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Short Communication

PERFORMANCES OF SOME RHIZOBIUM STRAINS ON LENTIL *(Lens culinaris)*

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Abstract

A pot-culture experiment was carried out to evaluate the performances of some rhizobium strains on nodulation and yield characters of lentil, cv. BARI Lentil-8 (*Lens culinaris*). Five newly isolated *Rhizobium* strains such as FALR114, FALR315 FALR317, FALR319, and FALR328 were tested against the reference strain BARIRLc107. All the yield contributing characters of lentil showed better performances with inoculated soil compared to uninoculated (control) soil. The highest seed yield (0.67 g plant^{-1} in 2020-21 and 1.76 g plant^{-1} in 2021-22) was recorded with *Rhizobium* strain FALR114. Performances of the *Rhizobium* strains need to be tested under field condition in lentil growing areas.

Keywords: Biofertilizer, Lentil, Nodulation, Rhizobium, Seed yield

1. Introduction

Biofertilizer is a special type of fertilizer that enhances soil nutrients by using microorganisms through its symbiotic relationships with the plants. Biofertilizers cannot meet the total nutrient needs. Nitrogen fixing biofertilizers make a net addition to nitrogen supplies by fixing atmospheric nitrogen in the soil-plant system (Juwarkar *et al.,* 2004). Nitrogen is needful for cellular synthesis of enzymes, proteins, chlorophyll, and nucleic acids and consequently important in plant enhancement and the outcome of food and feed. For legume plants, nitrogen is provided through symbiotic fixation of atmospheric N_2 by nitrogenase enzyme in rhizobial bacteroids (Senanayake *et al.,* 1987). This way of biological nitrogen fixation (BNF) accounts for 65% of the nitrogen utilized in agriculture, and will sustain to be important in future crop productivity, especially in sustainable systems (Viviene and Dakora, 2003).

Lentil is one of the age-old and popular pulses in Bangladesh which covers about 33% of the total pulse areas and they are, from the consumer's point of view, the most preferred pulse, popularly known as Masur daal. Leguminous crops such as pulses have played an important role in maintaining soil fertility in Bangladesh for centuries. Lentil plays a significant role in maintenance and improvement of soil fertility. Its cultivation enriches soil nutrient status by adding nitrogen, carbon and organic matter which promotes sustainable cereal-based systems of crop production (Sarker and Kumar, 2011). Nitrogen fixation capacity of lentil is significantly higher when grown in intercropping with other legume species than mono-cropping. The crop is generally grown in rotation with cereals to break cereal disease cycles and to maintain soil nitrogen, thus reducing the demand of other cereal crops for nitrogen fertilizers. Inoculation of *Rhizobium* appears to be very important for legume cultivation and sustainable agriculture. *Rhizobium* improves the root nodulation that ultimately enhances the total biomass and growth of lentil crop with augmenting the organic agriculture production (Das *et al.,* 2017). The objectives of this experiment was to evaluate some rhizobium strains on the yield of lentil.

3. Materials and Methods

3.1 Collection and Isolation of Rhizobium from nodules:

Nodules from lentil root (10 samples) were collected from different fields of Pabna, Faridpur and Jashore districts of Bangladesh using global positioning system (GPS) record along with crop history. Nodules were collected aseptically and crushed, streaked onto yeast extract mannitol agar (YEMA) plate and incubated at 30^0C for 2-3 days. Final selection of the bacterial strains of groundnut was done by comparing morphological (colony) characteristics and transferred to agar slants prepared with the corresponding plating media.

3.2 Purification and preservation of the isolated strains:

The isolated strains were purified by streak plate method. The purified strains were transferred to agar slants and preserved as stock culture in a refrigerator at 4^0C. In addition, the culture was mixed with 50% (v/v) glycerol and kept in -20^0C and -80^0C for long time preservation.

3.3 Inoculum preparation and biofertilizer production

Peat was used as carrier material for inoculant. In Bangladesh, there is a great reserve of peat soil in greater Faridpur, Khulna and Sylhet districts. After collection of the peat, soil materials were first air-dried thoroughly. The air-dry materials were ground to fine powder using a laboratory grinding mill so as to pass through an 80-mesh sieve. Powdered peats were neutralized with 5% $CaCO_3$ to raise the pH to 6.8. The inoculant materials were weighed and then taken in cotton plugged Erlenmeyer flasks and sterilized in an autoclave at 121^oC and 15 psi of steam pressure for 3 hours. The sterilized materials were then transferred into the polyethylene bags and sealed carefully to avoid any contamination.

To prepare the broth cultures, the *Rhizobium* strains were first sub-cultured in plates from the test tube stock. Yeast mannitol broth medium was taken in Erlenmeyer flasks and sterilized for 20 minutes at 121°C and a steam pressure of 15 psi. After cooling the medium, a small portion of *Rhizobium* culture was aseptically transferred from the plates to the liquid medium in the flasks with the help of an inoculating needle. The flasks were then placed on an electric shaker and shaken at a slow speed to enhance rhizobial growth. The broth culture was ready after 4-9 days depending on the rate of growth (Fast or slow growing) or when the medium in the flasks showed dense growth. It is called "mother culture". After checking the culture for purity and proper growth, the culture was transferred from tubes to large flasks containing sterile liquid medium for 4-9 days. Rhizobial broth was grown on YEM medium in large flasks on a horizontal shaker. After proper growth, the broth was checked for free from contamination. The broths were used having viable cells higher than 10^9/ml cells.

3.4 Pot Experiment

The experiment was conducted at the net-house of Soil Science Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur (24.00° N latitude, 90.25° E longitude and 8.4 m elevation) during rabi season of 2020-2021 and 2021-2022. The experiment was laid out in Complete Randomized Design (RCD) with three replications. There were 7 treatments viz. $T_{1:}$ *Rhizobium* strain FALR114, T_2: *Rhizobium* strain FALR315, T_3: *Rhizobium* strain FALR317, T_4: *Rhizobium* strain FALR319, T_5: *Rhizobium* strain FALR328, T_6: Reference strain BARIRLc107, T_7: Control. The test crop was lentil (cv. BARI Lentil-8). Seeds were sown on 15 November 2020 and 13 November 2021. The pot soil belongs to the Chiata series of Grey Terrace Soil. Peat based rhizobial inoculum containing 10^8 cells g^{-1} inoculum was used. Soil pH was 7.1, OM 0.5%, total N 0.026%, available P 9.9 mg kg^{-1}, exchangeable K 0.11 me%, available S 21.1 mg kg^{-1}, Zn 0.38 mg kg-1, and boron 0.22 mg kg-1. Seeds were mixed thoroughly with inoculum (20:1 ratio) before sowing. Six seeds were sown in each pot in 1 cm soil depth. Phosphorus, potassium, sulphur, zinc and boron @ $P_{22}K_{42}S_{20}Zn_5$ kg ha^{-1} were used in the form of TSP, MoP, gypsum, zinc sulphate and boric acid, respectively. All P, K, S, Zn, B were applied at the time of pot preparation. All the intercultural operations such as irrigation, weeding, insect control etc. were done as and when necessary.

Nodules were collected by uprooting carefully and five sample plants were selected randomly from each unit plot at the 50 percent flowering stage. Nodules were separated from the roots, counted and then oven-dried and weighed. Data on yield and yield components were recorded at maturity. The crop was harvested on 12 February 2021 and 22 February 2022. Chemical fertilizers were applied on soil test basis according to the method described in Fertilizer Recommendation Guide (BARC, 2018).

3. Results and Discussion

Different strains of *Rhizobium* biofertilizer showed significant effect on the yield components and yield of lentil under pot culture (Table 1).

For the first year (2020-2021), nodule no. was recorded as the highest (91.33) with T_5 (*Rhizobium* strain FALR328), which was significantly higher over all other treatments. The highest number (20.06) of pods plant^{-1} was recorded from T_1 (*Rhizobium* strain FALR114), which was significantly higher over all other treatments. The highest seed yield (0.67 g plant^{-1}) was observed in T_1, which was statistically similar to T_6 but significantly higher over rest of the treatments. The stover yield was found highest (0.57 g plant^{-1}) also with T_1, which was similar to T_6 and both of them were significantly higher over rest of the treatments (Table 1).

In case of second year (2021-2022), nodule number per plant was counted the highest (36.3) with *Rhizobium* strain FALR114, which was statistically superior to all other R. strains. No. of pods plant^{-1} was recorded as the highest (46.3) with *Rhizobium* strain FALR315, which was identical to T_1, and T_4 but differed with other treatments (Table 1). The highest seed yield (1.76 gm plant^{-1}) was obtained with *Rhizobium* strain FALR114, which was identical to T_2, T_3, T_4, and T_5 but significantly higher over T_6 (Reference strain *Rhizobium* strain BARIRLc107) and T_7 (control) differed with other treatments. Stover yield was recorded as the highest (1.66 gm plant^{-1}) with T_2 (*Rhizobium* strain FALR315), which was identical to T_1, T_3, and T_4 but differed with rest of the treatments (Table 2).

Table 1. Yield contributing characters of lentil (BARI Masur-8) as influenced by the application of *Rhizobium* biofertilizer during 2020- 2021 and 2021-2022

Treatments (*R.* strains)	No. of nodules plant^{-1}		No. of pods plant^{-1}		Seed yield (g plant^{-1})		Stover yield (g plant^{-1})	
	2020- 21	2021- 22	2020- 21	2021- 22	2020- 21	2021- 22	2020- 21	2021- 22
T_1	36.3a	45.7ef	20.1a	45.3a	0.67a	1.76a	0.57a	1.42ab
T_2	25.7c	45.0ef	10.9c	46.3a	0.40bc	1.67a	0.35b	1.66a
T_3	29.4b	65.30c	11.4c	32.1bc	0.42bc	1.62a	0.30b	1.49ab
T_4	18.9e	76.0b	12.8c	43.9a	0.41bc	1.36ab	0.32b	1.41ab
T_5	21.1d	91.3a	14.0bc	27.3cd	0.45abc	1.47ab	0.34b	1.07bc
T_6	10.8f	52.7d	17.7b	35.0b	0.62ab	0.97bc	0.55a	1.07bc
T_7	7.2g	40.0f	9.7c	22.3d	0.35c	0.47c	0.26b	0.70c
CV%	3.87	5.02	17.3	10.6	29.1	21.6	23.5	22.2

In a column figures having similar letter (s) do not differ significantly as per LSD at 5% level of significance.

CV= Co-efficient of Variation, T_1: *Rhizobium* strain FALR114, T_2: *Rhizobium* strain FALR315, T_3: *Rhizobium* strain FALR317, T_4: *Rhizobium* strain FALR319, T_5: *Rhizobium* strain FALR328, T_6: Reference strain BARIRLc107, T_7: Control

Although application of *Rhizobium* biofertilizer resulted in higher yield and yield attributes of lentil in both years, the results in irrespective of R. strains were remarkably higher in 2021-22 than in 2020-21. All the Rhizobium isolates including reference strain performed better on lentil cultivation. *Rhizobium* sp. FALR114 (T_1) produced the highest yield because the same treatment produced higher plant biomass, and the higher biomass was due to consequently higher rhizospheric microbiome activity and enhanced nutrient acquisition by lentil plant. *Rhizobium* inoculum with inorganic fertilizer showed better performance against only with inorganic fertilizer treatments (Bhuiyan *et al.,* 2015). Similar result was reported by Hossain (2018) that *Rhizobium* inoculation alone increased grain and Stover yields of lentil significantly compared to uninoculated control

4. Conclusions

Inoculation of *Rhizobium* strains exhibited better performances in terms of nodulation, pod set, grain yield and stover yield compared to uninoculated control. Inoculated with *Rhizobium* strain FALR114 performed the best grain yield of lentil. However, field study is needed to evaluate the performances of those R. strains in lentil.

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Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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