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ISOLATION OF SOME SOIL BACTERIA SHOWING POTENTIALITY FOR DISEASE CONTROL, GROWTH ENHANCEMENT AND PESTICIDE DEGRADATION IN *VIGNA UNGUICULATA* L.

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Abstract

Vigna unguiculata L. or cowpea is an important annual legume used as pulse, green vegetables, fodder and green manure. It has growing potential for sustainable agriculture in marginal lands and increasingly being cultivated in West Bengal. The crop suffers from charcoal rot, root rot and seedling blight disease caused by *Macrophomina phaseolina* (Tassi.) Goid. The present study involves isolation and evaluation of some soil bacteria for potentiality to control disease, enhance growth in plants as well as tolerate and degrade pesticide for bioremediation. Four rhizospheric soil bacteria namely Disha-A (BC1), Disha-B (BS-1), Tn-4 and Tn-6 were found tolerant to 0.1% methomyl, imidacloprid, carbendazim and Tn-1 was tolerant to 0.1% carbendazim and methomyl only. Some of the strains also degrade pesticides. Pathogenicity test of twenty isolates of *Macrophomina* was conducted and isolate R9 was found to be most virulent pathotype. Assessment of antagonistic capability of PGPRs against R9 *in vitro* exhibited maximum inhibition by Tn-6. Production of volatile and non-volatile organic compounds, cell wall degrading enzymes (CWDE), siderophore as well as IAA and phosphate solubilization. ascribed to the mechanism of biocontrol and plant growth promotion to all the rhizobacteria. Pot evaluation of the bioagents for disease control and growth enhancement resulted highest (86.4%) disease control by Tn-6. All the bacterial spp. also increased shoot and root length, nodule count, dry weight as well as yield except BS1. Therefore the selected PGPR strains Tn-1, Tn-4, Tn-6 and BCI have disease control, plant growth promotion together with pesticide tolerance and degrading ability which may be exploited for sustainable agriculture and bioremediation.

Key words : *Macrophomina phaseolina*, *Vigna unguiculata*, rhizobacteria, biocontrol, growth promotion, pesticide tolerance.

Introduction

Cowpea, lobia or black eyed pea [*Vigna unguiculata* (L.) Walp.] is an important pulse crop widely grown in tropics and subtropics of Asia, Africa, central and southern America and parts of southern Europe and USA (Tiwari and Shivare, 2016). In India, this legume is cultivated mainly in arid and semi arid tracts of Rajasthan, Karnataka, Kerala, Tamil nadu, Maharashtra, Gujarat and in some parts of north India as in Punjab, Haryana, Delhi, and West UP. Cowpea is commonly used as pulse, green vegetables, fodder and green manure having great potential for sustainable agriculture in marginal lands and increasingly being cultivated in West Bengal recently. The plant is highly nutritional for human consumption as well as forage crop containing Protein-22-24%, Calcium-0.08-0.11%, Iron-0.005%, essential amino acids (Lysine, leucine and phenylalanine).

The crop suffers from seed and soil borne disease complex including seed rot, seedling damping off, charcoal rot, dry root rot, leaf and stem blight caused by the fungal pathogen *Macrophomina phaseolina* (Tassi.) Goid. through all the growing stages from seedling till harvest. In mature plant, charcoal rot and root infection cause severe damage causing yield loss from 10-80% (Tiwari and Shivare, 2016). But control of the seed and soil borne pathogen is persistent problem. Indiscrminate use of chemical fertilizer and pesticide pose problem of resurgence giving rise to pesticide resistant strains of pathogen, lyses of beneficial organisms, environmental pollution and deterioration of soil health. Natural control with rhizosphere microorganisms and integrated management tactics play cardinal role in crop protection as well as increasing plant vigor.

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Plant growth promoting rhizobacteria (PGPR) are root colonizing bacteria able to enhance plant health and promote growth of a wide variety of crops (Kloepper, 1993; Liu *et al*., 2017). PGPRs improve plant growth by suppression of deleterious root colonizing microorganisms including plant pathogens through antibiosis, *i.e.* by production of fungi toxic compounds including volatile and non-volatile antibiotics and cell wall degrading enzymes (CWDE), competition with pathogenic microorganisms for nutrients by producing siderophores, production of plant growth regulators (PGRs) like auxin, gibberelin, cytokinin, atmospheric nitrogen fixation and solubilization of insoluble phosphates. PGPR can suppress diseases by inducing a systemic resistance (ISR) in the plants against both root and foliar pathogens. PGPRs also improve a plant's tolerance to stresses like drought (Ngumbi, and Kloepper, 2016), high salinity, metal toxicity, pesticide load as well as play role in bioremediation of contaminated soils.

These beneficial organisms are inadequate or non-persistent in the rhizosphere at effective level unless supplied exogenously. Moreover, their population and activities are hampered due to toxic pesticidal residues present in heavily infested soil. Some microorganisms develop resistance after a long term exposure to agrochemicals by using them as the source of nutrient and energy and can successfully be used for bioremediation of pesticide contaminated soils (Khan *et al*, 2009). Alternatively, these microbes may have plant growth promoting traits in addition to pesticide degradation and can be used in soil remediation process (Shahgoli, 2014) along with plant growth promotion. The pesticide tolerant plant growth promoting microbioagents may be established well in the soil when applied exogenously together with degrading pesticides, which are being used injudiciously by the vegetable growers. A comprehensive natural control of *Macrophomina* disease complex and improve plant health and yield with these pesticide tolerant PGPRs has not been attempted so far in *Vigna unguiculata*.

Therefore, in the present study, attempts have been made to endeavour biological control of charcoal rot and root rot disease of *Vigna unguiculata* caused by *Macrophomina phaseolina* (Tassi.) Goid. together with enhanced plant growth and pesticide degradation by application of chemo toxic pesticide tolerant strains of PGPR.

Materials and Methods

Host

Cowpea or *Vigna unguiculata* seeds of variety Kashi Kanchan, which is widely used cultivar in the local areas of North 24 parganas used in the experiment.

Isolation and identification of pesticide tolerant plant growth promoting rhizobacteria

All the rhizobacteria were isolated from pesticide infested rhizospheric soil of different districts of West Bengal, India. One gram of the soil was mixed with 9 ml of sterile distilled water, vortexed and centrifuged at 5,000 rpm for 5 min. The supernatant was collected and stored as a stock solution. One ml of stock solution was inoculated into the MS medium containing 0.01% pesticide and incubated at 37°C for 24 h. Pesticide-tolerant colonies were selected and used for further enrichment experiments (Tang and You, 2012). Optimum pesticide tolerance of these strains were tested in the presence of different concentration of methomyl, carbendazim and imidacloprid in nutrient broth with or without 2% agar.

The selected isolates were primarily screened for their antagonistic and PGP properties, such as production of volatile and non volatile compounds, HCN, CWDE like endoglucanase, pectinase and chitinase, indole acetic acid, siderophore and solubilisation of inorganic phosphate.

Isolation of pathogen

Total twenty isolates of *Macrophomina phaseolina* was collected from infected plant parts, rhizosphere and rhizoplane of *Vigna* plants and infected field soil from diverse locations of West Bengal. For isolation of pathogen from infected plant parts, small portion of diseased samples were surface sterilized with 0.1% mercuric chloride solution and placed in sterilized Petri dishes containing sterile moist blotting paper creating moist chamber and incubated in BOD incubator at $25^{o} \pm 1^{o}C$. After three days a small bit of whitish growing mycelium was placed on 20 ml Potato Dextrose Agar medium in Petri plate supplemented with Rose Bengal dye and incubated at $25\pm1^{o}C$ inside BOD incubator. After 5-7 days, the emerging fungal mycelium was transferred on fresh PDA slants. After repeated sub-culturing the cultures were purified, maintained in PDA slants and stored in 4^{o} C for further studies. For isolation of fungal pathogen from *Macrophomina* infested jute soil, dilution plate technique (Warcup, 1950) was employed.

Pathogenicity of *Macrophomina* isolates was evaluated by cross inoculation on *Vigna* seedlings of the variety Kashi Kanchan in moist chambers *in vitro*. Pathogenic isolates were grown in Czapeck Dox Broth for 15 days and the metabolites collected aseptically through Buchner funnel and sterilized again in bacteriological G-5 sintered glass filter. Ten *Vigna* seeds

were soaked in culture metabolite of each isolates for 24 hrs in sterilized Petri dishes and allowed to germinate on moist blotting paper circles contained in Petri dishes with three replicates. Seeds soaked in sterile distilled water served as control. Per cent seed germination, growth rate of seedling length and seedling dry weight were recorded.

Evaluation of pesticide tolerance by PGPRs

All the bacterial isolates were cultured separately in presence of different concentrations of methomyl (Roy and Das, 2017), imidacloprid and carbendazim in the MS medium, with or without glucose (carbon source) and growth curves were obtained. MS medium without pesticide served as control.

Evaluation of *in vitro* antagonistic activity of pesticide tolerant PGPRs against pathogen

In vitro evaluation of the selected pesticide tolerant PGPRs against *M. phaseolina* was conducted following dual culture plate technique (Bells *et al*., 1983). PDA and NA media mixed in 1:1 proportion was used for the experiment. Percentage and zone of inhibition of the pathogen by the antagonist colony was calculated after 3 to 7 days growth of both BCA and pathogen on the plate. Results were expressed in percent increase/decrease over control with formula: [(A-B)/A] X100, where A is the initial distance between the pathogen and antagonist and B is the distance covered by the pathogen towards the antagonist (Bandopadhyay, 2002; Nandy *et al*., 2013).

Biochemical characterization of the bacterial isolates

The bacterial isolates were biochemically characterized according to the methods described by Dubey and Maheswari (2012).

Evaluation of bacterial isolates for PGPR traits

The selected isolates were primarily screened for their antagonistic and PGP properties, such as

production of volatile and non volatile compounds, HCN, CWDE like endoglucanase, pectinase and chitinase, indole acetic acid, siderophore, and solubilisation of inorganic phosphate in presence or absence of pesticide.

Seed Germination Assay

All the selected PGPRs were grown in NB medium for 48 hours in incubator shaker at 32°C. The bacterial slurry were then centrifuged at 8000 rpm for 10 minutes and the supernatants were collected. The supernatants were then sterilized in bacteriological Millipore membrane filters (0.2µm). Cell free sterilized bacterial metabolites were used in further study. Healthy disease free, clean seeds of cowpea were taken and surface sterilized with 0.1% mercuric chloride solution. 10 seeds were taken in each sterile petridish containing blotting paper and soaked in bacterial metabolites for 6 hours. The petridishes containing seeds were then kept in seed germinator and observed for 7days. The plates were finally examined for germination rate and seedling growth in comparison to control with sterile distilled water and media only. The experimental set up was maintained in triplicates. Seedling vigour was analysed at the end of 7 days of incubation using the method of Abdul Baki and Anderson (1973). To assess vigour, the length of the root and shoot of an individual seedling was measured. The vigour index (VI) was calculated using the formula: VI = (mean root length + mean shoot length) (% germination).

Evaluation of PGPRs for disease control and plant growth promotion by pot culture in greenhouse

For pot culture, all the selected PGPRs were inoculated in nutrient broth medium. Fifty ml broth media contained in 250 ml flasks were inoculated with 1 ml of bacterial inoculums from mother culture initially prepared by inoculating a loopfull of bacterial cultures in 50 ml media and incubated for 3 days. The flasks were incubated for 3-5 days in incubator shaker at 30±1°C, at 200 rpm. Bacterial population was kept 20×10^6 cfu per ml of media. Field soil was steam sterilized in autoclave for 3 alternative days. In 30 cm diameter pots the soil was made sick with 50 g of *Macrophomina phaseolina* inoculum per pot prepared in sand maize medium. Fifty ml metabolites of bacterial slurry were mixed with the *Macrophomina* infested soil in each pot. *Vigna* seeds were sown in separate sets. Untreated control pots were kept under each set of experiments. Seven days old seedlings were thinned out to keep 10 seedlings per pot maintained in green house condition. Disease counts were taken at 15 days interval till harvest. Plant height, fresh weight, biomass dry weight, nodule count and pod weight per single plant was recorded from 10 plants in each treatment including control.

Assessment of disease

Assessment of charcoal rot and root rot disease incidence on *Vigna* plants was calculated per cent disease incidence using the following formula: Per cent disease incidence = (Number of infected plants Incidence × 100)/ Total number of plants observed.

Statistical analysis

Each data represented were means of three replicates. The data were analysed statistically by ANOVA. Mean values were compared at significance levels of 1% and 5%. Other data were analysed using

Microsoft office excel software 2007.

Results and Discussion

Identification of plant growth promoting rhizobacteria

The bacterial isolates Tn-1 and Tn-6 were preliminarily identified through biochemical characterization as *Pseudomonas* sp. Whereas, Tn-4 was identified as *Bacillus* sp (table 1). Disha-A (BC1) and Disha-B (BS1) were already identified as *Bacillus cereus* and *Bacillus safensis* and assigned accession no. as NCIM 5557 and NCIM 5558, respectively (Roy and Das, 2017).

Isolation and selection of virulent strain of pathogen *Macrophomina phaseolina*

Effect of secondary metabolites produced in the culture filtrates of twenty *Macrophomina phaseolina* isolates was studied for seed germination, seedling growth and disease development in *Vigna* plants *in vitro*. Culture metabolite of R9 allowed only 8% germination out of total 10 seeds taken in the experiment. Maximum inhibition of seed germination 91.67% and seedling growth 65.52% was exhibited by R9 followed by 89.58% and 45.98% in R11, respectively. Inhibition of seedling dry weight was maximum 93.75% in R9 followed by 84.71% in RM2. R11 inhibited seedling dry weight by 81.96%. RK1 inhibited seed germination by 62.5%, seedling growth by 39.31% and seedling dry weight by 52.5% over control. Isolates R12, R13, R19 and RJ3 inhibited seed germination by 50%, seedling growth by 16.09%, 54.02%, 56.32%, 57.47% and seedling dry weight inhibition 61.67%, 77.79%, 65.29% and 79.17%, respectively over control. Isolates RK3, RM1 and RS2 inhibited seed germination by 47.91%, seedling growth by 40.23%, 27.36%, 56.32% and seedling dry weight by 64.17%, 75%, and 68.75% respectively. Isolates R18 and RK4 inhibited seed germination by 45.83%, seedling growth by 33.33 and 53.33% and seedling dry weight by 61.12 and 58.33%, respectively. Isolates RS1, R15 and RS3 inhibited seed germination by 43.75%, 39.58% and 33.33%, seedling growth by 33.33%, 51.72% and 35.63%, and seedling dry weight 67.92%, 75% and 55.83%, respectively over control. Isolates RM2, R21, and R17 also inhibited seed germination to a little extent by 16.67%, 14% and 10.41% and seedling growth by 1.15%, 52.64% and 33.33%, respectively. Isolates R21 and R17 inhibited seedling dry weight by 60% and 61.12%, respectively. Metabolites of isolate RK2 was toxic to *Vigna* seed to very small extent showing seed germination inhibition of only 8.33%, seedling growth inhibition 49.42% and seedling dry weight inhibition 47.91% over control (table 2).

Pesticide tolerance of PGPR isolates

All the bacterial isolates were tolerant to pesticides Methomyl, imidacloprid and carbendazim at 0.1% concentration except Tn-1, which was intolerant to imidacloprid (table 3).

In-vitro evaluation of antagonistic activity

Bacterial isolates could inhibit the growth of pathogen R-9 to a great extent. Bacteria *Bacillus cereus*, *B. safensis*, Tn-4 and Tn-6 inhibited the mycelial and sclerotial growth of the pathogen as evident from figs. 1A, B, C and D.

Detection of fungitoxic and plant growth promoting principle compounds from metabolite of PGPRs *in vitro*

All the bacterial bioagents produced non volatile antifungal compound but only *B. cereus* produced volatile antifungal compound. HCN production was highest in Tn-6 followed by Tn-1. None of the bacterial isolates produced endoglucanase but *B. cereus*, Tn-1 and Tn-4 produced pectinases. All the isolates except Tn-6 produced chitinases. All the bacterial isolates solubilised insoluble phosphates, produced siderophores and IAA in varied amounts (table 4).

Seed Germination Assay

Evaluation of the PGPR metabolites for their effect on seed germination of *Vigna* showed maximum 86.6% seed germination by sterile distilled water. Seed germination rate was also high in metabolites of Tn-1, Tn-4, and Tn-6 *i.e.* 73.33%, 70% and 70%, respectively. Among the bacterial bioagents, Tn-6 showed highest seedling vigour index (VI) 2300.2 followed by Tn-1 2229.2. *Bacillus safensis* showed lowest seedling vigour index 596 (fig. 2).

Pot evaluation for disease suppression in *Vigna* by pesticide tolerant bacteria

Pot evaluation of the bacterial bioagents for control of *Macrophomina* charcoal rot and root rot incidence in *Vigna* showed significant percent of disease control ranging from 86.4% to 37.6%. Highest control of charcoal and root rot incidence in *Vigna* was exhibited by Tn-6 i.e. 86.4% followed by Tn-1 71.3%, Tn-4 68.7% and *B. cereus* 69.6% in comparison to control 50.9%. The disease incidence was maximum in *Macrophomina* R9 treated pots i.e. 84.3% (fig. 3).

Pot evaluation for growth promotion in *Vigna* by pesticide tolerant bacteria

Pot evaluation on impact of PGPRs on growth

Table 1 : Biochemical Characterization of the bacterial isolates.

Biochemical Tests	Microbioagents		
	Tn-1	Tn-4	Tn-6
MR Test	(+)ve	(-)ve	(+)ve
VP Test (for colour)	(-)ve	(+)ve	(-)ve
Catalase Test(H_2O_2)	(-)ve	(-)ve	(+)ve
Sugar Mannitol Fermentation	(-)ve	(-)ve	(+)ve
Fermentation of Glucose(Dextrose)	(-)ve	(-)ve	(-)ve
Fermentation of Sucrose	(-)ve	(-)ve	(-)ve
Fermentation of Lactose	(-)ve	(-)ve	(-)ve
Citric acid (Citrate) utrilization	(+)ve	(+)ve	(+)ve
Demonstration of Starch Hydrolysis (Amylase production)	(-)ve	(+)ve	(-)ve
Determination of Nitrate Reduction	(-)ve	(+)ve	(-)ve
Gram staining Morphology	(-)ve Cells: Small rod	(+)ve Cells: rod	(-)veCells: Small rod
Aerobically acid production	(+)ve	(+)ve	(+)ve
Anaerobically acid production	(+)ve	(-)ve	(+)ve
Phenyl alanine Deaminase Production	(-)ve	(-)ve	(-)ve
H_2S Production	(-)ve	(-)ve	(+)ve

Table 2 : Effect of culture metabolites of *M. phaseolina* isolates on seed germination and seedling growth of *Vigna*.

Treatment	No. of seeds germinated (50 seeds)	Seed germination (%)	Seed germination inhibition(%)	Seedling length (mm)	Seedling growth inhibition (%)	Seedling dry weight (mg)	Seedling dry weight Inhibition (%)
R9	4.00	8.00	91.67	15.00	65.52	1.50	93.75
R11	5.00	10.00	89.58	23.50	45.98	4.33	81.96
R12	24.00	48.00	50.00	36.50	16.09	9.20	61.67
R13	24.00	48.00	50.00	20.00	54.02	5.33	77.79
R15	29.00	58.00	39.58	21.00	51.72	6.00	75.00
R17	43.00	86.00	10.41	29.00	33.33	9.33	61.125
R18	26.00	52.00	45.83	22.00	49.42	9.33	61.125
R19	24.00	48.00	50.00	19.00	56.32	8.33	65.29
R20	42.00	84.00	12.50	27.00	37.93	9.40	60.83
R21	43.00	86.00	14.00	20.60	52.64	9.60	60.00
RJ3	24.00	48.00	50.00	18.50	57.47	5.00	79.17
RK1	18.00	36.00	62.50	26.40	39.31	11.40	52.50
RK2	44.00	88.00	8.33	22.00	49.42	12.50	47.91
RK3	25.00	50.00	47.91	26.00	40.23	8.60	64.17
RK4	26.00	52.00	45.83	20.30	53.33	10.00	58.33
RM1	25.00	50.00	47.91	31.60	27.36	6.00	75.00
RM2	40.00	80.00	16.67	43.00	1.15	3.67	84.71
RS1	27.00	54.00	43.75	29.00	33.33	7.70	67.92
RS2	25.00	50.00	47.91	19.00	56.32	7.50	68.75
RS3	32.00	64.00	33.33	28.00	35.63	10.6	55.83
Control	48.00	96.00	0.00	43.50	0.00	24.00	0.00
C.D. at 5%	12.48	30.05		10.90		4.78	
1%	16.08	38.71		14.05		6.16	

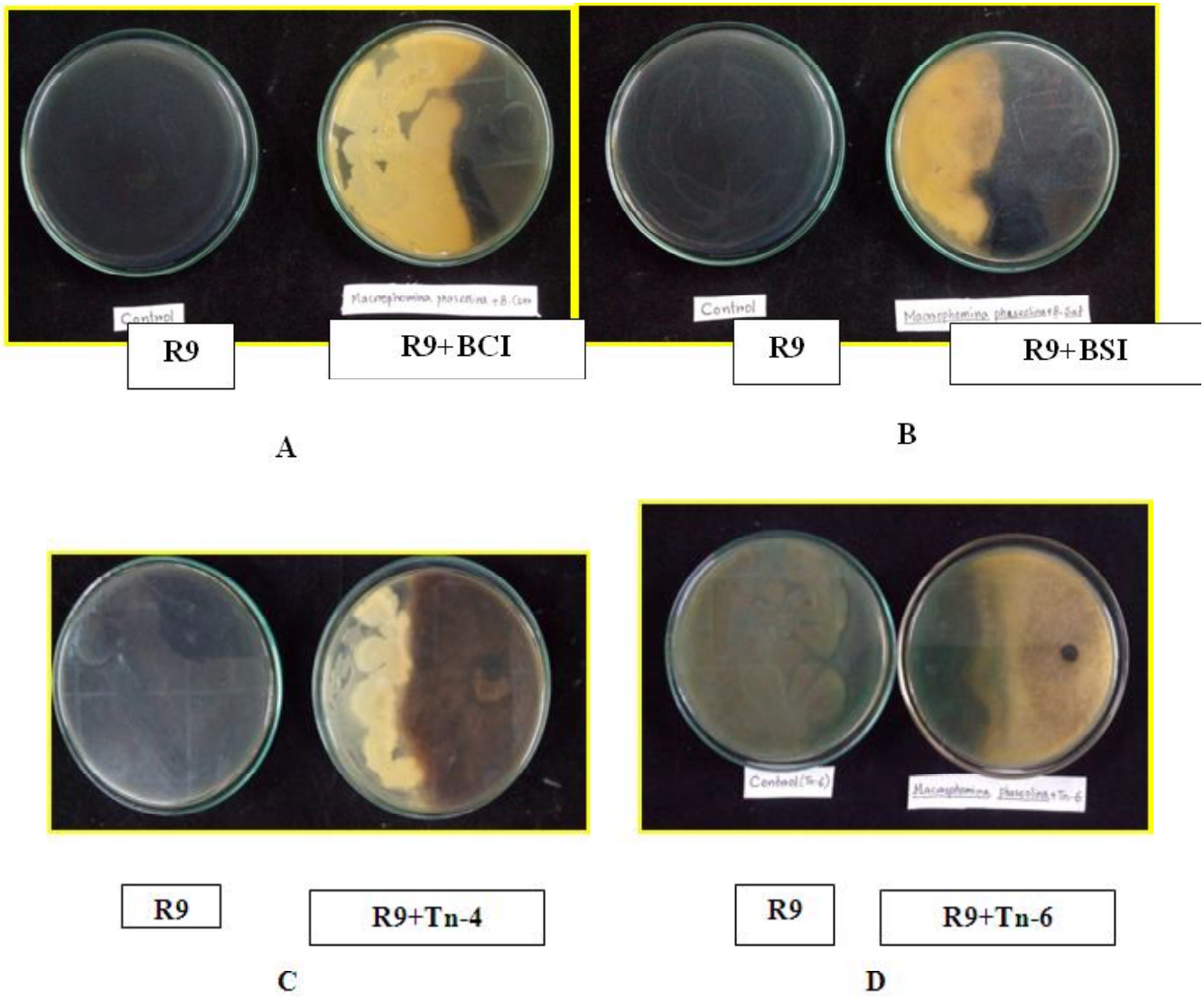


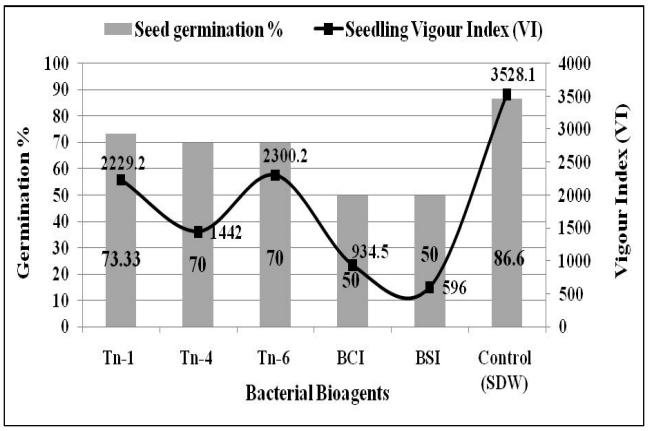
Fig. 1 : Interaction of pesticide tolerant PGPRs with *Macrophomina* pathotype R9 in dual culture *in-vitro*.

enhancement of *Vigna* plant showed effective results. Tn-6 increased maximum shoot length (21.7 cm) of *Vigna* , followed by Tn-1 (21.56 cm) (fig. 4). Among PGPRs Tn-1 exhibited maximum increase of root length up to 5.75 cm followed by Tn-6 (4.5 cm) and Tn-4 (4.38 cm) compared to control (3.67 cm) (fig. 5). Tn-6 showed maximum number of nodules (19.17) per plant followed by Tn-1 (17.84) and *B. cereus* (17.0) compared to control (14.17) (fig. 6) after 60 days of field sowing. Assessment of fresh weight and dry weight per plant showed that Bacteria Tn-6 improved both fresh and dry weight of plant *i.e.* 4.08 gm and 0.69 gm per plant compared to control 3.67 gm and 0.53 gm, respectively. Tn-1 showed 3.78 gm of fresh weight and 0.65 gm of dry weight per plant (fig. 7). Tn-6 yielded maximum amount of pod *i.e.* 17.03 gm of pods per plant followed by Tn-1 (16.71 gm) and Tn-4 (15.81 gm) (fig. 8). *Bacillus safensis* reduced the root length 3.13 cm per plant, shoot length 9.01 cm per plant, nodule count 4 per plant, fresh weight and dry weight 0.9 gm and 0.19 gm per plant, respectively compared to control. The yield of *B. safensis* treated plants were only 10 gm of pods per plant. *Bacillus safensis* deteriorated plant health and showed some

Table 3 : Pesticide tolerance of PGPR isolates.

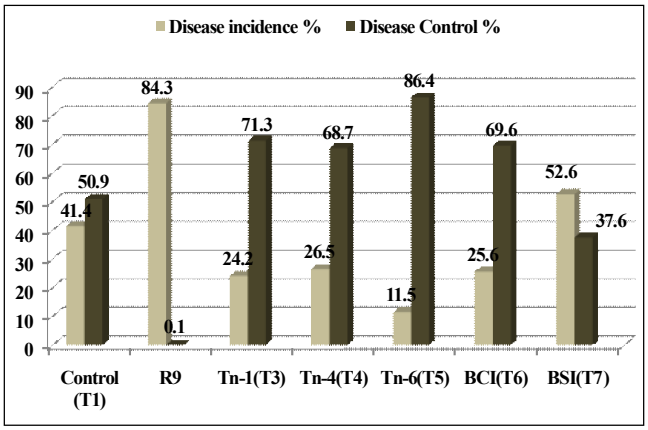
Bacterial isolates	Methomyl (0.1%)	Imidacloprid (0.1%)	Carbendazim (0.1%)
BCI	++	++	++
BSI	++	++	++
Tn-1	++	—	++
Tn-4	++	++	++
Tn-6	++	++	++

BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*).



BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*)

Fig. 2 : *Vigna* seed germination assay by bacterial bioagents.



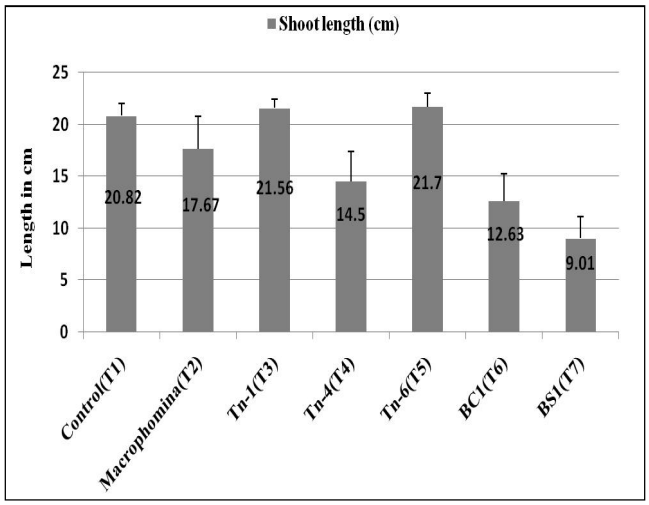
BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*)

Fig. 3 : Effect of Bioagents on disease incidence and control in *Vigna* in pot trial.

disease symptoms.

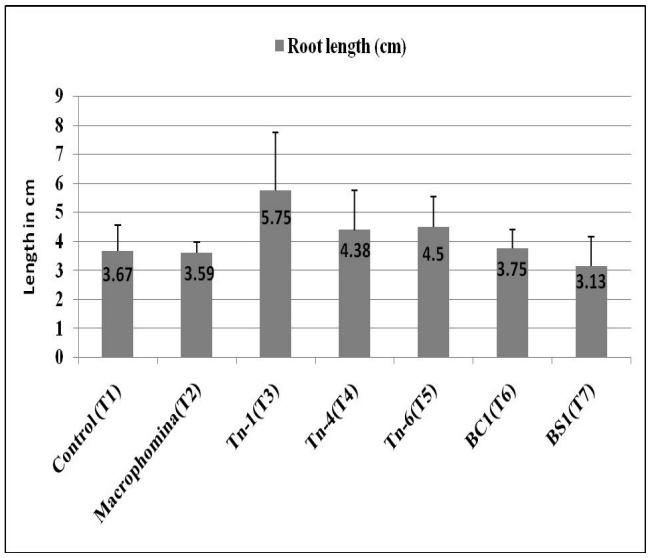
In this study, it is observed that pathogenicity and virulence of the fungal pathogen *Macrophomina phaseolina* are affected by application of certain bacteria that are effective as biofertilizer or biocontrol agents or both. Some bacteria have also been found to increase susceptibility of the host plant by synergistic effect along with the pathogen instead of reducing it by its antagonistic activity. Whereas, others have been found to induce an opposite reaction enhancing the growth of the host plant and imparting resistance in host against invasion of the pathogen.

This study also showed that the bacteria were tolerant to pesticides like methomyl, carbendazim and imidacloprid at varying concentrations. In case of pesticide tolerant rhizobacterial isolates, an increase in microbial growth was obtained by adding the pesticide up to 0.1%. This type of findings are supported by the finding of Ahmed and Ahmad (2006). The ability of bacterial strains to use pesticide for a carbon source has also been reported by



*Value represents average data of 15 plants
BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*)

Fig. 4 : Effect of PGPRs on shoot length of *Vigna* in pot trial.

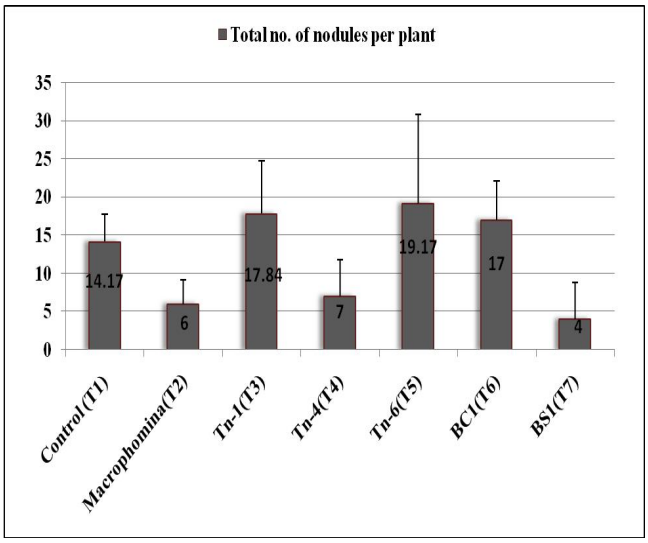


BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*)

Fig. 5 : Effect of PGPRs on root length of *Vigna* in pot trial.

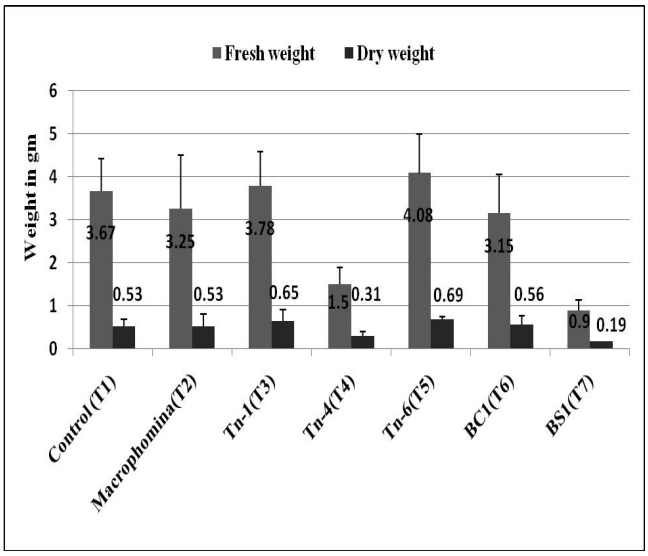
Chen *et al*. (2012) and Sarat and Barathi (2013).

The findings of the present investigation reveal that the pesticide tolerant bacterial bioagents *Bacillus cereus, Bacillus* sp. Tn-4, *Pseudomonas* sp. (Tn-1) and *Pseudomonas* sp. (Tn-6) are efficient plant growth promoting rhizobacteria (PGPRs) possessing all the required traits for plant growth promotion. The bioagents were found to produce non volatile antibiotics, volatile HCN, siderophores, cell wall degrading enzymes (CWDE) like cellulase, pectinase, chitinase, siderophore, plant growth promoting IAA and solubilised insoluble phosphates. The bioagents efficiently inhibited the selected virulent pathotype R9 *in vitro*. Non-volatile compounds from secondary metabolite of the selected



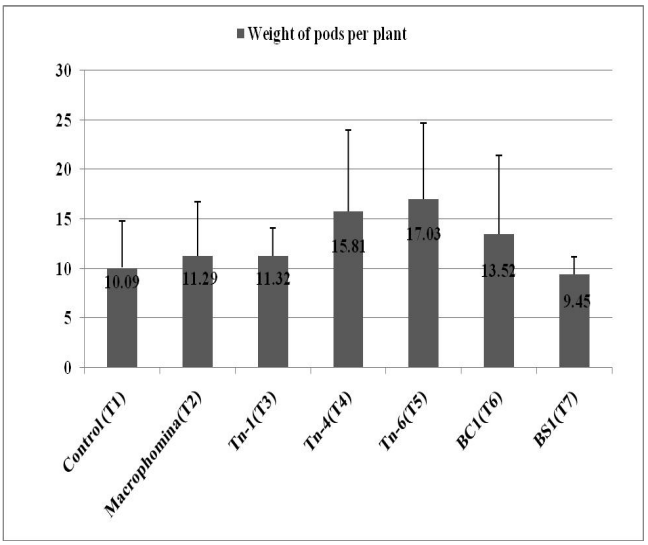
BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*)

Fig. 6 : Effect of PGPRs on total no. of root nodules per *Vigna* plant in pot trial.



BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*)

Fig. 7 : Effect of PGPRs on fresh weight and dry weight per plant of *Vigna* in pot trial.



BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*)

Fig. 8 : Effect of PGPRs on weight of pods per plant of *Vigna* in pot trial after 60 days.

bacteria inhibited mycelial and sclerotial growth of *Macrophomina*. Volatile toxic substances produced by antagonists can diffuse easily through the air filled pores of the soil and inhibit the soil borne pathogen specially suppressing the sclerotia formation. Thus actual physical contact between antagonist and pathogen was not necessary.

Evaluation of the PGPR metabolites for their effect on seed germination of *Vigna in vitro* showed minimum seedling vigour index (VI) by *Bacillus safensis* followed by *Macrophomina phaseolina*. Thus the metabolite of *Bacillus safensis* proved to be toxic to the *Vigna* seeds and inhibited the seedling growth. The seed germination rate in sterile distilled water revealed the viability and health condition of the seeds. Seed germination rate was also high in metabolites of Tn-1, Tn-4 and Tn-6 respectively which revealed their non toxic affect on

Table 4 : Detection of fungitoxic and plant growth promoting principle compounds from metabolite of PGPRs i*n vitro.*

Tests	BCI	BSI	Tn-1	Tn-4	Tn-6
Production of non-volatile antifungal compound	+	++	+	++	+++
Production of other volatile antifungal compound	+	—	—	—	—
HCN production	—	—	+	—	+++
Endoglucanase	—	—	—	—	—
Pectinase	+	—	+	+	—
Chitinase	+	+	+	++	—
Phosphatase	+	+	+	+	++
Siderophore	+	+	+	+	++
Indole Acetic Acid	++	+	+++	+	++

BCI = Disha-A (*Bacillus cereus*), BSI= Disha-B (*Bacillus safensis*). +, positive; -, negative; ++, moderate. +++High.

Vigna seeds. Maximum VI was obtained by Tn-6.

Pot application of the bioagents exposed affective control of *Macrophomina* charcoal rot and root rot disease incidence by the bioagents. Highest disease control exhibited by Tn-6, followed by Tn-1, and *B. Cereus*. This result is attributed to production of cell wall degrading enzymes, volatile and non volatile antibiotics, and siderophores by the BCAs. The investigation also revealed that Tn-1, Tn-4, Tn-6 and *B. Cereus* increased root length, shoot length, nodule count, fresh and dry weight (plant biomass) as well as total weight of pods per *Vigna* plant compared to control. Thus production of plant growth regulators like IAA, supply of siderophore sequestered iron and other micro nutrients to the plant, and solubilisation of P by BCAs increase plant growth and pod yield. Moreover, nodule counts were also enhanced by the PGPRs which in turn probably increased the activity of nitrogen fixation in the soil by the root nodule inhabiting *Rhizobium*. This could also have thrusted plant growth. However, *Bacillus safensis* was found to have an negative effect on the plant growth and vigour, thus increasing the susceptibility probably due to synergistic pathogenic effect along with *Macrophomina phaseolina*.

Thus, reduction of disease incidence in *Vigna* with fluorescent *Pseudomonas* and other PGPRs is evident from this study. The effective role of *Pseudomonas* has been observed in this study. The biocontrol and plant growth promoting potential of the organisms tested might have been possible due to simultaneous action of all the components like competition, parasitism, lysis and antibiosis. Siderophore mediated antibiosis and production Plant growth promoting substances (PGPs) like IAA, phosphate solubilisation and nitrogen fixation.

From the above study, it is concluded that the selected pesticide tolerant PGPR Disha-A (*Bacillus cereus*), Tn-4 *(Bacillus* sp) and Tn-1 (*Pseudomonas* sp.) and Tn-6 (*Pseudomonas* sp.) have disease control and plant growth promoting properties on *Vigna unguiculata*. Thus, with multifunctional PGPR activities and for multiple benefits of pathogen suppression sustaining in varied pesticides, plant nutrient supply and growth promotion, these PGPR strains have an enormous prospects in future broadening the spectrum of PGPR available for field application in agriculture. Therefore, in future, these may be exploited for development of suitable microbial bio-pesticide and biofertilizer formulations for disease management as well as plant growth promotion applicable to other crops also. It will be more economically sustainable, low cost eco-friendly technology.

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