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Lab and Field Level Potential of Endophytic Bacteria in Managing the Dry Root Rot Disease in Black Gram Incited by *Rhizoctonia bataticola* (Taubenh) Butler

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ABSTRACT

Background: The plant growth promotion and efficacy against phytopathogens by the endophytic bacteria are being focused now due to their ecofriendly nature.

Methods: Endophytic bacteria (24 nos) isolated from the roots, stems and leaves of black gram plants collected from different locations were tested for their potential to inhibit the growth of *R. bataticola* under *in vitro* and *in vivo conditions.*

Result: The leaf endophyte BLE 4 exhibited maximum inhibition (79.6%) of *R. bataticola* followed by BSE 4 (77.4%), BSE 7 (77.0%) and BLE 1 (74.0%). Among these 4 isolates tested as seed treatment and soil application, there was significant increase in dry weight (7.1 g), plant height (37.7 cm), number of branches (13.2) and number of pods (26.2) in BLE 4 treated plots. Whereas, the incidence of dry root rot and yield were insignificant. In the screening study of isolates for their phosphate solubilization potential, protease activity, siderophore and HCN production, no single isolate possessed all the properties, but siderophore production, protease activity and phosphate solubilization were found in BSE 4, BRE 3, BRE 5 and BRE 10 isolates.

Key words: Bacteria, Biochemical, Dry root rot, Endophyte, Field, Plant growth, Siderophore.

INTRODUCTION

Black gram [*Vigna mungo* (L.) Hepper] is the most important pulse crop of arid and semi-arid regions of India. The area under cultivation of black gram in India is around 5.2 m. ha. with the average production of 3.06 m.t. (Agristat, 2019). The production of the black gram was affected by various diseases, among which dry root rot (DRR) contributes to the yield loss of 25 to 28 per cent. Dry root rot of black gram is caused by the sclerotia forming necrotrophic fungi *Rhizoctonia bataticola* (Taubenh) Butler (pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid). *R. bataticola* is reported to incite DRR, charcoal rot, stalk rot disease in almost 500 species of plants (Sinclair, 1982). The complexity of the disease is being increased due to altered climate scenario where the plants are predisposed to moisture stress and high temperatures (Savary *et al.,* 2011; Sharma *et al.,* 2016).

The soil-inhabiting *R. bataticola*, infects the host plants at any stage of the growth, but most commonly at the post-reproductive stage under dry conditions (Sharma and Pande, 2013). Efforts to control *R. bataticola* through soil funmigation (Pearson *et al.,* 1984) and applying irrigation water to reduce the disease-promoting effects of drought (Kendig *et al.,* 2000) and biocontrol agents (Pal and Mc Spadden Gardener, 2006) are cited. The biocontrol agents with tolerance to variable conditions was suggested in the integrated disease management (IDM) for effective management of DRR considering economic feasibility to the farmers (Pankhurst and Lynch, 2005; Ghini *et al.,* 2008).

The endophytic microbiome influence the survival and health of plant under adverse conditions. Similar to plant growth-promoting rhizobacteria (PGPR), endophytes facilitate plant growth and protect the plants against several biotic and abiotic stresses and help in enhancing growth and yields (Lugtenberg *et al.,* 2016; Lata *et al.,* 2018) by direct and indirect mechanisms. Where the former includes the improvement of nutrient uptake by the plants and the later includes the inhibition of phytopathogens by producing antibiotics, hydrolytic enzymes, limiting the nutrient availability and priming the plant defense (Khare *et al.,* 2018).

Endophytic bacteria of *Pseudomonas, Bacillus, Stenotrophomonas, Serratia, Achromobacter, Pantoea* genus facilitate the plant growth promotion by *de novo* synthesis of hydrogen cyanide (HCN), protease, ammonia, Indole-3-acetic acid (IAA), gibberellins, 1-aminocyclo propane-1-

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carboxylate (ACC) deaminase, phytoalexins and also enhance biological nitrogen fixation by plants (Gao *et al.*, 2010; Waqas *et al.,* 2015; Ma *et al.,* 2016); Etminani and Harighi, 2018). The endophytic bacteria also produce low molecular weight siderophores that chelate iron and limit its availability to phytopathogens (Sabate *et al.,* 2018). Besides, they also possess the potential for solubilization of the unavailable form of inorganic phosphorous in the soil, making it available to the host plants in which they reside (Singh *et al.,* 2017).

They elicit induced systemic resistance (ISR) in host plants they reside on the onset of infection by phytopathogens (Kloepper and Ryu, 2006). Black gram plants colonized by endophytic *Pseudomonas fluorescens* stimulated the activities of phenylalanine ammonia-lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) in addition to the accumulation of lignin and phenolics upon inoculation with *M. phaseolina* (Karthikeyan *et al.,* 2005).

MATERIALS AND METHODS

Isolation of pathogen

The dry root rot sample was collected from the infected black gram plants in the fields of Regional Agricultural Research Station, Tirupati and was brought to the laboratory and washed with tap water. The diseased root bits without any lateral hairs were excised along with a healthy portion and were surface sterilized in sodium hypochlorite solution (1%) for 1 min. Later the excised root bits were consecutively washed thrice with sterile distilled water (SDW) and were aseptically placed in the Petri plates containing pre-sterilized potato dextrose agar (PDA) medium (Himedia, Mumbai); incubated at 26±1°C for 5 d. The emanating black coloured fungal colonies was confirmed as *R. bataticola* based on sclerotia formation. Subculturing was done to maintain pure culture and stored for further use.

Isolation of the endophytes

The endophytic bacteria were isolated from leaf, stem and root of black gram plants at different stages of growth as suggested by Bhowmik *et al.* (2002). Samples were surface washed well and the inner tissues of roots were carefully excised by removing the outer epidermis and washed under slow running tap water for 15 min followed by washing with Tween 20 (1 drop in 200 ml SDW) for 1 min. Later the tissues were surface sterilized with sodium hypochlorite (1%) for 1 min and were rinsed thrice with SDW. Such surface-sterilized tissue of 1.0 g was collected into pestle and mortar and macerated with PBS buffer (140 mM NaCl, 2.5 mM KCl, 10 mM Na_2HPO_4, 1.5 mM KH_2PO_4) of pH 7.4. The extract was serially diluted and 100 µl of 10^{-6} dilution was plated on nutrient agar (NA) medium and incubated at 25±2°C for 48 hr. Stem and leaf endophytes were isolated from stem sections collected at 20 cm from the stem base and small pieces of leaves, respectively and were washed and surface sterilized as described for the roots. To validate the effectiveness of surface sterilization, 1 ml of the rinsed extract was collected from the last Petri plate used in the surface sterilization (Schulz *et al.,* 1993) and serially diluted followed by plating 100 µl of 10^{-6} dilution on the NA medium and as no bacterial growth was observed it is confirmed that the bacterial colonies observed in the extract of macerated tissue are endophytes. These endophytes were collected and streaked separately on NA medium and preserved for further use by giving codes (BRE from the root, BSE from stem and BLE from leaf).

In vitro evaluation of endophytes against *R. bataticola*

The antifungal activity of each of the endophytic bacteria was screened for its antagonism against *R. bataticola* by dual culture technique (Dennis and Webster, 1971). A mycelial disc of *R. bataticola* (5 mm) was cut from actively growing culture and placed in the center of the Petri plate containing PDA. Two parallel streaks of bacteria (3.5 cm long) were made 2 cm apart on opposite sides of the mycelial disc. The plates without bacterial streaking were kept as control and the plates were incubated at 28°C for 5 d. The mycelial growth inhibition of the pathogen (mm) was calculated by the formula:

$$\frac{C-T}{C} \times 100$$

Where

C = Radial growth of the pathogen in control.

T = Radial growth of the pathogen in treatment given by Vincent (1927).

Biochemical characterization

All the endophytic bacteria were inoculated separately in the freshly prepared nutrient broth and incubated in the rotary shaker at 150 rpm for 48 hr at 25±2°C and these cultures were used for biochemical characterization.

Gram staining

Gram's property of the bacteria was determined by using Gram's staining procedure. The endophytic bacterial isolates were smeared on the glass slide, air-dried and heat-fixed. Crystal violet was flooded over the smear and allowed for 30 sec; washed with SDW. The smear was then flooded with Gram's iodine and allowed for 60 sec, later the smear was flooded with decolorizer (95% ethyl alcohol) and finally, the smear was flooded with safranin and allowed for 30 sec. At last, the smears were observed under the microscope using an oil immersion objective. The bacteria that appear in purple and pink colours were considered to be Gram-positive and Gram-negative, respectively.

Phosphorous solubilization potential

The bacteria was aseptically spot inoculated in the center of the Petri plates having Pikovskaya's agar medium (Pikovskaya, 1948) and incubated at 25±2°C for 5 d by placing the inoculated plates in the inverted position. Positive isolates developed transparent zones around the colonies. The diameter of the transparent halo zones around the colonies was measured (in mm) as phosphorous solubilization potential.

Siderophore production

The bacteria were inoculated on chrome azurol sulfone (CAS) blue agar as described by Schwyn and Neilands (1987). The succinate medium was prepared as per the procedure described by Barbhaiya and Rao, 1985. The two media are aseptically mixed in equal proportions and poured to Petri plates. The bacteria were aseptically placed on CAS blue agar plates and incubated at 25±2°C for 72 hr in the inverted position. Siderophore production was indicated by the appearance of yellow to orange zones around the colonies. The zone diameter was measured in millimeter scale.

Protease activity

The protease activity of bacteria was determined by using the skim milk agar plate method (Cui *et al.,* 2015). The bacteria were spot inoculated on skim milk agar and incubated at 25±2°C for 48 hr. The clear zone around the colony indicated positive for protease production. The diameter of the zone was measured and expressed in cm.

Hydrogen cyanide (HCN) production

The ability of the bacteria to produce HCN was tested by following the method described by Bakker and Schippers (1987). HCN production by the bacteria was confirmed by observing the colour change in the filter paper from yellow to light brown, brown or reddish-brown. The light brown coloured (weak) was recorded as +; brown coloured (moderate) is recorded as ++; reddish-brown (strong) is recorded as +++.

Mass multiplication of the pathogen and endophytic bacteria

Mass multiplication of *R. bataticola* was carried out on sorghum grains as per the method suggested by Patil *et al.* (2011). The presence of black growth on the sorghum grains, observation of the mycelium and microsclerotia under the microscope was taken as confirmation for *R. bataticola*.

The talc based formulation of endophytic bacteria was prepared by following the method described by Vidhyasekaran and Muthamilan, 1995. The population of the bacteria was calculated by using the formula given by Aneja (2003):

$$\text{Number of Colony-forming units gram}^{-1} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{Dilution used}}$$

The formulation with a population of 9×10^8 was used in the present study. After determining the population the formulation was stored for further use.

Field layout

The field trial was conducted at Regional Agricultural Research Station, Tirupati, Andhra Pradesh during *Rabi,* 2017-18 by using black gram cv. TBG 104. The potential bacteria BSE 4, BSE 7, BLE 1 and BLE 4 were given as seed treatment and soil application; the sterilized talc served as control and carbendazim 50% WP served as check. The trial was conducted with 7 treatments (3 replications for each treatment) laid in a randomized block design (RBD). The dimension of each of the treatment plots was 3 m (width) × 2.5 m (length), with 0.5 m between them and 1 m between blocks. The moisture smeared seeds were treated with talc based formulation of the respective bacteria @ 10.0 g kg^{-1} of seed and were sown immediately in the field with a spacing of 0.3 × 0.1 m. The talc formulations of the respective bacteria at the rate of 2.5 kg per 100 kg of well decomposed farmyard manure was mixed; soil incorporation to respective plots was made (Nakkeeran *et al.,* 2005). The disease incidence was recorded by using the following formula:

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Plant characteristics

At crop harvest plants were uprooted and observations on plant height, dry weight (fresh weight and dry weight), number of branches, number of pods and seed yield were recorded by following standard set of procedure.

Statistical analysis

The data of *in vitro* evaluation and biochemical properties were analyzed in complete randomized design (CRD), whereas, the field data was analyzed using RBD with univariate-one way ANOVA analysis followed by subjecting the significant data to post hoc using Tukey's HSD test. The treatments were compared at $P \leq 0.01$ and P-values less than 0.01 were considered significant. SPSS statistical software version 20.0 was employed for analysis (IBM, 2012).

RESULTS AND DISCUSSION

***In vitro* screening**

Of 24 bacterial endophytes screened against *R. bataticola*, the radial mycelial growth inhibition of *R. bataticola* ranged from 0 to 79.6 per cent. The leaf endophyte BLE 4 exhibited maximum inhibition of mycelial growth (79.6%) followed by stem endophytes BSE 4 (77.4%), BSE 7 (77.0%) and the leaf endophyte BLE 1 (74.0%). Whereas, the stem endophyte BSE 1 exhibited zero mycelial growth inhibition (Table 1, Fig 1a, 1b, 1c).

***In vivo* screening**

Field-level efficacy tests with four potential endophytes proved best in *in vitro* were tested as seed treatment as well as soil application. There was no significant difference in the incidence of dry root rot and grain yield among the treatments (Table 2). Whereas, the plant growth parameters [plant height (37.7 cm), dry weight (7.1 g), number of branches per plant (13.2 nos) and number of pods per plant (26.2 nos)] were significantly superior in the treatment (seed treatment and soil application) with leaf endophyte BLE 4.

Biochemical characterization

Among 24 endophytic bacteria, 8 bacteria (BSE 2, BRE 1, BRE 5, BLE 2, BLE 3, BLE 4, BLE 5 and BLE 6) were Gram -ve in reaction, whereas, the remaining 16 bacteria were Gram +ve. In the phosphate solubilization potential the solubilization zone diameter varied from 0 to 3.5 mm.

Table 1: *In vitro* evaluation of the bacterial endophytes against *Rhizoctonia bataticola*.

Isolate	Mycelial growth (in mm)	Mycelial growth inhibition (%)
BSE 1	90.0±0.0^{k}	0 (0.0)±0.0^{k}
BSE 2	79.3±3.0^{j}	11.8 (20.0)±2.9^{j}
BSE 3	31.7±1.5^{c}	64.8 (53.6)±1.02^{cd}
BSE 4	20.3±0.57^{ab}	77.4 (61.6)±0.43^{ab}
BSE 5	43.0±3.6^{d}	52.2 (46.2)±2.2^{e}
BSE 6	47.3±1.1^{de}	47.4 (43.5)±0.73^{ef}
BSE 7	20.7±1.1^{ab}	77.04 (61.3)±0.86^{ab}
BRE 1	33.0±2.0^{c}	63.3 (52.7)±1.3^{d}
BRE 2	46.3±0.57^{de}	48.5 (44.1)±0.36^{ef}
BRE 3	56.3±0.57^{gh}	37.4 (37.7)±0.38^{h}
BRE 4	54.7±2.0^{fgh}	39.2 (38.7)±1.36^{gh}
BRE 5	25.7±1.1^{b}	71.4 (57.7)±0.81^{bc}
BRE 6	67.7±7.0^{i}	24.8 (29.6)±5.2^{i}
BRE 7	51.3±3.7^{efg}	42.9 (40.9)±2.4^{fgh}
BRE 8	58.0±5.5^{h}	35.5 (36.5)±3.7^{h}
BRE 9	80.7±1.5^{j}	10.3 (18.7)±1.5^{j}
BRE 10	80.7±4.0^{j}	10.3 (18.4)±4.4^{j}
BRE 11	46.3±1.1^{de}	48.5 (44.1)±0.73^{ef}
BLE 1	23.3±1.1^{ab}	74.07 (59.4)±0.84^{ab}
BLE 2	58.0±4.5^{h}	35.5 (36.5)±3.07^{h}
BLE 3	56.7±7.6^{gh}	37.04 (37.4)±5.1^{h}
BLE 4	18.3±1.1^{a}	79.6 (63.1)±0.91^{a}
BLE 5	82.7±1.5^{j}	8.1 (16.5)±1.7^{j}
BLE 6	49.0±7.9^{def}	45.5 (42.4)±5.08^{efg}
Control	90.0±0.0^{k}	-
F-value	126.70**	123.15**
p-value (<0.01)	0.00	0.00

The root endophyte BRE 10 solubilized more phosphate (clear halo zone of 3.5 mm diameter) followed by BRE 2 (2.5 mm). The HCN production potential was observed in BSE 2, BSE 7 and BLE 2. The stem endophyte BSE 1 produced more protease activity with a clear halo zone of 3.5 cm diameter, followed by the root endophytes BRE 1 and BRE 11 (halo zone 3.0 cm). In siderophore production test orange coloured zone around the bacterial colony was observed with the range of 0-3.0 mm diameter (Fig 2). The endophyte BRE 3 produced the maximum amount of siderophores with a zone of 3.0 mm diameter, followed by BSE 4 and BLE 2 (zone diameter of 2.0 mm each) (Table 3).

Bacterial endophytes were considerably focused since 80's for their plant growth promotion ability by direct and indirect means. Former occurs by the acquisition of nutrients (nitrogen and phosphorus) and by the production of growth hormones (IAA and gibberellins). While later occurs by production of antimicrobial peptides, volatile organic compounds (VOCs) and cell wall degrading enzymes to counter phytopathogens (Glick, 2015). In the current study endophytic bacteria (24 nos) isolated from roots, stems and leaves of the black gram plants at different growth stages and of different locations were tested under *in vitro* and *in vivo*.

Under *in vitro* evaluation the leaf endophyte BLE 4 showed maximum mycelial growth inhibition followed by stem endophytes BSE 4, BSE 7 and the leaf endophyte BLE1. Similar studies conducted by Brunda *et al.* (2018) indicated that the stem endophyte SB-DG-11 and leaf endophyte LB-BiN-8 of soybean exhibited inhibition of 47.4% and 41.2%, respectively over control.

Literature reveals plenty of studies on the efficacy tests of endophytic bacteria; and good results were obtained in the laboratory as well as greenhouse studies. However, they failed to exhibit their potential under field conditions (Backer *et al.,* 2018). For instance, *Azospirillum brasilense* (Ab-V5 and Ab-V6) application in Wheat and Maize resulted in increased shoot dry weight, root dry weight, number of tillers, nitrogen content in leaves and shoots and chlorophyll

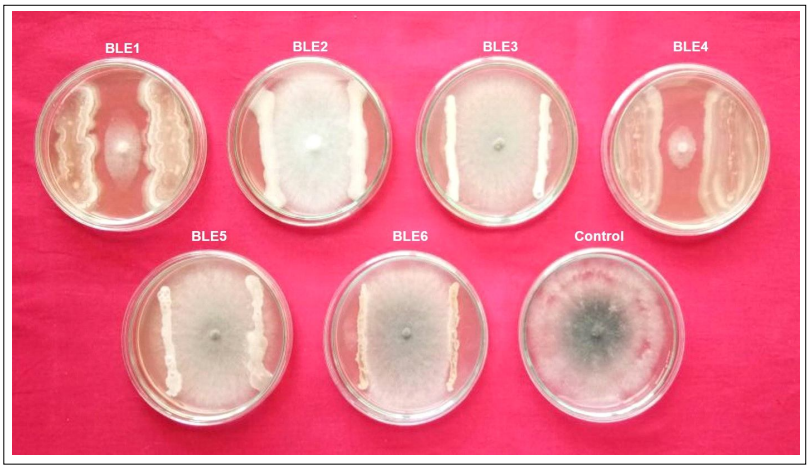


Fig 1a: Efficacy of leaf endophytes against *R. bataticola*.

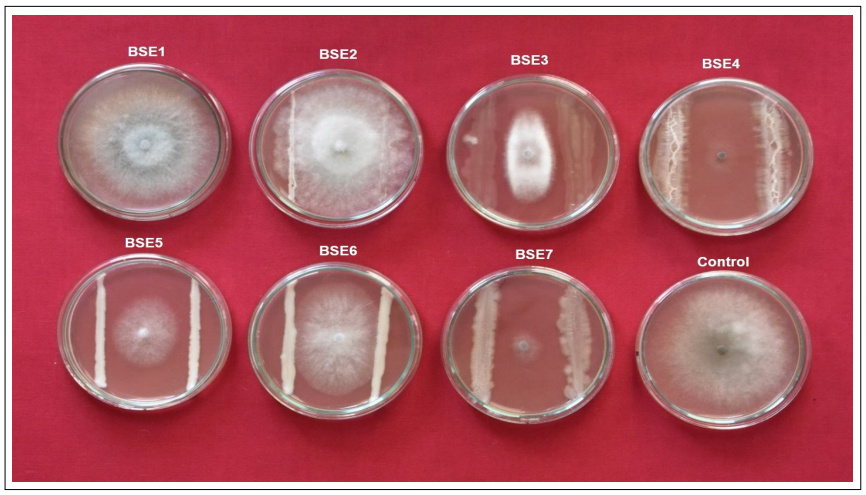


Fig 1b: Efficacy of stem endophytes against *R. bataticola*.

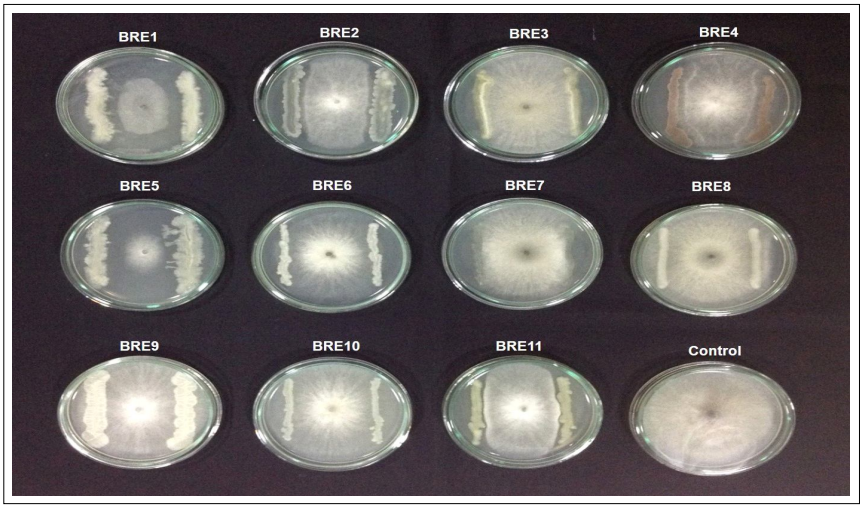
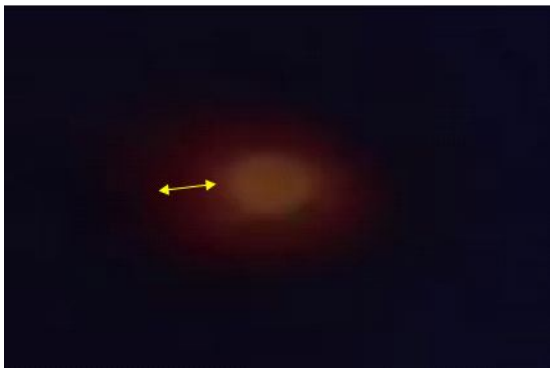


Fig 1c: Efficacy of root endophytes against *R. bataticola*.

Table 2: Evaluation of potential biocontrol agents against dry root rot of blackgram under field conditions.

Treatment	Shoot length (cm)	Root length (cm)	Plant height (cm)	Dry weight (g)	Number of branches	Number of pods	DRR incidence (%)	Yield (gm)
T1 (Seed treatment of BSE 4)	28.4^{b}	7.0^{d}	35.4^{b}	5.0^{c}	8.0^{ef}	12.5^{f}	28.8 (32.04)	161.6
T2 (T1 + soil application of BSE 4)	23.1^{ef}	6.0^{f}	29.2^{fg}	4.2^{d}	7.2^{g}	10.4^{g}	27.9 (31.7)	193.3
T3 (Seed treatment of BSE 7)	29.1^{b}	8.4^{a}	37.6^{a}	5.7^{b}	10.7^{b}	17.5^{b}	26.4 (30.6)	233.3
T4 (T3 + soil application of BSE 7)	22.5^{f}	6.2^{f}	28.8^{g}	4.2^{d}	7.8^{f}	10.06^{h}	24.8 (29.4)	195.0
T5 (Seed treatment of BLE 1)	24.5^{d}	6.3^{ef}	30.8^{ef}	3.5^{e}	8.4^{d}	13.8^{e}	24.2 (29.3)	160.0
T6 (T5 + soil application of BLE 1)	24.1^{de}	7.5^{b}	31.7^{de}	5.0^{c}	7.8^{f}	16.5^{c}	23.0 (27.7)	163.3
T7 (Seed treatment of BLE 4)	26.3^{c}	6.5^{e}	32.9^{cd}	5.7^{b}	11.0^{b}	15.6^{d}	31.6 (34.05)	270.0
T8 (T7 + soil application of BLE 4)	30.4^{a}	7.2^{cd}	37.7^{a}	7.1^{a}	13.2^{a}	26.2^{a}	30.0 (30.4)	243.3
T9 (Seed treatment + foliar spray at 30 DAS with carbendazim)	26.6^{c}	7.4^{bc}	34.07^{bc}	4.2^{d}	8.3^{de}	9.5^{i}	28.5 (31.7)	195.0
T10 (Uninoculated control)	26.2^{c}	7.2^{cd}	33.5^{bcd}	3.5^{e}	9.7^{c}	15.3^{d}	33.4 (35.02)	156.6
F-value	34.948**	43.366**	25.19**	243.26**	244.40**	1381.57**	0.156	0.754
p-value (<0.01)	0.00	0.00	0.00	0.00	0.00	0.00	0.996 (>0.05, NS)	0.658 (>0.05, NS)



Yellow line indicates the orange coloured halo zone.

Fig 2: Siderophore production potential by the endophytic bacteria.

Table 3: Biochemical properties of the endophytic bacteria.

Isolate	Gram reaction	HCN production	Protease	PSB	Siderophore
BSE 1	+	-	3.5^{a}	1.5^{bcd}	0.0^{d}
BSE 2	-	+	2.0^{abcd}	0.5^{cd}	0.0^{d}
BSE 3	+	-	0.0^{e}	0.0^{d}	0.0^{d}
BSE 4	+	-	1.5^{bcde}	0.5^{cd}	2.0^{b}
BSE 5	+	-	1.0^{cde}	0.0^{d}	0.0^{d}
BSE 6	+	-	2.5^{abc}	0.5^{cd}	0.0^{d}
BSE 7	+	+	2.0^{abcd}	0.0^{d}	1.0^{c}
BRE 1	-	-	3.0^{ab}	0.0^{d}	1.5^{bc}
BRE 2	+	-	1.0^{cde}	2.5^{ab}	0.0^{d}
BRE 3	+	-	1.0^{cde}	1.5^{bcd}	3.0^{a}
BRE 4	+	-	2.0^{abcd}	0.5^{cd}	0.0^{d}
BRE 5	-	-	2.0^{abcd}	0.5^{cd}	1.0^{c}
BRE 6	+	-	0.0^{e}	0.0^{d}	0.0^{d}
BRE 7	+	-	1.0^{cde}	0.5^{cd}	0.0^{d}
BRE 8	+	-	0.5^{de}	0.0^{d}	0.0^{d}
BRE 9	+	-	2.0^{abcd}	0.0^{d}	1.0^{c}
BRE 10	+	-	2.0^{abcd}	3.5^{a}	1.0^{c}
BRE 11	+	-	3.0^{ab}	2.0^{abc}	0.0^{d}
BLE 1	+	-	2.0^{abcd}	2.0^{abc}	0.0^{d}
BLE 2	-	+	1.5^{bcde}	0.0^{d}	2.0^{b}
BLE 3	-	-	0.0^{e}	1.5^{bcd}	0.0^{d}
BLE 4	-	-	0.5^{de}	0.0^{d}	1.0^{c}
BLE 5	-	-	1.0^{cde}	0.5^{cd}	0.0^{d}
BLE 6	-	-	0.0^{e}	1.0^{bcd}	0.0^{d}
F-value			3.26**	3.12**	7.72**
p-value (<0.01)			0.00	0.00	0.00

HCN: '+' present, '-' absent; PSB and siderophore production were expressed in mm; protease activity was expressed in cm.

content under greenhouse conditions, but, these parameters showed no significant difference when applied under field conditions (Fukami *et al.,* 2016). Among four endophytic bacteria (BSE 4, BSE 7, BLE 1, BLE 4) evaluated in field, seed treatment and soil application of BLE 4 recorded maximum growth promotion readings with non-significant impact on yield and dry root rot incidence. Microbiome is selected by the plant to have beneficial colonizers (Ryan *et al.,* 2008) and is applicable to the isolate BLE 4 in this study. The results are in corroboration with Lally *et al.* (2017) where endophytic *P. fluorescens* and the bacterial consortium were applied to *Brassica napus* and observed a significant increase in crop height, stem/leaf and pod biomass in consortium applied treatment with no significant difference in the seed and oil yield. The insignificance inter alia in the disease incidence and yield were attributed to lack of sufficient competition with resident microflora by the introduced bacteria (Samad *et al.,* 2017).

Bacteria that produce siderophores (Katiyar and Goel, 2004), IAA (Idris *et al.,* 2007), ACC deaminase (Mayak *et al.,* 2004), HCN (Olanrewaju *et al.,* 2017) and solubilize the unavailable form of phosphate (Rodriguez and Fraga, 1999) enhances plant growth. In the biochemical tests, BSE 4, BRE 3, BRE 5 and BRE 10 isolates showed protease activity, phosphate solubilization and siderophore production. Previous reports indicated the ability of the nitrogen-fixing and phosphate solubilizing strains in increasing nutrient uptake and yield of chickpea, paddy, wheat, maize, soybean and cucumber (Adesemoye and Kloepper, 2009; Adesemoye and Egamberdieva, 2013). Nevertheless, in the current study BRE 10 isolate produced the maximum amount of phosphate solubilization potential (3.5 mm), but it exhibited only 10 per cent mycelial growth inhibition under *in vitro*.

From the present study, it is evident that not all the bacteria exhibit all the functions and inconsistency in the results may be due to the usage of single strain (Van Veen *et al.,* 1997). It is also known that apart from all the biochemical functions, the competitive ability of the bacteria with the other microflora need to be understood before its application in the field as the conditions in the laboratory are unique and is dynamic in the field (Samad *et al.,* 2017). To overcome these, consortia of different microorganisms with different characters may be developed and applied for enhancing plant growth and reducing disease incidence (de Vrieze *et al.,* 2018).

CONCLUSION

Laboratory and field studies were conducted using the endophytic bacteria (24 nos) isolated from leaves, stems and roots of the black gram plants collected from different locations. Four isolates (BLE 4, BLE 1, BSE 4 and BSE 7) were found efficient in inhibiting the mycelial growth of *R. bataticola* under *in vitro*. Field efficacy tests revealed a significant increase in plant height, dry weight, number of branches and number of pods in the plots treated with BLE 4 (seed treatment as well as soil application) and there was no significant difference inter alia in disease incidence and yield. Future works on the usage of smart microbial consortia including multiple strains with different modes of action along with individual competitive ability are warranted for improving plant growth with reduced disease incidence.

Conflict of interest

All authors declared that there is no conflict of interest.

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