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Effect of rhizobium isolates and nitrogen on nodulation, growth, and yield in lentil

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Authors' Contribution **Tarek, M. and R. B. Billah** have been setup experiment, manage experiment, collected data and complied, statistical analysed; **S. Chowdhury** has been developed the concepts and also designed the experiments

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ABSTRACT

The experiments were conducted in three different locations, Magura, Rangpur and Mymensingh, Bangladesh to know the effect of two bacterial strains and 3 nitrogen doses on nodulation, yield and biomass production. The experimental material consists of one lentil popular variety viz., Binamasur-5 which was inoculated with two promising rhizobial isolates namely LB-21 and LB-40, and three levels of nitrogen viz., 25, 50, and 75 kg N ha^{-1}. The inoculant treatments were un-inoculated control (I_0), LB-21 (I_1), LB-40 (I_2), and a mixture of LB-21 and LB-40 (I_1+I_2). Results indicated that nodule number, nodule weight, straw yield, and seed yield at the three locations were greater in bacterial isolates treated plants than in control plants. Results further indicated that a mixture of two bacterial isolates had no superior effect against any single bacterial isolate. The both isolates (LB-21 and LB-40) had an almost similar greater effect on nodule number, nodule weight, physiological parameters and seed yield with being the highest in LB-40. Application of 25 kg N ha^{-1} under both bacterial isolates performed the best in seed yield. Application of nitrogen at the rate of 75 kg ha^{-1} had an adverse effect on nodulation, growth, and seed yield in lentil. Therefore, for getting higher seed yield in lentil, should apply N as basal dose at the rate of 25 kg N ha^{-1} and the bacterial isolate, LB-40 may be registered as bio-fertilizer after few more trials in the different lentil growing areas of Bangladesh.

Keywords: Bacterial isolates, nitrogen, nodulation, yield, lentil.

INTRODUCTION: Lentil (*Lens culinaris* L.) is an important leguminous crop with high nutritive value and a source of protein. Lentil being a legume crop can fix atmospheric nitrogen through root nodules by Rhizobium bacteria, which may reduce the pressure of nitrogenous fertilizer application to the crop. Pulse included in the cropping pattern helped to increase the organic matter in the soil (Corstanje *et al.*, 2007). Lentil crop is blessed with the ability of N-fixation. This N-fixation ability can be increased by 14-46% with Rhizobium Inoculation (Ali *et al.*, 2004; Tiwari *et al.*, 2018).

Similar to other legume crops, lentils can attain a part of their N requirements through symbiotic fixation of atmospheric N (N_2), the extent of which highly depends on the environmental conditions. Nevertheless, appropriate N management through the application of effective rhizobia inoculants and starter fertilizer can optimize and incentivize grain legume production (Huang *et al.*, 2017). Most producers in Bangladesh do not inoculate the seed or the soil with a rhizobial strain and provide little or no fertilizer N to their lentil crops. Due to the lag period between rhizobial root colonization infection and the onset of nodule functioning, the young lentil plants may require a small dose of additional N (i.e., starter-N) from external sources to achieve vigorous vegetative growth and establish N_2-fixing symbiosis. On the other hand, inappropriate use of starter N can interfere with seedling emergence and biological N fixation.

It is reported that when initial soil N was low, application of starter N increased dry pea yield in central Montana (Huang *et al.*, 2017). Gan *et al.* (2005) reported that lentil seed inoculation with rhizobium bacteria increased seed yield by 15% on silt loam soils and 70% on the heavy clay soils in the semiarid. Canadian prairies. They also found that application of starter N (15 kg ha^{-1}) increased seed yield by 13% for lentils grown on the heavy clay, but there was no effect on the silt loam. Little is known about N management for lentils in rain-fed conditions of Bangladesh. Nitrogen availability especially at the late growing stage also plays an important role in protein concentration in seeds of legume crops. It has been shown that insufficient N availability during grain filling can cause a reduction in grain N and protein concentration in legumes like peas. On the other hand, some researchers noticed that high N availability during grain-filling can stimulate legumes vegetative growth at the expense of harvest index (HI) and grain yield (Zakeri and Bueckert, 2015). Therefore, optimal N management can increase the quality (protein content) of the product without sacrificing the yield.

The successful growing of lentils is dependent on the availability of its microsymbiont bacteria in the soil. All effective Rhizobium strains are not present in all soils of Bangladesh. In this situation, inoculation can meet the challenge of providing superior strains in the soil, so that the most effective nodulation and nitrogen fixation are obtained. Thus, it was thought that there is a scope for utilizing the effective Rhizobial strains for obtaining more yield of lentil under field conditions which may also play a vital role in improving soil environment and agricultural sustainability. There is a need to identify appropriate rhizobium strain which will enhance nitrogen fixation attributes and yield of lentils under different nitrogen levels. However, the recommended rate of fertilizer might vary according to crop type (variety), location, soil type, etc (Meleta and Abera, 2019).

OBJECTIVES: This research was initiated to study the effect of rhizobium inoculants and N fertilizer rates on the growth and yield of lentils under sub-tropical conditions.

MATERIALS AND METHODS: The first experiment was set up in 2018 at two locations, Magura (23°3′ N 89°2′ E) and Rangpur (25°7′ N 89°3′ E) in Bangladesh to know the effect of two bacterial strains and 3 nitrogen doses on nodulation, yield, and biomass production. In the next year (2019), the 2nd experiment was set up at Mymensingh (24°8′ N 90°0′ E), Bangladesh to know the physiological causes of yield improvement in lentils due to inocula and N application. The experimental material consists of one lentil popular variety viz., Binamasur-5 which was inoculated with two promising rhizobial strains/isolates namely LB-21 and LB-40, and three levels of nitrogen viz., 25, 50, and 75 kg N ha^{-1}. The urea was used as a nitrogen source. The inoculant treatments were un-inoculated control (I_0), LB-21 (I_1), LB-40 (I_2), and the mixture of LB-21 and LB-40 (I_1+I_2). The experiment was laid out in a two-factor randomized complete block design (RCBD) with three replications where bacterial isolates were placed in the main plot and nitrogen doses were placed in the sub-plot. One treatment without urea and Rhizobium was taken as control.

The lands were fertilized with 22, 42, 20, and 5 kg ha^{-1} of P, K, S, and Zn in the form of Triple Superphosphate, Muriate of Potash, Gypsum, and Zinc Sulphate, respectively. Nitrogen was applied as urea according to the treatments. The size of each unit plot was 3.0m × 2.1m and the plot to plot distance was 1.0m. Strains were collected from the Biofertilizer Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA). Seeds of lentil were taken in a small polythene bag equal to each for 27. Out of 27 polythene packets, 24 packets were made ready for mixed with 40% gum acacia per 1kg seed. Then the selected inoculum was mixed with the seeds (@ 50g inoculum/kg seed) for each treatment and mixed well with the seeds by shaking the bag thoroughly. For each inoculant separate polythene bag was used and care was taken to avoid contamination of the inoculants. Seeds were sown on the furrow during the first week of November and the furrows were covered by soils soon after seeding. Uninoculated seeds were sown at the same time. The line-to-line (furrow to furrow) distance was maintained at 30 cm with a continuous distribution of seeds in the line. All the excess seedlings were thinned out at 15 days after sowing (DAS) by maintaining a spacing of 6-10 cm between two plants in a line. Intercultural operations were done as and when necessary.

During the present investigation, the influence of bacterial isolates and N on nodulation, plant growth, and at harvest seed yield and yield attributes were recorded. For growth parameters, two harvests were recorded at 60 and 80 DAS. The whole plants were oven drying at 80 ± 2 ^{0}C for 72 hours and dry weights were recorded. The growth analysis was carried out following the formulae of Beadle (1985). Nodule number was recorded at 60 DAS. The leaf area of each sample was measured at 80 DAS by automatic leaf area meter (Model: LI 3000, LI-COR Biosciences, USA). The yield contributing characters were recorded at harvest from 10 randomly selected plants of each plot. The whole plot yield was converted into kg ha^{-1}. All data were analyzed statistically as per the used design following the two-way analysis of variance technique and the mean differences were adjusted with Duncan's Multiple Range Test using the statistical computer package program, MSTAT-C

RESULTS AND DISCUSSION: The effect of bacterial isolates on nodule number, nodule weight, straw yield, and seed yield at both locations was significant (table 1). Results indicated that nodule number, nodule weight, straw yield and seed yield at both locations were greater in bacterial isolates treated plants than in control plants. These results indicated that the application of bacterial isolates had a tremendous effect on nodule production and seed yield in lentil plants. Results further indicated that a mixture of two bacterial isolates had no superior effect against any single bacterial isolate. The two isolates had almost similar greater effect on nodule number, nodule weight, straw yield, and seed yield. The seed and straw yield as was higher in two bacterial isolates, LB-21 and LB-40 due to increasing nodule production. These results indicate that seed yield in lentil is strongly correlated with nodule production in lentil plants.

The effect of nitrogen levels on nodules production was non-significant but was significant on nodule weight, straw, and seed yield (table 1). The nodule production plant^{-1} was not significantly influenced by nitrogen doses but significantly influenced on nodule weight plant^{-1} indicating the application of nitrogen has a positive effect on nodule size. The nodule weight plant^{-1} was significantly increased in nitrogen applied plants than control plant might be due to increasing size of nodules in nitrogen applied plants than control plants. Similar results were also reported by Iqbal *et al.* (2012) who opined that both nodule number and size increased in nitrogen applied plants than control plants in lentil. The higher number of nodule number and nodule weight plant^{-1} was observed in 25 and 50 kg nitrogen applied per hector with being the highest in 25 kg nitrogen per hector. Application nitrogen at the rate of 75 kg N ha^{-1} decreased nodule number and nodule weight plant^{-1}, straw and seed yield ha^{-1} over 25 and 50 kg N ha^{-1} might be due to overdoses of nitrogen application in soil for lentil cultivation that inhibit nodulation in lentil plants. Similar results were also reported by Huang *et al.* (2016) who observed that both nodule number and seed yield decreased in high doses of nitrogen application in lentil and mungbean plants. In low to medium fertility soils, a moderate amount of 'starter nitrogen' is required by the legume plant for nodule development, root and shoot growth before the onset of BNF. Amendment with nitrogen sources (vermicompost and urea) significantly increased legume productivity traits suggesting the nitrogen supplied played a role in legume plant growth before a symbiotic relationship of the host crop and rhizobia was fully functional. Although insignificant responses of soybean to starter N have been reported, positive responses by low doses of N have been reported by several studies (Hossain *et al.*, 2014; Huang *et al.*, 2016; Huang *et al.*, 2017) which demonstrates the need of low doses starter N, particularly in low fertility soils as it was the case in this study. In this experiment 25 kg N ha^{-1} performed best on plant growth and development.

The interaction effect between bacterial isolates and nitrogen doses on nodule number and nodule weight plant^{-1}, straw, and seed yield ha^{-1} was significant except seed yield at location-1 (table 2). The highest nodule number plant^{-1} and seed yield was recorded in LB-40 with 25 kg N ha^{-1} followed by LB-21 with 25 kg N ha^{-1}. The lowest number of nodules and nodule weight plant^{-1} as well as straw and seed yield ha^{-1} was observed in plots where no bacterial isolate and nitrogen was applied. In the 3rd experiment (2nd year), the growth parameters were recorded and the results were presented in tables 3 and 4. The effect of bacterial isolates on plant height, leaf area (LA) plant^{-1}, total dry mass (TDM) production plant^{-1} at 60 and 80 DAS, absolute growth rate (AGR) and nodule production plant^{-1} at Mymensingh was significant (table 3). The growth parameters like plant height, leaf area (LA), total dry mass (TDM) both at 60 and 80 days after sowing (DAS) and absolute growth rate (AGR) and nodule production plant^{-1} was significantly higher than that of uninoculated plant except AGR (table 3). However, amongst the bacterial isolates, there was no significant variation in growth parameters and nodule production. In case of nodule production, single bacterial isolate perform better than mixed isolates. The highest nodule production was recorded in LB-21 followed by LB-40. In contrast, the lowest number of nodules and growth was recorded in plants where no bacterial isolate was applied. Further effect of single bacterial isolate and mixture of two isolates on plant height, LA, TDM and AGR was almost same. Interesting thing is that control plant showed greater AGR than bacterial isolates treated though it produced fewer amount of TDM than isolate treated plants. Results further indicated that those plants which had higher LA and TDM also showed higher seed yield (table 5). This result indicates that higher TDM at flowering and fruiting stage is desirable for getting higher seed yield in lentil. Generally, high TDM and LA producing genotypes showed higher seed yield (Mondal *et al.*, 2013). In the present experiment, the Rhizobial strains, LB-21 and LB-40 treated plants showed higher TDM also showed higher seed yield. Seed yield are represented by the crop's early ability to intercept solar radiation and its subsequent utilization for biomass production (Mondal *et al.*, 2013). In field crop, increase interception of solar radiation at early seedling stages enable plant to make rapid early growth, resulting in high yield. In the present experiment, the isolate treated plants showed higher leaf area which intercept more solar radiation and also showed high TDM. Similar result was also reported by Dutta *et al.* (1998) in lentil who observed that the bacterial isolate treated genotypes which had capacity to early higher TDM and growth rate also showed higher seed yield. Further, the bio-fertilizer effect on growth and biomass production of legume crops has been studied (Iqbal *et al.*, 2012; Hossain *et al.*, 2014; Meleta and Abera, 2019) Haque *et al*., 2014 and found that bio-fertilizer treatment improve plant growth and development. In this study, bio-fertilizer treated plants produced higher TDM than non-biofertilizer treated plant that supported the earlier findings.

The effect of nitrogen levels on growth parameters like plant height, LA, TDM both at 60 and 80 DAS and AGR and nodule production plant^{-1} was significant (table 3). Results indicated that nitrogen applied plants performed greater than the plants where nitrogen was not applied. Results further indicated that plant height, LA, TDM, AGR and nodule number increased with increasing N doses until 50 kg ha^{-1} followed by a decline. The highest LA (433 cm^2 plant^{-1}), TDM plant^{-1} both at 60 and 80 DAS, AGR (1239 mg plant^{-1} day^{-1}) and nodule production (14.46 plant^{-1}) was recorded in 50 kg N ha^{-1} followed by 25 kg N ha^{-1} with same statistical rank. Similar results were also reported by Iqbal *et al.* (2012) who opined that both nodule number and size increased in nitrogen applied plants than control plants in lentil. Application nitrogen at the rate of 75 kg N ha^{-1} decreased plant height, growth parameters and nodule number plant^{-1} over 25 and 50 kg N ha^{-1} might be due to over doses of nitrogen application in soil for lentil cultivation that inhibit plant growth in lentil. This result indicates that application of N @ 75 kg ha^{-1} toxic for plant growth and development thereby seed yield. Similar results were also reported by Huang *et al.* (2016) and Hossain *et al.* (2014) who observed that plant growth and nodule number decreased in high doses of nitrogen application in lentil and mungbean plants.

The interaction effect between bacterial isolates and nitrogen doses on plant height, AGR, and nodule number was significant and non-significant effect on LA and TDM production plant^{-1} (table 4). The longest plant and nodule production recorded in LB-40 with 25 kg N ha^{-1}. The lowest number of nodules plant^{-1}, plant height, LA, TDM, and AGR was observed in plots where no bacterial isolate and nitrogen was applied.

The effect of bacterial isolates on yield contributing characters, straw, and seed yield was significant except 100-seed weight and harvest index (table 5). Results indicated that pod number, number of seeds pod^{-1}, straw and seed yield was greater in bacterial isolates treated plants than in control plants. This result indicated that application of bacterial isolates had tremendous effect on dry mass production and seed yield in lentil plants. Results further indicated that a mixture of two bacterial isolates had no superior effect on seed yield and yield contributing characters against any single bacterial isolate. The two isolates had almost similar greater effect on pod production, number of seeds pod^{-1}, straw and seed yield. The seed and straw yield were higher in two bacterial isolates, LB-21 and LB-40 due to increase pod production and increase number of seeds pod^{-1}. Haque *et al*. (2014) reported that application of biofertilizer increased seed yield in lentil that supported the present experimental results.

The effect of nitrogen levels on 100-seed weight and number of seeds pod^{-1} was non-significant but was significant on pod production, harvest index (HI), straw and seed yield (table 5). The seed weight was significantly increased in nitrogen applied plant than control plant might be due to increase pod production plant^{-1} in nitrogen applied plants than control plants (table 5).

Bradyrhizobium fix more atmospheric nitrogen (N_2) to the soil into ammonia (NH_3) or ammonium (NH_4^+) through the formation of root nodule which are readily available to the plant. Biofertilizer added Plant uptakes more available nitrogen for their growth and development. Readily available nitrogen helps to increase more photosynthesis and dry matter accumulation as well as increase leaf area index, thereby enhance biomass of root and shoot resulting bearing more pods and seeds pod^{-1}, 1000-grain weight of legume crops and performed more seed yield. Meleta and Abera (2019) stated that *Rhizobium* inoculants increased the grain yield of chickpea by 10.4-19.3% over uninoculated control. Tiwari *et al.* (2018) reported that inoculation of *Rhizobium* influenced maximum seed yield of lentil comparing with control. Hossain *et al.* (2014) reported that application of *Bradyrhizobium* inoculant produced significant effect on seed yields and 17-29% increase over control of Mungbean. Htwe *et al.* (2019) found that yields were more in soybean, cowpea and greengram inoculated with *Bradyrhizobium*. The highest seed yield with higher harvest index was observed in 25 kg N ha^{-1} followed by 50 kg N ha^{-1}.

The seed yield was the highest in 25 kg N ha^{-1} might be due to increased number of pods plant^{-1}. The lowest harvest index (26.54 %) was recorded in 75 kg N ha^{-1} indicating dry matter partitioning not favourible under high doses of N in lentil. Application nitrogen at the rate of 75 kg N ha^{-1} decreased seed yield and increased straw yield with lower harvest index indicating high doses of N application, plant behave vigorous growth which is not favourable in dry matter partioning for pulse crops. Similar results were also reported by Huang *et al.* (2016) and Hossain *et al.* (2014) who observed that seed yield decreased in high doses of nitrogen application in lentil and mungbean plants due to over growth under high doses of N.

Treatment	Nodules plant^{-1} (no.)		Nodule weight plant^{-1} (mg)		Straw yield (t ha^{-1})		Seed yield (kg ha^{-1})	
Inoculant	L-1	L-2	L-1	L-2	L-1	L-2	L-1	L-2
I_0	6.99 b	8.39 b	7.21 b	11.30 b	2.68 b	3.22 b	884 b	1218 b
I_1	12.84 a	11.27 a	14.28 a	16.53 a	3.30 a	3.56 a	1062 a	1339 a
I_2	12.34 a	13.45 a	13.90 a	18.87 a	3.28 a	3.37 a	1019 a	1324 a
I_1+I_2	11.96 a	12.63 a	14.07 a	16.33 a	3.02 a	3.37 a	1013 a	1306 a
F-test	**	**	**	**	**	*	**	**
Nitrogen level								
N_0	10.48	10.66	11.96 ab	14.14 b	2.87	2.99 b	929 c	1196 b
N_{25}	11.56	12.39	12.41 ab	17.21 a	3.18	3.54 a	1103 a	1369 a
N_{50}	12.00	11.59	13.40 a	16.75 a	3.18	3.52 a	1022 b	1378 a
N_{75}	10.09	11.10	11.69 b	14.93 b	3.03	3.47 a	918 c	1244 b
F-test	NS	NS	*	**	NS	**	**	**
CV (%)	27.79	32.44	28.22	25.69	11.71	13.43	10.22	11.29

Table 1: Effect of inoculant and nitrogen on nodule production and yield in lentil at Magura and Rangpur.

In a column, within treatment, figures with same letter (s) do not differ significantly at P ≤ 0.05; *, ** indicate significant at 5% and 1% level of probability, respectively, NS, not significant; L-1, location Magura; L-2, location Rangpur

Interaction		Nodules plant^{-1} (no.)		Nodule weight plant^{-1} (mg)		Straw weight (t ha^{-1})		Seed yield (t ha^{-1})	
Inocul.	N level	L-1	L-2	L-1	L-2	L-1	L-2	L-1	L-2
I_0	N_0	7.05 f	6.67 f	6.97 e	8.25 h	2.54 g	2.60 d	732	1005 d
	N_{25}	7.57ef	8.11 ef	7.01 e	11.22 g	2.67 fg	3.33 cd	942	1272 abc
	N_{50}	6.17 f	8.72 ef	7.33 e	13.51 ef	2.81 ef	3.38 bc	995	1365 ab
	N_{75}	7.17 ef	10.05 de	7.47 e	12.23 fg	2.69 fg	3.55 ab	865	1229 c
I_1	N_0	11.84 c	10.98 cd	13.2bcd	15.79cde	3.06 d	3.30 c	1075	1305 ab
	N_{25}	12.17 bc	12.88abc	14.05a-d	17.06 bc	3.32 ab	3.72 a	1195	1395 ab
	N_{50}	15.33 a	10.67 cd	16.22 a	16.93 bc	3.45 a	3.55 abc	1092	1367 ab
	N_{75}	12.00 bc	10.55 cd	13.6bcd	16.35bcd	3.35 ab	3.66 a	945	1287 ab
I_2	N_0	11.34 c	12.78abc	13.62bcd	17.97 bc	2.98 e	3.04 d	1002	1266 b
	N_{25}	14.50 ab	14.78 a	14.83abc	22.51 a	3.43 ab	3.62 ab	1083	1412 a
	N_{50}	13.84abc	13.83 ab	15.39 ab	18.72 b	3.50 a	3.50 ab	1014	1375 ab
	N_{75}	9.67 de	12.39 a-d	11.78 d	16.27 bc	3.12 cd	3.33 c	978	1244 b
I_1+I_2	N_0	11.67 c	12.22 a-d	14.05 a-d	14.54def	2.89 e	3.02 d	907	1207 c
	N_{25}	12.00 bc	13.80 ab	13.67bcd	18.04 bc	3.29 bc	3.48 abc	1190	1397 ab
	N_{50}	12.67 bc	13.82 ab	14.67 bc	17.85 bc	2.97 de	3.64 a	988	1404 a
	N_{75}	11.50 cd	11.39bcd	13.89bcd	14.87 d	2.94 de	3.52 abc	885	1315 ab
F-test		*	*	*	*	*	*	NS	**
CV (%)		27.79	32.44	28.22	25.69	11.71	13.43	10.22	11.29

Table 2: Interaction effect between inoculant and nitrogen on nodule production and yield in lentil at Magura and Rangpur

In a column, figures with same letter (s) do not differ significantly at P ≤ 0.05; *, ** indicate significant at 5% and 1% level of probability, respectively; NS, not significant; L-1, location Magura; L-2, location Rangpur

The interaction effect between bacterial isolates and nitrogen doses on pod number plant-1, straw and seed yield was significant and non-significant effect on number of seeds pod-1 and 100-seed weight (table 6). The highest and higher number of pods plant^{-1} as well as seed yield was recorded in both LB-21 and LB-40 with 25 kg N ha^{-1}. The lowest number of pods plant^{-1}, straw and seed yield was observed in plots where no bacterial isolate and nitrogen was applied. Results indicated that in both isolates, pod number plant^{-1} and seed yield decreased in in 75 kg N ha^{-1} than over 25 and 50 kg N ha-1 indicating 75 kg N ha^{-1} is over dose for lentil production. However, Harvest index was the highest in control plants where no isolate and N was applied. It means less growth is favourable for dry matter partitioning in lentil.

CONCLUSION: Application of both bacterial isolates (LB-21 and LB-40) increased nodule production and plant growth thereby seed yield in lentil with being the highest in LB-40. Application of 25 kg N ha^{-1} under both bacterial isolates performed the best in seed yield. Therefore, the bacterial isolate, LB-40 may be registered as biofertilizer after few more trials.

Treatment	Plant height (cm)	Leaf area plant^{-1} (cm^2)	Total dry mass plant^{-1} (g) 60 DAS	80 DAS	Absolute growth rate (mg g^{-1} d^{-1})	Nodules plant^{-1} (no.)
Inoculant						
I_0	55.9 b	286 b	2.68 b	4.60 b	1136 a	8.29 b
I_1	59.9 a	407 a	3.29 a	5.44 a	1073 b	15.10 a
I_2	59.7 a	391 a	3.36 a	5.47 a	1054 b	14.43 a
I_1+I_2	59.0 a	411 a	3.38 a	5.45 a	1036 b	13.33 a
F-test	*	**	**	**	**	**
Nitrogen level						
N_0	53.7 b	309 c	2.75 b	4.67 d	959 c	10.92 c
N_{25}	61.1 a	406 a	3.29 a	5.45 a	1079 b	13.70 ab
N_{50}	61.3 a	433 a	3.57 a	5.66 a	1239 a	14.46 a
N_{75}	58.4 a	347 b	3.13 a	5.17 c	1021 b	12.07 bc
F-test	**	**	**	**	**	**
CV (%)	8.92	12.34	13.20	7.80	14.30	17.11

Table 3: Effect of inoculant and nitrogen on growth and nodule production in lentil conducted during 2019-20 at Mymensingh.

In a column, within treatment, figures with same letter (s) do not differ significantly at P ≤ 0.05; ** indicates significant at 1% level of probability, respectively.

Interaction		Plant height (cm)	Leaf area plant^{-1} (cm^2)	Total dry mass plant^{-1} (g) 60 DAS	80 DAS	Absolute growth rate (mg g^{-1} d^{-1})	Nodules plant^{-1} (no.)
Inocul.	N level						
I_0	N_0	50.4	253	2.43	4.11	840 d	6.55 e
	N_{25}	56.3	271	2.55	4.68	1065 bc	8.00 de
	N_{50}	59.0 b	322	2.90	4.85	1672 a	8.50 de
	N_{75}	58.1 bc	296	2.82	4.75	965 cd	10.1 cd
I_1	N_0	53.7 d	330	2.88	4.87	995 cd	13.8 bc
	N_{25}	62.0 ab	443	3.41	5.70	1145 b	15.6 ab
	N_{50}	62.8 a	466	3.65	5.88	1115 b	15.8 ab
	N_{75}	61.3 ab	388	3.22	5.29	1035 bc	15.2 a
I_2	N_0	53.8 d	312	2.77	4.80	1015 bc	12.5 c
	N_{25}	62.3 ab	472	3.72	5.91	1095 bc	17.4 a
	N_{50}	61.8 ab	480	3.89	6.04	1075 bc	17.5 a
	N_{75}	60.7 ab	298	3.06	5.12	1030 bc	10.3 cd
I_1+I_2	N_0	57.0 c	340	2.93	4.90	985 cd	10.8 cd
	N_{25}	63.8 a	436	3.48	5.50	1010 bc	13.8 bc
	N_{50}	61.7 ab	465	3.67	5.86	1095 bc	16.0 ab
	N_{75}	53.5 d	404	3.42	5.53	1055 bc	12.7 c
F-test		**	NS	NS	NS	**	*
CV (%)		8.92	12.34	13.20	7.80	14.30	17.11

Table 4. Interaction effect between inoculant and nitrogen on growth and nodule production in lentil at Mymensingh.

In a column, figures with same letter (s) do not differ significantly at P ≤ 0.05; *, ** indicate significant at 5% and 1% level of probability, respectively, NS, not significant

Treatment	Pods plant^{-1} (no.)	Seeds pod^{-1} (no.)	100-seed weight (g)	Seed yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)
Inoculant						
I_0	126.2 b	1.59 b	2.72	1003 b	2345 b	30.47
I_1	136.0 a	1.78 a	2.81	1203 a	2732 a	30.57
I_2	137.3 a	1.81 a	2.84	1236 a	2857 a	30.30
I_1+I_2	132.0 ab	1.75 a	2.85	1152 ab	2862 a	29.63
F-test	*	**	NS	*	**	NS
Nitrogen level						
N_0	126.2 b	1.70	2.75	989 b	2131 b	31.97 a
N_{25}	139.5 a	1.77	2.85	1267 a	2790 a	31.77 a
N_{50}	137.0 a	1.76	2.84	1264 a	2854 a	30.69 a
N_{75}	132.0 ab	1.68	2.79	1075 b	3023 a	26.54 b
F-test	*	NS	NS	**	**	**
CV (%)	16.32	2.68	3.88	14.11	13.43	9.55

Table 5: Effect of inoculant and nitrogen on yield contribution characters and seed yield in lentil at Mymensingh.

In a column, within treatment, figures with same letter (s) do not differ significantly at P ≤ 0.05; *, ** indicate significant at 5% and 1% level of probability, respectively, NS, not significant

Treatment		Pods plant^{-1} (no.)	Seeds pod^{-1} (no.)	100-seed weight (g)	Seed yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)
Inocul.	N level						
I_0	N_0	116.4 b	1.56	2.64	724 c	1350 f	34.91 a
	N_{25}	132.0 a	1.70	2.76	1177 a	2540 de	31.66 ab
	N_{50}	137.1 a	1.54	2.78	1190 a	2781 cd	29.96 bc
	N_{75}	120.0 b	1.55	2.70	921 bc	2710 cd	25.36 d
I_1	N_0	133.5 a	1.77	2.75	1147 a	2420 e	32.15 ab
	N_{25}	142.2 a	1.80	2.87	1302 a	2784 cd	31.86 ab
	N_{50}	140.0 a	1.83	2.84	1291 a	2840 cd	31.25 b
	N_{75}	129.2 ab	1.70	2.78	1071 b	2892 c	27.02 cd
I_2	N_0	128.0 a	1.73	2.80	1142 a	2452 e	31.78 ab
	N_{25}	142.0 a	1.82	2.90	1324 a	2872 c	31.55 ab
	N_{50}	137.6 a	1.87	2.85	1269 a	2910 bc	30.42 bc
	N_{75}	134.3 ab	1.80	2.82	1208 a	3195 ab	27.43 cd
I_1+I_2	N_0	126.4 b	1.75	2.81	942 bc	2300 e	29.06 bc
	N_{25}	140.0 a	1.77	2.88	1263 a	2965 b	31.99 ab
	N_{50}	134.6 ab	1.80	2.88	1304 a	2886 c	31.12 b
	N_{75}	128.0 ab	1.68	2.87	1180 a	3296 a	26.36
F-test		*	NS	NS	*	**	*
CV (%)		16.32	2.68	3.88	14.11	13.43	9.55

Table 6. Interaction effect between inoculant and nitrogen on yield contribution characters and seed yield in lentil at Mymensingh.
In a column, figures with same letter (s) do not differ significantly at $P \leq 0.05$; *, ** indicate significant at 5% and 1% level of probability, respectively, NS, not significant

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REFERENCES: Ali, M. A., M. Hussain, W. H. Malik and M. Tahir, 2004. Evaluation of the effect of rhizobium inoculation and phosphorus, grain yield and nodulation behaviour of lens culinaris. Pakistan journal of life social science, 22: 112-114.

Beadle, C., 1985. Plant growth analysis. In: Techniques in bioproductivity and photosynthesis. Elsevier: pp: 20-25.

Corstanje, R., K. Reddy, J. Prenger, S. Newman and A. Ogram, 2007. Soil microbial eco-physiological response to nutrient enrichment in a sub-tropical wetland. Ecological indicators, 7(2): 277-289.

Dutta, R., M. Mondal and A. Podder, 1998. Response of lentil to *Bradyrhizobial* strains in relation to nitrate reduction and energy. Lentil experimental news service(25): 55-59.

Gan, Y., K. Hanson, R. Zentner, F. Selles and C. McDonald, 2005. Response of lentil to microbial inoculation and low rates of fertilization in the semiarid C*canadian prairies*. Canadian journal of plant science, 85(4): 847-855.

Hossain, M. E., I. F. Chowdhury, M. Hasanuzzaman, S. Mazumder, M. A. Matin and R. Jerin, 2014. Effect of nitrogen and bradyrhizobium on growth and yield of mungbean. Journal of biosciences and agriculture research, 1(02): 79-84.

Htwe, A. Z., S. M. Moh, K. M. Soe, K. Moe and T. Yamakawa, 2019. Effects of biofertilizer produced from bradyrhizobium and streptomyces griseoflavus on plant growth, nodulation, nitrogen fixation, nutrient uptake, and seed yield of mung bean, cowpea, and soybean. Agronomy, 9(2): 77.

Huang, J., R. Keshavarz Afshar and C. Chen, 2016. Lentil response to nitrogen application and rhizobia inoculation. Communications in soil science plant analysis, 47(21): 2458-2464.

Huang, J., R. Keshavarz Afshar, A. Tao and C. Chen, 2017. Efficacy of starter n fertilizer and rhizobia inoculant in dry pea (*Pisum sativum Linn.*) production in a semi-arid temperate environment. Soil science plant nutrition, 63(3): 248-253.

Iqbal, M. A., M. Khalid, S. M. Shahzad, M. Ahmad, N. Soleman and N. Akhtar, 2012. Integrated use of rhizobium leguminosarum, plant growth promoting rhizobacteria and enriched compost for improving growth, nodulation and yield of lentil (*Lens culinaris Medik.*). Chilean journal of agricultural research, 72(1): 104.

Meleta, T. and G. Abera, 2019. Effects of rhizobium inoculation and phosphorus fertilizer rates on growth, yield and yield components of chickpea (*Cicer arietinum* L.) at goro, bale zone, oromia regional state. International journal of applied agricultural sciences, 5(3): 62.

Mondal, M., A. Puteh, M. Malek and M. Rafii, 2013. Contribution of morpho-physiological attributes on yield in lentil. Plant omics, 7: 1503-1507.

Tiwari, A., V. Prakash, A. Ahmad and R. Singh, 2018. Effect of biofertilizers and micronutrients on nutrient uptake, growth, yield and yield attributes of lentil (*Lens culinaris* L.). International journal of current microbiology applied sciences, 7(02): 3269-3275.

Zakeri, H. and R. J. C. S. Bueckert, 2015. Post-flowering biomass and nitrogen accumulation of lentil substantially contributes to pod production. Crop science, 55(1): 411-419.