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Pravin Patel
Department of Plant Pathology College of Agriculture JNKVV Jabalpur, Madhya Pradesh, India

AR Wasnikar
Department of Plant Pathology College of Agriculture JNKVV Jabalpur, Madhya Pradesh, India

Gyanendra Singh
Department of Plant Breeding and Genetics, College of Agriculture JNKVV Jabalpur, Madhya Pradesh, India

M Surya Prakash Reddy
Department of Plant Pathology College of Agriculture JNKVV Jabalpur, Madhya Pradesh, India

Corresponding Author:
M Surya Prakash Reddy
Department of Plant Pathology College of Agriculture JNKVV Jabalpur, Madhya Pradesh, India

Evaluation of non-rhizospheric endophytic bacteria for the management of dry root rot of chickpea

Pravin Patel, AR Wasnikar, Gyanendra Singh and M Surya Prakash Reddy

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Abstract
Chickpea (*Cicer arietinum* L.) is an important pulse crop in India and chief source of dietary protein in the vegetarian diet. The important Destructive disease in chickpea is dry root rot caused by necrotrophic fungus *Rhizoctonia bataticola* (Taub.) Butler emerging as a serious threat to the production worldwide. The screening of endophytes of leaf and stem isolates under dual culture used as two media PDA and TSA. The 12 isolates are significant on but three isolates are EBS-2, EBS-3, EBS-4 higher suppression towards on *Rhizoctonia bataticola*. These three isolates used as further methods that roll towel method (vitro) and net house (Pot Cultivation). The roll towel observations to be recorded as Germination percentage, Root, shoot lengths and vigour index. The EBL-3 and EBL-4 with highest germination percentage, Root length were EBL-2, 3 and shoot length, vigour index of EBL-3 highest percentage. The effect of EB on *Rhizoctonia bataticola* under pot culture observations were recorded that phenological parameters are Germination percentage, Pre-Post, Total mortality, Shoot, Root lengths and Fresh dry weights with different cohabitation on disease incidence. The cohabitation of T8EBL (2+3+4) T7EBL (4+2) are significant on four genotypes JG14, JG16, JG62 and JG315 with inoculation of pathogen. The EBL-3 isolate is that significant correlates in field and lab conditions.

Keywords: Chick pea root rot, *Rhizoctonia bataticola*, non rhizosphere, endophytic bacteria phonological parameters

Introduction
Chickpea (*Cicer arietinum* L.) commonly known as “Chana” in Hindi belongs to family Leguminosae and is believed to be originated from south west Asia (Singh, 1993) [19]. It is an important pulse crop and grown in temperate as well as subtropical regions of the world. It is one of the most important food legumes grown worldwide (Saxena, 1990) [18]. India is one of the leading producers accounting a share of about 70 percent in global chickpea production and a leading consumer of chickpea in the world (Reddy and Mishra, 2010) [16]. In India, chickpea is mainly grown in six Indian states *viz.*, Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh together contributes 91% of the total production and 90% of the total area of the country. Madhya Pradesh contributes 3.01 mha area, 3.35 mt of production and an average productivity of 1115 kg/ha (Anonymous, 2016) [3]. The important Destructive disease in chickpea is dry root rot caused by necrotrophic fungus *Rhizoctonia bataticola* (Taub.) Butler emerging as a serious threat to the production worldwide (Pande and Sharma, 2010) [13]. Dry root rot generally appears during late flowering and pod filling stages and the infected plants appear completely dried. Among the several constraints affecting the productivity of chickpea, 10-35% yield losses are due to wilt and dry root rot diseases (Pal, 1998) [12]. *Rhizoctonia bataticola* is a polyphagous soil borne pathogen infecting over 500 plant species worldwide causing huge losses in several crop species. The fungus is seed and soil borne in nature (Dhingra and Sinclair, 1994) [7], however soil borne inoculum is predominant in causing infection and disease development. Pleban *et al*. (1995) [14] tested bacteria from various seeds and plant tissues and reported that a strain of *Pseudomonas fluorescens* and *Bacillus spp.* effectively inhibited growth of one or more of the plant pathogens *viz.*, *Rhizoctonia solani* (46-56%, in bean), *Pythium ultimum* and *Sclerotium rolfsii*

(26-79%). More evidence on protection against fungal pathogens, using *Pseudomonas and Bacillus spp.* as introduced endophytes, comes from studies conducted on crops such as cotton, oilseed rape, tomato, cucumber and peas (Chen *et al*., 1995; Liu *et al.*, 1995; Sturz *et al.*, 1999) [6, 10, 20]. Looking toward the findings on use of endophytic bacteria to control disease of different crops and opportunities to use these bacteria as a cost-effective and eco-friendly measure of disease control, the present research was aimed with the following objectives.

Material and Methods

The following material and methods were used for "Evaluation of non-rhizospheric endophytic bacteria for the management of dry root rot of chickpea". Experiment was conducted at All India Co-ordinated Research Project (AICRP) Lab on Chickpea, Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwa Vidhyalaya (JNKVV), Jabalpur.

Test pathogens: *Rhizoctonia bataticola* causal organism of dry root rot of chickpea.

Seed source

A total of four improved varieties JG14, JG16, JG62 and JG315 of chickpea were selected for the present study. The Seeds were taken from Chickpea Breeding Unit, JNKVV, Jabalpur M.P.

Isolation of endophytic bacteria

Endophytic bacteria were isolated from healthy leaf and stem of chickpea. Leaves and stem were thoroughly washed under running tap water to remove the traces of dust. Leaves and stem were separated from plant and cut into 2-3 cm long. Then surface sterilized for 3-5 times with 2% sodium hypochlorite for 2 times with 70% ethanol and 4 times in sterile distilled water and 1ml of last wash was plated on Tryptic Soya Agar medium (TSA) for sterility check. The tissue was macerated in 9ml of phosphate buffer and 1 ml of macerate was serially diluted upto 10^{-6} dilutions and 1ml of each dilution was pour plated on TSA plates. Single colonies were isolated and streaked on TSA plate and glycerol stocks were prepared and stored at -20 °C for further use (Bhavani *et al.*, 2015) [5].

Experimental details

Table 1: Isolates of Endophytic bacteria

S. No.	Plant Part	No. of isolates
1	**Leaf** (Endophytic bacteria Leaf-EBL)	*Bacillus* sp. (EBL 1)
2		*Pseudomonas* sp. (EBL 2)
3		*Bacillus* sp. (EBL 3)
4		*Pseudomonas* sp. (EBL 4)
5		*Bacillus* sp. (EBL 5)
6		*Xanthomonas* sp. (EBL 6)
7	**Stem** (Endophytic bacteria Stem-EBS)	*Bacillus* sp. (EBS 1)
8		*Pseudomonas* sp. (EBS 2)
9		*Xanthomonas* sp. (EBS 3)
10		*Pseudomonas* sp. (EBS 4)
11		*Xanthomonas* sp. (EBS 5)
12		*Pseudomonas* sp. (EBS 6)

The main objective of this investigation was to evaluate the effect of non-rhizospheric endophytic bacteria on management of dry root rot of chickpea. A total of 12 endophytic bacteria were isolated from leaf and stem of chickpea plant. Among these best three endophytic bacteria were selected based on results of dual culture experiment. Hence, a total of nine treatments combinations of three endophytic bacteria including treated and untreated controls were designed to study their effect on disease incidence and severity as well as other plant characters of chickpea in net house condition. The details of the treatments used are given here as under.T1-Treated control, T2- EBL- 2,T3-EBL- 3,T4-EBL- 4,T5-EBL (2+3) T6- EBL (3+4),T7-EBL (4+2),T8-EBL (2+3+4),T9- (Control) untreated.

Multiplication of endophytic bacteria

Culture of individual isolate to be tested, were grown on TSA for at least 3 days. A loop full if these culture was then transferred to flasks containing 150 ml of King's B Broth and incubated at room temperature on an orbital shaker for 24 hours at 200 rpm. Bacterial cell were harvested by centrifuging at 7000 rpm for 10 minutes and re-suspended in 10 ml $MgSO_4$. The separated cell were used for assay of endophytes (Rangeshwaran *et al.*, 2002) [15].

Purification and identification of test pathogen

The cultures of *Rhizoctonia bataticola* was purified by sub culturing the hyphal tip method and maintained by mass transfer on potato dextrose agar medium at room temperature. *Rhizoctonia bataticola* produced radial hyaline colonies, which later become carbonaceous brown to black mycelium was septate and dark brown in colour, typical right angled branching of mycelium was observed. Sclerotia were black, varied from spherical to irregular in shape and measured 80 to 85 µm in diameter. Pycnidial production was not observed in culture plates. The colony characters and morphological characters of mycelium and sclerotia were in agreement with the descriptions of Sajeena *et al.* (2004) [17].

Pathogenicity test and mass multiplication of *Rhizoctonia bataticola*

Pathogenicity test was conducted by soil infestation method. The bags containing sorghum seeds were autoclaved at 15 psi for 20 min. The pathogen was mass multiplied on sterilized sorghum grains in 250 ml conical flasks. Then the flasks were inoculated with 4 discs of 5.0 mm diameter mycelial growth of three days old culture of *Rhizoctonia bataticola* grown on PDA plate. The flasks were incubated at 28 ± 2 °C for seven days. Then the inoculums was mixed with sterilized soil @ 10 g kg^{-1} soil and filled in the pots (30 cm diameter). The seeds of chickpea were sown simultaneously with pathogen inoculation @ 10 seeds per pot and an un-inoculated control was maintained. The plants were observed for root rot symptoms. Each treatment replicated three times (Nene *et al.*, 1981) [11].

Dual culture test

Isolated endophytic bacteria were tested for their antifungal activities against *Rhizoctonia bataticola* in dual culture method. A fungus was inoculated at one end of the TSA and PDA plate and at the other end endophytic bacteria was streaked. The plate with only fungi inoculated on one end without bacteria was kept as control. These plates were incubated at 30 °C for 7days and radial growth was record. Percentage of inhibitions was calculated using the formula given by Bhavani *et al.*, (2015) [5].

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

Where,
I = Per cent inhibition in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment.

Roll-Towel Test
A roll towel method (lSTA, 1976) regularly used for seed vigour testing was used for testing bioefficacy of endophytes. Healthy seeds were first surface sterilized in sodium hypochlorite (1.05%) followed by three changes/washing in sterile water and then inoculated with the bacterial isolate. After air-drying, the seeds were again dipped in mycelial suspension of *Rhizoctonia bataticola* which was replicated in potato dextrose growth. Three replications of fifty seeds with pathogen and four varieties each were randomly counted and placed in coarse blotter paper sheets and covered with a moistened blotter and rolled. Rolls were kept on a butter paper sheet and rolled as a single bundle and incubated in a growth chamber at 25 °C with 80% RH for 8 days. After incubation, germination percentage was noted along with root and shoot length and vigour index was calculated. Vigour index was calculated by multiplying per cent plant stand with some of shoot and root length (Rangeshwaran *et al.*, 2002) [15].

Effect of endophytic bacteria on growth parameters
Data on germination percentage was recorded after 10 days and at the time of maturity plant height (cm), dry weight and fresh weight (g/plant) was calculated the vigor index percentage as follows (Abdul Baki and Anderson 1973) [1].

$$\textbf{Germination (\%)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Vigour index (%) = Germination% × Seedling length on the day of final count

Pre and post-emergence mortality (%)
Pre-emergence mortality was recorded immediate after complete emergence of the plants and post emergence mortality was recorded at 90 days after sowing. The pre- and post-emergence mortality was calculated using the following formula;

$$\text{Pre-emergence mortality (\%)} = \frac{\text{No. of diseased ungerminated seed}}{\text{Number of sown seed}} \times 100$$

$$\text{Post-emergence mortality (\%)} = \frac{\text{No. of seedling collapsed}}{\text{Total no. seedling emerged}} \times 100$$

Total Mortality (%) =Pre-emergence mortality (%) + Post-emergence mortality (%)

Root and shoot length
The plants were carefully uprooted to measure root and shoot length using scale.

Fresh and dry weight (g/plant)
Observations were recorded at the time of maturity. Mature plants were carefully uprooted for measuring fresh weight and dry weight. After measuring length and fresh weight, the seedling were placed between blotting paper and kept at 45ºC for 2-3 days in an oven for drying. The dry weight was recorded in an electronic balance.

Results and Discussions

Table 1: Screening of endophytic bacterial isolates against *Rhizoctonia bataticola* under dual culture

Isolated Number	Isolate Name	Percent growth inhibition on PDA	Percent growth inhibition on TSA	Fungal radial growth (sq. mm) on PDA	Fungal radial growth (sq. mm) on TSA	Bacterial growth (sq. mm) on PDA	Bacterial growth (sq. mm) on TSA
EBL 1	*Bacillus* sp.	8.60 (16.30)	9.09 (17.26)	60.30	55.4	0.40	0.53
EBL 2	*Pseudomonas* sp.	32.00 (34.28)	47.33 (43.10)	53.00	18.00	5.00	4.00
EBL 3	*Bacillus* sp.	21.33 (26.95)	53.00 (46.34)	64.00	15.00	0.67	3.33
EBL 4	*Pseudomonas* sp.	44.67(41.81)	55.33(47.44)	43.67	14.67	3.00	4.67
EBL 5	*Bacillus* sp.	5.80 (10.20)	5.90 (13.10)	65.33	56.80	0.46	0.64
EBL 6	*Xanthomonas* sp.	6.76 (12.34)	7.91 (15.20)	68.30	70.64	0.54	0.71
EBS 1	*Bacillus* sp.	5.20 (9.70)	6.00 (11.80)	61.69	65.70	0.62	0.72
EBS 2	*Pseudomonas* sp.	5.67(11.50)	7.20(13.56)	59.70	61.00	0.82	0.97
EBS 3	*Xanthomonas* sp.	7.35 (14.69)	8.00(17.75)	58.90	62.34	0.59	0.68
EBS 4	*Pseudomonas* sp.	8.00(15.87)	8.50(15.60)	64.74	67.60	0.52	0.81
EBS 5	*Xanthomonas* sp.	5.19(10.00)	7.00(14.50)	62.75	65.62	0.58	0.69
EBS 6	*Pseudomonas* sp.	5.96(12.09)	7.00(16.40)	66.92	69.90	0.67	0.97
	Control	0.00	0.00	80.67	72.00	0.00	0.00
SEm±		0.42	0.53	1.28	0.85	0.44	0.37
C.D.		1.38	1.76	4.24	2.81	1.46	1.23

Figures in parentheses are angular transformed values, PDA- Potato Dextrose Agar, * TSA- Tryptic Soya Agar

Table 2: Evaluation of selected endophytic bacteria for chickpea plant growth under *Rhizoctonia bataticola* (Roll towel method)

S. No.	Treatment	Germination%					Root length (cm)				
		JG14	JG16	JG62	JG315	Mean	JG14	JG16	JG62	JG315	Mean
1	Treated control	66.67	63.33	56.67	63.33	62.50	5.00	5.00	3.67	5.00	4.67
2	EBL -2	80.00	66.67	60.00	66.67	68.34	6.00	6.00	5.00	6.00	5.75
3	EBL -3	83.33	70.00	63.33	70.00	71.67	6.33	5.67	5.33	5.67	5.75
4	EBL -4	80.00	70.00	63.33	70.00	70.83	6.67	5.00	4.67	5.00	5.34
5	Untreated Control	73.33	66.67	60.00	66.67	66.67	5.33	5.33	4.33	5.33	5.08
Mean		76.67	67.33	60.67	67.33	68.00	5.87	5.40	4.60	5.40	5.32
SEm±		4.47	4.30	3.65	4.20	4.27	0.68	0.56	0.47	0.54	0.57
C.D.		13.41	12.90	10.95	12.6	12.81	2.04	1.68	1.41	1.62	1.71

Table 3: Evaluation of selected endophytic bacteria for chickpea plant growth under *Rhizoctonia bataticola* (Roll towel method)

S. No.	Treatment	Shoot length (cm)					Vigour index				
		JG14	JG16	JG62	JG315	Mean	JG14	JG16	JG62	JG315	Mean
1	Treated control	5.00	4.00	3.33	4.00	4.08	663.33	566.67	393.33	566.67	547.50
2	EBL -2	6.67	5.67	3.67	5.67	5.42	1013.33	776.67	546.67	776.67	778.34
3	EBL -3	7.00	5.33	4.67	5.33	5.58	1110.00	770.00	670.00	770.00	830.00
4	EBL -4	7.33	4.67	4.33	4.67	5.25	1126.67	673.33	573.33	673.33	761.67
5	Untreated Control	5.67	4.33	3.67	4.33	4.50	813.33	636.67	480.00	636.67	641.67
Mean		6.33	4.80	3.93	4.80	4.97	945.33	684.67	532.67	684.67	711.83
SEm±		0.70	0.40	0.62	0.42	0.56	-	-	-	-	-
C.D.		2.1	1.20	1.86	1.26	1.68	-	-	-	-	-

Table 4: Effect of EB and their combination on Dry root rot (*Rhizoctonia bataticola*) disease incidence (Pot culture)

Treatment Number	Treatment Combination	Germination (%)					Pre-emergence mortality (%)				
		JG14	JG16	JG62	JG315	Mean	JG14	JG16	JG62	JG315	Mean
T1	Treated control	73.33	73.33	66.67	70.00	70.83	26.67	26.67	33.33	30.00	29.17
T2	EBL -2	76.67	76.67	70.00	73.33	74.17	23.33	23.33	30.00	26.67	25.83
T3	EBL -3	80.00	80.00	70.00	76.67	76.67	20.00	20.00	30.00	23.33	23.33
T4	EBL -4	83.33	83.33	76.67	80.00	80.83	16.67	16.67	23.33	20.00	19.17
T5	EBL (2+3)	86.67	86.67	80.00	83.33	84.17	13.33	13.33	20.00	16.67	15.83
T6	EBL (3+4)	83.33	83.33	76.67	83.33	81.67	16.67	16.67	23.33	16.67	18.34
T7	EBL (4+2)	86.67	86.67	83.33	86.67	85.84	13.33	13.33	16.67	13.33	14.17
T8	EBL (2+3+4)	96.67	96.67	86.67	93.33	93.34	3.33	3.33	13.33	6.67	6.67
T9	Untreated control	80.00	80.00	73.33	76.67	77.50	20.00	20.00	26.67	23.33	22.50
Mean		82.96	82.96	75.93	80.37	80.56	17.04	17.04	24.07	19.63	19.44
SEm±		4.01	4.01	4.30	4.01	4.08	4.01	4.01	4.30	4.01	4.08
C.D.		12.00	12.00	N/A	12.00	12.00	12.00	12.00	N/A	12.00	12.00

Table 5: Effect of EB and their combination on Dry root rot (*Rhizoctonia bataticola*) disease incidence (Pot culture)

Treatment Number	Treatment Combination	Post-emergence mortality (%)					Total mortality (%)				
		JG14	JG16	JG62	JG315	Mean	JG14	JG16	JG62	JG315	Mean
T1	Treated control	27.19	27.19	69.84	32.86	39.27	46.67	46.67	80.00	50.00	55.84
T2	EBL -2	26.00	21.43	28.97	22.43	24.71	43.33	40.00	50.00	43.33	44.17
T3	EBL -3	25.26	25.00	23.41	26.19	24.97	40.00	40.00	46.67	43.33	42.50
T4	EBL -4	24.07	24.07	26.19	25.07	24.85	36.67	36.67	43.33	40.00	39.17
T5	EBL (2+3)	11.58	11.58	28.97	12.04	16.04	23.33	23.33	43.33	26.67	29.17
T6	EBL (3+4)	12.04	12.04	17.36	24.07	16.38	30.00	30.00	36.67	40.00	34.17
T7	EBL (4+2)	11.58	15.28	24.07	19.91	17.71	23.33	26.67	36.67	30.00	29.17
T8	EBL (2+3+4)	10.37	10.37	11.49	10.74	10.74	13.33	13.33	23.33	16.67	16.67
T9	Untreated control	26.26	25.26	36.02	26.57	28.53	40.00	40.00	53.33	43.33	44.17
Mean		19.37	19.14	29.59	22.21	22.58	32.96	32.96	45.93	37.04	37.22
SEm±		1.13	1.94	2.57	2.64	2.07	3.85	3.69	3.51	4.16	3.80
C.D.		3.38	5.82	7.69	7.91	6.20	11.53	11.03	10.52	12.45	11.38

Table 6: Effect of EB and their combinations on Dry root rot disease and phenotypic parameters (Pot culture)

Treatment number	Treatment Combination	Shoot length (cm)					Root length (cm)					Fresh weight (g/plant)				
		JG14	JG16	JG62	JG315	Mean	JG14	JG16	JG62	JG315	Mean	JG14	JG16	JG62	JG315	Mean
T1	Treated control	38.67	38.00	37.00	38.00	37.92	7.67	7.67	6.67	7.33	7.34	11.50	2.33	8.00	8.67	7.63
T2	EBL -2	46.67	44.33	46.67	43.33	45.25	10.33	9.67	10.33	9.67	10.00	14.95	14.33	12.33	12.33	13.49
T3	EBL -3	45.00	43.33	45.00	43.33	44.17	10.00	10.00	9.67	10.00	9.92	14.00	14.67	12.37	14.00	13.76
T4	EBL -4	46.00	43.67	46.00	43.67	44.84	9.33	9.33	9.33	9.33	9.33	14.33	15.42	11.00	13.67	13.61
T5	EBL (2+3)	50.67	47.00	50.67	47.00	48.84	11.67	11.00	10.67	10.00	10.84	15.67	14.00	12.67	13.00	13.84
T6	EBL (3+4)	49.67	47.00	49.67	45.33	47.92	11.33	10.67	10.33	10.67	10.75	15.00	16.33	13.00	13.33	14.42
T7	EBL (4+2)	52.00	49.33	52.00	49.33	50.67	12.00	10.33	11.33	10.33	11.00	15.00	16.00	12.07	13.67	14.19
T8	EBL (2+3+4)	54.33	51.00	54.33	50.33	52.50	13.67	12.33	12.33	11.67	12.50	16.33	17.33	13.00	15.00	15.42
T9	Untreated control	40.00	40.00	40.00	39.33	39.83	8.67	8.33	8.00	8.67	8.42	13.50	13.75	10.67	11.33	12.31
Mean		47.00	44.85	46.82	44.41	45.77	10.52	9.93	9.85	9.74	10.01	14.48	13.80	11.68	12.78	13.18
SEm±		0.88	2.50	0.91	2.44	1.68	0.40	0.78	0.71	0.75	0.66	1.22	1.17	0.82	0.90	1.03
C.D.		2.62	7.48	2.72	7.30	5.03	1.20	2.33	2.13	2.26	1.98	N/A	3.51	2.44	2.70	2.88

Table 7: Effect of EB and their combinations on Dry root rot disease and phenotypic parameters (Pot culture)

Treatment number	Treatment Combination	Dry weight (g/plant)					Vigour Index (%)				
		JG14	JG16	JG62	JG315	Mean	JG14	JG16	JG62	JG315	Mean
T1	Treated control	2.62	2.98	2.00	2.04	2.41	3400.00	3360.00	2916.67	3173.33	3212.50
T2	EBL -2	3.78	4.07	2.83	4.00	3.67	4370.00	4153.33	3996.67	3896.67	4104.17
T3	EBL -3	3.81	4.20	3.00	3.33	3.67	4386.67	4253.33	3830.00	4076.67	4136.67
T4	EBL -4	4.14	5.37	3.00	3.07	3.92	4616.67	4406.67	4236.67	4500.00	4440.00
T5	EBL (2+3)	4.91	4.78	3.20	3.33	4.06	5400.00	5016.67	4910.00	4733.33	5015.00
T6	EBL (3+4)	5.00	4.44	3.33	3.67	4.20	5086.67	4813.33	4593.33	4680.00	4793.33
T7	EBL (4+2)	4.22	4.17	3.00	3.67	3.77	5556.67	5166.67	5276.67	5166.67	5291.67
T8	EBL (2+3+4)	6.05	5.86	4.00	4.33	5.14	6573.33	6140.00	5773.33	5806.67	6073.33
T9	Untreated control	3.43	4.00	2.00	2.67	3.28	3880.00	3856.67	3520.00	3673.33	3732.50
Mean		4.22	4.43	3.00	3.14	3.79	4807.78	4574.07	4339.26	4411.85	4533.24
SEm±		0.44	0.41	0.61	0.37	0.46	-	-	-	-	-
C.D.		1.30	1.23	1.82	1.11	1.21	-	-	-	-	-

Dual culture with endophytic bacterial isolates against *Rhizoctonia bataticola*

The results of dual culture experiment with *Rhizoctonia bataticola* revealed that the three EBs were reported to be effective in inhibition of *Rhizoctonia bataticola.* Out of these three, EBL-4 was recorded highest percent inhibition (44.67%) followed by EBL-2 (32.00%) and EBL-3 (21.33%) on PDA whereas there was no inhibition recorded in control. Similar trend of percent inhibition was recorded on TSA (Table 7). The mean fungal radial growth of *Rhizoctonia bataticola* was 43.67, 53.00 and 64.00 mm^2 grown with EBL-4, EBL-3 and EBL-2, respectively on PDA whereas the fungal growth of 80.67 mm in control. The fungal growth of 14.67, 15.00 & 18.00 mm^2 was recorded in EBL-4, EBL-3 and EBL-2, respectively on TSA whereas it was 72.00 mm^2 in control. The highest bacterial growth on PDA was recorded by EBL-2 (5.00 & 4.00 mm^2) followed by EBL-4 (3.00 & 4.67 mm^2) and EBL-3 (0.67 & 3.33 mm^2) on PDA and TSA, respectively whereas there was no bacterial growth observed in control under both the media (Table 1) The antagonistic activity of Pseudomonas spp. against *Rhizoctonia solani* in-vitro plate assay by dual culture was done by Toppo and Tiwari (2015) [21]. They have reported that four Pseudomonas isolates PKS10, PKM11, PKJ25, PKB27 and Pmtcc (standard check) with better inhibition potential against the hyphal growth of Rhizoctonia spp.

Table (2 and 2.a): Effect of EB's on plant growth parameters under *Rhizoctonia bataticola*

Among the treatments, highest germination percent was recorded in EBL-3 (71.67%) followed by EBL-4 (70.83%) as compared to dry root rot treated (62.50%) and untreated control (66.67%). Among the varieties JG14 has recorded highest germination percent (80 to 80.33%) whereas the lowest germination for all the EBs were recorded in JG62 (60 to 63.33%) whereas highest germination was recorded in JG14 (80 to 83.33%) (Table 2). The genotypes JG14 has recorded highest root and shoot length (6.67 to 7.33 cm) for EBL-4 followed by EBL-3 (6.33 & 7.00 cm) (Table 4.7a &b). However, the lowest root and shoot length was recorded in JG62. The highest vigor index was recorded in EBL-3 (830.00) followed by EBL-2 (778.34) and EBL-4 (761.67) as compared to treated (547.50) and untreated control (641.67). Among the varieties, highest vigor index was recorded by JG14 followed by JG16 whereas the lowest vigor index was recorded for JG62 (Table 2). Gaurkhede *et al.* (2015) [9] investigated to know the antagonistic potential of *Pseudomons fluorescens* against *Sclerotium rolfsii in vitro*. Combined application of soil and seed treatment of *P. fluorescens* was found best for increasing germination percent in different chickpea varieties *viz*., JG62 (96.6%), JG63 (90%), JG315 (100%) and JG74(86.6%) as compared to treated and untreated controls.

Effect of endophytic bacteria against dry root rot disease incidence

Effect of individual EBs and their combinations were evaluated against dry root rot disease incidence in four different chickpea varieties. The results are mentioned below.

Germination per cent

Germination percentage of different treatments across the four varieties is presented in Table 3. Germination percentage among treatment ranged from 70.83 to 93.34%. The treatment T_5, T_7 and T_8 had significantly higher germination percentage as compared to T_1 (Treated control). Among the treatment with endophytic bacteria, the highest germination percent was recorded in T_8 (93.34%) followed by T_7 (85.84%) and T_5 (84.17%) whereas minimum germination percent was recorded in T_2 (74.17%) followed by T_3 (76.67%) in comparison to dry root rot pathogen treated (T_1-70.83%) and untreated control (T_9-77.50%). Among the varieties, the highest germination percent was recorded in JG14 and JG16 (82.96%) whereas minimum germination percent was observed in JG62 (75.93%) (Table 3).

Pre-emergence mortality (%)

The treatments T_5, T_7 and T_8 had significantly reduced the pre-emergence mortality as compared to T_1 (Treated control). Maximum pre-emergence mortality was recorded in T_1 (29.17%) followed by T_2 (25.83%) and T3 (23.33%) whereas minimum pre-emergence mortality was recorded in T_8 (6.67%) followed by T_7 (14.17%) and T_5 (15.83%). Among the genotypes, the highest pre-emergence mortality was recorded in JG62 (24.07%) followed by JG315 (19.63%) whereas lowest pre-emergence mortality was recorded in JG14 and JG16 (17.04%) (Table 3).

Post-emergence mortality (%)

All the treatments had significantly reduced the post-emergence mortality as compared to T_1 (Treated control). Maximum post-emergence mortality was recorded in T_1 (39.27%) followed by T_9 (28.53%) and T_4 (24.85%) whereas minimum post-emergence mortality was recorded in T_8 (10.74%) followed by T_6 (16.38%) and T_7 (17.71%). Among the genotypes, the highest post-emergence mortality was recorded in JG62 (29.59%) followed by JG315 (22.21%)

whereas lowest post-emergence mortality was recorded in JG16 (19.14%) and JG14 (19.37%) (Table 4).

Total mortality (%)

All the treatments had significantly reduced the total mortality as compared to dry root rot pathogen treated control (T_1). Maximum total mortality was recorded in T_1 (55.84%) followed by T_9 (44.17%) and T_2 (44.17%) whereas minimum total mortality was recorded in T_8 (16.67%) followed by T_5 (29.17%) and T_7 (29.17%) indicated that T_8 followed by T_5 and T_7 has significantly reduced disease incidence of dry root rot across the four varieties. Among the genotypes, the highest total mortality was recorded in JG62 (45.93%) followed by JG315 (37.04%) whereas minimum mortality was recorded in JG16 (32.96%) and JG14 (32.96%) (Table 4).Abed *et al*. (2016) studied the ability of several isolates belonging to Rhizobacteria (Pseudomonas and Bacillus) collected from several chickpea growing areas in Algeria, to control the mycelium growth of *Fusarium oxysporum f. sp. ciceris*.

Effect of endophytic bacteria and their combination on dry root rot disease and phenotypic parameters

Shoot length

All the treatments have recorded significantly higher shoot length as compared to treated control (T_1) under dry root rot disease incidence. The highest shoot length was reported in T_8 (52.50 cm) followed by T_7 and T_5 (50.67 and 48.84 cm, respectively) whereas the lowest shoot length was reported in T_1 (37.92 cm) followed by T_9 (39.83 cm). Among the varieties, JG14 (47.00 cm) followed by JG62 (46.82 cm) has recorded maximum shoot length whereas it was minimum in JG315 (44.41 cm). (Table 5).

Root length

All the treatments has recorded significantly higher root length as compared to treated control (T_1) under dry root rot disease incidence. The highest root length was reported in T_8 (12.50 cm) followed by T_7 and T_5 (11.00 cm and 10.84 cm, respectively) whereas the lowest root length was reported in T_1 (7.34 cm) followed by T_9 (8.42 cm). Among the varieties, JG14 (10.52 cm) has recorded maximum root length followed by JG16 (9.93 cm) whereas it was minimum in JG 315 (9.74 cm) (Table 5).

Fresh weight per plant

All the treatments have recorded significantly higher fresh weight per plant as compared to treated control (T_1). The highest fresh weight per plant was reported in T_8 (15.42 g) followed by T_6 (14.42 g) and T_7 (14.19 g) whereas the lowest fresh weight was reported in T_1 (7.63 g) followed by T9 (12.31 g). Among the varieties, JG14 (14.48 g) has recorded highest fresh weight per plant followed by JG16 (13.80 g) whereas it was lowest in JG62 (11.68 g) (Table 5).

Dry weight per plant

All the treatments have recorded significantly higher dry weight per plant as compared to treated control (T_1) showed a positive indirect effect of EBs on growth of the chickpea plants. The highest dry weight per plant was reported in T_8 (5.14 g) followed by T_6 (4.20 g) and T_5 (4.06 g) whereas the lowest dry weight was reported in T_1 (2.41 g) followed by T_2 and T_3 (3.67 g). Among the varieties, JG16 (4.43 g) followed by JG14 (4.22 g) recorded highest dry weight per plant whereas it was lowest in JG315 (3.14 g) (Table 6).

Vigor index

All the treatments have recorded significantly higher vigor index as compared to treated control (T_1) shows a positive indirect effect of EBs on growth and vigor of the chickpea plants across the varieties. The highest vigor index was reported in T_8 (6073.33) followed by T_7 (5291.67) and T_5 (5015.00) whereas the lowest vigor index was reported in T_1 (3212.50) followed by T_9 (3732.50). Among the varieties, JG14 (4807.78) followed by JG16 (4574.07) recorded highest vigor index whereas minimum was in JG62 (4339.26) (Table 6). Egamberdieva *et al*. (2017) [8] reported that endophytic bacterial isolates with best plant growth promoting traits which were capable to reduce the infection rate of root rot in chickpea, effective in growth stimulation and offering resistance to salt stress.

Conclusion

The effect of all endophytic bacteria isolates are significant but EBL-3 isolate that correlates with *in vivo* and *in vitro* conditions on genotypes JG14, JG16, JG62 and JG315 of chickpea with inoculated with *Rhizoctonia bataticola*

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