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Research Paper

Nitrogen, *Rhizobium* and molybdenum for higher soil bacterial population, N uptake and productivity of lentil (*Lens culinaris*) grown under rice (*Oryza sativa*)-fallow conditions

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ABSTRACT

A field experiment was conducted during the winter (*rabi*) seasons of 2014–15, 2015–16 and 2016–17 in loamy soils of the Regional Agricultural Research Station, Assam Agricultural University, Shillongani, Assam, to study the effect of varying doses of nitrogen, *Rhizobium* and molybdenum on bacterial population in soil, N uptake, yield attributes, grain yield and economics of lentil (*Lens culinaris* Medikus spp. *culinaris*) grown after the harvesting of winter rice (*Oryza sativa* L.) variety 'Gitesh'. Soil bacterial population increased with the increase in nitrogen doses. The crop grown using seeds inoculated with *Rhizobium* @ 50 g/kg seed and sodium molybdate @ 1 g/kg seed along with 100% of recommended dose (RD) of N (15 kg/ha) accrued in the highest bacterial population in the soil at harvesting. Seed inoculation with *Rhizobium* and sodium molybdate led to considerably higher bacterial population build up in soil than seed inoculation with *Rhizobium* and sodium molybdate separately. Nitrogen uptake by lentil got reduced with decreasing N doses, being 28.54 kg/ha under RD of N and 28.03 kg/ha under 75% of recommended N. The treatment combinations involving RD and 75% of RD of N (11.25 kg/ha) and seed inoculation with *Rhizobium* and sodium molybdate recorded 35.16 kg/ha and 34.97 kg/ha N uptake respectively. These 2 treatment combinations ultimately accrued in higher values for plant height, pods/plant, seeds/pod and 100-seed weight and significantly higher grain yield (928.90 and 868.89 kg/ha respectively) as compared to all other treatment combinations. Benefit: cost ratios under these 2 treatment combinations were 2.18 and 2.09 respectively. The treatment combinations were tested for maximizing lentil production utilizing rice-fallows and farmers' acceptability in farmers' fields during *rabi* 2017–18 through 3 Krishi Vigyan Kendras (KVK) in Upper Brahmaputra Valley (UBV) in Jorhat district, Central Brahmaputra Valley (CBV) in Nagaon district and Lower Brahmaputra Valley (LBV) in Nalbari district of the state. The RD of N + seed inoculation with *Rhizobium* and sodium molybdate yielded the highest (5.8–8.2 q/ha) at all the locations closely followed by 75% RD of N + *Rhizobium* + sodium molybdate (5.93–7.25 q/ha).

Key words: Bacterial population, Benefit: cost ratio, Grain yield, Lentil, N dose, N uptake, Rice-fallows, *Rhizobium*, Sodium molybdate

Lentil (*Lens culinaris* Medikus) is an important pulse crop of India cultivated on 1.46 million ha area with total production of 1.22 million tonnes and a productivity of 838 kg/ha (2016–17). It is grown in 28,185 ha with production and productivity of 21,018 t and 746 kg/ha, respectively, in Assam (2017–18). However, there is vast scope for increasing area under lentil in rice (*Oryza sativa* L.)-fallows. An estimate showed that almost 82% of the rice-fallow area is found in only seven Indian states, viz. Chattisgarh, Jharkhand, Madhya Pradesh, Assam, Bihar, Odisha and West Bengal (Pande *et al*., 2012). In North-Eastern region of India, where a large part of the area remains fallow after the rainy (*kharif)* season rice (Das *et al*., 2012), there exists a scope for area expansion under pulse crops like lentil in rice-fallows (Das *et al*., 2013). Conservation tillage provides better ecosystem to crop than that of conventional tillage (Lal, 2013). Lentil is mostly grown in rice relay system (Ghosh *et al*., 2016). However, in lowlands, due to delayed disappearance of ponded water, it can be grown under zero tillage conditions with retention of rice stubbles. Winter crops in rice fallows often confront a series of abiotic and biotic stresses. They experience variable degrees of nutrient deficiencies. As a result of poor soil physical conditions and low native *Rhizobium* population

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in typical rice-fallow ecosystem, nutrient mobilization is substantially reduced (Ali *et al*., 2014). Layek *et al.,* (2014) reported that, foliar application of diammonium phosphate or urea at pre-flowering stage enhances productivity of pulses in rice-fallows. This showed the importance of right dose of N fertilization and that of seed inoculation with *Rhizobium* in rice-fallows. Deficiency of micronutrients is also very common and there is a decreasing trend of soil micronutrients in rice-fallows. Therefore, supplementary application of micronutrients particularly of molybdenum is necessary in acidic rice-fallow soils (Ghosh *et al*., 2016). Molybdenum is directly involved in nitrogen-fixing enzymes nitrogenase and N reduction enzyme, nitrate reductase especially for legumes forming root nodules (Rahman *et al*., 2008). Keeping these in view, the present investigation was undertaken to find out right dose of N along with seed inoculation with *Rhizobium* and sodium molybdate for maximizing lentil production in rice-fallows under sequential cropping.

MATERIALS AND METHODS

A field experiment was conducted during the winter (*rabi*) seasons of 2014–15, 2015–16 and 2016–17 at the Regional Agricultural Research Station, Assam Agricultural University, Shillongani, Nagaon, Assam (92’65°E, 26.21°N and 50.2 m above mean sea-level). The technologies generated, viz. recommended dose (RD) of N (15 kg/ha) + seed inoculation with *Rhizobium* @ 50 g/kg seed and sodium molybdate @ 1 g/kg seed, and 75% of RD of N + seed inoculation with *Rhizobium* @ 50 g/kg seed and sodium molybdate @ 1 g/kg seed, were tested through Krishi Vigyan Kendras (KVKs) for farmers’ acceptability in 3 agro-climatic zones of the state during *rabi* 2017–18. Four levels of N, viz. RD (15 kg/ha), 75% of RD, 50% of RD and 25% of RD and, 4 seed inoculation treatments, viz. no seed inoculation, *Rhizobium* @ 50 g/kg, sodium molybdate @ 1 g/kg and *Rhizobium* + sodium molybdate (Table 1), were evaluated in factorial randomized block design with 3 replications. Recommended doses of P_2O_5 (35 kg/ha) and K_2O (15 kg/ha) were common to all the treatments. The soil was loamy, having pH 5.6, organic C 0.78%, available N 273.4 kg/ha, available P_2O_5 21.2 kg/ha and available K_2O 133.8 kg/ha in 2014–15; pH 5.6, organic C 0.78%, available N 274.0 kg/ha, available P_2O_5 22.3 kg/ha and available K_2O 135.5 kg/ha in 2015–16, and *p*H 5.6, organic C 0.79%, available N 276.4 kg/ha, available P_2O_5 22.7 kg/ha and available K_2O 138.2 kg/ha in 2016–17. Lentil variety ‘HUL 57’ was broadcast on 30, 28 and 30 November in respective year of study using a seed rate of 30 kg/ha. The crop was sown 2 days after harvesting of winter rice variety ‘Gitesh’ with rice-stubble retention to a height of about 30 cm under no-till conditions. The soil moisture at the time of sowing was 18.50, 20.35 and 17.66% in respective year of experimentation. This level of soil moisture (72.5–83.5% of field capacity) was sufficient for germination of the seeds. The field capacity of the soil was 24.37%. The crop duration was 118, 120 and 117 days, respectively, in the 3 years of study. The total rainfall received during crop-growth period of rice was 961.3 mm in 2014, 876.2 mm in 2015 and 981.0 mm in 2016. The mean maximum and minimum temperature recorded were in the range of 25.48 to 26.78°C and 14.46 to 14.98°C, respectively (mean of 3 years); mean relative humidity ranged from 84.2 to 86.2% (morning) and 57.5 to 59.6% (evening) during the crop-growth period, while rainfall was 30.3 mm in 2014–15, 143.9 mm in 2015–16 and 87.8 mm in 2016–17. The soil analyses for initial nutrient status and plant analyses for N uptake were done by using standard methods. The B:C was computed by dividing gross return by cost of cultivation based on prevailing market prices of inputs and outputs.

The enumeration of soil bacterial population was done on agar plates containing appropriate media following serial dilution technique and pour-plate method (Pramer and Schmidt, 1966). Yeast mannitol agar medium was used for counting bacterial population. The *p*H of the medium was maintained at 6.8 ± 0.2 and the medium was sterilized at 15 lbs (6.80 kg) steam pressure for 20 minutes. Plates were incubated at 30°C. The counts were recorded on day 5 of incubation as number of cell/g soil. For this, soil samples were collected 90 days after sowing (DAS).

Table 1. Soil microbial population (Bacteria, 10^7 cfu/g soil)

N dose	Seed inoculation				
	No	*Rhizobium* 50 g/kg	Sodium molybdate 1 g/kg	*Rhizobium* + Sodium molybdate	Mean
Recommended dose (15 kg/ha)	38	57	39	63	49.25
75% Recommended dose	32	52	35	58	44.25
50% Recommended dose	29	48	31	54	40.50
25% Recommended dose	26	43	29	49	36.75
Mean	31.25	50.00	33.50	56.00	

RESULTS AND DISCUSSION

Soil bacteria

Total bacterial population in soil increased with gradual increase in N dose (Table 1). Zhou and Jiang (2017) reported that, N addition consistently decreased bacterial diversity and altered bacterial community composition. Nitrogen fertilization significantly improves the availability of N in soil, and it stimulates the growth of soil-dwelling microorganisms (Strachel *et al*., 2017). Increased nitrogen fertilization increases the numbers of bacteria in the rhizosphere (Kolb and Martin, 1988). Liljeroth *et al.,* (1990) also found that, total number of bacteria, counted on a relatively poor medium, was larger at higher nitrogen concentrations.

There was no considerable increase in soil bacterial population when seed inoculation was done with sodium molybdate 1g/kg. However, seed inoculation with *Rhizobium* 50 g/kg accrued in considerably higher bacterial population in rhizophere than no inoculation. Further, when seeds were inoculated with both *Rhizobium* and sodium molybdate, bacterial count in the rhizospheric soil was the highest. The maximum number of soil bacteria (63×10^7 cfu/g soil) was observed with combination of 100% RD of N + seed inoculation with *Rhizobium* and sodium molybdate. In rice-fallows, because of flooded condition during the most part of the rice crop cycle, native *Rhizobium* population was drastically low. It makes it mandatory to use *Rhizobium* for seed inoculation while growing any leguminous crop in rice lands. To increase the activity of *Rhizobium*, application of molybdenum is of great significance. It plays an important role in N metabolism and protein synthesis in plants. During symbiotic N_2-fixation, it acts as a cofactor for nitrogenase enzymes to catalyze the redox reaction (Alam *et al*., 2015).

Nitrogen uptake

Nitrogen uptake by lentil was directly proportional to N dose up to 75% of RD (Table 2). Further increase in N dose by 25% led to marginal increase in N uptake (28.54 kg/ha) over 75% RD (28.03 kg/ha). Kabir *et al.,* (2019) also reported higher N uptake with the application of higher N dose in lentil. Amongst the seed inoculation treatments, *Rhizobium* (50 g/kg) + sodium molybdate (1 g/kg) resulted in the highest N uptake (27.53 kg/ha) by lentil. The success of pulse production depends on biological N_2 fixation affected by *Rhizobium*, and efficacy of *Rhizobium* is determined by availability of Mo besides other factors. This led to higher N uptake by lentil. Similar was the observation of Swarnalakshmi *et al*. (2016). The treatment combinations of RD of N + *Rhizobium* + sodium molybdate (35.16 kg/ha) and 75% RD of N + *Rhizobium* + sodium molybdate (34.97 kg/ha) recorded almost equal values of N uptake. Higher plant N content and uptake were also reported by Carlos *et al*., (2019) in peanut (*Arachis hypogaea* L.) crop grown in a no-tillage system after 20 years of pasture under the combination of Rhizobial inoculation and Mo fertilization.

Yield attributes, crop yield and economics

Grain yield of lentil in rice-fallows was significantly affected by varying N doses and seed inoculation treatments (Table 7). There was significant increase in grain yield with increasing N dose up to 75% of RD. Though the highest grain yield (787.47 kg/ha) was recorded under RD of N, it was statistically at par with that under 75% RD of N (730.88 kg/ha). Under these 2 treatments, plant height, pods/plant, seeds/pod and 100-seed weight were also higher as compared to the lower doses of N (Tables 3, 4, 5, 6). Singh *et al*., (2017) reported that, the RD of NPK accelerated crop growth, chlorophyll pigment *a* and *b*, nodulation, yield attributes and above all, grain yield (over 50% of RD) of chickpea (*Cicer arietinum* L.) grown in rice-fallows. Amongst the seed inoculation treatments, *Rhizobium* + sodium molybdate accrued in the highest grain yield (771.96 kg/ha), a resultant of the highest values for plant height, pods/plant, seeds/pod and 100-seed weight, which was significantly higher than *Rhizobium* and sodium molybdate inoculation separately. Rhizobial inoculation and

Table 2. nitrogen uptake (kg/ha) in lentil (pooled data of 3 years)

N dose	Seed inoculation				
	No	*Rhizobium* 50 g/kg	Sodium molybdate 1 g/kg	*Rhizobium* + Sodium molybdate	Mean
Recommended dose (RD) (15 kg/ha)	22.11	30.25	26.62	35.16	28.54
75% Recommended dose	20.43	30.10	26.54	34.97	28.03
50% Recommended dose	19.02	21.46	21.23	22.05	20.93
25% Recommended dose	16.80	17.18	17.50	18.00	17.36
Mean	19.60	24.75	22.98	27.53	
CD (P=0.05)	N Dose: 2.27	Inoculation: 2.27	Interaction: 5.04		

Table 3. Plant height (cm) of lentil (pooled data of 3 years)

N dose	Seed inoculation				
	No	*Rhizobium* 50 g/kg	Sodium molybdate 1 g/kg	*Rhizobium* + Sodium molybdate	Mean
Recommended dose (RD) (15 kg/ha)	34.20	41.00	38.30	42.50	39.00
75% Recommended dose	33.90	38.80	38.40	40.60	37.93
50% Recommended dose	32.70	36.20	33.50	37.10	34.88
25% Recommended dose	31.80	34.40	32.20	35.00	33.35
Mean	33.15	37.60	35.60	38.80	
CD (P=0.05)	N Dose: 2.33	Inoculation: 2.33	Interaction: NS		

Table 4. Pods/plant in lentil (pooled data of 3 years)

N dose	Seed inoculation				
	No	*Rhizobium* 50 g/kg	Sodium molybdate 1 g/kg	*Rhizobium* + Sodium molybdate	Mean
Recommended dose (RD) (15 kg/ha)	55.20	84.87	75.60	89.67	76.33
75% Recommended dose	54.27	77.20	74.53	80.13	71.53
50% Recommended dose	50.93	66.00	52.80	70.60	60.08
25% Recommended dose	48.00	56.73	49.60	60.53	53.78
Mean	52.10	71.20	63.13	75.23	
CD (P=0.05)	N Dose: 3.62	Inoculation: 3.62	Interaction: 7.25		

Table 5. Seeds/pod in lentil (pooled data of 3 years)

N dose	Seed inoculation				
	No	*Rhizobium* 50 g/kg	Sodium molybdate 1 g/kg	*Rhizobium* + Sodium molybdate	Mean
Recommended dose (15 kg/ha)	1.93	2.00	1.87	2.00	1.95
75% Recommended dose	1.87	1.93	1.93	2.00	1.93
50% Recommended dose	1.73	1.87	1.80	1.87	1.87
25% Recommended dose	1.73	1.80	1.73	1.80	1.77
Mean	1.82	1.90	1.83	1.92	
CD (P=0.05)	N Dose: 0.08;	Inoculation: 0.08;	Interaction: NS		

Table 6. 100-seed weight (g) in lentil (pooled data of 3 years)

N dose	Seed inoculation				
	No	*Rhizobium* 50 g/kg	Sodium molybdate 1 g/kg	*Rhizobium* + Sodium molybdate	Mean
Recommended dose (RD) (15 kg/ha)	2.13	2.28	2.18	2.32	2.23
75% Recommended dose	2.11	2.25	2.15	2.29	2.20
50% Recommended dose	2.09	2.24	2.12	2.28	2.18
25 % Recommended dose	2.10	2.20	2.08	2.26	2.16
Mean	2.11	2.24	2.13	2.29	
CD (P=0.05)	N Dose: 0.03;	Inoculation: 0.03;	Interaction: NS		

Table 7. Grain yield (kg/ha) of lentil

Treatment	2014–15	2015–16	2016–17	Pooled
N dose				
Recommended dose of N (15 kg/ha)	654.14	886.11	831.11	787.47
75% Recommended dose of N	576.39	820.11	796.11	730.88
50% Recommended dose of N	530.56	629.17	675.56	611.74
25% Recommended dose of N	387.50	495.14	521.67	468.08
CD (P=0.05)	30.03	52.08	40.93	59.03
Seed inoculation				
No inoculation	434.03	620.11	563.89	539.34
Rhizobium 50 g/kg	590.28	729.86	740.56	686.90
Sodium molybdate 1 g/kg	475.01	658.33	666.67	600.00
Rhizobium + Sodium molybdate	640.28	822.22	853.33	771.96
CD (P=0.05)	30.03	52.08	40.93	59.03
CV (%)	7.04	8.83	7.31	

Mo fertilization could potentially be combined in effort to recover degraded areas, enhance biological N_2-fixation, etc. These 2 in combination had exerted positive effects on seed yield of various leguminous crops in Brazil, such as soybean [*Gycine max* (L.) Merr.], common bean, and peanut. Inoculation of seeds led to greater development of

Table 8. Interaction effect on grain yield (kg/ha) of lentil (pooled data of 3 years)

N dose	Seed inoculation			
	No	*Rhizobium* 50 g/kg	Sodium molybdate 1 g/kg	*Rhizobium* + Sodium molybdate
Recommended dose (15 kg/ha)	678.90	822.97	719.06	928.90
75% Recommended dose	595.52	785.36	673.70	868.89
50% Recommended dose	477.60	639.80	585.36	744.26
25% Recommended dose	405.35	499.43	545.74	545.74
CD (P=0.05)	92.51			

Table 9. Economics of different treatment combinations in lentil

Treatment	Cost of cultivation (₹/ha)	Gross return (₹/ha)	Net return (₹/ha)	Benefit: cost ratio
RD of N (15 kg/ ha) + no seed inoculation	22,505	40,734	18,229	1.81
RD of N (15 kg/ ha) + Seed inoculation with *Rhizobium* @ 50 g/kg	24,813	49,378	24,565	1.99
RD of N (15 kg/ ha) + Seed inoculation with sodium molybdate @ 1 g/kg seed	24,795	43,144	18,349	1.74
RD of N (15 kg/ ha) + Seed inoculation with *Rhizobium* @ 50 g/kg + sodium molybdate @ 1 g/kg seed	25,616	55,734	30,118	2.18
75% RD of N + no seed inoculation	22,332	35,731	13,399	1.60
75% RD of N + Seed inoculation with *Rhizobium* @ 50 g/kg	24,171	47,122	22,951	1.94
75% RD of N + Seed inoculation with sodium molybdate @ 1 g/kg seed	23,898	40,422	16,524	1.69
75% RD of N + Seed inoculation with *Rhizobium* @ 50 g/kg + sodium molybdate @ 1 g/kg seed	24,971	52,133	27,162	2.09
50% RD of N + no seed inoculation	22,160	28,656	6,496	1.29
50% RD of N + Seed inoculation with *Rhizobium* @ 50 g/kg	23,531	38,388	14,857	1.63
50% RD of N + Seed inoculation with sodium molybdate @ 1 g/kg seed	23,002	35,123	12,121	1.53
50% RD of N + Seed inoculation with *Rhizobium* @ 50 g/kg + sodium molybdate @ 1 g/kg seed	24,331	44,656	20,325	1.83
25% RD of N + no seed inoculation	21,989	24,321	2,332	1.11
25% RD of N + Seed inoculation with *Rhizobium* @ 50 g/kg	22,890	29,966	7,076	1.31
25% RD of N + Seed inoculation with sodium molybdate @ 1 g/kg seed	22,107	32,744	10,637	1.48
25% RD of N + Seed inoculation with *Rhizobium* @ 50 g/kg + sodium molybdate @ 1 g/kg seed	23,689	32,744	9,055	1.38

Price of lentil grain, ₹ 60/kg; *Rhizobium* culture, ₹ 75/kg; sodium molybdate, ₹ 1,152/100 g

Table 10. Grain yield and benefit: cost ratio under on-farm testing

Treatment	Nagaon		Jorhat		Nalbari	
	Grain yield (q/ha)	Benefit: cost ratio	Grain yield (q/ha)	Benefit: cost ratio	Grain yield (q/ha)	Benefit: cost ratio
RD of N (15 kg/ha) + Seed inoculation with *Rhizobium* @ 50 g/kg+ Sodium molybdate @ 1 g/kg seed	5.80	2.76	7.7	2.39	8.20	2.50
75% RD of N + Seed inoculation with *Rhizobium* @ 50 g/kg + Sodium molybdate @ 1 g/kg seed	5.93	2.65	7.2	2.18	7.25	2.38
Farmer's practice (RD of N)	4.93	2.11	6.7	2.08	5.9	2.15

plant root system and nodulation and, consequently greater uptake of nutrients and water (Carlos *et al*., 2019). The combination of RD of N and seed inoculation with *Rhizobium* and sodium molybdate led to the highest grain yield (928.90 kg/ha), which was significantly higher than those under all the other treatment combinations barring that (868.89 kg/ha) affected by the combination of 75% RD of N and seed inoculation with *Rhizobium* and sodium molybdate (Table 8). Because, the former treatment combination recorded the highest plant height, pods/plant, seeds/pod and 100-seed weight. The B: C ratio with these 2 treatment combinations were 2.18 and 2.09 respectively (Table 9). The RD of N + seed inoculation with *Rhizobium* and sodium molybdate yielded the highest (5.8–8.2 q/ha) at all the locations, closely followed by 75% RD of N + *Rhizobium* + sodium molybdate (5.93–7.25 q/ha) in the verification study at farmers' fields of Nagaon, Jorhat and Nalbari districts of Assam through the respective KVKs (Table 10).

From this study, it can be concluded that, for sustainable and profitable lentil production in rice fallows under sequential cropping, the crops should be fed with 75–100% RD of N (15 kg/ha) besides seed inoculation with *Rhizobium* culture @ 50 g/kg and sodium molybdate @ 1 g/kg seed.

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