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Phenotypic, bio-chemical and molecular characterization of potential endophytic bacterial isolates and evaluation of endophytic bacterial formulations and extracted antibiotic substances under glass house conditions

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Abstract
A total of 22 chickpea dry root rot causing pathogen *Rhizoctonia bataticola* isolates and 40 antagonistic endophytic bacteria were isolated from chickpea roots which are collected from different chickpea growing districts of Andhra Pradesh. Cultural and morphological variability among the pathogen isolates were studied by growing on PDA and observed variation in colony growth rate, colour, texture, time taken to grow full plate, Sclerotial initiation, Sclerotial density, Sclerotial size and shape. Among the 14 potential endophytic bacterial isolates, Gram negative (7) and Gram positive (7) were recorded in equal proportion and all were rod shaped bacteria. Out of the 14 isolates, a total of 9, 7, 2, 1, 2, 3, 3, 13, 6, 3 and 0 showed positive for amylolytic activity (starch hydrolysis), proteolytic activity (Gelatin hydrolysis), lactose utilization, indole production, methyl red test, Voges-Proskeur test, citrate utilization, presence of catalase activity, ammonia production, HCN production and siderophore production respectively. 16S r DNA sequence analysis was used to identify the potential bacterial isolates such as CREB 9, CREB 21 and CREB 37 and which were exhibited close similarity of 97.75, 98.48 and 93.28 percent with *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. Under glasshouse conditions, among the treatments T7 (seed treatment with potential bacterial antagonist (talc formulation) @10 g kg-1 + soil application with talc based formulation of potential bacterial antagonist @100 g pot-1) was found to be superior as it recorded maximum germination percent, plant height, root length, dry weight and least percent disease incidence compared to other treatments.

Keywords: Chickpea, sclerotial density, indole production, glass house and antagonist

1. Introduction
Chickpea (*Cicer arietinum* L.) is widely cultivated in the world covering more than 50 countries spread over Asia, Africa, Europe, Australia, North America and South America and it is the third most important pulse crop, after Dry bean and Peas, produced in the world. It accounts for 20 percent of the world pulses production (Gaur *et al*., 2014) [20]. Among chickpea growing states of India, Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Andhra Pradesh, Karnataka, Chhattisgarh, Bihar and Jharkhand contribute more than 95 percent to the total production. In recent years, the country has witnessed remarkable increase in area, production and productivity of chickpea. India contributes to a major share of the world's chickpea area (70%) and production (67%) and continues to be the largest chickpea producing nation (Dixit *et al*., 2019) [15].

In India, chickpea occupies an area about 105.60 L ha, production 113.79 L T and productivity 1078 kg ha^{-1} (Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Government of India, 2017-18) [13, 14]. Chickpea area (35.90 L ha), production (45.95 L T) and productivity (1280 kg ha^{-1}) is maximum in Madhya Pradesh (Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Government of India, 2017-18) [13, 14].

In Andhra Pradesh, chickpea occupied an area of 5.20 L ha, production 5.88 L T and productivity 1132 kg ha^{-1} (Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Government of India, 2017-18) [13, 14]. Chickpea is a highly nutritious grain legume crop and widely appreciated as a health food.

It is a protein-rich supplement to cereal-based diets, especially to the poor in developing countries, where people are vegetarians or cannot afford animal protein. Diseases are the most serious limitations to chickpea production causing up to 100 percent losses. Ecological, environmental and physiological factors and intensity of biotic stresses are known to aggravate the occurrence and severity of the diseases. Though many diseases are reported, only a few such as collar rot (*Sclerotium rolfsii*), wilt (*Fusarium oxysporum* f. sp. ciceri), dry root rot (*Rhizoctonia bataticola*), wet root rot (*Rhizoctonia solani*), blight (*Ascochyta rabiae*), grey mould (*Botrytis cinerea*) and chickpea stunt may cause severe losses and prevent farmers from realizing the potential yield. Among these, dry root rot and wilt causes severe losses in almost all major chickpea growing states of India. Dry root rot causes 20-30 percent crop losses in major chickpea growing states of India (Nene *et al*., 2012) [32].

Among the several soil-borne fungal diseases, dry root rot caused by R. bataticola (Taub.) Butler is the most severe disease of chickpea especially in the central and southern zones, where the crop is mostly grown in Rabi season under rainfed conditions. Predominantly, disease appears around flowering and podding stage. The first symptom is yellowing and sudden drying of the plants. The tap root becomes dark brown quite brittle in dry soil and shows extensive rotting resulting in the loss of lateral roots. The lower portion of the tap root is often left in the soil when plant is uprooted (Nene *et al*., 2012) [32].

In A.P, major chickpea growing districts includes Kurnool, Y.S.R Kadapa, Anantapuramu, Prakasam and Guntur and the dry root rot occurrence is severe in these areas. This commences to work on this disease to generate basic information on percent disease incidence, to search for an alternate possible approach other than use of fungicides for the management of the dry root rot.

Chickpea diseases may cause yield losses of up to 100 percent depending on time of infection. The recommended method of managing the diseases is to use resistant varieties. A number of improved chickpea disease-resistant varieties have been multiplied and disseminated to farmers in many districts of India. However, their current prevalence in farmers' fields has not been documented (Ghosh *et al*., 2013) [21].

Besides, increase in pathogen resistance to the fungicides and environmental concerns regarding fungicide usage led to the alternative methods of chickpea dry root rot management such as biological control. Biological control of dry root rot using antagonistic bacteria has been demonstrated earlier. However, consistent and satisfactory control is yet to be obtained for their use on a large scale by farmers.

Endophytes are those that colonize and living inside the plant tissue without showing any external sign of infection or any adverse effect on their host (Schulz and Boyle, 2006) [41]. The precise and specific role of endophytes in plants is not yet known. Although, their survival capability within the host tissues away from microbial competition and environmental adaptation has made endophytes as potential candidates for use in agriculture.

Plants are ubiquitously colonized by endophytic microorganisms which contribute significantly to plant health through production of plant growth regulators or disease suppression. Endophytes have been reported to possess the potential to produce an array of bio-active metabolites with antifungal properties which enables the host plants to meet possible challenges generated by phytopathogenic fungi. Many endophytic bacteria have been reported to exhibit excellent natural product biosynthetic potential and hence much interest has been generated in recent years to develop bacterial based commercial formulations (Jasim *et al*., 2016) [24].

Endophytic competence of antagonistic bacteria in roots of many crops is a vital area of research that requires elaborate exploration for successful management of plant diseases by biocontrol agents. Hence, the present investigation was undertaken to exploit endophytic microbes and their antibiotic compounds antagonistic to R. bataticola, and to study the feasibility of using them in chickpea ecosystem against dry root rot management.

Endophytic bacteria have been isolated from a large range of plants. Bacteria like Bacillus, Klebsiella, Pseudomonas, Burkholderia, Pantoea, Agrobacterium, Enterobacter and Methylobacterium spp. constitute the endophytes commonly isolated from diversified plants such as rice, wheat, maize, clover, potato, cotton, sugarcane, cucumber, tomato and wild grasses (Bacon and Hinton, 2006) [5]. Role of endophytic bacteria in plant disease management is of recent interest and some endophytes may be found in specific plant parts such as leaves, roots and twigs, while others may infect several of these parts (Stone *et al*., 2004) [48].

Majority of the endophytic bacteria showed beneficial effects like enhancement of biological N2-fixation, production of phytohormones, cell wall degrading enzymes and siderophores, solubilization of phosphate and inhibition of ethylene (C2H2) biosynthesis in response to biotic and abiotic stresses and have bio-control activity. Bacterial endophytes are known to protect the plants from pathogens by production of antibiosis, competition and induced systemic resistance (Sessitsch *et al*., 2004) [43].

The information on activity of secondary metabolites produced by endophytic bacteria having antiphytopathogenic properties is meagre. Hence, in the present investigation an attempt was made to explore the feasibility of using endophytic bacteria and their antibiotic substances (secondary metabolites) for management of dry root rot of chickpea incited by R. bataticola.

2. Material and Methods

2.1 Cultural and morphological characterization of different isolates of R. bataticola

A total of 22 pathogen isolates were collected from major chickpea growing areas of Andhra Pradesh. Cultural and morphological characterization of pathogen isolates was done based on colony colour, growth pattern, growth rate, mycelial character, sclerotial initiation time, sclerotial intensity and morphology of the sclerotia (sclerotial size and colour).

All the 22 isolates of R. bataticola were characterized for morphological and cultural characteristics. The mycelial discs of 5 mm diameter were cut from the edge of a three days old culture and transferred aseptically to 80 mm Petri dish containing 15 ml PDA. These plates were incubated at 35°C with 12 hrs photoperiod. Each treatment was replicated thrice. The colonies of isolates were characterized for growth rate at 42 hrs after incubation. Seven days old cultures were used to record texture, colour and presence or absence of aerial mycelium. At the seventh day after incubation number of sclerotia/microscopic field (10x), sclerotia size and shape were recorded by using Q-capture image analyzer. All the cultures were observed daily for recording the time taken for sclerotial initiation.

2.2 Phenotypic, biochemical and molecular characterization (16s rDNA sequence analysis) of potential endophytic bacteria

Phenotypic and biochemical characterization was performed to the isolates which exhibited 50 percent above inhibition against virulent R. bataticola isolate in dual culture technique.

2.2.1 Phenotypic characterization of potential endophytic bacterial isolates

Endophytic bacteria were tentatively grouped based on phenotypic characteristics such as colour, shape, elevation, margin, texture, cell morphology and gram reaction (Zvyagintsev, 1991) [59].

2.2.2 Biochemical characterization of potential endophytic bacterial isolates

Biochemical tests such as amylolytic activity (Hydrolysis of starch), proteolytic activity (Hydrolysis of Gelatin), lactose fermentation, in dole production from tryptophan, methyl red, Voges-Proskauer, citrate utilization, presence of catalase activity, NH3 production and HCN production and siderophore production were performed for potential endophytic bacterial isolates as described by Zvyagintsev (1991) [59].

Out of 11 bio chemical tests performed, the isolates which showed positive for 5 and above bio chemical tests were considered as most potential and used for molecular characterization.

2.2.3 Molecular characterization of potential endophytic bacteria using 16s rDNA sequence analysis

The 16S rDNA sequence was used for identification of potential endophytic bacterial biocontrol agents which performed well in biochemical analysis (Julian *et al*., 1998) [25].

2.2.3.1 Isolation of DNA

A loopful of 24 hrs grown endophytic bacteria were taken and suspended in TE buffer. The samples were transferred into Eppendorff tubes and centrifuged at 10, 000 rpm for 3 min, supernatant was discarded and collected the pellet. 200 µl of lysis buffer was added to the pellet and re-suspended it. Then proteinase K of 10 µl (10 mg/ml) was added to it, the tubes were vertexed, added 7 µl of 20% sodium dodecyl sulphate (SDS) and kept it in water bath at 60oC for 1 hr. The tubes were inverted for every 15 min, then centrifuged at 10, 000 rpm for 5 min at 20^{o}C, collected the supernatant, discarded the pellet and added equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) to it. Tubes were inverted for 5 min and centrifuged at 10, 000 rpm for 5 min. Supernatant was taken, added 0.1 volume of 3 M sodium acetate and 2 volumes of ice cold ethanol/ice cold isopropanol to it. Then these tubes were incubated overnight for precipitation and centrifuged at 13, 000 rpm for 15 min at 4oC. Pellets were washed with 70% ethanol at 13, 000 rpm for 10 min. Ethanol was discarded and tubes were air dried. Then the extracted DNA was dissolved in 50 µl of TE buffer (Kumar *et al*., 2004) [26].

2.2.3.2 Preparation of gels

Gel plates (13 x 14 cm, Genei, Bangalore) were washed thoroughly with cleaning solution followed by distilled water and dried. The two open sides of the plates were sealed with cellophane tape. Gel solution was prepared by mixing 1 g of agarose (Fermentas, Bangalore) in 100 ml of 1x TBE buffer (1.0% gel) in a conical flask and boiled in an oven until a clear solution was obtained and 4 µl of ethidium bromide (10 mg μl^{-1}) was added. The solution was poured onto the sealed gel casting tray, with suitable comb inserted and allowed topolymerize.

2.2.3.3 Loading and running of gels

The inserted comb was gently removed from the gel after polymerization. The gel plate was placed in horizontal apparatus and fixed with 1x TBE buffer. The samples were loaded in the wells with the help of micro pipettes. After loading, the electrophoretic unit was connected to power pack (Genei, Bangalore) with a regulated electric power supply of 100V. At the end of run, the gel was carefully removed and analyzed.

2.2.3.4 Qualitative and quantitative verification of DNA from different isolates of potential endophytic bacteria

DNA samples (5 µl) from each isolate mixed with 4 µl of 1x loading dye were loaded on the wells of the 1% agarose gel along with 5 µl of DNA marker in order to verify the quality and quantity of DNA. Alternatively the quality and quantity of DNA was also verified by spectrophotometer.

2.2.3.5 Amplification of 16S rDNA from potential endophytic bacteria

The 16S rDNA sequence has been selected for identification of potential endophytic bacterial isolates. The 16S rDNA from potential isolates of endophytic bacteria has been amplified by using 27F and 1492R primers. The primer sequences are as given below.

27F- 5^{1} AGAGTTTGATCACTGGCTCAG - 3^{1}

1492R- 5^{1} TACGGACTTACCTTGTTACGACTT-3^{1}

2.2.3.6 Gel extraction and purification

The amplified 16S rDNA of the potential endophytic bacterial isolates was eluted from the gel using the kit Qualigens, Bangalore and then purified using the kit supplied by Qiagen, Bangalore and sent for sequencing to Medauxin sequencing company, Bangalore.

2.3. Evaluation of efficacy of talc based formulation of potential endophytic bacteria and crude antibiotic extract under glasshouse conditions

To evaluate the efficacy of endophytic bacterial bioformulation and extracted antibiotic substances under glasshouse conditions, pot culture experiment was carried out. Cultivar used for this study – L550 (Dry root rot susceptible variety)

Table 1: Treatments used for the study

S. No.	Treatments
T1	Seed treatment with potential bacterial antagonist (Talc formulation) @10 g kg^{-1}
T2	Seed treatment with antibiotic extract @100 ppm
T3	Seed treatment with antibiotic extract @200 ppm
T4	Seed treatment with antibiotic extract @500 ppm

T5	Soil application with talc based formulation of potential bacterial antagonist @100 g pot^{-1}
T6	Foliar spray with antibiotic extract @500 ppm
T7	T1+T5
T8	T3+T6
T9	T4+T6
T10	Control (Treatment with distilled water)

Design: CRD; Replications: Three

2.3.1 Mass multiplication of virulent *R. bataticola* isolate
The test pathogen isolate of *R. bataticola* was mass multiplied on sterilized sorghum seeds for pot culture studies. For this, 100 g of sorghum seeds were washed thoroughly in tap water and soaked in water overnight in 250 ml conical flask with addition of 20 ml of 4 percent dextrose. After removing the water, the flasks were autoclaved for 20 min at 15 psi and inoculated with 2-3 discs of 4 days old culture of test pathogen. After 7 days, the inoculum was mixed with sterilized soil in pots @100 g kg^{-1} soil.

2.3.2 Preparation of talc based formulation of potential endophytic bacterial isolate
Talc based formulation of potential endophytic bacterial isolate was prepared by following the method as described by Vidhyasekaran and Muthamilan (1995) [55].
The initial population of the bacterial biocontrol agent was evaluated by serial dilution method and used in integrated disease management.

2.3.3 Seed treatment with talc based formulation of potential endophytic bacteria and crude antibiotic
Chickpea seeds were treated with talc based formulation of potential endophytic bacteria @10 g kg^{-1} of seed and crude antibiotic solution @100, 200 and 500 ppm, then the treated seeds were sown in the pathogen infested soil present in the pots.

2.3.4 Soil application of talc based formulation of potential endophytic bacteria
The talc based formulation of potential endophytic bacteria was applied to the soil @100 g pot^{-1} before sowing.

2.3.5 Foliar spray with crude antibiotic
Ten days after sowing crude extract of antibiotics @500 ppm was sprayed on the chickpea plants with atomizer.
Observations were taken on germination percentage, plant height, Root length, dry weight of the plant and PDI.

3. Results
3.1 Cultural and morphological variability in *R. Bataticola*
Variability in the cultural and morphological characters of 22 isolates of *R. bataticola* isolated from major chickpea growing areas of Andhra Pradesh were studied by growing on potato dextrose agar medium.
The colony growth of the *R. bataticola* isolates measured at 48 hrs after inoculation. The size of the sclerotia was measured using Q-capture image analyzer software at 100x objective of the microscope. Observations on various cultural and morphological characters were recorded and the results obtained were presented in Table 2 and Plate 1.

Table 2: Cultural and morphological variability among the isolates of R. bataticola

S. No.	Name of the district	Name of the mandal	Name of the village	Isolate code	Time taken to grow full plate (Days)	Growth rate (Radial growth (mm))	Colony texture	Colony colour	Sclerotial initiation (Days)	Sclerotial density per 10x microscopic field	Sclerotial size (dia. (µm))	Sclerotial shape
1.	Kurnool	Kurnool (Rural)	L. Peta	CRb 1	2.77^{lmn}	75.25^{ab}	Appressed	Jet black	1.97^{ijklm}	13.00^{lm}	107.92^{c}	Round
			Peddapadu	CRb 2	3.40^{de}	62.49^{qr}	Fluffy	Jet black	3.00^{bc}	27.67^{bc}	48.50^{op}	Irregular
		Kodumooru	Kodumooru	CRb 3	2.87^{klm}	72.19^{def}	Appressed	Jet black	2.00^{ijkl}	33.00^{a}	45.35^{qrs}	Ovoid
			Venkatagiri	CRb 4	3.30^{efg}	65.50^{lmn}	Appressed	Jet black	2.60^{def}	23.33^{fg}	58.61^{kl}	Ovoid
		Nandyal	Ayyalur	CRb 5	3.60^{bc}	61.30^{qrs}	Appressed	Jet black	3.03^{b}	18.00^{ij}	68.50^{g}	Ovoid
			Chapirevula	CRb 6	2.83^{lmn}	73.16^{cd}	Appressed	Jet black	1.97^{ijklm}	11.00^{mn}	119.32^{a}	Ovoid
2.	Y.S.R Kadapa	Jammalamadugu	Dharmapuram	CRb 7	3.40^{de}	62.56^{pq}	Appressed	Jet black	2.67^{de}	15.00^{kl}	104.22^{d}	Round
			Devagudi	CRb 8	2.83^{lmn}	72.74^{cde}	Appressed	Light black	1.97^{ijklm}	12.00^{mn}	112.46^{b}	Irregular
		Prodduturu	Dhorasanipalli	CRb 9	3.10^{hij}	67.92^{ij}	Appressed	Jet black	2.10^{ij}	23.00^{fg}	59.58^{k}	Round
			Ramapuram	CRb 10	2.50^{o}	75.78^{a}	Appressed	Jet black	1.70^{n}	24.00^{ef}	58.19^{kl}	Round
3.	Prakasam	Ongole	Cheruvukommapalem	CRb 11	3.63^{b}	61.28^{qrst}	Appressed	Jet black	2.50^{efg}	26.00^{bcde}	55.47^{m}	Ovoid
			Chejerla	CRb 12	3.17^{ghi}	66.50^{jkl}	Appressed	Light black	2.03^{ijk}	28.00^{b}	47.36^{opq}	Round
		Tangutoor	Kandukuru	CRb 13	3.37^{def}	64.32^{mnop}	Velvetty	Grey	2.50^{efg}	15.00^{kl}	93.65^{e}	Round
			Vallooru	CRb 14	3.10^{hij}	67.42^{ijk}	Appressed	Jet black	2.13^{i}	18.33^{i}	63.56^{hi}	Ovoid
4.	Anantapuramu	Gooty	Peddadoddi	CRb 15	3.00^{jk}	68.39^{i}	Fluffy	Light black	2.13^{i}	16.00^{ijk}	86.49^{f}	Ovoid
			Basinapalle	CRb 16	2.83^{lmn}	74.35^{abc}	Appressed	Light black	1.83^{klmn}	33.33^{a}	44.27^{rs}	Ovoid
		Guntakal	Ayyavaripalli	CRb 17	3.53^{bcd}	62.00^{qrs}	Appressed	Jet black	2.83^{bcd}	22.67^{fgh}	62.14^{ij}	Round
			Nakkanadoddi	CRb 18	4.13^{a}	57.46^{u}	Appressed	Jet black	3.33^{a}	26.67^{bcd}	49.33^{no}	Round
5.	Guntur	Vinikonda	Brahmanapalli	CRb 19	3.30^{efg}	65.43^{lmno}	Appressed	Jet black	2.43^{fgh}	26.00^{bcde}	51.19^{n}	Round
			Dondapadu	CRb 20	3.00^{jk}	70.32^{gh}	Appressed	Jet black	2.10^{ij}	22.00^{fgh}	62.65^{ij}	Ovoid
		Rompicherla	Gogulapadu	CRb 21	2.90^{kl}	71.42^{g}	Appressed	Jet black	1.83^{klmn}	18.00^{ij}	65.55^{h}	Round
			Daseripalem	CRb 22	3.20^{gh}	65.62^{klm}	Appressed	Jet black	2.50^{efg}	28.00^{ij}	45.47^{qr}	Irregular
C.D (P=0.05 %)					0.16	1.81			0.23	2.44	2.08	
SEm (±)					0.06	0.63			0.08	0.85	0.73	
SE (d)					0.08	0.90			0.11	1.21	1.03	
C.V					3.03	1.63			5.89	6.77	1.83	

Fig 1: Plate 1; Pure cultures of different isolates of R. bataticola from major growing areas of chickpea

3.1.1 Cultural characters of different isolates of *R. bataticola*

The cultural characters *viz*., growth rate, time taken to occupy full plate, texture of colony, colony colour, etc. were observed in all 22 isolates of *R. bataticola*.

Time taken to grow full plate

Among all the isolates CRb 10 taken significantly least time (2.50 days) to occupy the full plate followed by CRb 1 (2.77 days) CRb16 and CRb 8 (2.83 days). Higher time was taken by CRb 18 (4.13 days) which was significantly differed with all other remaining isolates.

Radial growth rate (mm)

The data on radial growth rate revealed that there was higher radial growth was observed with CRb 10 isolate (75.78 mm) after 42 hrs which was at par with CRb 1 (75.25 mm) and CRb 16 (74.35) and significantly differed with all other remaining isolates.

Similarly, significantly the least radial growth was recorded with isolate CRb 18 (57.46 mm) followed by CRb 11 (61.28 mm), and CRb5 (61.30 mm).

Colony texture

Among the 22 isolates, 19 isolates produced appressed colony while 2 isolates had shown fluffy texture, whereas only one isolate had produced velvety growth.

Fluffy colony was produced by isolates CRb 2 and CRb 15 while velvety colony texture was observed with CRb 13 and remaining all the isolates showed appressed colony texture (CRb 1, CRb 3, CRb 4, CRb5, CRb6, CRb7, Crb8, CRb9, CRb10, CRb11, CRb12, CRb14, CRb16, CRb 17, CRb 18, CRb 19, CRb 20 CRb 21 and CRb 22).

Colony colour

Based on visual observations on colony colour, the cultures were divided into three groups. Jet black colour was observed in 17 isolates (CRb1, CRb2, CRb3, CRb4, CRb5, CRb6, CRb7, CRb9, CRb10, CRb11, CRb14, CRb17, CRb18, CRb19, CRb20, CRb21 and CRb22). While, light black mycelium was recorded in four isolates (CRb 8, CRb 12, CRb 15 and CRb 16). Grey colour was observed in only one isolate (CRb 13).

3.1.2 Morphological characters of different *R. Bataticola* isolates

Morphological characters *viz*., number of days taken for initiation of sclerotia, sclerotial density, size of sclerotia, sclerotial shape etc. were studied among all 22 isolates of *R. bataticola*.

Sclerotial initiation (Days)

Among all the isolates, for sclerotial initiation significantly maximum time was taken by CRb 18 isolate (3.33 days) followed by CRb 5 (3.03 days), CRb 2 (3.00 days) and CRb 17 (2.83 days). Least time was taken by CRB 16 (1.83 days)

and which was significantly differed with all other remaining isolates.

Sclerotial density

Among the isolates, higher number of sclerotia per microscopic field (10 x) was observed with CRb 16 isolate (33.33) which was at par with CRb 3 (33) and significantly differed with all other remaining isolates and lower sclerotial density was observed with CRb 6 (11) which was at par with CRb8. 12) and CRb 1 (13) and significantly differed with all other remaining isolates.

Sclerotial size (µm)

Among the 22 isolates, large sclerotial size (diam.) was observed with CRb 6 (119.32 µm) followed by CRb 8 (112.46 µm) CRb1 (107.92µm), CRb 7 (104.22 µm) and CRb 13 (93.65 µm), CRb 15 (86.49 µm) and CRb 5 (68.50 µm). These isolates were significantly differed with each other and with remaining isolates. Small sclerotial size (diam,) was observed with CRb 16 (44.27 µm) which was at par with CRb 3 (45.35 µm) and CRb 22 (45.47 µm) and significantly differed with all other remaining isolates.

Sclerotial shape

The isolates were categorized into irregular, round and ovoid groups based on shape of sclerotia. Irregular shaped sclerotia were observed in three isolates (CRb 2, CRb 8 and CRb 22), while ovoid shaped sclerotia were observed in 9 isolates (CRb 2, CRb 3, CRb 4, CRb 5, CRb 11, CRb 14, CRb 15, CRb 16, and CRb20), round shape of sclerotia was observed in 10 isolates (CRb 1, CRb 7, CRb 9, CRb 10, CRb 12, CRb 13, CRb 17, CRb 18, CRb 19 and CRb 21).

3.2 Phenotypic, biochemical and molecular characterization (16s rDNA sequence analysis) of potential endophytic bacterial isolates

The endophytic bacterial isolates *viz*., CREB 1, CREB 8, CREB 9, CREB 10, CREB 11, CREB 15, CREB 16, CREB 17, CREB 19, CREB21, CREB 36, CREB 37, CREB 38 and CREB 39 which showed 50% or above inhibition against virulent pathogen isolate (CRb 9) in dual culture were considered as potential and studied for phenotypic and bio-chemical characters.

3.2.1 Phenotypic characterization of potential endophytic bacterial isolates

Total of 14 endophytic bacterial isolates *viz*., CREB 1, CREB 8, CREB 9, CREB 10, CREB 11, CREB 15, CREB 16, CREB 17, CREB 19, CREB 21, CREB 36, CREB 37, CREB 38, and CREB 39 which showed 50% or above inhibition against pathogen (*R. bataticola*) in dual culture were considered as potential and studied for phenotypic characters.

All the potential endophytic bacterial isolates were grown on NA medium at 28± 2o C for 48 hrs to record phenotypic (morphological) characters such as colony colour, shape, elevation, margin, texture, cell shape and Gram staining reaction (Table 3). Among the isolates, CREB 8 and CREB 36 produced yellow colour, circular, concave, entire moist colonies and rod shaped cells with Gram negative reaction. While, CREB 19 and CREB 38 produced yellow colour, circular, concave, entire moist colonies and small rods with Gram negative reaction. CREB1 produced creamy white, circular, concave, undulate, dry moist colonies and big rods with Gram positive reaction. While, CREB 9 produced creamy white, irregular, convex, undulate, dry colonies and rod shaped cells with Gram negative reaction. CREB 10 showed dull white, circular, flat, undulate, dry colonies and small rods with Gram positive reaction. Whereas, CREB 11 showed creamy white, irregular, raised, undulate, dry colonies and rod shaped cells with Gram positive reaction. CREB15 produced creamy white, irregular, flat, undulate dry colonies with rod shaped cells and with Gram positive reaction. While, CREB 16 showed dull white, circular, convex, entire, moist colonies and small rods with Gram positive reaction. CREB 17 exhibited creamy white, circular, raised, undulate, moist colonies and small rods with Gram positive reaction. Whereas, CREB 21 showed greenish white, circular, convex, entire, moist and rod shaped cells with Gram negative reaction. CREB 37 produced creamy white, circular flat undulate, dry colonies and rod shaped cells with Gram positive reaction. While, CREB 39 showed greenish white, irregular, flat, undulate, moist colonies and small rods with Gram negative reaction.

Among the 14 isolates Gram negative (7) and Gram positive (7) were present in equal proportion and all were rod shaped bacteria.

Table 3: Phenotypic characterization of potential endophytic bacterial isolates

S. No.	Isolate	Phenotypic characters						
		Colony colour	Colony shape	Elevation	Margin	Texture	Bacterial shape	Gram reaction
1.	CREB 1	Creamy white	Circular	Convex	Undulate	Moist	Big rods	+
2.	CREB 8	Yellow	Circular	Convex	Entire	Moist	Rods	-
3.	CREB 9	Creamy white	Irregular	Convex	Undulate	Dry	Small rods	-
4.	CREB 10	Dull white	Circular	Flat	Undulate	Dry	Small rods	+
5.	CREB 11	Creamy white	Irregular	Raised	Undulate	Dry	Rods	+
6.	CREB 15	Creamy white	Irregular	Flat	Undulate	Dry	Rods	+
7.	CREB 16	Dull white	Circular	Convex	Entire	Moist	Small rods	+
8.	CREB 17	Creamy white	Circular	Raised	Undulate	Moist	Small rods	+
9.	CREB 19	Yellow	Circular	Convex	Entire	Moist	Small rods	-
10.	CREB 21	Greenish white	Circular	Convex	Entire	Moist	Rods	-
11.	CREB 36	Yellow	Circular	Convex	Entire	Moist	Rods	-
12.	CREB 37	Creamy white	Circular	Flat	Undulate	Dry	Rods	+
13.	CREB 38	Yellow	Circular	Convex	Entire	Moist	Small rods	-
14.	CREB 39	Greenish white	Irregular	Flat	Undulate	Moist	Small rods	-

3.2.2 Biochemical characterization of potential endophytic bacterial isolates

Fourteen potential endophytic bacterial isolates which showed 50% or above inhibition in dual culture against virulent pathogen isolate (CRb 9) were selected for biochemical tests such as starch hydrolysis (test for amylolytic activity), gelatin

hydrolysis (test for proteolytic activity), lactose utilization, indole production, methyl red, Voges Proskeur, citrate utilization, presence of catalase, NH3 production, HCN production and siderophore production. The observations pertaining to different biochemical tests were presented in Table 4.

Table 4: Bio chemical characterization of potential endophytic bacterial isolates

S. No.	Isolate	Bio chemical tests											Total no. of positive tests
		Starch hydrolysis (Amylolytic activity test)	Gelatin hydrolysis (Proteolytic acivity test)	Lactose utilization	Indole production	Methyl red (MR)	Voges Proskauer (VP)	Citrate utilization	Catalase activity	NH_3 production	HCN production	Siderophore production	
1.	CREB 1	-	-	-	-	-	-	-	-	+	-	-	1
2.	CREB 8	+	-	-	-	+	-	-	+	-	-	-	3
3.	CREB 9	+	-	-	-	-	+	+	+	+	+	-	6
4.	CREB 10	-	+	-	-	-	-	-	+	+	-	-	3
5.	CREB 11	-	-	-	-	-	-	-	+	+	-	-	2
6.	CREB 15	-	+	-	-	-	-	-	+	-	-	-	2
7.	CREB 16	+	-	-	+	-	-	-	+	-	-	-	3
8.	CREB 17	+	-	-	-	-	-	-	+	-	-	-	2
9.	CREB 19	+	+	-	-	-	-	-	+	-	-	-	3
10.	CREB 21	-	+	-	-	-	-	+	+	+	+	-	5
11.	CREB 36	+	+	-	-	-	+	-	+	-	-	-	4
12.	CREB 37	+	+	+	-	-	+	+	+	+	+	-	8
13.	CREB 38	+	-	-	-	+	-	-	+	-	-	-	3
14.	CREB 39	+	+	+	-	-	-	-	+	-	-	-	4
15.	Control (Sterile distilled water)	-	-	-	-	-	-	-	-	-	-	-	
Total no. of isolates showed positive		9	7	2	1	2	3	3	13	6	3	0	

Starch hydrolysis (Amylolytic activity) test

In the present study, the endophytic bacterial isolates were tested for amylolytic activity by spot inoculating on the plates containing starch casein agar medium and incubated at 37oC for 24 hrs. Out of the 14 isolates, nine *viz.*, CREB 8, CREB 9, CREB 16, CREB 17, CREB 19, CREB 36, CREB 37, CREB 38 and CREB 39 showed positive for amylolytic activity (starch hydrolysis) by producing clear zone around the growth after pouring iodine reagent over the plates and remaining showed negative including control.

Proteolytic activity (Gelatin hydrolysis) test

In the present investigation, all the bacterial isolates were spot inoculated on the Gelatin agar medium containing plates for testing the proteolytic activity and incubated for 24 hrs at 30-37oC. Among the 14 isolates, seven *viz*., CREB 10, CREB 15, CREB 19, CREB 21, CREB 36, CREB 37 and CREB 39 showed positive for proteolytic activity (Gelatin hydrolysis) by liquifying gelatin agar medium around the growth after ice cold treatment and remaining showed negative including control.

Lactose utilization test

To test the ability of bacteria to utilize lactose in the medium the endophytic bacterial isolates were inoculated in the test tubes containing lactose broth medium and incubated at 37oC for 24 hrs. Among the 14, only two isolates *viz*., CREB 37 and CREB 39 exhibited positive for lactose utilization by turning media colour from red to yellow upon incubation and remaining isolates showed negative reaction (no colour change) including control.

Indole production test

To test the in dole production ability of bacterial isolates they were inoculated in the tryptophan broth tubes and incubated for 48 hrs. Among the isolates tested, only one (CREB 16) was showed positive reaction for in dole production by producing red colour ring at the surface of the broth after adding kovac's reagent and remaining isolates exhibited negative reaction including control.

Methyl red (MR) test

To test ability of endophytic bacterial isolates to produce stable acid end product upon fermentation. They were inoculated in glucose phosphate broth tubes and incubated for 4 days at 30ºC then added 5 drops of methyl red solution to 5 ml broth. Out of 14 isolates tested only two isolates *viz*., CREB 8 and CREB 38 produced positive reaction for methyl red test by turning media colour to distinct red colour after adding methyl red solution. Remaining isolates showed negative reaction including control.

Voges Proskeur (VP) test

To determine the ability of potential bacterial isolates to produce neutral end product from glucose fermentation, they were inoculated in glucose phosphate broth tubes and incubated at 30oC for 1-2 days, then added barrit reagent and 40% KOH with time gap. Among the tested isolates three (CREB 9, CREB 36 and CREB 37) were found to be produce positive reaction for Voges-Proskeur test by forming cherry red colour in the medium after addition of reagents. The remaining isolates showed negative reaction including control.

Citrate utilization test

In this study the endophytic bacterial isolates were tested for citrate utilization ability by streaking on Simon's citrate agar medium. Among the 14 isolated tested, only three (CREB 9, CREB 21 and CREB 37) showed positive for citrate utilizations by turning media colour from green to blue and remaining showed negative including control.

Test for presence of catalase activity

To determine the presence of catalase activity in selected bacterial endophytes loofull of bacteria placed on slide and added few drops of 8% hydrogen peroxide and taken the observations. Among all the isolates, except CREB 1 and control, CREB 8, CREB 9, CREB 10, CREB 1, CREB 15, CREB 16, CREB 17, CREB 19, CREB 21, CREB 36, CREB 37, CREB 38 and CREB 39) showed positive for presence of catalase activity by immediate streaming of oxygen bubbles and a surface froth accumulation after adding H_2O_2.

Ammonia production test

To test the ability of endophytic bacterial isolates to produce ammonia, which were inoculated in peptone broth tubes then incubated for 72 hrs at 28±2oC and added Nessler's reagent. Among the 14 isolates, six isolates *viz*., CREB 1, CREB 9, CREB 10, CREB 11, CREB 21 and CREB 37 produced positive reaction for ammonia production by developing yellow colour in the medium after adding Nessler's reagent and remaining showed negative reaction including control.

Hydrogen cyanide (HCN) production test

Potential endophytic bacterial isolates were evaluated for the HCN production by streaking on nutrient agar medium amended with glycine. A change of the filter paper from yellow to orange or brown was indicated positive for ammonia production. Among all the isolates, CREB 9, CREB 21 and CREB 37 showed positive for HCN production and remaining were negative including control.

Siderophore production test

The endophytic bacterial isolates were spot inoculated on the chrome azurol S agar medium amended with ferric chloride, upon incubation (5 days) no one produced halo zone around the growth indicated all the isolates showed negative reaction for siderophore production.

A total of 11 biochemical tests were performed for characterization of 14 endophytic bacterial isolates. Among the 14 isolates CREB 1 for only one test, CREB 11, CREB 15 and CREB 17 for two tests, CREB 8, CREB 10, CREB 16, CREB 19 and CREB 38 for three tests, CREB 36 and CREB 39 for four tests, CREB 21 for five tests, CREB 9 for six tests and CREB 37 for eight tests showed positive reaction.

Based on the above results, out of 11 biochemical tests performed, the isolates such as CREB 21, CREB 9 and CREB 37 showed positive for 5 or above biochemical tests. So, which were considered as most potential and used for molecular characterization.

3.2.3 Molecular characterization of potential endophytic bacterial isolates

Recently molecular techniques have gained importance in characterization and diagnosis of microbial population. Moreover, these techniques are growth independent, not influenced by environment and are reproducible when compared to conventional methods. Hence, molecular characterization of potential endophytic isolates by 16S rDNA was carried for identification of bacterial endophytes. The Endophytic bacterial isolates *viz.,* CREB 9, CREB 21 and CREB 37 which showed positive for most of the bio-chemical tests (5 or more tests out of 11) were considered as potential and studied for molecular characterization.

3.2.3.1 Qualitative and quantitative verification of DNA from potential endophytic bacterial isolates

The genomic DNA from the isolates was extracted as per the standard method given in material and methods. The quality of DNA was analyzed by running 2 µl of each sample on 1 percent agarose gel and quantity of DNA was assessed by spectrophotometer (Nanodrop technologies). The spectrophotometer ratio 260/280 was more than 1.9 in the isolated DNA of endophytic bacterial isolates and the DNA was used for PCR amplification. The agarose gel analysis and spectrophotometer results confirmed the quality and quantity of DNA as pure. The DNA concentration was adjusted to 100 ng μl^{-1} for the 16S rDNA amplification.

3.2.3.2 Characterization of potential endophytic bacterial isolate by 16S rDNA sequence analysis

16S rDNA region of potential isolates was amplified using primers 27F and 1492R. Both primers produced amplified gel product having size of approximately 1400 bp observed on 1 percent Agarose gel (Plate 2) and indicated that the potential antagonistic isolates were of bacteria and belong to the kingdom prokaryotes.

The PCR product was sent for sequencing to Medauxin, Bangalore. 16S rDNA gene sequence of the selected isolate was compared with gene sequence available at NCBI database through BLASTN search (https://www.ncbi.nlm.nih.gov). Multiple sequence alignment of CREB 9, CREB 21 and CREB 37 was performed by using Clustal Omega. CREB 9, CREB 21 and CREB 37 exhibited close similarity of 97.75, 98.48 and 93.28 percent with *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. The sequence of the isolates was submitted to NCBI gene Bank and got Accession numbers such as MN 400679, MN 400682 and MN 400680 for CREB 9 (*E. cloacae*), CREB21 (*P. aeruginosa*) and CREB 37 (*B. subtilis*) respectively.

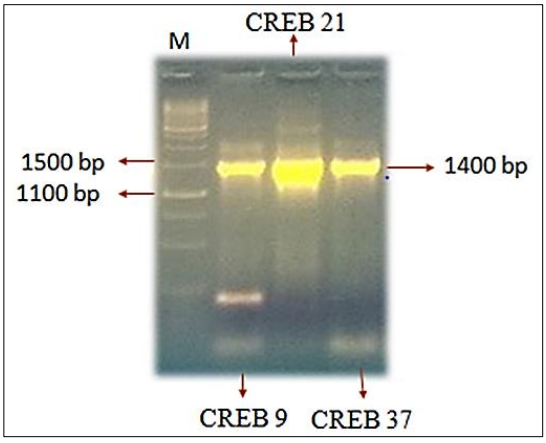

Fig 2: Plate 2: Amplification product of 16S rDNA with 27F and 1492R ribosomal DNA primers from CREB-9, CREB 21 and CREB 37 isolates

3.3 Evaluation of the efficacy of endophytic bacterial bioformulation and extracted antibiotic substances under glasshouse conditions

3.3.1 Mass multiplication of *R. Bataticola*

The virulent pathogen isolates (CRb-9) which exhibited maximum percent disease incidence (100%) during pathogenecity test in pot culture was selected for glass house studies. The pathogen isolate (CRb-9) was mass multiplied on sorghum seeds and added to soil @100 g kg^{-1} at the time of sowing.

3.3.2 Preparation of talc based formulation of potential endophytic bacterial antagonist *B. Subtilis* (CREB37)

The bacterial endophytic isolate (CREB 37-Bacillus subtillis) which exhibited maximum number of positive reactions to performed biochemical tests were considered as a effective isolate and used for glass house studies.

Talc based formulation of potential bacterial biocontrol agent B. subtilis (CREB37) was prepared by following the standard procedure (Plate 3). The population was estimated in talc based formulation and it was 1.0 x 108 cfu g-1. The talc based formulation of potential biocontrol agent B. subtilis (CREB 37) was applied @100 g per pot before sowing.

Fig 3: Plate 3; Talc based formulation of potential endophytic bacterial isolate CREB-37

3.3.3 Effect of different treatments on disease incidence and plant growth parameters (Potculture)

The data on percent germination, plant height, root length, dry weight of the plant and percent disease incidence in each of the treatment was recorded and presented in Table 5.

Table 5: Evaluation of the efficacy of endophytic bacterial bioformulation (talc based) and extracted antibiotic substances under greenhouse conditions in pot culture

S. No.	Treatments	Germination (%)	Plant height (cm)	Root length (cm)	Dry weight (g)	Disease incidence (%)
1.	T_1: Seed treatment with potential bacterial antagonist (Talc formulation) @ 10 g kg^{-1}	86.67^{abc} (72.27)	16.50^{g}	7.60^{f}	0.29^{h}	53.33^{bc} (46.90)
2.	T_2: Seed treatment with antibiotic substances @ 100 ppm	100.00^{a} (90.00)	17.60^{f}	8.30^{e}	0.35^{g}	46.67^{cd} (43.06)
3.	T_3: Seed treatment with antibiotic substances @ 200 ppm	100.00^{a} (81.14)	18.47^{e}	9.80^{d}	0.38^{f}	40.00^{de} (39.22)
4.	T_4: Seed treatment with antibiotic substances @ 500 ppm	100.00^{a} (90.00)	18.70^{e}	9.97^{d}	0.41^{e}	40.00^{de} (39.22)
5.	T_5: Soil application with talc based formulation of potential bacterial antagonist @ 100 g pot^{-1}	93.33^{ab} (81.14)	23.70^{b}	12.67^{b}	0.50^{b}	20.00^{f} (26.55)
6.	T_6: Foliar spray with antibiotic substances @ 500 ppm	80.00^{bcd} (68.05)	14.87^{h}	6.90^{g}	0.24^{i}	60.00^{b} (50.75)
7.	T_7: T_1+T_5	100.00^{a} (90.00)	25.93^{a}	13.60^{a}	0.56^{a}	6.66^{g} (0.00)
8.	T_8: T_3+T_6	100.00^{a} (90.00)	20.63^{d}	11.13^{c}	0.44^{d}	40.00^{de} (39.22)
9.	T_9: T_4+T_6	100.00^{a} (90.00)	21.90^{c}	12.57^{b}	0.47^{c}	26.67^{f} (30.78)
10.	T_{10}: Control (treatment with sterile distilled water)	66.67^{d} (54.97)	11.63^{i}	4.93^{h}	0.15^{j}	100.00^{a} (90.00)
C.D (P=0.05)		16.57 (18.49)	0.39	0.33	0.01	10.85 (6.47)
SEm (±)		5.58 (6.22)	0.13	0.11	0.01	3.65 (2.18)
C.V.		10.50 (13.35)	1.21	1.97	2.09	14.82 (9.29)

Percent germination

Maximum percent germination (100%) was recorded in T2 (seed treatment with antibiotic substances @100 ppm), T3 (seed treatment with antibiotic substances @200 ppm, T4 (seed treatment with antibiotic substances @500 ppm, T7 (seed treatment with potential bacterial antagonist (Talc formulation @10 g kg-1, T8 (seed treatment with antibiotic substances @200 ppm + foliar spray with antibiotic substances @500 ppm, T9 (seed treatment with antibiotic substances @500 ppm + foliar spray with antibiotic substances @500 ppm which were at par among them and with the treatments T5 (93.33%), T1 (86.67%).

It was evident from the Table 5 that least germination percentage (66.66%) was recorded in T10 (control), which was at par with T6 (80.00).

Plant height (cm)

It was evident from the data (Table 5) maximum plant height (25.93 cm) was recorded in treatment T7 (seed treatment with potential bacterial antagonist Talc formulation @10 g kg-1 + soil application with talc based formulation of potential bacterial antagonist @100 g pot-1) followed by T5 (23.70 cm), T9 (21.90 cm) and T8 (20.63 cm) which were significantly differed among them. The least plant height (11.63 cm) was recorded in control (T10).

Root length (cm)

It was evident from the data (Table 5) significantly maximum root length was recorded in Treatment T7 (30.60 cm) followed by T5 (12.67 cm), T9 (12.57 cm), T8 (11.13 cm), T4 (9.97cm), T3 (9.80 cm), T2 (8.30 cm), T1 (7.60 cm) and T6

(6.90 cm) and least (4.93 cm) was recorded in control (T10).

Plant dry weight (g)

The Maximum plant dry weight (0.56 g) was recorded in T7 (seed treatment with potential bacterial antagonist Talc formulation @10 g kg-1 + soil application with talc based formulation of potential bacterial antagonist @100 g pot-1) followed by T5 (0.50 g), T9 (0.47g), T8 (0.44), T4 (0.41 g), T3 (0.38), T2 (0.35g), T1 (0.289g), T6 (0.24g). From the data least dry weight (0.15 g) was recorded in inoculated control (T10). All the treatments were significantly differed among them.

Disease incidence (%)

From the data (Table 5) it was evident that all the treatments were superior over control in reducing percent disease incidence. Significantly least percent disease incidence (6.66) was recorded in T7 (seed treatment with potential bacterial antagonist talc formulation @10 g kg-1 + soil application with talc based formulation of potential bacterial antagonist @100 g pot-1) followed by T5 (20.00), T9 (26.67), T8 (40.00), T4 (40.00), T3 (40.00), T2 (46.67), T1 (53.33) and T6 (60.00) and highest (100%) disease incidence was observed in inoculated control (T10).

Percent disease incidence was low when antagonists were applied in combination (seed treatment + soil application) when compared to seed treatment with antagonist or antibiotic substances and foliar spray with antibiotic substances. This might be due to the application of a greater number of antagonist propagules which have multiplied at faster rate, persist for longer period in the soil and inhibited the growth of the pathogen.

Similarly, germination percent, plant height, root length and dry weight of plants were recorded maximum when the antagonist (talc formulation) or antibiotic applied treatments compared with control. This might be due to the antagonists and their antibiotic compound have some stimulator effect on seed treatment due to the presence of some hormones in their secondary metabolites in addition to antibiotic compounds.

Thus, overall the treatment T7 (seed treatment with potential bacterial antagonist talc formulation @10 g kg-1 + soil application with talc based formulation of potential bacterial antagonist @100 g pot-1) showed maximum germination percent, plant height (cm), root length (cm), dry weight (g) and least disease incidence compared with other treatments this might be due to application of more number of propagules in the form of seed treatment and soil application which have multiplied rapidly, persistence for longer period in the soil and presence of some hormones in their secondary metabolites which helps in plant growth.

4. Discussion

The isolates of R. bataticola collected from different agroclimatic zones had shown huge variation in their morphological and cultural parameters *viz*., growth pattern, growth rate, colony colour, mycelial characters, morphology of the sclerotia and sclerotial initiation time (Sharma *et al*. 2004). The morphological and cultural variation in R. bataticola has been exhibited in different hosts *viz*., sunflower, cowpea, pearl millet, groundnut, and bean (Ndiaye, 2007., Fernandez *et al*. 2006., Suriachandraselvan and Seetharaman, 2003., Atiq *et al*. 2001., Okwulehie, 2001 and Rantoo *et al*. 1997) [4, 19, 31, 34, 39, 51].

Similar studies on cultural and morphological characters of R. bataticola were conducted by Sharma *et al*. (2012) [32] and Gupta *et al*. (2012) [23]. Sharma *et al*. (2012) [32] reported the variation among 94 isolates of R. bataticola collected from chickpea and concluded that the light black (52.12%) colony colour was more predominant. Different shapes of sclerotia *viz*., oblong, ellipsoid, irregular and round type were also recorded. The isolates varied for length and width of sclerotia. Most of the isolates grew very fast and covered the plate within 96 hrs, while other isolates such as RB 21, RB 31, RB 7, RB 84 and RB 87 grew slowly and showed appressed growth. Sclerotial time of initiation varied from 36 to 48 hrs after inoculation. Sclerotial intensity varied from very less to high and was moderate in majority of the isolates. These findings were in accordance with observations recorded in the present study.

Similarly, Gupta *et al*. (2012) [23] when worked with forty isolates of R. bataticola of chickpea collected from Madhya Pradesh, Chhattisgarh, Gujarat and Maharashtra found the sclerotial size ranging from 103.3-117.2 x 90.1-106.5 µm (bigger size) to 72.7-87.5 x 57.1-73.5 µm (smaller size). Based on sclerotial morphology, they categorized the isolates into two groups' *viz*., oblong shape with irregular edges and round with regular edges. These results were in agreement with the present work of difference in sizes and shapes of sclerotia and results revealed that the morphological characters of the R. bataticola vary with the isolate and age of the culture. Similar studies were also done by Byadgi and Hegde (1985) [9], Vishwadar and Sarbhoy (1993) [57], Shekhar *et al*. (2006) [47], Sundravadana *et al*. (2011) [50] and Dhakar *et al*. (2019) [12].

In the present study, Among the 14 isolates of endophytic bacterial isolates Gram negative (7) and Gram positive (7) were present in equal proportion and all were rod shaped bacteria.

The results are in line with Vetrivelkalai *et al*. (2010) [54] who found that Gram negative and Gram positive endophytic bacterial isolates were present in almost equal proportion in the roots of different crops. While, in contrary, Sgroy *et al*. (2009) [44] reported that presence of 68.9% Gram positive bacteria and 31.1% Gram negative bacteria in the root of Prosopis strombulifera.

Similarly, Padder *et al*. (2017) [35] reported that resulting of the colony characterization of 81 endophytic bacterial isolates circular forms dominated, likewise the colonies with entire margins and convex elevation dominated among all the isolates. Similar studies were also done by Aravind *et al*. (2009), Goryluk *et al*. (2009) [22], Zhang *et al*. (2012) [12] and Mbai *et al*. (2013) [27].

In this study, out of 11 biochemical tests performed, the isolates such as CREB 21, CREB 9 and CREB 37 showed positive for 5 or above biochemical tests. So, which were considered as most potential and used for molecular characterization. The results are in accordance with the findings of Bhutani *et al*. (2018) [8] who reported that among 22 endophytic bacterial isolates tested all the isolates produced ammonia and only four isolates showed positive for siderophore production. While, Chhabra and Sharma (2019) [10] reported that among the 263 non-rhizobial endophytic bacterial isolates 74.4%, 12.6%, 37.4% and 55.7% showed positive for catalase, citrate utilization, methyl red and Voges Proskeur tests respectively. Ferchichi *et al*. (2019) [18] observed that among the 22 bacterial endophytes, all the isolates exhibited IAA production, 12 isolates showed positive for siderophore production and only four isolates

produced HCN. Similar reports were also obtained by Bernal *et al*. (2002) [6], Aravind *et al*. (2009) and Goryluk *et al*. (2009) [22].

Based on the 16S rDNA sequence of the potential isolates they were identified as a E. cloacae, P. aeruginosa and B. subtilis. The results are in line with the Misbah *et al*. (2005) [28] who identified the Acineto bacter of clinical isolates by amplification of 16S rDNA genome region consisting of approximately 1500 nucleotides using three primer pairs *viz*., 27F, 780R; 529F, 1099R; 925F and 1491R. While, El-Deeb *et al*. (2012) have isolated a total of 38 bacterial endophytes and 16S rDNA sequence analysis revealed that they were phylogenetically closely related to genus Acetobacter, Acinetobacter, Methylococcus, Bacillus, Micrococcus and Planococcus.

Similarly, Paul *et al*. (2013) [36] have isolated a diverse range of bacterial taxa from leaves, stems and roots of chilli pepper plants and identified as *Actinobacter*, *Arthrobacter*, *Enterobacter*, *Escherichia*, *Kitasatospora*, *Pandoraea*, *Pantoea*, *Rhizobium*, *Ralstonia*, *Paenibacillus* and *Serratia* based on 16S rDNA sequence analysis. While, Bhavani *et al*. (2015) [7] conducted 16S rDNA sequence analysis for identification of potential endophytic bacterial isolate B5 and phylogenetic analysis revealed that the isolate was showing 99% similarity with the Pseudomonas aeruginosa PAO1. The similar reports were also obtained by Nhu and Diep (2017) [33], Rosales *et al*. (2017) [40], Abbas *et al*. (2018) [1], Abdallah *et al*. (2018) [2], Vinayarani and Prakash (2018) [56] and Ferchichi *et al*. (2019) [18].

Rajeswari *et al*. (1999) [37] and Durai, (2004) [16] reported that sterilized sorghum seeds were found to be suitable for mass multiplication of R. bataticola. Aghakhani and Dubey (2009) [3] and Veena and Reddy (2016) [53] also used sterilized sorghum grains for mass multiplication of R. bataticola. Rangeshwaran *et al*. (2001) [38] also prepared talc based formulations of Pseudomonas fluorescens (PDBCA 2) and Pseudomonas putida (PDBCAB 19) which were effective in inhibiting the wilt and root rot of chickpea in vitro. While, Sundaramoorthy and Balabaskar (2012) [49] prepared the talc based formulation of endophytic B. subtilis EPCO16 and rhizospheric P. fluorescens for testing the bio efficacy against early blight of tomato incited by Alternaria solani under greenhouse conditions. Similarly, Veena and Reddy (2016) [53] mass multiplied the potential root endophytic bacterial isolate CREB-16 in nutrient broth and prepared the talc-based formulation for using in integrated disease management.

In the present study T7 (seed treatment with potential bacterial antagonist talc formulation @10 g kg-1 + soil application with talc based formulation of potential bacterial antagonist @100 g pot-1) showed maximum germination percent, plant height (cm), root length (cm), dry weight (g) and least disease incidence. This results were in line with the reports of Nautiyal (1997) [30] who reported that seed bacterization with Pseudomonas fluorescens NBRI1303 increased the germination of chickpea by 25%. Whereas, Kim *et al*. (2012) found that a purified antibiotic namely FP-1 was effective in inhibiting fusarium wilt of tomato at 10 µg ml-1, with no phytotoxicity effects even at 500 µg ml-1 under pot culture conditions.

The results were also in agreement with the findings of Nassif *et al*. (2015) [29] who reported that the inoculated plants with endophytic bacterial isolates showed significant differences in all examined vegetative parameters (root length, shoot length, root fresh and dry weight and shoot fresh and dry weight) as compared to control. While, Tamreihao *et al*. (2016) [52] conducted a pot trial experiment to test the efficacy of talcum powder formulation of Streptomyces corchorusii strain UCR3-16 against sheath blight of rice caused by R. solani. The results showed that significant enhancement in shoot length, weight of shoot and root, total grain yield and weight of grains in rice plants. Talcum formulation also significantly reduced the sheath blight disease in rice leaves.

Similarly, Dey *et al*. (2019) [11] observed that HR1 isolate of endophytic bacteria significantly increased the germination percentage, shoot and root length, shoot and root dry weight in the treated plants compared to the healthy control and the M. phaseolina infected plant. While, Sendhilvel *et al*. (2005) [42] used Pseudomonas fluorescens isolate SVPF2 in combination of seed treatment and soil application against cowpea root rot and recorded less disease incidence and maximum root length (22.38 cm) under greenhouse conditions.

5. Summery and Conclusion

Variability in the cultural and morphological characters of 22 isolates of R. bataticola were studied by growing on potato dextrose agar medium. Among all the isolates CRb 10 taken significantly least time (2.50 days) to occupy the full plate and higher radial growth at 42 hrs was observed with CRb 10 isolate (75.78 mm). However, fluffy colonies were produced by CRb 2 and CRb 15 isolates while, velvety colony texture was observed with CRb 13 and remaining all the isolates showed appressed colony texture. Similarly, out of 22, jet black, light black and grey colour mycelia was observed in total of seventeen, four and one isolates respectively. Among all the isolates, for sclerotial initiation significantly maximum time was taken by CRb 18 isolate and least time was taken by CRb 16 (1.83 days). Among the isolates higher number of sclerotia per 10 x microscopic field and sclerotial size was observed with CRb 16 (33.33) and CRb 6 (119.32 µm) respectively. Similarly, among 22 isolates, irregular, ovoid and round shaped sclerotia were observed in total of three, nine and ten isolates respectively.

Among the 14 potential isolates of endophytic bacteria, Gram negative (7) and Gram positive (7) were present in equal proportion and all were rod shaped bacteria.

Out of the 14 endophytic potential bacterial isolates, a total of 9, 7, 2, 1, 2, 3, 3, 13, 6, 3 and 0 showed positive for amylolytic activity (starch hydrolysis), proteolytic activity (Gelatin hydrolysis), lactose utilization, indole production methyl red test, Voges-Proskeur test, citrate utilization, presence of catalase activity, ammonia production, HCN production and siderophore production respectively.

16S r DNA sequence analysis was used to identify the potential bacterial isolates such as CREB 9, CREB 21 and CREB 37 and which were exhibited close similarity of 97.75, 98.48 and 93.28 percent with Enterobacter cloacae, Pseudomonas aeruginosa and Bacillus subtilis respectively. The sequence of the isolates was submitted to NCBI gene Bank and got Accession numbers such as MN 400679, MN 400682 and MN 400680 for CREB 9 (E. cloacae), CREB 21 (P. aeruginosa) and CREB 37 (B. subtilis) respectively.

Talc based formulation of potential bacterial biocontrol agent B. subtilis (CREB 37) was prepared and the bio efficacy of formulation and crude antibiotic extract was tested under glasshouse in pot culture.

Maximum percent germination (100%) was recorded in T2

(seed treatment with antibiotic substances @100 ppm), T3 (seed treatment with antibiotic substances @200 ppm, T4 (seed treatment with antibiotic substances @500 ppm, T7 (seed treatment with potential bacterial antagonist (Talc formulation @10 g kg-1, T8 (seed treatment with antibiotic substances @200 ppm + foliar spray with antibiotic substances @500 ppm, T9 (seed treatment with antibiotic substances @500 ppm + foliar spray with antibiotic substances @500 ppm and least (66.66%) was recorded in T10 (control).

Higher plant height (25.93 cm), root length (30.60 cm), plant dry weight (0.56 g) and least disease incidence (6.66%) was recorded with T7 (seed treatment with potential bacterial antagonist talc formulation @10 g kg^{-1} + soil application with talc based formulation of potential bacterial antagonist @100 g pot^{-1}) compared with inoculated control.

In conclusion, endophytic bacteria is the source for plenty of secondary metabolites and the potential source of antibiotics and plant growth regulators, which helps in the disease suppression and plant growth promotion. Exploitation of endophytes in the agriculture improve the plant health and ultimately leads to potential yields.

6. References

1. Abbas H, Patel RM, Parekh VB. Culturable endophytic bacteria from halotolerant *Salicornia brachata* L: Isolation and plant growth promoting traits. Indian Journal of Applied Microbiology 2018;21(1):10-21.
2. Abdallah RAB, Jabnoun-Khiareddine H, Nefzi A, Daami-Remadi M. Evaluation of the growth-promoting potential of endophytic bacteria recovered from healthy tomato plants. Journal of Horticulture 2018;5(234):1-10.
3. Aghakhani M, Dubey SC. Morphological and pathogenic variation among isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea. Indian Phytopathology 2009;62(2):183-189.
4. Atiq M, Shabeer A, Ahmed I. Pathogenic and cultural variation in *Macrophomina phaseolina*, the cause of charcoal rot in sunflower. Sarhad Journal of Agriculture 2001;2:253-25.
5. Bacon CWH, Hinton A. Growth-inhibiting effects of concentrations of fusaric acid on the growth of Bacillus mojavensis and other biocontrol Bacillus species. Journal of Applied Microbiology 2006;100(1):185-194.
6. Bernal G, Illanes B, Ciampi L. Isolation and partial purification of a metabolite from a mutant strain of Bacillus sp. with antibiotic activity against plant pathogenic agents. Electronic Journal of Biotechnology 2002;5(1):12-20.
7. Bhavani DA, Kumari MA, Kumar AK. Chickpea endophytic bacteria inhibiting dry root rot fungus *Rhizoctonia bataticola*. International Journal of Scientific and Engineering Research 2015;6(2):83-85.
8. Bhutani N, Maheshwari R, Suneja P. Isolation and characterization of plant growth promoting endophytic bacteria isolated from *Vigna radiata*. Indian Journal of Agricultural Research 2018;52(6):596-603.
9. Byadgi AS, Hegde RK. Variation among the Isolates of *Rhizoctonia bataticola* (Taub.) Butler, *Macrophomina phseolina* (Tassi.) Goid from different host Plants. Indian Phytopathology 1985;38(2):297-301.
10. Chhabra D, Sharma P. Non rhizobial endophytic bacteria from chickpea (*Cicer arietinum* L.) tissues and their antagonistic traits. Journal of Applied and Natural Science 2019;11(2):346- 351.
11. Dey S, Dutta P, Majumdar S. Biological control of *Macrophomina phaseolina* in *Vigna mungo* L. by endophytic Klebsiella pneumoniae HR1. Jordan Journal of Biological Sciences 2019;12(2):219-227.
12. Dhakar MK, Prajapati RK, Chadar V, Karcho S. Prevalence and incidence of dry root rot disease in chickpea caused by (*Rhizoctonia bataticola* (Taub) Butler at Tikamgarh district of Madhya Pradesh. Techno Fame-A Journal of Multidisciplinary Advance Research 2019;8(1):39-44.
13. Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Government of India. Area, production and productivity of gram in India 2017-2018 2018. www.indiastat.com.
14. Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Government of India. Selected state-wise area, production and productivity of gram in India 2017-2018 2018.
15. www.indiastat.com.
16. Dixit GP, Srivastava AK, Singh NP. Marching towards self-sufficiency in chickpea. Current Science 2019;116(2):239-242.
17. Durai M. Management of charcoal rot of sesame (*Sesamum indicum* L.) incited by *Macrophomina phaseolina* (Tassi.) Goid. M.Sc. (Ag.) Thesis. Acharya N.G. Ranga Agricultural University Hyderbad, Andhra Pradesh, India 2004.
18. El-Deeb B, Bazaid S, Gherbawy Y, Elhariry H. Characterization of endophytic bacteria associated with rose plant *(Rosa damascena trigintipeta*) during flowering stage and their plant growth promoting traits. Journal of Plant Interactions 2012;7(3):248-253.
19. Ferchichi N, Toukabri W, Boularess M, Smaoui A, Mhamdi R, Trabelsi D. Isolation, identification and plant growth promotion ability of endophytic bacteria associated with lupine root nodule grown in Tunisian soil. Archives of Microbiology 2019, 1-16.
20. Fernandez RB, Santiago AS, Delgado SH, Mayek-Perez N. Characterization of Mexican and Non-Mexican isolates of *Macrophomina phaseolina* based on morphological characteristics, pathogenicity on bean seeds and endoglucanase genes. Journal of Plant Pathology 2006;88(1):53-60.
21. Gaur PM, AJukanti KA, Sushil SSK, Chaturvedi S, Basu PS, Babbar A *et al*. Climate change and heat stress tolerance in chickpea. In Tuteja, N. and Gill, S.S (eds.) Climate Change and Plant Abiotic Stress Tolerance, Wiley-VCH Verlag GmbH & Co KGaA, Weinheim, Germany 2014, 839–855.
22. Ghosh R, Sharma M, Telangre R, Pande P. Occurrence and distribution of chickpea diseases in central and southern parts of India. American Journal of Plant Sciences 2013;4:940-944.
23. Goryluk A, Burlaga HR, Miec SJ. Isolation and characterization of bacterial endophytes of *Chelidonium majus* L. Polish Journal of Microbiology 2009;58(4):355-361.
24. Gupta O, Patel S, Mishra M. Diversity in isolates of *Rhizoctonia bataticola* causing dry root rot in chickpea from Central India. JNKVV Research Journal 2012;46(3):376-381.
25. Jasim B, Benny R, Sabu R, Mathew J, Radha Krishnam EK. Metabolite and mechanistic basis of antifungal

property exhibited by endophytic Bacillus amyloliquefaciens BMB 1. Applied Biochemistry and Biotechnology 2016;7:1-15.
26. Julian Marchesi R, Takuchi Sato, Weightman JW, Tracey A, Martin JC, Fry Hiom SJ *et al*. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial rRNA. Applied and Environmental Microbiology 1998, 795-799.
27. Kumar A, Sarma YR, Anandaraj M. Evaluation genetic diversity of *Ralstonia solanacearum* causing bacterial wilt of ginger using Rep-PCR and RFLP-PCR. Current Science 2004;87:1555–1561.
28. Mbai FN, Magiri EN, Matiru VN, Nyambati VCS. Isolation and characterization of bacterial root endophytes with potential to enhance plant growth from Kenyan Basmati rice. American International Journal of Contemporary Research 2013;3(4):25-40.
29. Misbah S, Hassan Yusof MY, Hanifah YA, Abu Bakar S. Genomic species identification of Acinetobacter of clinical isolates by 16S rDNA sequencing. Singapore Medical Journal 2005;46(9):461-466.
30. Nassif FA, Ata AM, Mahmoud MA, Hassan EA, Soltan HA. Phylogenetic relationship, growth promotion and anti-phytopathogenic activity of endophytic bacteria isolated from tomato. Minia Journal of Agricultural Research and Development 2015;35(1):177-194.
31. Nautiyal CS. Selection of chickpea rhizosphere competent Pseudomonas fluorescens NBRI1303 antagonistic to *Fusarium oxysporum* f.sp. ciceri, *Rhizoctonia bataticola* and *Pythium* spp. Current Microbiology 1997;35:52-58.
32. Ndiaye M. Ecology and management of charcoal rot (*Macrophomina phaseolina*) on cowpea in the Sahel. Ph. D Thesis Wageningen University, Netherlands 2007.
33. Nene YL, Reddy MV, Haware MP, Ghanekar AM, Amin KS, Pande S *et al*. Field diagnosis of chickpea diseases and their control. Information Bulletin 2012;28:1-58.
34. Nhu VTP, Diep CN. Isolation and characterization of endophytic bacteria in soybean (*Glycine max* L. (Merrill)) cultivated on alluvial soil of Can Tho city, Vietnam. International Journal of Innovations in Engineering and Technology 2017;8:202-221.
35. Okwulehie IC. Physiological studies in groundnuts (*Arachis hypogeal* L.) infected with *Macrophomina phaseolina* (Maub.) Ashby. International Journal of Tropical Plant Diseases 2001;19:25-37.
36. Padder SA, Dar GH, Bhat ZA, Verma K, Wani AB. Morphological metabolic and biochemical characterization of bacterial root endophytes associated with brown sarson (*Brassica rapa* L.). Journal of Pharmacognosy and Phytochemistry 2017;6(2):226-232.
37. Paul NC, Ji SH, Deng JX, Yu SH. Assemblages of endophytic bacteria in chili pepper (*Capsicum annuum* L.) and their antifungal activity against phytopathogens *in vitro*. Plant Omics Journal 2013;6(6):441-448.
38. Rajeswari B, Chandrasekhara Rao K, Pramod Chandra Kumar C. Efficacy of antagonists and carbendazim against dry root rot of mungbean incited by *Macrophomina phaseolina* (Tassi.) Goid under glasshouse conditions. Journal of Biological Control 1999;13:93-99.
39. Rangeshwaran R, Prasad RD, Anuroop CP. Field evaluation of two bacterial antagonists, Pseudomonas putida (PDBCAB 19) and Pseudomonas fluorescens (PDBCAB 2) against wilt and root-rot of chickpea. Journal of Biological Control 2001;15(2):165-170.
40. Rantoo RS, Jain KL, Bhatnagar MK. Variations in *Macrophomina phaseolina* isolates of Ash-gray stem blight of Cowpea. Journal of Mycology and Plant Pathology 1997;27:91-92.
41. Rosales EP, Meléndez LA, Puente ME, Juarez RV, Guzman EQ, Savin TZ *et al*. Isolation and characterization of endophytic bacteria associated with roots of jojoba (*Simmondsia chinensis* (Link) Schneid). Current Science 2017;112(2):396-401.
42. Schulz B, Boyle C. The endophytic continuum. Mycological Research 2006;109:661-686.
43. Sendhilvel V, Bhuvaneswari D, Kanmozhi S, Kavitha K, Raguchander T. Management of cowpea root rot caused by *Macrophomina phaseolina* (Tassi.) Goid using plant growth promoting *Rhizobacteria*. Journal of Biological Control 2005;19(1):41-46.
44. Sessitsch A, Reiter B, Berg G. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. Canadian Journal of Microbiology 2004;50(4):239-249.
45. Sgroy V, Cassan F, Masciarelli O, Papa MF, Lagares A, Luna V. Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera. Applied Microbiology and Biotechnology 2009;85(2):371-381.
46. Sharma YK, Gaur RB, Bisnoi HR. Cultural, morphological and physiological variability in *Macrophomina phaseolina*. Journal of Mycology and Plant Pathology 2004;34:532-534.
47. Shekhar M, Sharma RC, Rakshit S, Yadav P, Singh L, Dutta R. Genetic variability in Macrophomina phaseolina (Tassi.) Goid. incitant of charcoal rot of maize in India. Indian Phytopathology. 2006;59(4):453-459.
48. Stone JK, Polishook JD, White JF. Endophytic fungi. (In Mueller, G.M., Bills, G.F and Foster, M.S. eds) Biodiversity of fungi. Inventory and monitoring methods. Elsvier Academic Press, San Diego, U.S.A. 2004, 241-270.
49. Sundaramoorthy S, Balabaskar P. Consortial effect of endophytic and plant growth promoting rhizobacteria for the management of early blight of tomato incited by Alternaria solani. Journal of Plant Pathology and Microbiology 2012;3(7):1-4.
50. Sundravadana S, Alice D, Thirumurugan S. Exploration of variability in colony morphology and virulence of Rhizoctonia bataticola isolates causing dry root rot of pulses. Global Journal of Biosciences and Biotechnology. 2012;1(1):91-97.
51. Suriachandraselvan M, Seetharaman K. Effect of culture media on growth and sclerotial production of different isolates of Macrophomina phaseolina infecting sunflower. Journal of Mycology and Plant Pathology 2003;33:226-229.
52. Tamreihao K, Debanand S, Nimaichand S, Singh ES, Reena P, Singh SH *et al*. Biocontrol and plant growth promoting activities of a Streptomyces corchorusii strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. Microbiological Research. 2016;192:260-270.
53. Veena GA, Reddy NPE. Integrated disease management of dry root rot of chickpea. International Journal of

Applied Biology and Pharmaceutical Technology. 2016;7(2):45-54.
54. Vetrivelkalai P, Sivakumar M, Jonathan EI. Biocontrol potential of endophytic bacteria on Meloidogyne incognita and its effect on plant growth in bhendi. Journal of Biopesticides. 2010;3(2):452-457.
55. Vidhyasekaran P, Muthamilan M. Development of formulations of Pseudomonas fluorescens for control of chickpea wilt. Plant Disease. 1995;79(8):782-786.
56. Vinayarani G, Prakash HS. Growth promoting rhizospheric and endophytic bacteria from Curcuma longa L. as biocontrol agents against rhizome rot and leaf blight diseases. Plant Pathology Journal. 2018;34(3):218-235.
57. Vishwadar, Sarbhoy AK. An a typical isolate of Rhizoctonia bataticola. Indian Phytopathology, 1993, 245-246.
58. Zhang YZ, Chen WF, Li M, Sui XH, Liu HC, Zhang XX *et al*. Bacillus endoradicis sp. nov., an endophytic bacterium isolated from soybean root. International Journal of Systematic and Evolutionary Microbiology. 2012;62:359-363.
59. Zvyagintsev DG. Methods for Soil Microbiology and Biochemistry. Moscow: Moscow State University [in Russian] 1991.