019-20, respectively. The increase in the microbial population of organically treated chrysanthemum plots is attributed to the use of biofertilizers in combination with liquid organic manures such as Panchagavya and Jivamrita.

Figure 1. A. Control (T_1) has less growth of microbial population (Actinomycetes, Fungal and Bacterial), B. 6 % Panchagavya + CBD *(T_7) has excellent growth of microbial population (Actinomycetes, Fungal and Bacterial).

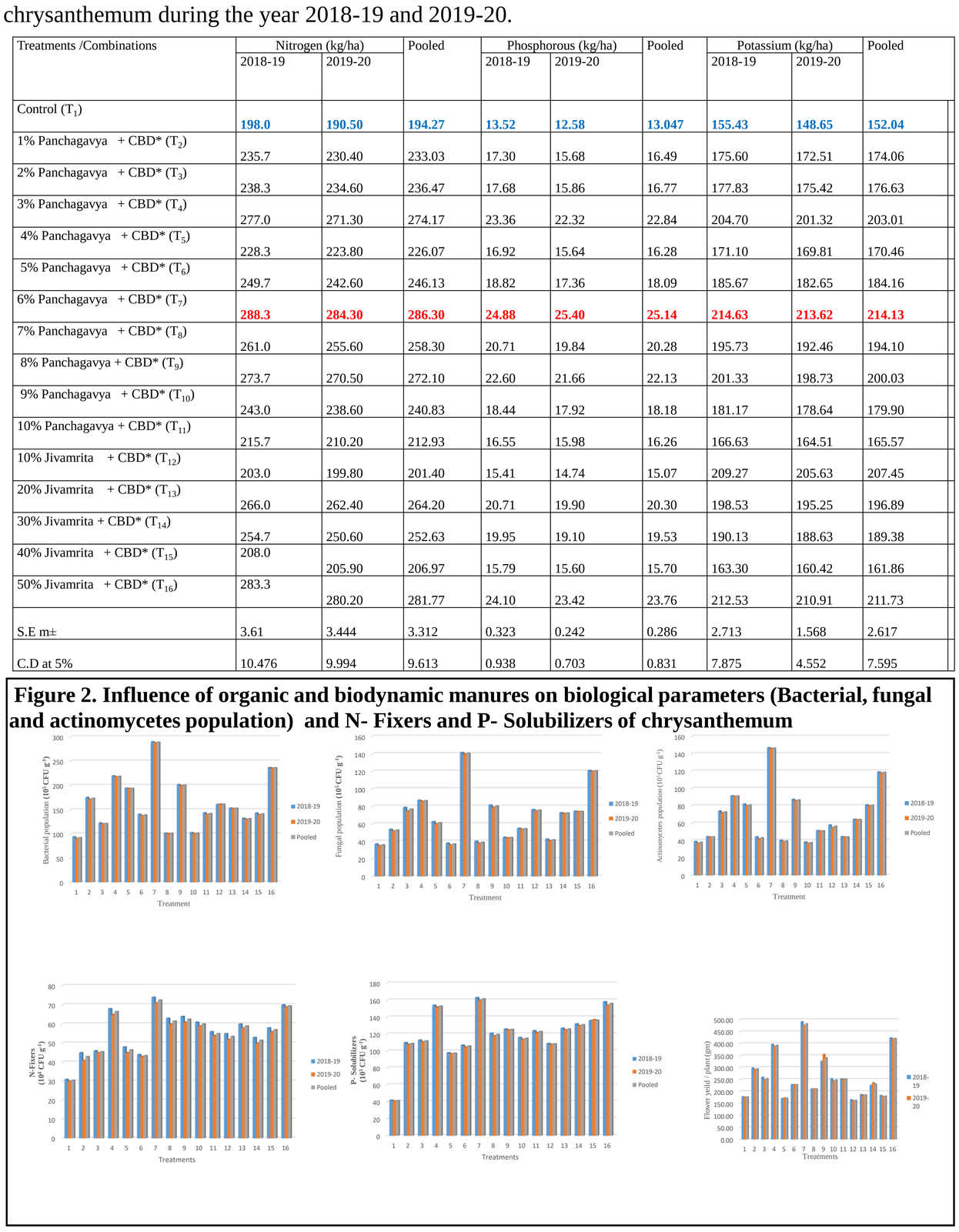
Table 1. Effect of organic and biodynamic manures on biological parameters (Bacterial, fungal and actinomycetes population) of chrysanthemum

Combinations / Treatments	Bacterial Population (10^5 CFU g^{-1})		Pooled	Fungal Population (10^5 CFU g^{-1})		Pooled	Actinomycetes Population (10^5 CFU g^{-1})		Pooled
	2018-19	2019-20		2018-19	2019-20		2018-19	2019-20	
Control (T_1)	94	91	93	37	35	36	39	37	38
1% Panchagavya + CBD* (T_2)	176	172	174	54	52	53	45	44	44
2% Panchagavya + CBD* (T_3)	123	121	122	79	75	77	74	72	73
3% Panchagavya + CBD* (T_4)	220	218	219	87	86	86	92	91	91
4% Panchagavya + CBD* (T_5)	195	194	194	63	60	61	82	80	81
5% Panchagavya + CBD* (T_6)	141	138	140	38	36	37	44	42	43
6% Panchagavya + CBD* (T_7)	290	288	289	142	140	141	147	146	146
7% Panchagavya + CBD* (T_8)	102	101	101	40	38	39	40	39	40
8% Panchagavya + CBD* (T_9)	202	200	201	82	79	80	88	86	87
9% Panchagavya + CBD* (T_{10})	103	101	102	45	44	44	38	37	38
10% Panchagavya+ CBD* (T_{11})	144	141	143	55	54	54	52	51	51
10% Jivamrita + CBD* (T_{12})	161	162	162	76	75	76	58	55	57
20% Jivamrita + CBD* (T_{13})	154	153	153	43	41	42	45	44	44
30% Jivamrita + CBD* (T_{14})	133	131	132	73	72	72	65	64	64
40% Jivamrita + CBD* (T_{15})	144	141	142	76	74	75	71	69	70
50% Jivamrita + CBD* (T_{16})	237	236	237	121	120	121	119	118	119

Table 2. Effect of Panchagavya and Jivamrita on soil physical properties and nutrient parameters in chrysanthemum during the year 2018-19 and 2019-20.

Treatments /Combinations	Nitrogen (kg/ha)		Pooled	Phosphorous (kg/ha)		Pooled	Potassium (kg/ha)		Pooled
	2018-19	2019-20		2018-19	2019-20		2018-19	2019-20	
Control (T_1)	198.0	190.50	194.27	13.52	12.58	13.047	155.43	148.65	152.04
1% Panchagavya + CBD* (T_2)	235.7	230.40	233.03	17.30	15.68	16.49	175.60	172.51	174.06
2% Panchagavya + CBD* (T_3)	238.3	234.60	236.47	17.68	15.86	16.77	177.83	175.42	176.63
3% Panchagavya + CBD* (T_4)	277.0	271.30	274.17	23.36	22.32	22.84	204.70	201.32	203.01
4% Panchagavya + CBD* (T_5)	228.3	223.80	226.07	16.92	15.64	16.28	171.10	169.81	170.46
5% Panchagavya + CBD* (T_6)	249.7	242.60	246.13	18.82	17.36	18.09	185.67	182.65	184.16
6% Panchagavya + CBD* (T_7)	288.3	284.30	286.30	24.88	25.40	25.14	214.63	213.62	214.13
7% Panchagavya + CBD* (T_8)	261.0	255.60	258.30	20.71	19.84	20.28	195.73	192.46	194.10
8% Panchagavya + CBD* (T_9)	273.7	270.50	272.10	22.60	21.66	22.13	201.33	198.73	200.03
9% Panchagavya + CBD* (T_{10})	243.0	238.60	240.83	18.44	17.92	18.18	181.17	178.64	179.90
10% Panchagavya + CBD* (T_{11})	215.7	210.20	212.93	16.55	15.98	16.26	166.63	164.51	165.57
10% Jivamrita + CBD* (T_{12})	203.0	199.80	201.40	15.41	14.74	15.07	209.27	205.63	207.45
20% Jivamrita + CBD* (T_{13})	266.0	262.40	264.20	20.71	19.90	20.30	198.53	195.25	196.89
30% Jivamrita + CBD* (T_{14})	254.7	250.60	252.63	19.95	19.10	19.53	190.13	188.63	189.38
40% Jivamrita + CBD* (T_{15})	208.0	205.90	206.97	15.79	15.60	15.70	163.30	160.42	161.86
50% Jivamrita + CBD* (T_{16})	283.3	280.20	281.77	24.10	23.42	23.76	212.53	210.91	211.73
S.E m±	3.61	3.444	3.312	0.323	0.242	0.286	2.713	1.568	2.617
C.D at 5%	10.476	9.994	9.613	0.938	0.703	0.831	7.875	4.552	7.595

Figure 2. Influence of organic and biodynamic manures on biological parameters (Bacterial, fungal and actinomycetes population) and N- Fixers and P- Solubilizers of chrysanthemum



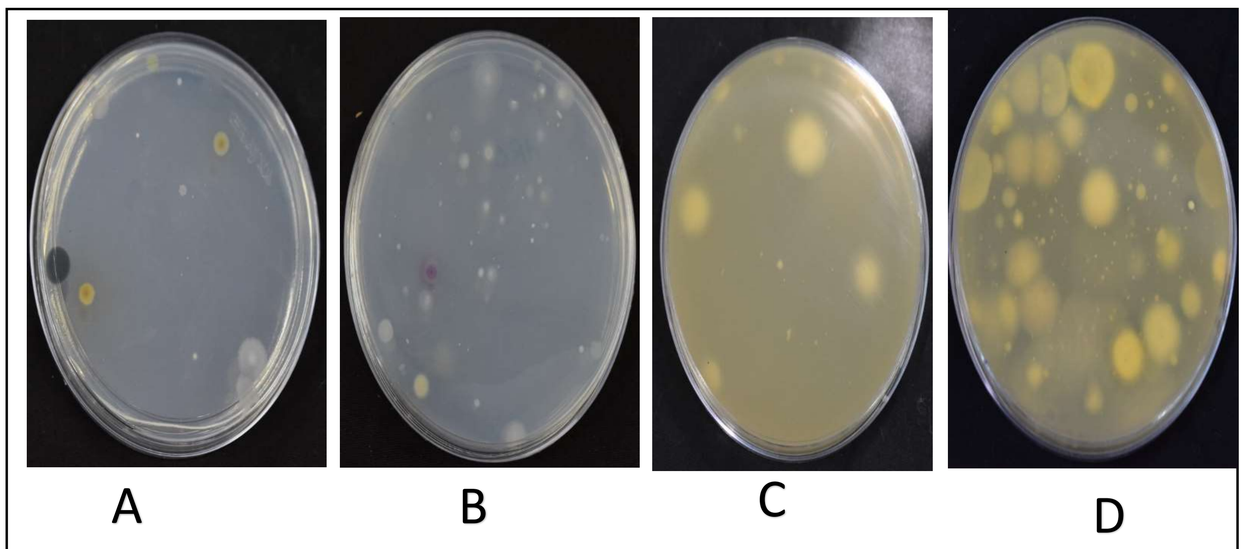


Figure 3. A. Control (T_1) showed minimum growth for N-fixers, B. 6 % Panchagavya + CBD *(T_7) showed excellent growth of N-fixers, C. Control (T_1) showed minimum growth for P-Solubilizers, D. 6 % Panchagavya + CBD *(T_7) showed excellent growth of P-solubilizers

Conclusion

The standard agricultural practices degrade crops and soil in commercial chrysanthemum growing. These issues can be resolved by organic farming, as chemical fertilisers impair soil health, growth, and productivity, ultimately affecting human health and the environment. The analysis indicated that the application of T_7 (6 % Panchagavya + Common basal dose) increased microbial growth and had a significant effect on soil physical and nutritional parameters in chrysanthemum cultivation when compared to the control and other treatments

Synergistic effect of vermicompost and bioaugmentation of liquid based biofertilizer on growth of *Cucumis sativus* var. Green sikhar SPL. (Cucumber)

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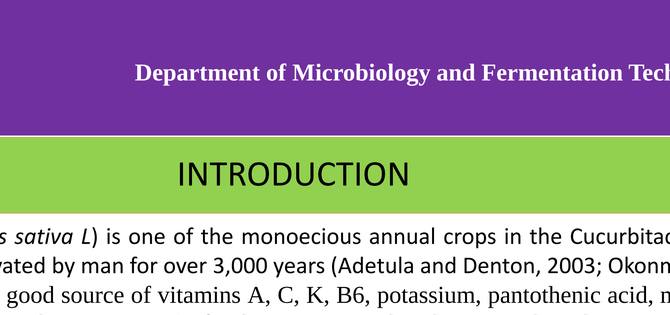
INTRODUCTION

Cucumber (*Cucumis sativa L*) is one of the monoecious annual crops in the Cucurbitaceae family that has been cultivated by man for over 3,000 years (Adetula and Denton, 2003; Okonmah, 2011). Cucumber is a very good source of vitamins A, C, K, B6, potassium, pantothenic acid, magnesium, phosphorus, copper and manganese.Biofertilizer improve the plant growth and protect from pests and disease. Improves soil fertility.Vermicompost is a mesophilic biodegradation product resulting from interaction between earthworm and microorgnism in the breakdown of organic wastes. Vermicompost, is a mesophilic biodegradation product where biofertilizers are commonly known as microbial inoculants that enhance plant growth.

METHODOLOGY

The experiment was conducted in the Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad,U.P. to find out Green sikhar the most popular cucumber variety with high productivity can be imparted for enhancing the plant growth and protect from pests and disease by inoculating with *Pseudomonas aeruginosa* .

Pure culture *Pseudomonas aeruginosa* were used for in vitro and field experiments in the Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad,U.P.



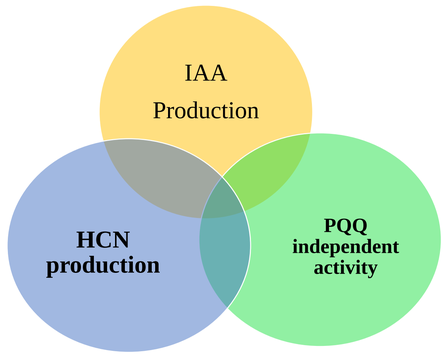
Details of treatments

Abbre.	TREATMENTS
T_0	Control
T_1	Vermicompost treated soil + Untreated seed
T_2	Vermicompost treated soil + Seed treatment with *Pseudomonas aeruginosa* (liquid based)
T_3	Vermicompost treated soil + Seed treatment with *Pseudomonas aeruginosa* (Carrier based)
T_4	Vermicompost treated soil + Seed treatment *Pseudomonas aeruginosa* (liquid based) + *Pseudomonas aeruginosa* (Carrier based)
T_5	Vermicompost untreated soil + Seed treatment with *Pseudomonas aeruginosa* (liquid based)
T_6	Vermicompost untreated soil + Seed treatment with *Pseudomonas aeruginosa* (Carrier based)
T_7	Vermicompost untreated soil + Seed treatment with *Pseudomonas aeruginosa* (Carrier based) + *Pseudomonas aeruginosa* (liquid based)

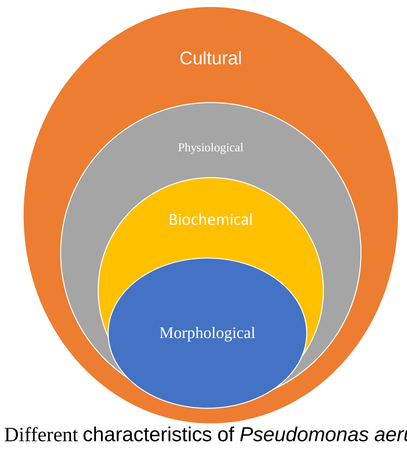
Variety : Green sikhar

Design : Randomized Block Design

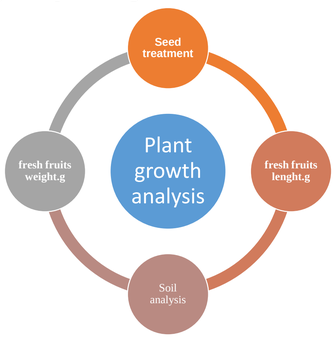
Replications : 03



Plant growth promoting activities of inoculants



Different characteristics of *Pseudomonas aeruginosa*



Different plant parameters of field experiment

Experimental details

Crop	Cucumber (*Cucumis sativus*)
Season	Zaid
Variety	Green sikhar SPL.
Fruit color	Green
Plot size (L× B)	10 x 06m

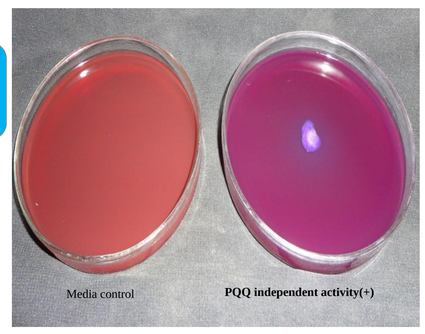
RESULTS AND DISCUSSION

In this experiment, the isolated cultures *Pseudomonas aeruginosa* were assessed for plant growth promoting characters. *Pseudomonas aeruginosa* produce high amount of IAA, HCN , Auxin and PQQ . Plant parameters include fruits weight. The field used in this study was from a site situated of Allahabad School of Agriculture, University campus of SHIATS. Before starting an experiment, composite of soil samples from the surface 0 to 20 cm depth were collected and analyzed for physical and chemical characteristics. The data recorded during course of investigation were analyzed statistically using sample standard deviation one way analysis of variance.Vermicompost, is a mesophilic biodegradation product where biofertilizers are commonly known as microbial inoculants that enhance plant growth. *Pseudomonas aeruginosa* was characterized for cultural, morphological and biochemical characterization as per Bergey's Manual of Systematic Bacteriology **Holt *et al.* (1984).**

Pseudomonas aeruginosa was characterized as a gram negative rod shaped bacteria showing positive biochemical test. *Pseudomonas aeruginosa* was assayed for phosphate, Zinc, Potassium solubilization in different media it produce IAA, Auxin, HCN and it showed PQQ independent activity. Phosphate solubilization index was higher in NBRIP (3.44±0.19) as compared to PVK media (1.51±0.02). *Pseudomonas aeruginosa* had a shelf life of 70 days. Physico-chemical analysis of vermicompost had increase in Carbon%, Nitrogen%, Calcium from 0 to 60 days of maturation as (4.10, 0.66, 0.08 and 1.60) pH changed from 7.5 to 6.9. Physico- chemical properties were recorded as Nitrogen (0.041%), Potassium (0.073%), Organic carbon (0.066%), and Phosphorus (0.650%). Vermicompost + Carrier Based *Pseudomonas aeruginosa* Biofertilizer showed best Cucumis sativus for all growth parameters viz seed germination (%), shoot length (10.83±0.2, 10.53±0.3), leaf length (5.69±0.07), number of fruit (1.13±0.11) and root length (21.33±2.80). In conclusion use of vermicompost and *Pseudomonas aeruginosa* as biofertilizers enhanced growth of yield of Cucumis sativus.

Table: IAA, HCN, and PQQ independent activity by *Pseudomonas aeruginosa*

PGP traits	*Pseudomonas aeruginosa*
IAA production	+
HCN production	+
Auxin production	+
PQQ independent activity	+



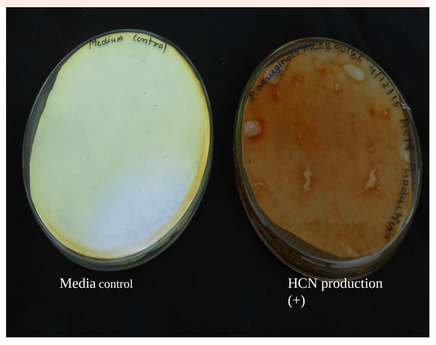
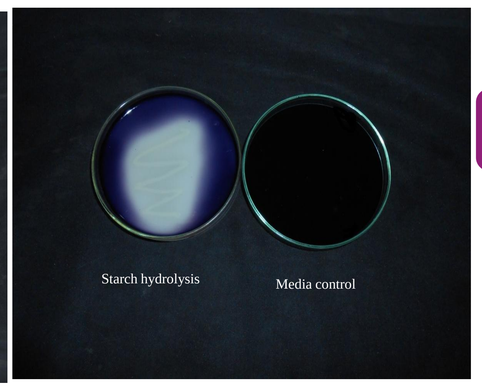
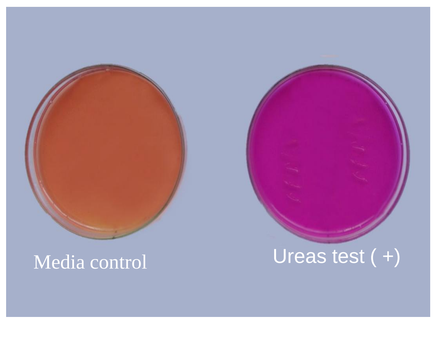


Table: Carbohydrate fermentation test for *Pseudomonas aeruginosa*

	Characteristics	*Pseudomonas aeruginosa*
Carbohydrate fermentaion	D-xyloase	A^-G^-
	D-fructose	A^-G^-
	Glucose	A^-G^-
	Sucrose	A^-G^-
	Manitol	A^-G^-
	Sorbitol	A^-G^-
	Lactose	A^-G^-
	Trehaluose	A^-G^-
	D-galactose	A^-G^-





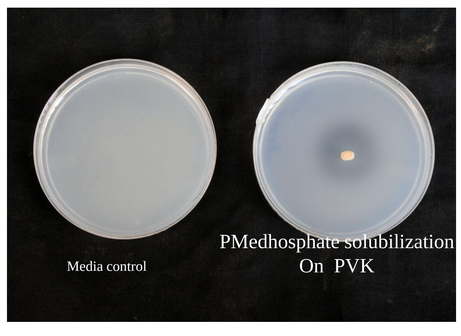
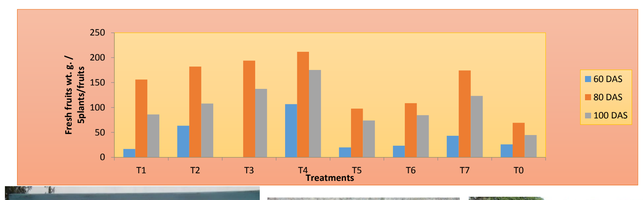
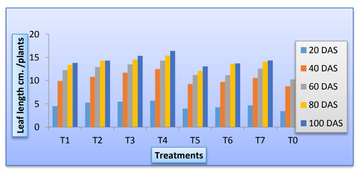
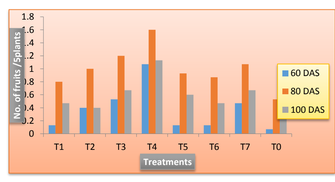


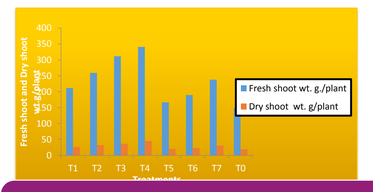
Table Variation in fresh fruits wt.g. among treatments of *Cucumis sativus* Green Sikhar SPL

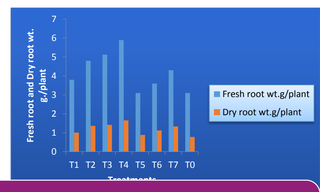
Abbre.	Treatments	Fresh fruit wt.g (DAS)		
		60 DAS	80 DAS	100 DAS
T_1	Vermicompost	16.67±28.87 a	156±16.37 bc	86±15.09 cde
T_2	Vermicompost + liquid based biofertilizer	63.33±40.41 a	182±29.46 ab	108±10.58 bcd
T_3	Vermicompost + Carrier based biofertilizer	86.67±77.67 a	194±37.36 ab	137.33±30.66 ab
T_4	Vermicompost + Carrier based+ liquid based biofertilizer	106.67±92.91 a	**211.67±30.13 a**	**175.33±26.65 a**
T_5	liquid based biofertilizer	20.0±34.64 a	97.67±37.09 d	74±14.42 de
T_6	Carrier based biofertilizer	23.33±40.41 a	108.67±40.06 cd	84.67±9.87 cde
T_7	Carrier based + liquid based biofertilizer	43.33±45.09 a	174.33±27.42 ab	123.33±50.96 bc
T_0	Control	26.0±22.53 a	69.33±17.92 d	44.67±17.32 e
	$F_{cal.}$	1.224	8.389	8.143
	$F_{tab.}$	2.119	2.119	2.119
	F_{test}	NS	S	S
	CD-(0.05%)	62.909	52.986	42.958
	S.Ed.(±)	43.30	24.992	20.261











CONCLUSION

In conclusion utilization of carrier based biofertilizer of *Pseudomonas aeruginosa* along with use of vermicompost the best way to enhance growth and yield of *Cucumis sativus* Green sikhar simultaneously to maintain fertility of soil. Carrier based biofertilizer is more effective and liquid based biofertilizer under in *vivo* condition.

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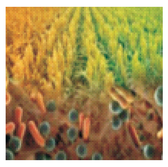

Registration Number – **4.18**

DEVELOPMENT AND CHARACTERIZATION OF IN-SILICO BASED EST-SSR MARKERS IN *Withania somnifera* & *Centella asiatica*

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1. INTRODUCTION

Withania somnifera (WS) and *Centella asiatica* (CA) are used in Ayurvedic as well as indigenous drugs for more than 30 centuries.

S. No.	Name of Plant	Active ingredients	Biological activities
1.	*Withania somnifera* (Ashwagandha) (Solanaceae family)	❑ **Alkaloids** (isopelletierine, anaferine, etc.) ❑ **Steroidal lactones** (withanolides and withaferins) ❑ **Saponins**	❑ Antidepressant ❑ Antifungal ❑ Antimicrobial ❑ Apoptotic ❑ Chondroprotective ❑ Cardioprotective ❑ Immunomodulator ❑ Neuroprotective, etc.
2.	*Centella asiatica* (Brahmi) (Apiceae family)	❑ **Triterpenoid** ❑ **Saponins**	Treating various skin conditions such as – ❑ Leprosy ❑ Lupus ❑ Varicose ulcers ❑ Diarrhoea ❑ Amenorrhea diseases of the female genitourinary tract ❑ Fever, etc.

Table 1.1: Active components and biological functions of WS and CA.

1.1 EST-SSR

EST markers are preferred because of the following reasons:

- ❑ It is a simple and quick strategy to study the transcribed parts of various genomes.
- ❑ Useful in cloning of specific gene of interest and mapping of functional genes.
- ❑ Used for assaying variation in transcribed and known-function genes.
- ❑ EST derived markers are highly conserved and more transferable between species.
- ❑ EST is a cost-effective.

1.2 The objectives of the work

- ❑ To search the EST database for the presence of SSR-containing sequences in Withania somnifera (Ashwagandha) and Centella asiatica (Indian pennywort).
- ❑ Insilco Development of gene specific SSR primers based on data analysis by different software.
- ❑ Prepare a comparative analysis of different gene(s) presence in different tissue of Withania somnifera (Ashwagandha) and Centella asiatica (Indian pennywort) .

2. MATERIALS & METHODOLOGY

- ❑ For the improvement of medicinal plants, molecular markers are most popularly used for estimation of polymorphisms, relatedness & mating system parameters, genotype characterization & marker-assisted selection (MAS).
- ❑ Hence, EST-SSR was developed for Withania somnifera & Centella asiatica.
- ❑ Therefore, the EST SSR markers were constructed for the same.

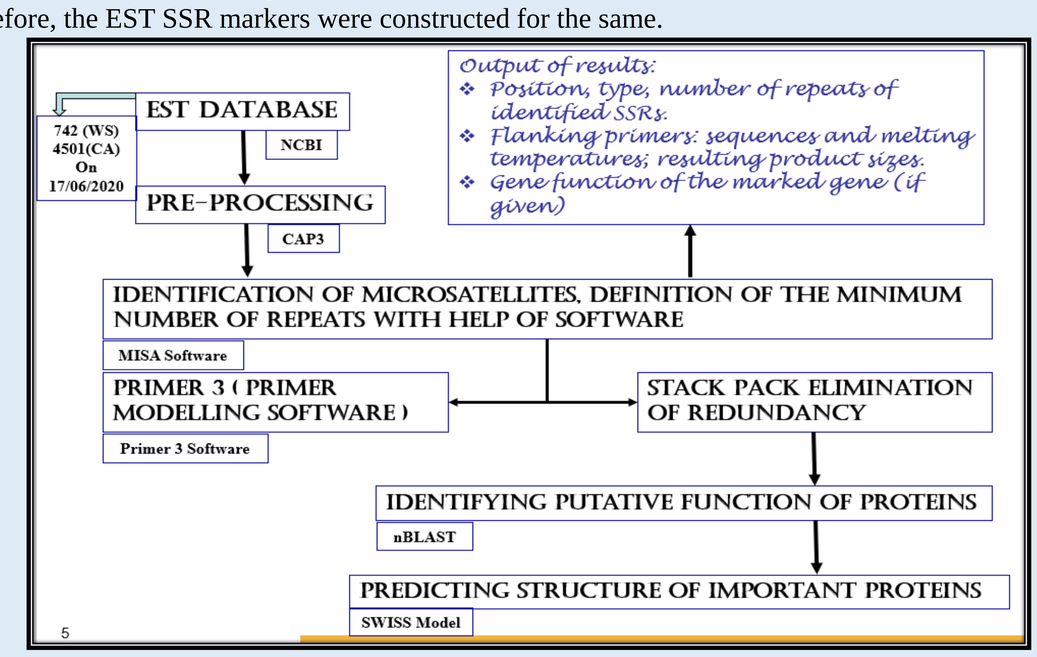

Figure 2.1: Flow chart of methodology

5. REFERENCES

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3. RESULTS

Parameters used in screening	Data generated by MISA
Total number of sequences examined	661
Total size of examined sequences (bp)	408746
Total number of identified SSRs	335
Number of SSR containing sequences	286
Number of sequences containing more than 1 SSR	42
Number of SSRs present in compound formation	29
Mononucleotide	306
Dinucleotide	11
Trinucleotide	18

Table 3.1: Summary of repeat units for all the SSR"s in *Withania somnifera*

Parameters used in screening	Data generated by MISA
Total number of sequences examined	4306
Total size of examined sequences (bp)	2717834
Total number of identified SSRs	1389
Number of SSR containing sequences	1025
Number of sequences containing more than 1 SSR	207
Number of SSRs present in compound formation	285
Mononucleotide	1122
Dinucleotide	171
Trinucleotide	91
Tetranucleotide	5

Table 3.2: Summary of repeat units for all the SSR"s in *Centella asiatica*

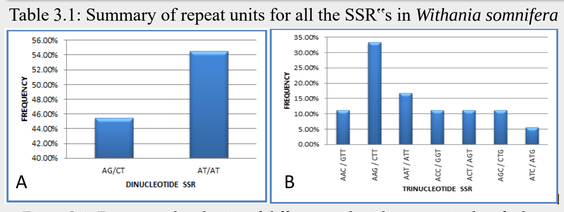

Figure 3.1: Frequency distribution of different nucleotide repeats in identified SSRs sequences in W. somnifera: (A) Dinucleotide, (B) Trinucleotide

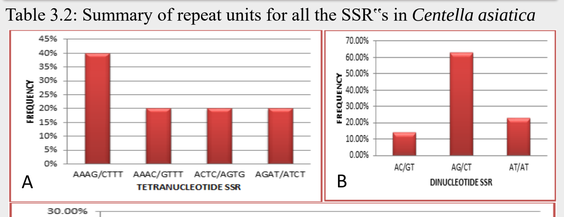

Figure 3.2: Frequency distribution of different nucleotide repeats in identified SSRs sequences in C. asiatica: (A) Dinucleotide, (B) Trinucleotide, (C) Tetranucleotide

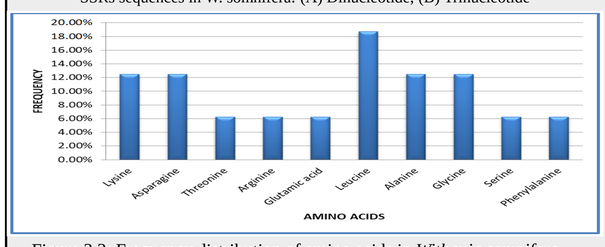

Figure 3.3: Frequency distribution of amino acids in *Withania somnifera*

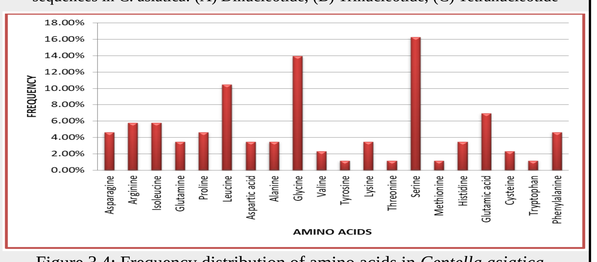

Figure 3.4: Frequency distribution of amino acids in *Centella asiatica*

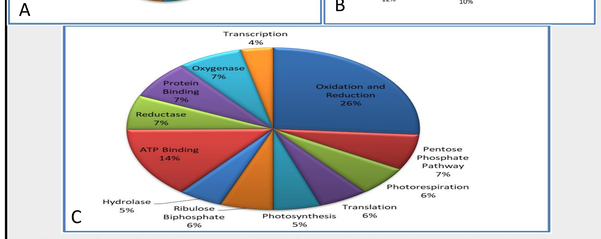

Figure 3.5: Classification of EST-SSRs of *Withania somnifera* based on significant match by BLAST analysis based on (A) Biological functions, (B) Protein functions and (C) Enzymatic functions.

S.NO.	PROTEIN FUNCTION	STRUCTURE OF PROTEIN
1.	PREDICTED: Solanum tuberosum auxin-induced protein PCNT115-like (LOC102581138), mRNA	
2.	PREDICTED: Arachis hypogaea auxin-responsive protein IAA14 (LOC112711184), mRNA	
3.	PREDICTED: Daucus carota subsp. sativus gibberellin receptor GID1C-like (LOC108223835), mRNA	
4.	PREDICTED: Nicotiana tabacum glycine-rich RNA-binding protein-like (LOC107809589), mRNA	
5.	PREDICTED: Coffea arabica vesicle-associated membrane protein 727 (LOC113730657), transcript variant X4, mRNA	
6.	PREDICTED: Daucus carota subsp. sativus pathogenesis-related protein 5 (LOC108217858), mRNA	
7.	PREDICTED: Prunus x yedoensis var. nudiflora major allergen Mal d 1-like (LOC103937956), mRNA	

Table 3.3: 3D structure of some biologically important proteins

S.NO.	ID	SSR Type	SSR Motif	Tm (°C)	GC% (±5)	Forward Primer	Reverse Primer	Expected Product Size (bp)
LK1	GR923293	p3	(GGT)5	60.03	50	CACCTCGTAGGTTGGTTCGT	GATCCGAGGAACGACGATGAT	222
LK2	GR923350	p3	(AAT)5	59.96	50	ATCCTGGATCCCAAGCTTCT	CAGGGGTTACTGGTCCTTCA	102
LK3	GR923386	p3	(CTT)5	59.85	50	TTAACTGCCATGCTGCACTC	AGTTTGCGTTTCCTTCCTGA	275
LK4	GR923395	c	(TA)6	59.89	55	TGCTATTGAAGCGATGAACG	ACCGTAACCCACCTTCACGAC	226
LK5	GR923405	p3	(GCA)5	59.93	50	TCCCTGCTGCATCCTATACC	TATTTCCCAACCGAGTCGTC	211

Table 3.4: Primers developed from SSR containing ESTs of *Withania somnifera*

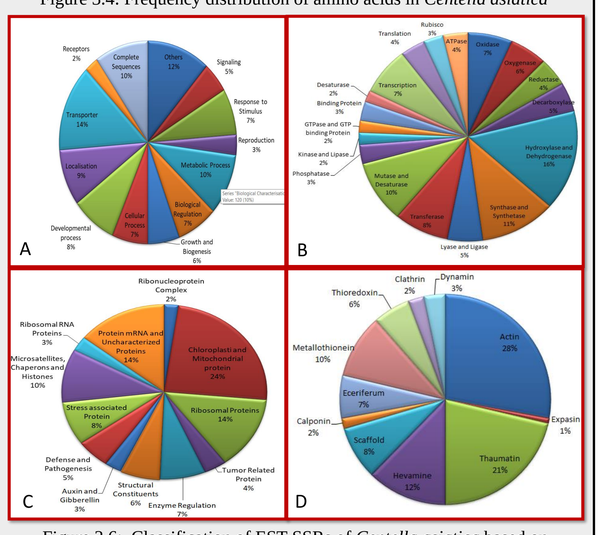

Figure 3.6: Classification of EST-SSRs of *Centella asiatica* based on significant match by BLAST analysis based on (A) Biological functions, (B) Enzymatic functions (C) Protein functions and (D) Specific proteins.

S. NO.	ID	SSR Type	SSR Motif	Tm (°C)	GC% (±5)	Forward Primer	Reverse Primer	Expected Product Size (bp)
LK1	GT714961	p3	(AGG)7	60.03	55	CAGGAATGGTTAAGGCTGGA	AGAGGACAGGGTGCTCTTCA	273
LK2	JK513968	p3	(TGA)5	59.90	50	GGGAGGAAAAGGGATTTCTG	ACGACAAGATTGTCCGTTCC	105
LK3	JK514168	p3	(TAC)6	60.00	50	GATACTGCCGGTCAAAGGAA	GCCTCCACATTCACCAATCT	116
LK4	JK514231	p3	(GAA)5	59.99	55	AGAACAACTCCGATGCTGCT	GGCGTTTGAGTGGAGAGAAG	162
LK5	JK514427	p3	(TCT)5	59.97	50	TATTGCAATTGCTGCCAGAG	CGGTGGAGGAATGCTGTTAT	124
LK6	JK514655	p3	(GAA)5	59.96	55	AAGTCGTTCGTAACCGGATG	GGAGCATCACCAAGAAGAGC	157
LK7	JK514663	p3	(CTC)7	60.09	55	GCCAAGGTCGCTAATGCTAC	TCTCCAGTTCCTCCAACGAC	275
LK8	JK514887	p3	(GAC)5	59.96	50	GCATTGGAGGAGCAGAACTC	GCGTGTCCACCTCGATATTT	171
LK9	JK515229	p3	(CCG)5	60.05	55	GGCTGGGTGTAGGCTTATGA	CGCAAGTAGAAGCCAAAAGG	107
LK10	JK515231	p3	(CAA)5	60.03	50	CTCACGGTCATCGGAAACTT	CGATCACCGTCCTTTTTGTT	149

Table 3.5: Primers developed from SSR containing ESTs of *Centella asiatica*

4. CONCLUSION & FUTURE PROSPECTS

- ❑ In Withania somnifera 40 contigs & 621 single tones were identified, and in Centella asiatica 255 contigs & 3751 single tones were identified
- ❑ The most abundant repeat motifs found in this study are AG/CT (58.5%), AAG/CTT (29.5%) & AAAG/CTTT (40%) for dinucleotide, trinucleotide & tertranucleotide repeats, respectively.
- ❑ In Withania somnifera 18 trinucleotide repeat motifs were identified, which results in approximately 10 amino acids. Whereas, in Centella asiatica 91 trinucleotide repeat motifs were identified, which represents around all 20 amino acids.
- ❑ Total of 260 primer pairs were developed in *Withania somnifera* and 780 primer pairs were developed in *Centella asiatica*. Primers also includes the Gene specific primers to facilitate PGPR Growth which further facilitates the growth of plants.
- ❑ BLASTN analysis suggested that the sequences belong to different categories of function such as-

Plant	Total No. of Sequences	Biological functions	Protein Functions	Enzymatic functions
Withania somnifera	661	250	177	234
Centella asiatica	4306	1220	1115	1350

Table 4.1: Functional Characterization of genes

- ❑ A total of 52 protein structures were predicted, which includes biologically important proteins.
- ❑ In conclusion the results of this study demonstrate that genotyping Withania somnifera & Centella asiatica accessions with microsatellite markers can quickly reveal the genetic diversity among accessions as the polymorphic EST-SSR markers constructed in this study will considerably enhance the number of informative microsatellite markers available for genetic analysis.

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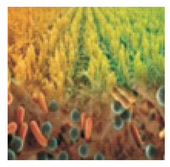


4.19 Deciphering the potential of PGPR and their consortium on wheat productivity improvement grown under different geographical locations

Amir Khan, Ajay Veer Singh and Viabhav Kumar Upadhayay
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Introduction

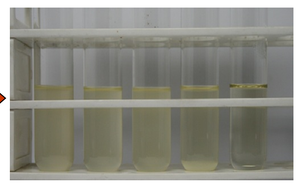
In present day world, agriculture is suffering from miscellaneous problems. At higher attitude temperature is low and some nutrients are less accessible for plants. Moreover, planes agriculture is also deteriorating through chemical fertilizers application. Hence there it drastically affects agriculture productivity and quality. Various techniques are practicing to combat this problem but they are associated with many limitations. Therefore, current research targets for the isolation, characterization and application of potential plant growth promoting rhizobacteria and their consortium as they are inexpensive and effective alternative approach of chemical fertilizers with ultimate solution for assisting plants through enhancing their growth and yield in a sustainable manner.

Objective

Isolation and characterization of potential plant growth promoting rhizobacteria and their consortium development to promote plant growth and yield in wheat in order to develop psychrotolerant biofertilizer

Methodology

Isolation of cold adaptive bacteria



Screening through Macro and Micro nutrient solubilization potential

Antagonistic property assessment

Determination of morphological and Biochemical behavior

Compatibility testing Consortium development

In Vitro assessment of bioinoculants potential for Plant growth promoting potential

Assessment of bioinoculants potential for Plant growth promotion at different altitude

Results and Discussion

1. Soil samples were collected from Uttarakhand and based on different morphology eighty four bacteria were isolated on different medium

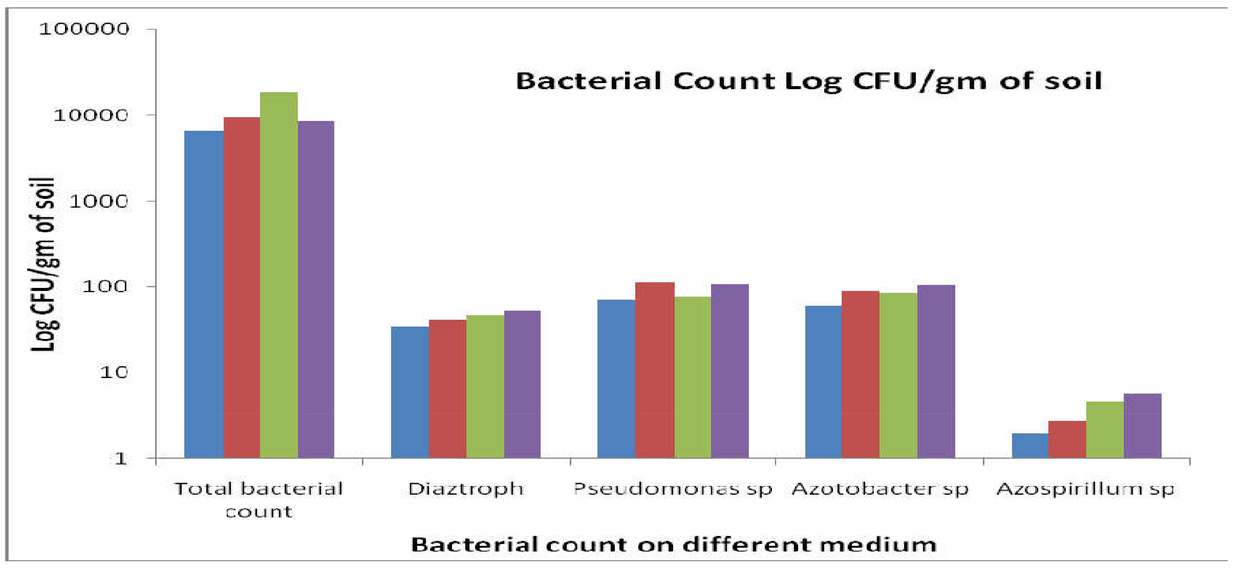
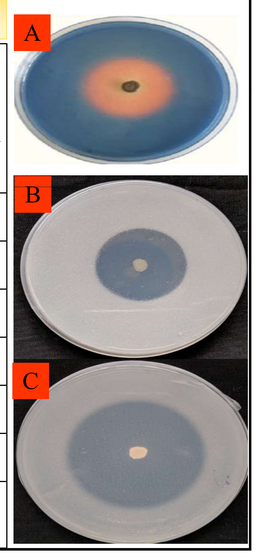


Fig 1: (A) Culturable bacterial counts (CFU/g dry weight soil) on different nutrient medium

2. Macro and Micro nutrient solubilization

Table 1: Nutrient solubilization characteristics of selected potential bacterial isolates

SN	Bacterial Isolates	Nitrogen Fixation	Phosphorous Solubilization	Potassium Solubilization	Zinc Solubilization	Iron Solubilization	IAA production	EPS production
1.	DHB 5	Positive	Positive	Positive	Positive	Positive	Negative	Positive
2.	NID 5	Positive	Positive	Positive	Positive	Positive	Positive	Positive
3.	ST 14 2	Positive	Positive	Positive	Positive	Positive	Positive	Positive
4.	PAU 12	Positive	Positive	Positive	Positive	Positive	Positive	Positive
5.	KU 13	Positive	Positive	Positive	Positive	Positive	Positive	Positive
6.	NID 2	Positive	Positive	Negative	Positive	Positive	Negative	Positive
7.	DHA PA 1	Positive	Positive	Negative	Positive	Positive	Negative	Positive



3. Antagonistic property assessment of bacterial isolates

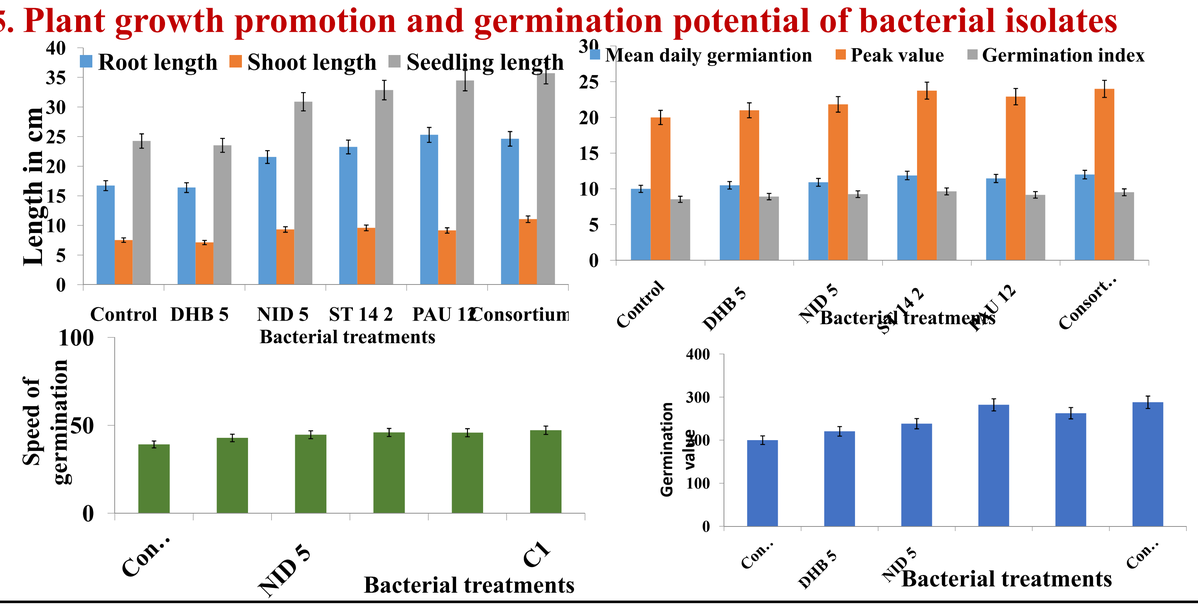
SN	Bacterial isolates	*Fusarium oxysporum*	*Colletotrichum lindemuthianum*	*Alternaria alternata*
1.	DHB 5	+++	+++	+++
2.	NID 5	+++	-	++
3.	ST 14 2	+++	++	+++
4.	PAU 12	-	-	-
5.	KU 13	+++	++	++
6.	NID 2	+++	+++	+++
7.	DHA PA 1	-	-	-

4. Biochemical behavior of bacterial isolates

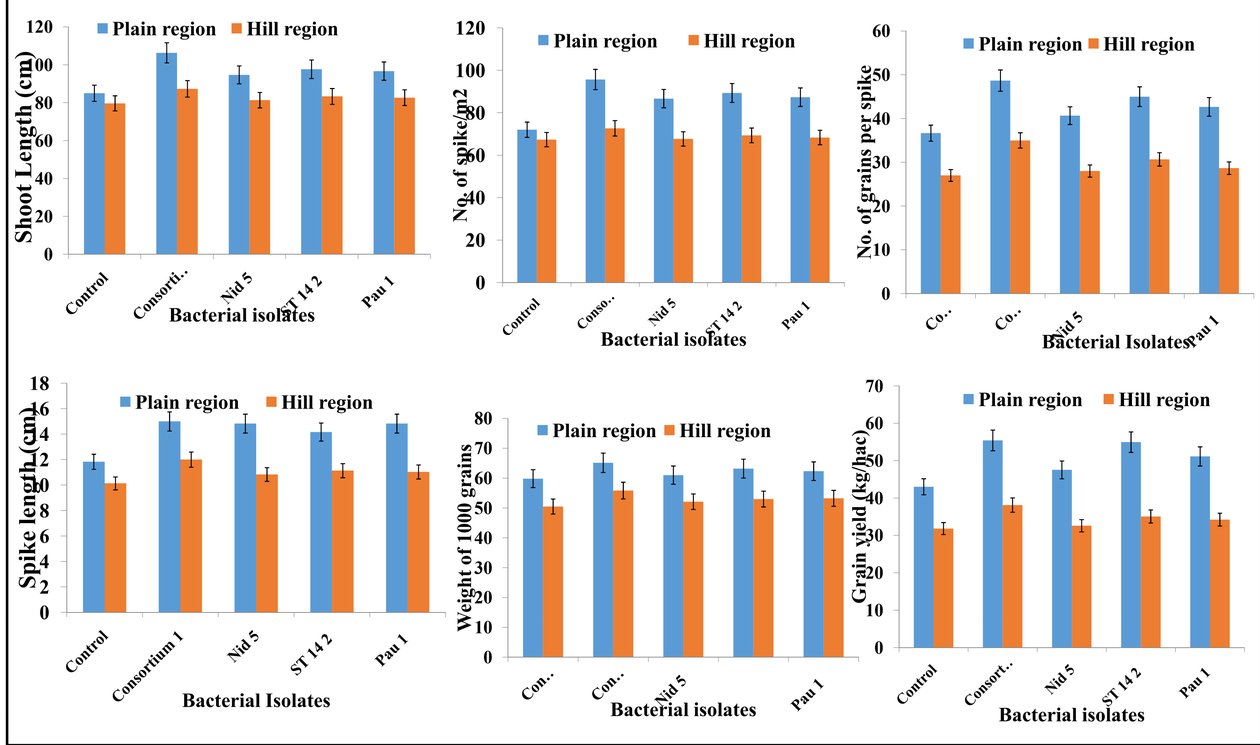
SN	Bacterial isolates	HCN production	Ammonia production	Xylanase production	Pectinase production
1.	DHB 5	-	+	-	-
2.	NID 5	-	+	-	-
3.	ST 14 2	-	+	-	-
4.	PAU 12	-	+	-	-
5.	KU 13	-	+	-	-
6.	NID 2	-	-	-	-
7.	DHA PA 1	-	-	-	-

On the basis of PGPR potential NID 5, Pit 4, ST 14 2 and PAU 1 2 were selected for consortium preparation through compatibility testing and these bacterial isolates along with consortium 1 were then tested for germination and plant growth promotion potential though towel paper assay

5. Plant growth promotion and germination potential of bacterial isolates



6. Assessment of bioinoculants potential for Plant growth promotion at different altitude



Conclusion

Bacterial isolates were shown EPS, Ammonia, IAA, Siderophore production and can solubilize zinc, potassium and phosphate under *In vitro* condition. The outcomes of seed germination assay confirmed the efficiency of bacterial bioinoculants and consortium 1 through enhanced seedling germination and agronomical parameters. Moreover, field demonstration showed that consortium 1 were the best bioinoculants at both the altitude and hence may be utilized as bio-protective agents to enhance crop productivity and quality in a more eco-friendly manner in plains as well as in hill regions.

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Bacterial Mediated Zinc Biofortification of Two Rice Varieties Grown in Terai Region of Uttarakhand

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Registration No. 4.20

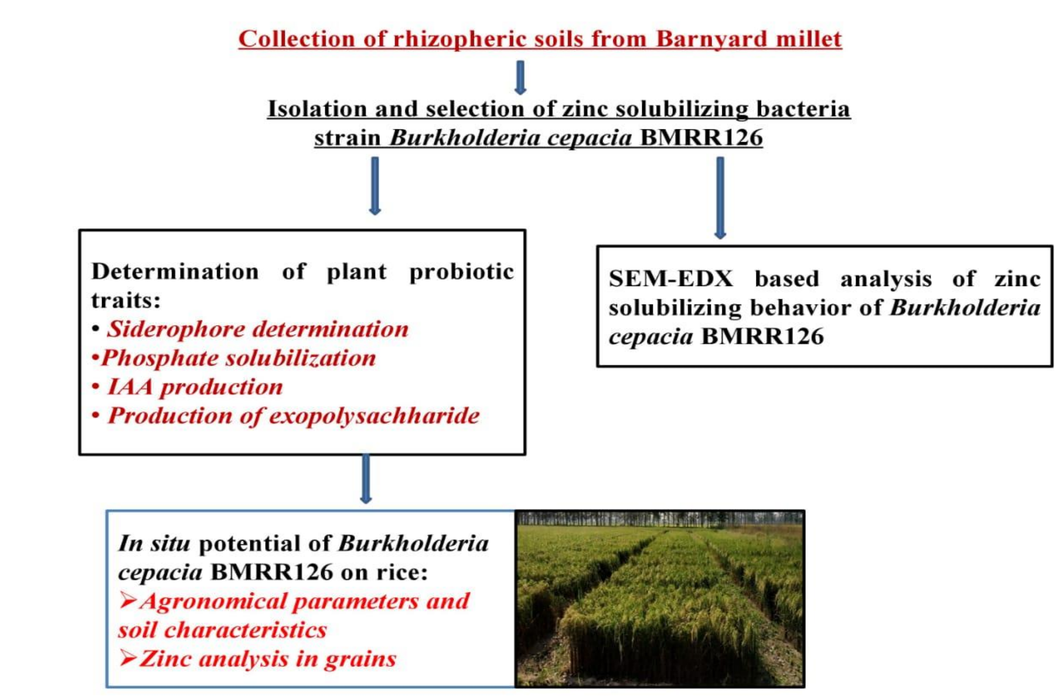
Introduction

Increasing risks of zinc (Zn) malnutrition have led to the studies on zinc solubilizing bacteria and their auspicious annotation for crop biofortification and overall crop growth (2). 'Bacterial assisted biofortification method' is more convenient and environmental friendly approach over other more expensive tactics such as agronomic, plant breeding, and biotechnological approaches (3). However, there are very limited studies are available on microbial mediated zinc biofortification of rice (4). Therefore, the present study was conducted to decode the prolific role of *Burkholderia cepacia* BMRR126 in Zn-biofortification of grains of two paddy varieties (*Pusa Basmati-1* and *Pant Dhan-18*) under field conditions.

Aims and Objectives

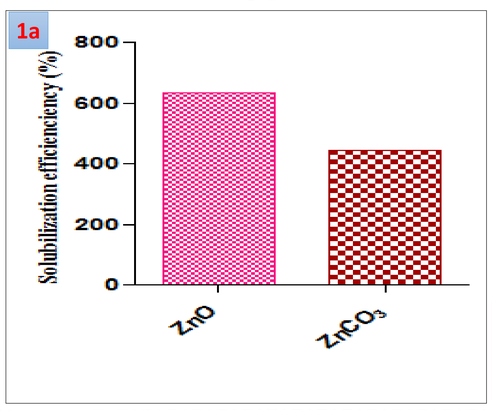
❖Assessment of zinc solubilizing potential and plant probiotic traits of *Burkholderia cepacia* BMRR126 under in vitro conditions.
❖In situ effect of *Burkholderia cepacia* BMRR126 with and without ZnO supplement on growth of plant and zinc content in grain of paddy under field trial.

Methodology



Results

1. Zinc solubilizing potential



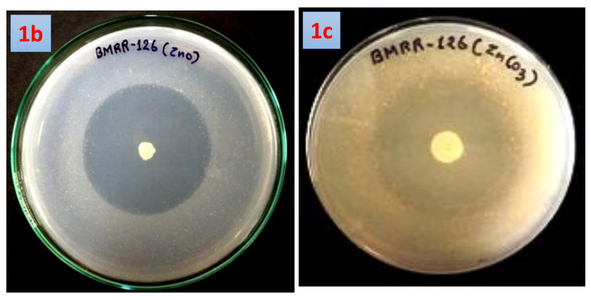
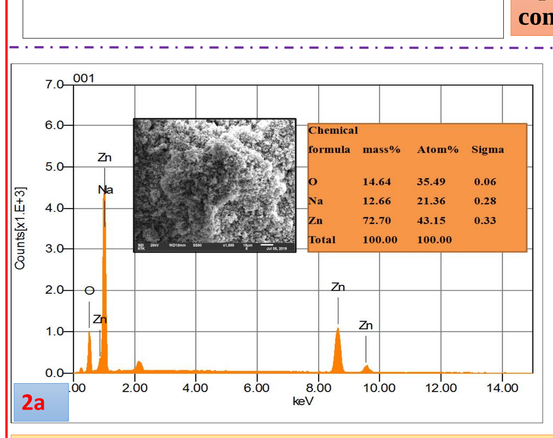


Fig 1: Zinc solubilization efficiency of *Burkholderia cepacia* BMRR126 using two insoluble zinc compounds (ZnO, and $ZnCO_3$) (1a,1b and 1c)



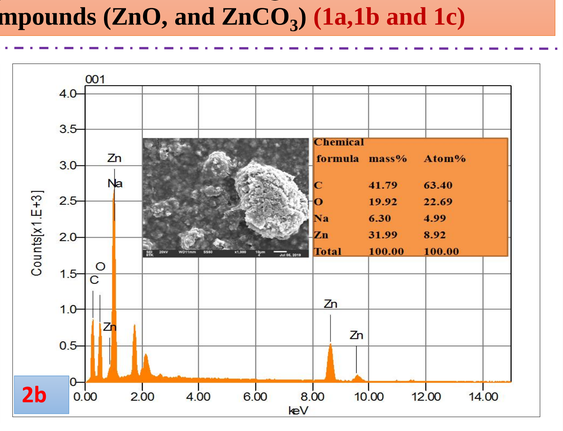


Fig. 2: w-SEM and EDX spectrum based analysis of ZnO mineral residue in uninoculated sample (control) (2a) and *Burkholderia cepacia* BMRR126 (2b). *Bacterial inoculated sample showed a lesser value of zinc (31.99%) (through EDX analysis) in comparison of control (72.70%) indicates that strain solubilized zinc oxide efficiently.*

2. Plant probiotic traits

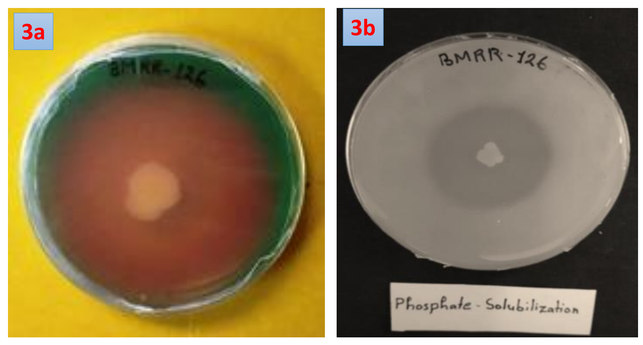


Fig 3: 3a. Qualitative estimation of siderophore production on CAS agar medium plates (zone diameter: 5.87±1.03 cm); 3b. Phosphate solubilization potential of *Burkholderia cepacia* BMRR126 (halo zone diameter : 3.20±0.20 cm)

Table. 1: Plant probiotic traits of *Burkholderia cepacia* BMRR126

Name of the test	Values
1. Siderophore production (% siderophore unit)	*66.20*
2 . Phospahte solubilization (µg/ml)	*302.67*
3. IAA production (µg/ml)	*23.45±1.18*
4. EPS Production (mg/ml)	*2.80±0.10*

3. Agronomic traits

Table. 2: Treatments used for field trial

T1	Control
T2	ZnO suplement@60 kg/hectare
T3	*Burkholderia cepacia* BMRR126
T4	*Burkholderia cepacia* BMRR126 + ZnO suplement@60 kg/hectare

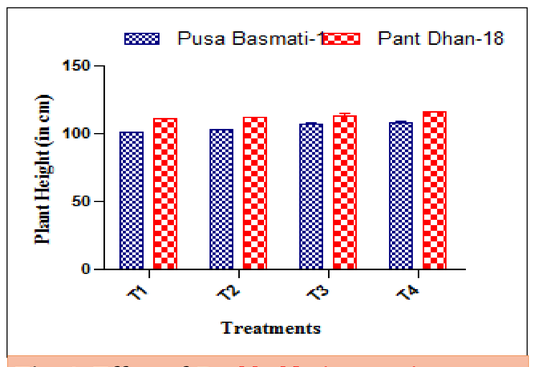


Fig. 4: Effect of *Burkholderia cepacia* BMRR126 on plant height of two rice varieties (Pusa Basmati-1 and Pant Dhan-18)

Table. 3: Effect of *Burkholderia cepacia* BMRR126 on different yield parameters (grain yield, straw yield, biological yield and harvest index) of two rice varieties (A) Pusa Basmati-1 and (B) Pant Dhan-18

Treatments	Grain yield (q/ha)		Straw yield (q/ha)		Biological yield (q/ha)		Harvest Index	
	A	B	A	B	A	B	A	B
T1	37.94	42.83	50.31	69.22	90.74	112.05	41.83	40.19
T3	38.53	42.98	52.29	68.46	90.82	111.44	42.44	39.76
T4	40.77	47.96	57.69	67.68	98.45	115.65	41.41	43.47
T10	42.52	49.15	63.03	72.87	98.46	122.02	43.48	43.22

Table. 4: Effect of *Burkholderia cepacia* BMRR126 on different yield parameters (effective tillers, spike length, number of grains per spike and 1000 grain weight) of two rice varieties (A) *Pusa Basmati-1* and (B) *Pant Dhan-18*

Treatments	Effective tillers		Spike length (in cm)		No. of grain/spike		1000 grain weight (g)	
	A	B	A	B	A	B	A	B
T1	9.20	10.57	28.92	26.59	130.87	131.03	16.60	25.16
T3	9.67	10.47	29.32	27.19	137.20	24.17	16.81	25.48
T4	11.20	11.50	31.14	29.09	159.93	25.61	18.56	26.43
T10	12.33	11.80	32.44	29.38	172.40	26.86	19.85	28.16

Table. 5: Effect of *Burkholderia cepacia* BMRR126 on Chemical properties of experimental soils of both locations of two rice varieties (A) *Pusa Basmati-1* and (B) *Pant Dhan-18*

Treatments	pH		EC		Organic carbon (%)		Nitrogen (kg/ha)		Phosphorus (kg/ha)		Potassium (kg/ha)	
	A	B	A	B	A	B	A	B	A	B	A	B
T1	7.76	7.72	0.4	0.40	0.72	0.87	220.56	242.52	16.45	22.91	114.5	189.32
T3	7.75	7.63	0.4	0.38	0.73	0.84	222.2	250.88	16.53	22.73	117.79	192.83
T4	7.62	7.52	0.3	0.33	0.79	0.98	245.21	280.15	17.28	24.41	139.81	201.04
T7	7.45	7.41	0.3	0.32	0.81	0.94	245.67	321.96	17.85	195.44	158.59	214.93

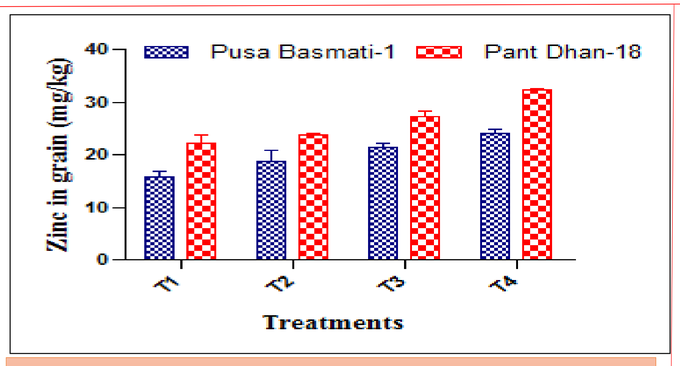


Fig. 5: Response of *Burkholderia cepacia* BMRR126 on Zn content of both rice varieties

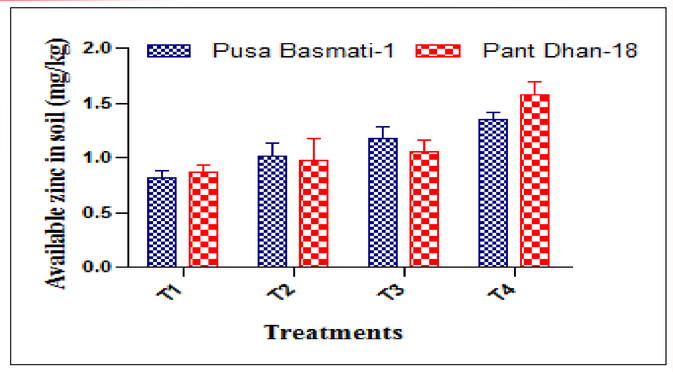


Fig. 6: Response of *Burkholderia cepacia* BMRR126 on available Zn content in soils of both rice varieties

Conclusion

➢*Burkholderia cepacia* BMRR126 was annotated on the basis of zinc solubilization potential and plant probiotic traits.
➢*Burkholderia cepacia* BMRR126 + ZnO supplement (@60kg/hectare) augmented overall plant growth and yield of both rice varieties *Pusa Basmati-1* and *Pant Dhan-18* and also improved soil quality.
➢The increased zinc content in grain part of rice provided the benefit of Zn-biofortification under the response of *Burkholderia cepacia* BMRR126.
➢Thus, this bacterial strain can be used as biostimulant for crop biofortification in future studies.

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Acknowledgement

1. Department of Microbiology, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar (India)
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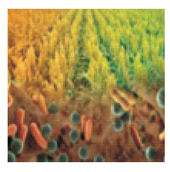


Enhanced production of native AMF in sorghum pot cultures amended with organic substrate and *Burkholderia arboris* as assessed through AM-signature lipids

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Introduction

•Due to obligate nature of AMF, its large scale multiplication is mainly being undertaken through root organ culture (Fortin et al., 2002) under invitro and substrate –based pot cultures involving trap plants (Agnihotri et al. 2021).
•The production of AMF in pots is directly influenced due to type of substrate used. For example the use of organic wastes and amendments in pot cultures has enhanced production of AMF (Chaiyasen et al. 2016).
•In our earlier study we reported that combination of soybean hulls and vermicompost with organic soil in sorghum pot cultures has enhanced AM production.
•Further the use of *Burkholderia arboris* as mycorrhiza helper bacteria (Garbaye 1994) in promoting AMF have been reported (Wang et al (2011). However, its role in AMF production in organic substrate pot cultures have not been investigated.
•The quality of AM inocula produced from different modes mainly being assessed through microscopic methods and that lacks reproducibility among the observers (Gange et al.1999). The use of biochemical methods (AM signature phospho and neutral lipids e.g., 161ω5cis) have been used to measure AM biomass in soil and roots (Olson 1999; Sharma and Buyer 2015).

Methods

Experimental Details:
•**Potting Substrate & Host:** Soil: Sand mix (3:1) with 3:1:1, soil-sand mix vermicompost-hulls; Sorghum black gusseted polyethylene bags (10 Kg capacity)
•**AMF and dose:** AMF (soil-based inoculum dominant in *Rhizophagus irregularis*) @ 2000 spore per pot
•**Bacteria-***Burkholderia arboris* (JF 792427; MTCC-10752) applied with 1 OD culture as seed treatment
•**Treatments and Design:** 8 (4×2 factorial)-04 Inoculations (AMF, *B. arboris*, AMF+*B. arboris*, control) under sterilized and unsterilized conditions in three replications in a completely randomized design
Parameters:
•AM colonization in roots (Philips and Hayman, 1970; Biermann and Lindeman, 1981); Spore density in soil (Gerdeman and Nicolson, 1963)
•16:1ω5cis EL-FAME in soil and roots (Sharma and Buyer, 2015)
• 16:1ω5cis NLFA and PLFA in soil at harvest (Sharma and Buyer, 2015)
•Glomalin (Easily extractable +Total glomalin) at harvest (Write and Upadhyay, 1996) .

Results

•Out of all combinations, incorporation of AMF+ *B. arboris* under unsterlized conditions has significantly enhanced higher number of spores (50.33(nos./g soil)), MCP (74.20%), glomalin content in soil when compared to AM alone pots.
•The amendment of *B. arboris* to AM pots has also tremendously increased the biomass of AM signature lipids i.e., 16:1ω5c NLFA and PLFA in soil and roots.
•The quantification of AM signature fatty acids (either 16:1ω5cis PLFA or NLFA) in AM inoculum can be used as potential biochemical tool to assess the quality of AM inocula and live-biomass of AMF

Objective

•To examine the role of *B. arboris* in AM production in soil: sand-organic amended sorghum pots under sterilized and unsterilized condition and assess the AM biomass through AM signature fatty acid biomarkers and microscopic methods

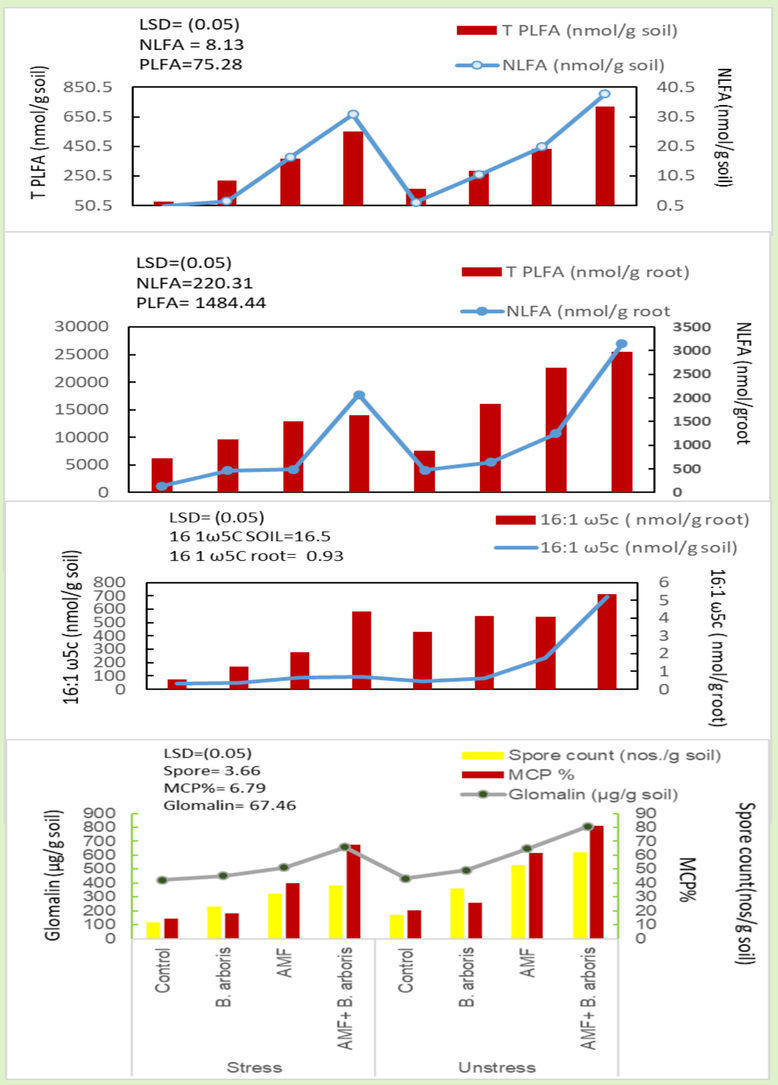

Table1: Inoculation responses of B *arboris* in AM pot cultures on spore count.MCP%, AM signature lipids

Take Home Message

The incorporation of *B. arboris* to AMF organic substrate-based pot cultures under unsterilized conditions has tremendously enhanced AM inoculum production

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Use of fermented substrate refuge as potential carrier in developing PGPM

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4.22

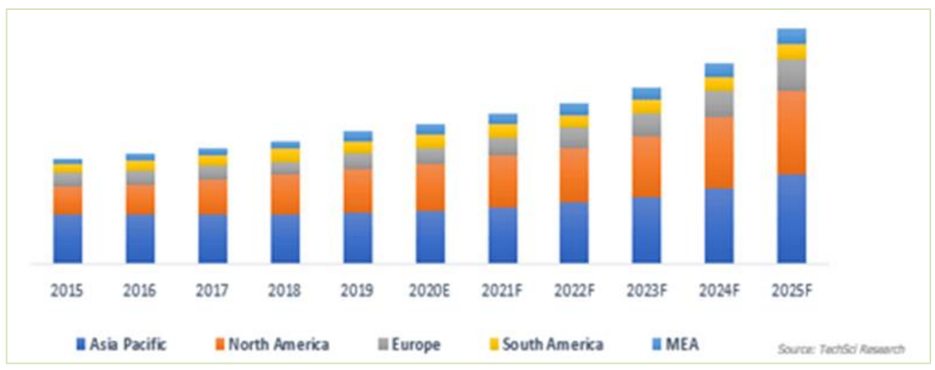
Introduction

- For a sustainable agriculture system, it is essential to use renewable inputs which benefit the plant and cause no or minimal damage to the environment. One possible way is to reduce the use of chemical fertilizers and pesticides.
- Excess use of chemical fertilizer or pesticides have increased the food production yields but complete loss of soil fertility and their health.
- Due to hazardous nature of chemical fertilizer causes various health problem in human. Ex- Cancer, liver or kidney damage.
- The Plant Growth Promoting Microbes (PGPM) is a viable solution both for promotion of plant growth and control of soil born pathogens. It helps the farmers to increase soil fertility and thereby increase the yield of the crops.
- Plant growth-promoting microorganisms are free-living soil, rhizosphere, rhizoplane, and phylosphere bacteria that are beneficial for plants.

Global demand of Biofertilizers

- The global biofertilizers market size was valued at USD 1.0 billion in 2019 and is anticipated to witness a compound annual growth rate (CAGR) of 12.8% from 2020 to 2027.
- The increasing usage of microbes in biofertilizers proves the potential for sustainable farming methods and food safety.

Global Biofertilizers Market size, 2015-2025F



Aim

A study on transformation of the fermented refuge into biofertilizers and understanding their impact on agricultural improvement.

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Acknowledgement

I would like to thank Department of Biotechnology, Jaypee Institute of Information Technology for providing me the opportunity to present our study in 6th National Asian PGPR conference.

Why Biofertilizers?

- Directly affect the metabolism of the plants by providing substances such as Nitrogen, Phosphorus, vitamins, hormones etc.
- Improve the plant tolerance to stresses, such as drought, high salinity, metal toxicity, and pesticide load.
- Act as biocontrol PGPM indirectly promote plant growth by preventing deleterious effects of phytopathogenic bacteria, fungi, nematodes and viruses.

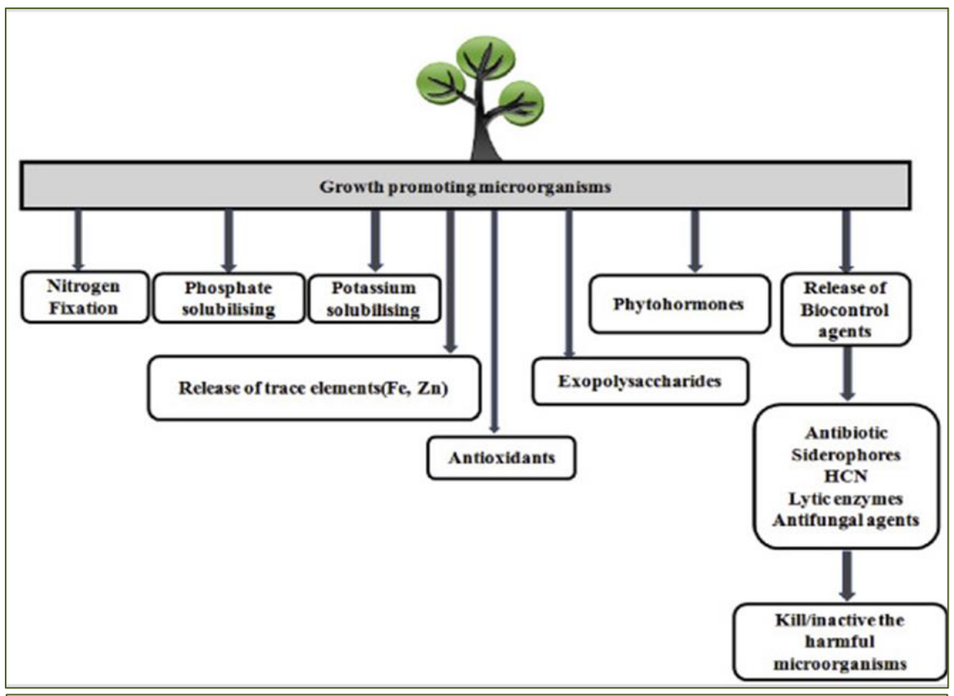

Fig1: The various means through which plant growth is promoted by effective microorganisms.(Naik et al, 2019)

Methodology

- Agro-wastes include plant, leaves, food and food residues which are used as a substrate for microbial fermentation, synthesizes various bioactive compounds including biofuels, biomass, enzymes, and bio-supplements.
- Post-fermentation, the fermented refuge were again used as a biofertilizers to promote plant growth promotion properties.

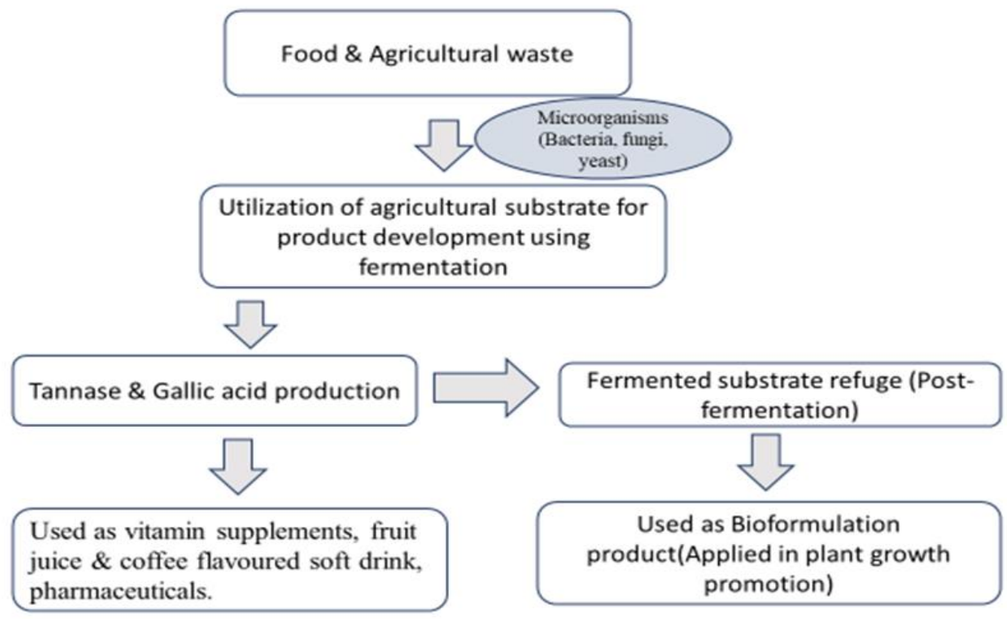

Fig2: Schematic representation of use of fermented refuge as a carrier in developing PGPM

Biofertilization in Agricultural Practices

- Biofertilizers are composed of agriculturally beneficial microorganisms that can improve the soil condition and plant growth through mobilizing the available nutrients with their biological activities.
- The microbes present secrete many health and nutrient enhancement compounds, which will promote plant growth. These microorganisms also contribute to the life cycle of plants through the decomposition of organic matter, nitrogen fixation, and supply to plants as well as the solubilization of insoluble phosphates.
- The biological fertilization provides benefits to soil and crops production, but this practice also has its limitations, and its feasibility needs to be studied to evaluate its potential use in the future.

Conclusions & Future Prospects

- Growing concerns on environmental and ecological impacts associated with agriculture activities have created the need for more sustainable agriculture practices.
- Biological fertilizers derived from fermented refuge are studied for its potential as an alternative source of fertilizer. The biofertilizers has shown great advantages to soil and plant growth.
- With a higher demand of biofertilizers, the cost for biofertilizer will eventually reduce as the higher production rate will ease the production cost.
- It is vital to develop more efficient management processes to fully utilize the valuable compounds that can be extracted from these biomass waste and realize the commercialization of bio-products from biowaste.





A study on the ground status of Bioformulations use and applications by farmers in two states from the Northern part of India

(4.23)

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Introduction

Agriculture is an important sector in developing countries because most of the part of economy relies totally on agriculture. Agriculture is under pressure to complete food demand, and several types of synthetic chemicals are used in agriculture to increase agricultural productivity. Several types of pests such as insects, mites, fungi, nematodes, etc. affect 30-40% of crops and pesticides are used to manage these pests. However, due to the widespread usage of pesticides in agriculture, the environment is adversely affected. Therefore, safer alternatives to chemical pesticides are much needed and which are also safe for human beings, easy to handle, non-toxic, and target-specific.

Bioformulations include biofertilizers, biopesticides, nutrients (major and minor), hormones, and plant activators which are environmental friendly and play an important role in sustainable agriculture production. PGPR are beneficial microorganisms and PGPR based bioformulations (biopesticides and biofertilizers) used as an alternative for synthetic chemicals such as pesticides and fertilizers, which are capable to enhance plant growth and control several agricultural pests. The aim of the study was to collect information from Uttar Pradesh and Haryana farmers about how they control pests in agriculture and which pesticides are more commonly used, chemical pesticides or biopesticides (PGPR based).

Methods

Figure.1. Field survey in different areas

Results

Table.1. Frequently used pesticides in the study area

S. No.	Pesticide/ Active Ingredient	WHO Toxicity Class *	Pesticides class	Pesticides types
1	Phenthoate	II	Organothiophosphate	Insecticide
2	Propargite	III	-	Insecticide/ Acaricide
3	Profenofos	II	Organophosphate	Insecticide
4	2,4-D (Ethyl Ester)	II	Dichlorophenoxy acetic acid	Herbicide
5	Phorate	Ia	Organophosphate	Insecticide
6	Dimethioate	II	Organophosphate	Insecticide
7	Acetamiprid	U	Neonicotinoid,	Insecticide
8	Chlorpyriphos	II	Organophosphate	Insecticide
9	Monocrotophos	Ib	Organophosphate	Insecticide
10	Cypermethrin	II	Synthetic pyrethroid	Insecticide
11	Quinolphos	II	Organophosphate	Insecticide
12	Carbendazim	Ib	Carbamate	Fungicide
13	Mancozeb	U	Carbamate	Fungicide
14	Glyphosate	III	Nphosphonomethyl Glycine	Herbicide
15	Paraquate	II		Herbicide
16	Azoxystrobin	U	B-methoxyacrylate	Fungicide

Table.2. Commercial PGPR based Biopesticides

PGPRs	Target pest/Disease	Action	Brand name	Producer
A. radiobacter	Crown galls	Antagonist	Galltrol-A Dygall	AgBioChem Agbioresearch
B. pumilus	Rust, downy and powdery mildews	Fungicide	Ballad Sonata AS Astona	Agraquest Inc. Gustafson LLC
B. subtilis	*Rhizoctonia, Fusarium,* and *Alternaria*	Fungicide and antagonist	Serenade Rhapsody Cease	AgraQuest, Inc. Gustafson, Inc.
B. subtilis FZB24	*Rhizoctonia, Fusarium,* and *Alternaria*	Fungicide	Rhizo-Plus Rhizo-Plus Konz	FZB Biotechnik, GmbH
B. thuringiensis	Caterpillars	Insecticide	Baritone Larvect 50	-
P. cepacia	Soil pathogenic fungi	Toxic	Intercept	Soil Tech
P. chlororaphis *P. flourescens*	Pathogenic fungi *P. tolasi* and *Erwinia*	- Antibacterial	Cedomon Conquer Blight Ban A506	BioAgri AB Mauri Foods NuFarm Inc
Pseudomonas + Azospirillum	Brown patch	Antagonist	BioJet	Eco-Soil

PGPR based bioformulations may either directly enhance plant growth by facilitating the use of resources or by modulating the levels of plant hormones, or indirectly by reducing the inhibitory effects of various pathogenic agents.

Application of biopesticides is still limited to only a few percent of all pesticides used for crop protection.

During the survey, some farmers admitted that they use *Bacillus* and *Pseudomonas* based pesticides to control pests when we talked about biopesticides but the majority of farmers rely on chemical based pesticides.

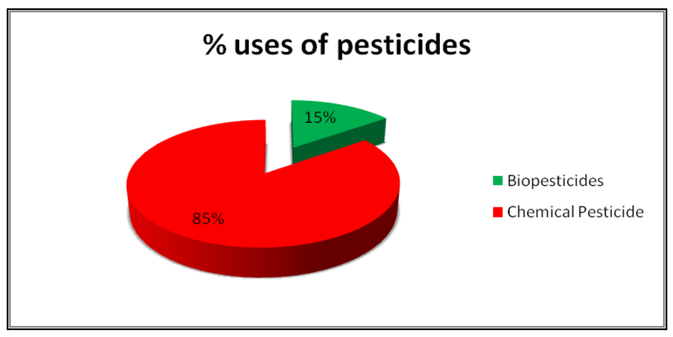

Figure.2. Type of pesticides use in agriculture

Conclusion

The use of chemical pesticides in agriculture is effective but there are several negative effects with the use of chemical pesticides. Therefore, safer alternatives to pesticides are much needed. Biopesticides used in agriculture are one of the safe methods, which are safe to human, environment friendly, and target-specific, they can be a novel alternative for crop protection use. Such studies serve as an eye-opener for researchers working in the area of PGPR research. It is required to develop strategies to take the bioformulation based products to the door-step of farmers.

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