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Synergistic Effect of Endophytic Bacteria and *Bradyrhizobium* sp. on Growth of Greengram (*Vigna radiata* L.)

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ABSTRACT

The present study was conducted to know the Synergistic effect of endophytic bacteria and Bradyrhizobium sp. on growth of Greengram. Endophytic bacteria were isolated from three different parts of green gram namely, root, nodule and seed. After the characterization and compatibility studies three efficient endophytes were taken for further pot culture studies to study their effect on growth of greengram. There was a significant difference between the treatments. The values for plant height, germination percentage, root and shoot length, dry matter of greengram, nodule number, nodule fresh and dry weight were recorded highest in T_{13} *which received the inoculation of root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + Bradyrhizobium sp. when compared to* T_1 *(control) and* T_2 *(Bradyrhizobium). It is evident that the co-inoculation of root, nodule and seed endophyte along with Bradyrhizobium sp. encourages more robust crop with better plant health, disease resistance, biomass and ability to cope with environmental stresses.*

Keywords: *Synergistic effect, Endophyte, Bradyrhizobium.*

INTRODUCTION

Microbes colonize living, internal tissues of plants such as leaf, root, stem and seeds without causing any immediate, over-negative effects are termed as endophytes (Bacon & White, 2000). Plant-microbe interactions that promote the plant development and plant health have been the subject of considerable interest. Plants constitute vast and diverse niches for endophytic microorganisms. The term endophyte (Gr. Endon, within; *phyton*, plant) was first coined by (De Bary, 1866). An endophyte belongs to either bacteria or fungi, which spends the whole or part of its life cycle colonizing inter- and / or intracellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Wilson, 1995).

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The endophytic bacteria were studied in terrestrial plants which are found to possess antibacterial, antifungal activities. Endophyte carrying plants grow more vigorously and are toxic to herbivores, such plants are more drought tolerant than non-infected plants. Also salt tolerance is observed in plants infected with endophytes. Endophytes acts as biological triggers to activate the stress response more rapidly and strongly than non-symbiotic plants.

A renewed interest in internal colonization of healthy plants by endophytic bacteria has arisen as their potential for exploitation in agriculture. Exploration of endophyte plant interactions can result in the promotion of plant growth and it can play a significant role in low input sustainable agriculture application. However, research works have not been undertaken on the effect of Co-inoculation of endophytic bacteria and *Bradyrhizobium* in plant growth promotion of greengram. Hence the present study was undertaken to observe the synergistic effect of endophytic bacteria and *Bradyrhizobium* on growth of greengram.

MATERIALS AND METHODS

Synergistic effect of endophytic bacteria with reference strain

Synergistic effect of endophytic bacteria with *Bradyrhizobium* on growth of greengram was studied by pot culture experiment under greenhouse condition. Variety KKM 3 used in the study, which was collected from National seed project (NSP), UAS, GKVK, Bengaluru.

Preparation of endophytic bacterial isolates and *Bradyrhizobium* sp. (Mother culture preparation)

The endophytic bacteria were grown on nutrient broth and *Bradyrhizobium* sp. in YEMA broth with constant shaking at 150 rpm for 48 hours at room temperature to get a population of approximately 10^6 CFU/ ml. The bacterial cells harvested by centrifugation at 10000 rpm for 15 minute and carrier based formulations were made using talc. This was used as bacterial inoculum for greengram seed treatment.

Treatment of endophytic bacteria *Bradyrhizobium* sp.

Endophytic bacterial isolates and *Bradyrhizobium* sp. were inoculated according to treatment combinations. A total of 13 treatments with 3 replications were formulated for the study under Completely Randomized Design. The pot culture experiment was conducted in the green house of ZARS, UAS, GKVK, and Bengaluru, where in T_1 was control, **T_2:** *Bradyrhizobium* sp., **T_3:** Nodule endophytic bacteria, **T_4:** Seed endophytic bacteria, **T_5:** Root endophytic bacteria, **T_6:** Root endophytic bacteria +*Bradyrhizobium* sp., **T_7:** Nodule endophytic bacteria + *Bradyrhizobium* sp., **T_8:** Seed endophytic bacteria +*Bradyrhizobium* sp., **T_9:** Root endophytic bacteria + nodule endophytic bacteria, **T_{10}:** Root endophytic bacteria + seed endophytic bacteria, **T_{11}**: Nodule endophytic bacteria + seed endophytic bacteria, **T_{12}**: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria, **T_{13}:** Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Bradyrhizobium* sp. The inoculation was done by seed treatment and soil drenching methods using sterile distilled water after 15 days of sowing.

The surface sterilized seeds were treated with single and combination of organisms and dibbled in pot and allowed to grow. Following observations *viz.*, plant height, root and shoot length of the plant, number of nodules, nodule fresh weight and dry weight, nitrogen content of plant were recorded. Values obtained were means of three replications ± standard deviation and were statistically analysed using Duncan's multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

Synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. on plant height of greengram at different intervals

The outcome of the synergistic effect of endophytic bacteria with *Bradyrhizobium sp.* on plant height at different growth stages of greengram is presented in Table 1.

Plant height was recorded at 30, 60 days after sowing (DAS) and at harvest and the treatment means were found to be significantly different.

The seeds treated with T_{13} (Root endophytic bacteria +Nodule endophytic bacteria +Seed endophytic bacteria+ *Bradyrhizobium* sp.) showed good response at 30 DAS, where it showed 22.80 cm height of greengram. T_6 (Root endophytic bacteria + Bradyrhizobium sp.; 20.50 cm) and T_{12} (Root endophytic bacteria +Nodule endophytic bacteria +Seed endophytic bacteria; 20.73 cm) were next best treatments and were found to be on par with each other. Lowest plant height was observed in treatment T_1 (control; 11.33 cm).

At 60 DAS the maximum height was recorded in treatment T_{13} (34.13 cm) and the lowest plant height was found in T_1 (23.50 cm). The Next best treatment to T_{13} was T_6 with a plant height of 30.36 cm and it was followed by the treatments T_7 (29.13 cm), T_8 (29.00 cm) and T_2 (28.60 cm) which were found to be on par with each other.

At harvest, the plants treated with treatment T_{13} showed maximum height of 46.93 cm. Treatment T_1 showed lowest plant height of 31 cm. Next best treatments to T_{13} were T_6 and T_7 which recorded 44.96 and 44.36 cm respectively. Treatments T_{11} (40.76 cm) and T_{12} (41.03 cm) were found to be on par with each other.

The increase in plant height could be attributed to increased cell elongation and multiplication because of enhanced nutrient content in plants following inoculation of *Bradyrhizobium* sp. and endophytic bacteria. Similar results have been reported earlier (Struz et al., 1997, Tomar et al., 1996).

Synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. on root and shoot length of greengram.

The observation recorded on the influence of endophytic bacteria and *Bradyrhizobium* sp. on root and shoot length of greengram is presented in Table 2.

Root length of greengram plant was recorded after harvesting the crop, highest root length of 26.20 cm was recorded in treatment T_{13} (Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria + *Bradyrhizobium* sp.) it was followed by treatment T_6 (Root endophytic bacteria + *Bradyrhizobium* sp.) with a root length of 25.00 cm. Treatments, T_{12} (Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria), T_8 (Seed endophytic bacteria + *Bradyrhizobium* sp.) and T_7 (Nodule endophytic bacteria + *Bradyrhizobium* sp.) showed root length of 23.80, 23.00 and 22.10 cm respectively. Treatments T_9 (21.33 cm) and T_{10} (21.00 cm) were on par with each other. Lowest root length was observed in treatment T_1 (control; 17.33 cm).

In shoot length of greengram, plants treated with T_{13} showed maximum shoot length of 46.90 cm. The lowest shoot length was observed in treatment T_1 where it recorded shoot length of 31.00 cm. Next best treatments after $T1_3$ were T_6 and T_7 which recorded 44.90 and 44.50 cm respectively. Treatments T_{12} (41.00 cm) and T_{11} (40.67 cm) were on par with each other.

The increased root and shoot length was due to nitrogen fixation by *Bradyrhizobium* sp. and more assimilation of solubilized phosphorus and plant growth promoting substances produced by endophytic bacteria. These plant growth promoting substances play a role in shoot and root elongation (Brown, 1975). Similar results have been reported by earlier workers in other leguminous crops (Menaria et al., 2013, Prabakaran et al., 2000, Verma et al., 2013).

Synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. on shoot and root dry weight and total dry matter of plant (Biomass of plant)

The data pertaining to the synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. on root and shoot dry weight of greengram is depicted in Table 3.

Shoot dry weight

Shoot dry weight was recorded after harvesting, the plants were dried under hot air oven. The shoot dry weight was recorded maximum in co- inoculation of endophytic bacteria and *Bradyrhizobium* sp. Treatment T_{13} (Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria + *Bradyrhizobium* sp. ; 8.19 g plant $^{-1}$) followed by T_6 (Root endophytic bacteria and *Bradyrhizobium* sp; 7.60 g plant ^{1}) and T7 (Nodule endophytic bacteria and *Bradyrhizobium* sp. 7.50 g plant $^{-1}$) which were on par with each other. Lowest shoot dry weight of 3.51 g plant $^{-1}$ was recorded in treatment T_1 (control).

Root dry weight

Root dry weight was recorded after harvest of the crop, the plants were dried in hot air oven. Maximum root dry weight was observed in T_{13} (Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria + *Bradyrhizobium* sp.*;* 1.287 g plant^{-1}) which was significantly highest among all other treatments. While, lowest root dry weight was observed in T_1 (control; 0.303 g plant^{-1}). The next best treatment to T_{13} was T_6 (Root endophytic bacteria+*Bradyrhizobium* sp.*;*1.02g plant $^{-1}$) followed by T_2 (*Bradyrhizobium sp.*; 0.977 g plant $^{-1}$), T_{12} (Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria; 0.673 g plant $^{-1}$), T8 (Seed endophytic bacteria + *Bradyrhizobium* sp.*;* 0.610 g plant $^{-1}$) and T_7 (Nodule endophytic bacteria + *Bradyrhizobium* sp.*;* 0.590 g plant $^{-1}$), where in T_8 and T_7 were found be on par with each other.

Biomass of plant

The same trend was followed for the biomass or total dry weight of the plant, where in the highest data was recorded in T_{13} (Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria + *Bradyrhizobium* sp.*;* 9.48g plant $^{-1}$) which was significantly highest among all other treatments it was followed by T_6 (Root endophytic bacteria and *Bradyrhizobium* sp; 8.62 g plant ^{1}) and the least data was obsereved in untreated control T_1 3.80 g plant $^{-1}$.

The increase in shoot dry weight, root dry weight and total biomass could be attributed to increased nutrient availability and their uptake which in turn resulted in increased number of leaves and branches per plant, by producing various metabolites and growth promoting hormones these are the main contributing characters for total dry matter production. Similar results were reported in cowpea (Ranjana et al., 2015, Verma et al., 2013).

Synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. on number of nodules per plant and fresh and dry weight of nodule

The observations recorded on the influence of endophytic bacteria and *Bradyrhizobium sp.* on nodule number along with fresh and dry weight of nodule of greengram plants are presented in the Table 4.

The highest number of nodules were observed in treatment T_{13} (Root endophytic bacteria + Seed endophytic bacteria + Nodule endophytic bacteria + *Bradyrhizobium* sp.*;* 30.66) followed by T_6 (Root endophytic bacteria + *Bradyrhizobium* sp*; 28),* T_7 *(*Nodule endophytic bacteria + *Bradyrhizobium* sp.*:* 27) and T_8 (Seed endophytic bacteria + *Bradyrhizobium* sp.*; 27),* these three treatments were found to be on par with each other. However, treatments T_2 (*Bradyrhizobium* sp.; 25.33), T_{12} (Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria; 25) and T_{11} (Nodule endophytic bacteria + Seed endophytic bacteria; 24.33) were also found to be on par with each other and lowest nodule number (13.33) was observed in T_1 (control).

Fresh weight of nodules

The plants treated with treatment T_{13} (Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria + *Bradyrhizobium* sp.) showed maximum nodule fresh weight of 556.97 mg per plant followed by treatment T_6 (Root endophytic bacteria + *Bradyrhizobium* sp.*;* 477.0 mg plant^{-1}). The plants treated with treatments T_7 (Nodule endophytic bacteria and *Bradyrhizobium* sp.) and T_8 showed 441 and 434 mg of nodule fresh weight respectively and were found to be on par with each other. Lowest nodule fresh weight of 292.6 mg per plant was recorded in T_1 (control).

Dry weight of nodules

Treatment T_{13} showed maximum nodule dry weight of 36.67 mili grams per plantthis was followed by T_7 (31 mili gram plant^{-1}) and T_6 (30.67 mili gram plant^{-1}) which were found to be on par with each other. Next best treatment was T_8 (29.46 mili gram plant^{-1}). T_{12} (Nodule endophytic bacteria+ Root endophytic bacteria + Seed endophytic bacteria; 27.33 mg plant^{-1}) and T_2 (*Bradyrhizobium* sp.*;* 27.02 mg plant^{-1}) were found to be on par with each other. Least nodule dry weight of 15.87 mg per plant was observed in T_1 (control).

Significant increase in nodule number, nodule fresh weight and nodule dry weight was due to increased nitrogen and phosphorus availability. Availability of more number of *Bradyrhizobium* in the rhizosphere inoculated through seed treatment and also by continuous supply of phosphorus in soil stimulates multiplication of rhizobia and development of their motile forms which are essentially required for migration through soil towards the root system. These probably have helped in the formation of more number of nodules and maintain higher activity of bacteria in the nodules. These results are in confirmation with earlier findings in blackgram (Balyan et al., 2002, Prabakaran et al., 2000), cowpea (Verma et al., 2013). and greengram (Perveen et al., 2002).

Synergistic effect of endophytic bacteria and *Bradyrhizobium sp.* on available nitrogen content in rhizosphere soil of greengram at the time of harvest

The data obtained on the synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. on available nitrogen content in rhizosphere soil of greengram at the time of harvest of crop is represented in table 5.

The Plants treated with treatment T_{13} (Root endophytic bacteria+ Nodule endophytic bacteria +Seed endophytic bacteria and *Bradyrhizobium* sp.) showed maximum nitrogen content of 183.01 kg/ha it was followed by T_6 (Root endophytic bacteria + *Bradyrhizobium* sp.) with a nitrogen content of 182.67 kg/ha and they were found to be on par with each other. Treatments T_8 (Seed endophytic bacteria + *Bradyrhizobium* sp.; 181.01 kg/ha), T_7 (Nodule endophytic bacteria + *Bradyrhizobium* sp.; 180.46 kg /ha) also showed a good amount of N content in soil. And treatments T_{12} (Root endophytic bacteria+ Nodule endophytic bacteria+ Seed endophytic bacteria; 179.83 kg/ha) and T_2 (*Bradyrhizobium* sp.*;* 179.82 kg/ha) were observed to be on par with each other. Least N content of 165 kg/ha was observed in T_1 (control).

This may be due to nitrogen fixation by *Bradyrhizobium* sp. which improves plant nitrogen content when it is inoculated in combination with endophytic bacteria the nitrogen fixing ability increases and increase soil nitrogen uptake due to improved root growth by phosphorus nutrition. Similar results were recorded by (Verma et al., 2013). in chickpea crop and by (Ranjana et al., 2015).

Table 1: Synergistic effect of endophytic bacteria and *Rhizobium* sp. on plant height of greengram at different intervals

Treatments	Plant height (cm)		
	30 DAS	60 DAS	At harvest
T_1: Control	11.33^{i} (±0.10)	23.50^{i} (±0.43)	31.00^{k} (±0.20)
T_2: *Bradyrhizobium* sp.	19.30^{d} (±0.34)	28.60^{c} (±0.45)	42.26^{e} (±0.35)
T_3: Nodule endophytic bacteria	13.96^{h} (±0.65)	24.20^{h} (±0.30)	33.23^{j} (±0.41)
T_4: Seed endophytic bacteria	14.96^{g} (±0.15)	24.93^{gh} (±0.15)	3450^{i} (±0.45)
T_5: Root endophytic bacteria	15.66^{g} (±0.15)	25.46^{fg} (±0.56)	35.63^{h} (±0.30)
T_6: Root endophytic bacteria + *Bradyrhizobium* sp.	20.50^{b} (±0.86)	30.36^{b} (±0.45)	44.96^{b} (±0.15)
T_7: Nodule endophytic bacteria + *Bradyrhizobium* sp.	19.76^{cd} (±0.32)	29.13^{c} (±0.30)	44.36^{c} (±0.41)

T_8: Seed endophytic bacteria + *Bradyrhizobium* sp.	20.20^{bc} (±0.26)	29.00^{c} (±0.10)	43.83^{d} (±0.15)
T_9: Root endophytic bacteria + Nodule endophytic bacteria	16.73^{f} (±0.30)	26.00^{ef} (±0.20)	40.73^{f} (±0.25)
T_{10}: Root endophytic bacteria + Seed endophytic bacteria	17.63^{e} (±0.70)	26.73^{de} (±1.10)	39.66^{g} (±0.40)
T_{11}: Nodule endophytic bacteria + Seed endophytic bacteria	16.506 ^{f} (±0.20)	25.23f^{g} (±0.25)	40.76^{f} (±0.40)
T_{12}: Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria	20.73^{b} (±0.30)	27.30^{d} (±0.60)	41.03^{f} (±0.15)
T_{13}: Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria + *Bradyrhizobium* sp.	22.80^{a} (±0.20)	34.13^{a} (±0.41)	46.93^{a} (±0.15)

Table 2: Synergistic effect of endophytic bacteria and Bradyrhizobium sp. on shoot and root length of green gram

Treatments	**Shoot length (cm)**	**Root length (cm)**
T_1: Control	31.00^{k} (±0.20)	17.33^{j} (±0.45)
T_2: *Bradyrhizobium* sp.	42.33^{e} (±0.35)	20.67^{g} (±0.20)
T_3: Nodule endophytic bacteria	33.33^{j} (±0.41)	18.00^{i} (±0.10)
T_4: Seed endophytic bacteria	34.50^{i} (±0.45)	19.33^{h} (±0.15)
T_5: Root endophytic bacteria	35.67^{h} (±0.30)	19.33^{h} (±0.15)
T_6: Root endophytic bacteria + *Bradyrhizobium* sp.	44.90^{b} (±0.15)	25.00^{b} (±0.20)
T_7: Nodule endophytic bacteria+ *Bradyrhizobium* sp.	44.50^{c} (±0.41)	22.10^{e} (±0.41)
T_8: Seed endophytic bacteria + *Bradyrhizobium* sp.	43.83^{d} (±0.15)	23.00^{d} (±0.10)
T_9: Root endophytic bacteria + Nodule endophytic bacteria	40.67^{f} (±0.25)	21.33^{f} (±0.41)
T_{10}: Root endophytic bacteria + seed endophytic bacteria	39.67^{g} (±0.40)	21.00^{fg} (±0.52)
T*11* : Nodule endophytic bacteria + seed endophytic bacteria	40.67^{f} (±0.40)	22.00^{e} (±0.10)
T_{12}: Root endophytic bacteria + Nodule endophytic bacteria + seed endophytic bacteria	41.00^{f} (±0.15)	23.80^{c} (±0.92)
T_{13}: Root endophytic bacteria + Nodule endophytic bacteria + seed endophytic bacteria + *Bradyrhizobium* sp.	46.90^{a} (±0.15)	26.20^{a} (±0.17)

Table 3: Synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. on shoot and root dry weight and total dry matter of green gram

Treatments	Shoot dry weight (gm)	Root dry weight (gm)	Biomass (gm)
T1: Control	0.30[j] (±0.007)	3.51[i] (±0.07)	3.80[i] (±0.11)
T2: *Bradyrhizobium* sp.	0.98[c] (±0.032)	6.56[d] (±0.14)	7.53[d] (±0.25)
T3: Nodule endophytic bacteria	0.42[h] (±0.010)	4.53[h] (±0.10)	4.99[h] (±0.11)
T4: Seed endophytic bacteria	0.37[i] (±0.015)	4.68[h] (±0.07)	5.17[h] (±0.08)
T5: Root endophytic bacteria	0.43[h] (±0.020)	5.03[g] (±0.02)	5.46[g] (±0.20)
T6: Root endophytic bacteria + *Bradyrhizobium* sp.	1.02[b] (±0.036)	7.60[b] (±0.16)	8.62[b] (±0.09)
T7:Nodule endophytic bacteria+ *Bradyrhizobium* sp.	0.59[e] (±0.010)	7.01[c] (±0.08)	7.60[d] (±0.24)
T8: Seed endophytic bacteria + *Bradyrhizobium* sp.	0.61[e] (±0.010)	7.50[b] (±0.11)	8.11[c] (±0.24)
T9: Root endophytic bacteria + Nodule endophytic bacteria	0.49[g] (±0.030)	6.00[e] (±0.21)	6.49[e] (±0.10)
T10: Root endophytic bacteria + seed endophytic bacteria	0.45[h] (±0.020)	5.61[f] (±0.13)	6.06[f] (±0.25)
T11:Nodule endophytic bacteria + seed endophytic bacteria	0.53[f] (±0.015)	4.96[g] (±0.20)	5.49[g] (±0.03)
T12: Root endophytic bacteria + Nodule endophytic bacteria + seed endophytic bacteria	0.67[d] (±0.025)	6.96[c] (±0.16)	7.64[d] (±0.07)
T13: Root endophytic bacteria + Nodule endophytic bacteria + seed endophytic bacteria + *Bradyrhizobium* sp.	1.29[a] (±0.015)	8.19[a] (±0.04)	9.48[a] (±0.18)

Table 4: Synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. on nodule number, fresh and dry weight of nodules

Treatments	Nodule number	Fresh weight of nodules (mg)	Dry weight of nodules (mg)
T_1: Control	13.33[g] (±0.57)	292.67[j] (±2.32)	15.87[j] (±0.30)
T_2: *Bradyrhizobium* sp.	25.33[d] (±0.57)	413.00[e] (±2.55)	27.02[d] (±0.48)
T_3: Nodule endophytic bacteria	14.66[g] (±0.057)	300.00[ij] (±2.27)	17.87[i] (±0.41)
T_4: Seed endophytic bacteria	17.00[f] (±1)	304.00[i] (±1.64)	18.75[h] (±0.21)
T_5: Root endophytic bacteria	18.66[f] (±0.0.57)	316.00[h] (±5.16)	20.97[g] (±0.10)
T_6: Root endophytic bacteria + *Bradyrhizobium* sp.	28.00[b00] (±1)	471.00[b] (±1.22)	30.67[b] (±0.36)

T$_7$: Nodule endophytic bacteria + *Bradyrhizobium* sp.	27.00^{bc} (±1)	441.00^{c} (±1.23)	31.00^{b} (±0.02)
T$_8$: Seed endophytic bacteria + *Bradyrhizobium* sp.	27.00^{bc} (±1)	434.00^{cd} (±0.93)	29.46^{c} (±1.20)
T$_9$: Root endophytic bacteria + Nodule endophytic bacteria	22.33^{e} (±1.52)	356.00^{f} (±3.09)	24.33^{f} (±0.44)
T$_{10}$: Root endophytic bacteria + Seed endophytic bacteria	24.00^{de} (±1)	321.00^{h} (±0.80)	25.79^{e} (±0.23)
T$_{11}$: Nodule endophytic bacteria + Seed endophytic bacteria	24.33^{d} (±1.52)	338.02^{g} (±0.66)	26.13^{e} (±0.63)
T$_{12}$: Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria	25.00^{d} (±1.73)	431.00^{d} (±17.09)	27.33^{d} (±0.41)
T$_{13}$: Root endophytic bacteria + Nodule endophytic bacteria + Seed endophytic bacteria + *Bradyrhizobium* sp.	30.66^{a} (±1.52)	556.98^{a} (±3.44)	36.67^{a} (±0.30)

Table 5: Synergistic effect of endophytic bacteria and *Bradyrhizobium* sp. nitrogen content in plant after harvest of crop

Treatments	% of N content in plants
T$_1$: Control	0.833^{i}(±0.020)
T$_2$: *Bradyrhizobium* sp.	1.873^{d} (±0.580)
T$_3$: Nodule endophytic bacteria	1.230^{g} (±0.010)
T$_4$: Seed endophytic bacteria	1.250^{g} (±0.036)
T$_5$: Root endophytic bacteria	1.150^{h} (±0.036)
T$_6$: Root endophytic bacteria + *Bradyrhizobium* sp.	2.100^{b} (±0.036)
T$_7$: Nodule endophytic bacteria+ *Bradyrhizobium* sp.	1.640^{e} (±0.010)
T$_8$: Seed endophytic bacteria + *Bradyrhizobium* sp.	2.043^{c} (±0.051)
T$_9$: Root endophytic bacteria + Nodule endophytic bacteria	1.410^{f} (±0.036)
T$_{10}$: Root endophytic bacteria + seed endophytic bacteria	1.407^{f} (±0.015)
T$_{11}$: Nodule endophytic bacteria + seed endophytic bacteria	1.423^{f} (±0.015)
T$_{12}$: Root endophytic bacteria + Nodule endophytic bacteria + seed endophytic bacteria	1.870^{d} (±0.043)
T$_{13}$: Root endophytic bacteria + Nodule endophytic bacteria + seed endophytic bacteria + *Bradyrhizobium* sp.	2.327^{a} (±0.020)

CONCLUSION

Healthy plants carry populations of endophytic bacteria and the plant kingdom represents a vast and relatively unexplored ecological niche for these organisms. The co-inoculation of endophytic bacteria and *Bradyrhizobium* sp. on greengram are not only desirable for enhancing the growth of crop and also in future course of time they minimize the usage of agricultural inputs, thus saving the costs and reducing pollutants to the environment. In organic agriculture where we avoid the use of chemicals, the seed bacterization with endophytic bacteria with *Bradyrhizobium* is a cheap and viable option to increase the plant growth parameters and total biomass. It is better to incorporate the non rhizobial endophytic bacteria with *Bradyrhizobium* sp. which improves the growth and development of the plant than uninoculated plants and inoculation of *Bradyrhizobium* alone.

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