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Symbiotic Efficiency of Native and Exotic *Rhizobium* Strains Nodulating Lentil (*Lens culinaris* Medik.) in Soils of Southern Ethiopia

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Abstract: Lentil plays a major role in the food and nutritional security of low income Ethiopian families because of the high protein content of their seed; however, their productivity typically is low largely due to soil fertility limitations. Field and pot experiments were conducted during the 2011 cropping season to determine the effectiveness of *Rhizobium* strains on two cultivars of lentil in Southern Ethiopia. Six rhizobial inoculant treatments (four indigenous and two commercial inoculants), a nitrogen (N) fertilizer treatment (50 kg· urea· ha^{-1}) and an absolute control (non-inoculated non-fertilized) were used. Inoculated plants produced significantly higher nodule number, nodule dry weight, grain yield and yield components than non-inoculated non-fertilized plants. Inoculation of field grown lentil with rhizobia strain Lt29 and Lt5 enhanced seed yield by 59% and 44%, respectively. Whereas urea fertilization enhanced yields by 40%. Similarly, grain yields were increased during the pot experiment by 92% and 67% over the control treatments by inoculation with Lt29 and Lt5, respectively. The highest levels of N fixation were achieved in plants inoculated with Lt29 (65.7% Ndfa). Both field and pot investigations indicate that inoculation of lentil with native rhizobial strains replace the need for inorganic N fertilization to optimize lentil yields.

Keywords: Lentil; N_2 fixation; rhizobial inoculant; nodulation; fixed N

1. Introduction

Lentil (*Lens culinaris* Medik.) is an important cool-season food legume crop in Ethiopia and is considered vital to food and nutritional security. It is a popular ingredient in every day diets in the majority of households in Ethiopia, used in "misirwot" (a sauce of split or whole seeds) and in soup (from whole seeds or flour). For those who cannot afford animal products, lentil is a vital protein source, with a mean grain protein content of 23.25% [1]. Additionally, lentil seed typically contains important macro and micronutrients (Ca, P, K, Fe, and Zn), vitamins (Niacin, Vitamin A, ascorbic acid, and inositol), fiber, and carbohydrates for balanced nutrition [2]. Lentil also is rich in lysine, an essential amino acid found only at low levels in cereal protein [3]. Lentil straw also is a valued animal feed.

In Ethiopia, lentil is grown on small-scale farms as a food and cash crop. Ethiopia ranks first in its production and hectareage in Africa [4] with 123.7 thousand hectares sown to lentil in 2012/13, among which only 685 ha (0.56%) was grown in the southern regional state. The average yield of lentil in the region is 0.82 tonnes· ha^{-1}, which is low compared to the national average of 1.22 tonnes· ha^{-1} [5].

The low average yield of the crop in the region might be attributable to a lack of adapted high yielding cultivars, inadequate agronomic management, planting on marginal lands without any fertilizer application, and the lack of indigenous rhizobial symbionts of lentil in the soil as a result of continuous cereal mono-cropping.

Low soil fertility is partly responsible for declining crop yields and food security concerns for smallholder farms in many parts of Ethiopia. Crop residues are used as livestock feed and manure as fuel resulting in a net export of nutrients without adequate replenishment, and cropping practices have contributed to severe topsoil erosion [6,7]. Nitrogen deficiencies, in particular, limit agricultural production whereas lentil, as a legume, has the ability to fix atmospheric N through symbiotic association with rhizobia. The inclusion of lentil, along with other pulse crops, can benefit succeeding crops by improving soil health as a result of biological nitrogen fixation (BNF).

Rhizobial inoculants are now widely used in various parts of the world. They are inexpensive, environment-friendly, and easy to use with no side effects in most cases [8]. The *Rhizobium*-legume association can be manipulated, through inoculation under N-limiting field conditions, to enhance crop production easily and inexpensively [9]. Where soils do not contain the specific rhizobia needed to establish an effective association, inoculation is essential, ensuring that a large and effective rhizobial population is available in the rhizosphere of the plant [10]. Several reports demonstrated significant improvement of yield and yield components in lentil with *Rhizobium* inoculation [11–13].

This study was conducted to evaluate the efficacy of indigenous *Rhizobium* strains previously isolated from root nodules of lentil (cultivar TESHALE) grown in soils collected from lentil growing fields from diverse agro-ecological locations within the southern and central parts of Ethiopia. These strains were assessed in a preliminary experiment in which 48 *Rhizobium* strains were assessed for their ability to enhance nodulation and biomass yield under controlled conditions in modified Leonard jar (unpublished). Selected indigenous strains were subsequently compared to commercial inoculants and inorganic N fertilizer for the ability to enhance growth parameters including nodulation and seed yield under greenhouse and field conditions in southern Ethiopia.

2. Material and Method

2.1. Estimation of Indigenous Rhizobia

Soil samples were collected from Canadian International Food Security Research Fund (CIFSRF) project sites at Huletegna Choroko (latitude 07°20′ N and longitude 38°06′ E), Taba (latitude 07°01′ N and longitude 37°53′ E), Ele (latitude 08°10′ N and longitude 38°00′ E) and Jole Andegna (latitude 08°12′ N and longitude 38°27′ E). All sites are from Southern Nations, Nationalities and Peoples Regional State of Ethiopia. A most-probable number (MPN) count was used to determine the number of viable and infective rhizobia at each site [14]. Briefly, 10 g of soil were diluted in 90 mL sterilized distilled water, followed by serial dilution to 10^{-10} and subsequently used to inoculate lentil seedlings grown in acid treated and sterilized sand using four replications. Nodule observations were made 45 days after inoculation. The enumeration of indigenous rhizobial populations by the MPN method [15] revealed that the population size of indigenous rhizobia compatible to this crop varied at different locations, ranging from 0 at Ele and Huletegna Choroko, to 1.7×10^3 and 1.7×10^4 cell$\cdot$ g^{-1} of soils at Taba and Jole, respectively. Based on the need for inoculation, Huletegna Choroko was selected for the field experiments and soil for pot experiments was collected from this site.

2.2. Description of Field Experiment Study Site

A field experiment was conducted during the lentil growing season of 2011 at Huletegna Choroko (latitude 07°20′ N and longitude 38°06′ E, at an altitude of 1650 m above sea level). The average annual rainfall at Halaba (2002 to 2011), the nearby metrological station, is 974 mm having minimum and maximum temperatures of 13.6 and 28.1 °C, respectively (Table 1). Before sowing, soil samples were taken from representative points from the 0–30 cm depth to make one composite surface soil sample for analysis of soil texture and some chemical properties as results depicted below (Table 2), according to standard methods [16]. Soil analysis indicated that the textural class of the surface soil was clay loam and the soil was slightly acidic (pH: 6.5) with low organic carbon and total nitrogen N contents [17]. The cation exchange capacity (CEC) of the soil was medium and Ca, Mg and K concentrations in the soil were adequate for crop production. Available phosphorus (P) content (10.8 mg· kg^{-1} soil) was low and application of P fertilizer may be required for optimum crop production. The availability of Mn, Zn, Fe and Cu was sufficient to meet crop needs [18]. The major soils of Huletegna Choroko area were classified as Andic Lixisols and Andic Cambisols [19].

Table 1. Average rainfall, maximum and minimum temperature during the 2011 growing season, annual and long-term average (2002–2011) at Halaba, nearby metrological station of the study area.

Year		Rainfall	[a] Max. T	[b] Min. T
		mm	°C	°C
	July	118.6	24.1	14.3
2011	August	139.4	25.4	14.2
	September	98.7	27.4	14.4
	Annual	968.3	26.5	13.4
10 years (2002–2011)	Annual Average	973.9	28.1	13.6
Altitude	m above sea level		1650	

[a] Maximum Temperature; [b] Minimum Temperature.

Table 2. Soil physicochemical characteristics of the 0 to 30 cm soil layer of the experimental sites in Huletegna Choroko at the initiation of the experiments in 2011.

Soil Characteristic	Value	Soil Characteristic	Value
Texture class [a]	Clay Loam	K (cmolc· kg^{-1}soil)	1.62
pH-H_2O (1:2.5)	6.5	Mg (cmolc· kg^{-1}soil)	2.34
EC (ms· cm^{-1}) (1:2.5)	0.15	Ca (cmolc· kg^{-1}soil)	11.35
Organic Carbon (%) [b]	0.91	Micronutrients [g]	
Total Nitrogen (%) [c]	0.08	Cu (mg· kg^{-1}soil)	0.51
Available P (mg· P· kg^{-1}soil) [d]	10.8	Fe (mg· kg^{-1}soil)	3.17
CEC (cmolc· kg^{-1}soil) [e]	25.3	Mn (mg· kg^{-1}soil)	2.43
Exchangeable bases [f]	0.91	Zn (mg· kg^{-1}soil)	3.32
Na (cmolc· kg^{-1}soil)	1.62		

Method: [a] Hydrometer; [b] Walklay and Black; [c] Kjeldahl; [d] Olsen; [e&f] Ammonium acetate; [g] diethylenetriaminepentaacetic acid (DTPA).

2.3. Treatments and Experimental Design

The treatments included two varieties of lentil (Alemaya and Teshale). Six rhizobial inoculants were used: the four best indigenous strains Lt5 (isolated from Jole Andegna, Mesqan District, Southern Ethiopia), Lt29 (isolated from Ele, Mesqan District, Southern Ethiopia), Lt87 (isolated from Halaba District, Southern Ethiopia), and Lt136 (isolated from Dodola District, West Arsi, Ethiopia); LtNSTC (National Soils Testing Center inoculant); and LtSK (a Canadian commercial inoculant). Additionally, a N fertilizer treatment (50 kg urea per hectare, no inoculant), and an absolute control (no fertilizer and inoculation) were included. All plots received the equivalent of 46 kg· P_2O_5· ha^{-1} as TSP (100 kg TSP) as a broadcast application. The experiment was laid out in randomized complete block design (RCBD) with three replications. The size of each experimental plot was 3 m × 2 m (6 m^2), with a total of 48 plots. Spacing between plants, rows, plots, and blocks was 5 cm, 20 cm, 0.5 m, and 1 m respectively. For pot experimentation, each pot was filled with 4 kg of soil, planted with six seeds per pot, and thinned to four plants at the two-leaf stage. The experiment was laid out using a completely randomized design (RCD) with three replications for nodulation and grain yield and a total of 6 pots per treatment. All other treatments and management practices were similar to the field experiment. A non-nodulating chickpea (cultivar PM233) from the International Center for Agricultural Research in the Dry Area

(ICARDA) (received from the Ethiopian Institute of Agriculture Research, Holleta Research Center) was used as a reference crop and seeded in one plot within each replication in the field experiment and in three separate pots in the pot experiment. The reference crop was used for assessing percentage N derived from the atmosphere (%Ndfa). With the exception of the commercial inoculant (in the form of a peat-based powder), rhizobial inoculants were prepared from yeast manitol broth cultures grown for 5 days foreach of the strains, and mixing 10 mL with 125 g lignite based carrier. Seeds were inoculated with the respective rhizobial strains just before planting and kept in shade to maintain the viability of the cells. Seeds were allowed to air dry for a few minutes before planting.

2.4. Data Collection

Nodulation was assessed at the mid-flowering stage of lentil. Five representative plants were randomly taken from the second border row on each side of the plot for nodule number, nodule dry weight, and shoot dry weight determination. At physiological maturity, plants from the central six rows were manually harvested close to the ground surface. Ten plants were randomly selected from the central rows of each plot and plant height, the number of pods and branches per plant were recorded. For the pot experiment, intact plants were collected at 50% flowering stage from each treatment and nodule number, nodule dry weight, and shoot dry weight data were determined from three replicate. At physiological maturity, the remaining treatment replicates were harvested from the surface of the soil to determine plant height, the number of pods and branches per plant. The harvested plants were weighed to determine the biomass yield and were threshed to determine the grain yield of each plot and pot, respectively. One hundred seed weight was determined.

2.5. Plant and Seed Analysis

At physiological maturity five plants from within each plot including the reference crop were harvested and separated into straw and grain. These samples were used to determine seed N, total N, N derived from the atmosphere (Ndfa), and protein content. The sample materials were oven dried at 60 °C to a constant weight and ground to pass through a 2 mm sieve. Plant tissue N was determined using a LECOCNS-2000 carbon, N, and sulfur analyzer. Ground seed samples were further pulverized to a fine powder in a ball mill and subsamples (approximately 3 mg) were pelleted into 6 × 8 mm tin caps. Samples were then analyzed using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) coupled to a Delta V mass spectrometer with a ConFlo IV interface (Thermo Scientific, Bremen, Germany), at the Stable Isotope Facilities, Department of Soil Science, University of Saskatchewan. The total protein content was calculated using a factor of 6.25 [20]. The amount of seed N fixed was calculated as (%Ndfa × seed yield × seed N concentration)/100 [21]. Natural ^{15}N abundance and percent N derived from the atmosphere (%Ndfa) based on the ^{15}N Natural Abundance Method (δ^{15}N) was calculated using the following equation [22]:

$$\delta 15\text{N} = \frac{\text{atom \% 15 N sample} - \text{atom \% 15N atmosphere}}{\text{atom \% 15N atmosphere}} \times 1000 \quad (1)$$

where the standard was atmospheric N_2 (0.3663 atom % ^{15}N).

$$\%\text{Ndfa} = \frac{\text{\% 15N of reference plant} - \text{\%15N of N2 fixing legume}}{\text{\%15N of reference plant} - \text{B}} \times 100 \quad (2)$$

where B the δ^{15}N of the N_2-fixing plant grown in N-free medium. The value of B for lentil was assumed to be 0.0 [22].

2.6. Statistical Analysis

Treatment effects were analyzed using the General Linear Model (GLM) procedure (SAS/STAT, version 9.3). Mean values were separated according to Duncan's multiple range test (DMRT) at $p = 0.05$ [23].

3. Result and Discussion

The effect of *Rhizobium* inoculation and N fertilization on nodule number plant^{-1}, nodule dry weight (mg· plant^{-1}), shoot dry weight (g· plant^{-1}), plant height (cm), number of branches· plant^{-1}, number of pod· plant^{-1}, straw yield (kg· ha^{-1}), seed yield (kg· ha^{-1}), and 100-seed weight (g) are presented in Table 3 for the field experiment and Table 4 for the pot experiment. Inoculated plants produced significantly higher nodule numbers and nodule dry weights than non-inoculated plants in both the field and pot experiments ($p < 0.001$). Nitrogen fertilization and seed inoculation produced significantly higher pod numbers and straw and seed yields compared to non-inoculated non-fertilized control treatments, whereas no significant impact on shoot dry weight, plant height, or number of branches were detected ($p < 0.05$). The 100-grain weight was significantly higher in inoculated treatments in the pot experiment but not in the field trial.

Nodulation Test: Highly significant differences ($p < 0.001$) in nodule number and nodule dry weight between rhizobial inoculants were observed in both pot and field conditions; however, N application at sowing did not affect the nodulation status of the crop. Plants inoculated with *Rhizobium* strain Lt5 produced the highest number of nodules per plant (64.0), followed by strain Lt29 (62.2) under field conditions. When grown in pots, plants inoculated with Lt29 produced the highest number of nodules per plant (56.5), followed by those treated with the LtSK (48.3). Uninoculated controls had the lowest nodule numbers in both field (2.5 nodules per plant) and pot (6.4 nodules per plant) experiments. In the field experiment plants inoculated with *Rhizobium* strain Lt29 produced the highest nodule dry weight per plant (43.7), followed by those treated Lt5 (36.7). Similarly, in the pot experiment plants inoculated with Lt29 produced the highest nodule dry weight per plant (89.3), followed by those treated with Lt87 (54.3). The lowest nodule dry weight per plant (2.8) was measured in uninoculated lentil under field conditions, but in the pot experiment the N fertilized pot produced the lowest nodule dry weight per plant (12.2). The current results are similar to previous reports [22,24,25] conducted on lentil and other grain legumes in which inoculation increased nodule number and dry weight.

Table 3. Effect of *Rhizobium* inoculation and N fertilizer application on nodule number (NN), nodule dry weight (NDW), shoots dry weight (SDW), grain yield, and yield component in Huletegna Choroko soil under field conditions.

Description	SDW	NDW	NN	NB	NP	PlH	Straw	GY	100GW
	(g· plt^{-1})	(mg· plt^{-1})		(plt^{-1})		(cm)	(kg· per· ha)		(g)
Treatment									
Control	1.02	2.8^{e}	2.5^{d}	6.11	59.26	23.39	704.3^{c}	636.4^{c}	3.24
Nitrogen	1.14	3.1^{de}	2.5^{d}	7.06	83.6	23.22	1120.2^{a}	890.3^{ab}	3.27
Lt 5	1.15	36.7^{b}	64.0^{a}	6.22	68.67	23.78	912.4^{abc}	918.2^{ab}	3.28
Lt29	1.19	43.7^{a}	62.2^{a}	7.61	77.76	22.39	1034.4^{ab}	1013.2^{a}	3.24
Lt87	1.04	3.6^{cde}	7.5^{d}	6.72	71.96	22.22	837.6^{bc}	839.2^{abc}	3.22
Lt136	1.17	7.7^{cd}	9.0^{cd}	6.33	67.59	23.06	823.9^{bc}	761.9^{bc}	3.22
LtSK	1.09	6.2^{cde}	19.5^{b}	6.17	63.67	23.33	766.0^{c}	698.6^{bc}	3.24
LtNSTC	1.09	8.2^{c}	15.8^{bc}	6.39	72.02	22.56	737.8^{c}	738.9^{bc}	3.07
Variety									
Alemaya	1.01	13.11	22.83	6.56	74.4	21.24^{b}	793.95^{b}	759.29	2.74^{b}
Teshale	1.21	14.9	22.93	6.6	66.73	24.75^{a}	940.21^{a}	864.85	3.70^{a}
Treatment	NS	***	***	NS	NS	NS	*	*	NS
Variety	NS	NS	NS	NS	NS	***	*	NS	***
TreatmentXVariety	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV	29.1	25.9	28.8	18.2	21.1	7.6	23	23.4	6.9

Note: plt-per plant, PlH-plant height, NB-number of branch, NP-number of pod, GY-grain yield, GW-grain weight. Mean values followed by the same letters in each column and treatment showed no significant difference by DMRT ($p = 0.05$). *, **, ***, denotes significance at $p = 0.05, 0.01, 0.001$, respectively, and NS denotes no significant difference.

Grain yield and yield components: Nitrogen fertilizer application significantly affected the number of pods per plant compared to the non-inoculated control. Inoculation treatments significantly affect the number of pods per plant over the non-inoculated control under field conditions, but all of the inoculation and N fertilized treatments showed significantly higher number of pods per plant than the non-inoculated control in the pot experiment. Hundred-grain weight differences in all of the inoculated plants were found to be non-significant under field conditions, but in the pot experiment there were significant differences in 100-grain weight among treatments. Plants inoculated with Lt29 and the LtNSTC inoculant had the highest 100-grain weight (3.6 g).

The straw and grain yield suggest that rhizobial inoculation as well as N application increased the straw and grain yield of the crop in both the field and pot experiments (Tables 3 and 4). During the field experiment N fertilizer application enhanced straw yields by 59%, 47% and 29% over the control treatments, and inoculation with Lt29, and Lt5, respectively. Similarly, in the pot experiment straw yields were increased by 87%, 72% and 64% over the control treatments by inoculation with Lt29, Lt87, and with N fertilization, respectively. Grain yields in the field experiment were increased by 59%, 44% and 40% over the control treatments, when inoculated with Lt29, Lt5, and with N fertilizer, respectively. Similarly, grain yields were increased during the pot experiment by 92%, 74% and 67% over the control treatments, by inoculation with Lt29, Lt87, and Lt5, respectively. These results are similar to another report [22] that inoculation alone increased seed yield of lentil by 135%. Our results are also in line with a recent study [26] that indicates inoculation with elite indigenous isolates of common bean nodulating rhizobia improved the use efficiency of N and reduced the need of exogenous N application to get maximum yield in eastern Ethiopia. Experiments conducted in Western Canada failed to demonstrate any yield advantage from applying N fertilizer to lentil relative to the current practice of relying on N_2 fixation. These results indicate that biological N_2 fixation is a sustainable and more economical means of supplying N to the lentil crop in Western Canada without the need of applying N fertilizer, particularly with high yielding early maturing cultivars [27]. Other reports from Italy also indicated that two isolates of rhizobia induced a significant improvement of grain yield of lentil from 37% to 40% with respect to non-inoculated plants [28]. Moreover, several reports demonstrated that significantly higher yield in lentil was due to N_2 fixation [13,29,30].

Table 4. Effect of *Rhizobium* inoculation and N fertilizer application on nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), grain yield, and yield component in Huletegna Choroko soil under greenhouse conditions.

Descriptions	SDW	NDW	NN	NB	NP	PlH	Straw	GY	100 GW
	$g\cdot plt^{-1}$	$mg\cdot plt^{-1}$		plt^{-1}		(cm)	(kg/ha)		(g)
Treatment									
Control	1.04	14.2^{c}	6.4^{d}	6.6	17.5^{c}	34.7	11.6^{c}	10.1^{c}	3.3^{bc}
Nitrogen	1.51	12.2^{c}	10.7^{d}	10.7	22.0^{b}	35.8	19.0^{ab}	16.0^{ab}	3.1^{c}
LT05	1.22	45.3^{b}	42.7^{bc}	8.3	24.6^{ab}	37.2	17.8^{ab}	16.9^{ab}	3.4^{ab}
LT29	1.59	89.3^{a}	56.5^{a}	8.2	27.4^{a}	38.3	21.7^{a}	19.4^{a}	3.6^{a}
LT87	1.64	54.3^{b}	35.0^{c}	9.3	23.6^{ab}	37.7	20.0^{ab}	17.6^{ab}	3.1^{bc}
LT136	1.46	49.4^{b}	47.0^{ab}	8.9	22.9^{ab}	36	16.1^{b}	14.2^{bc}	3.4^{abc}
LtSK	1.57	44.1^{b}	48.3^{ab}	9.7	26.9^{a}	35.6	15.5^{bc}	13.2^{bc}	3.4^{ab}
LtNSTC	1.41	43.1^{b}	42.3^{bc}	9.7	25.5^{ab}	36.1	16.6^{b}	12.9^{bc}	3.6^{a}
Variety									
Alemaya	1.4	44.7	38.6^{a}	8.8	24.8	34.2^{b}	16.4	14.5	3.1^{b}
Teshale	1.45	43.3	33.6^{b}	9	22.8	38.7^{a}	18.1	15.6	3.6^{a}
Treatment	NS	***	***	NS	***	NS	***	*	**
Variety	NS	NS	*	NS	NS	***	NS	NS	***
TreatmentXVariety	NS	NS	NS	NS	NS	**	NS	NS	NS
CV	24.4	23.1	21.9	22.7	14.5	9.6	19.8	23.3	7.9

Note: plt-per plant, PlH-plant height, NB-number of branch, NP-number of pod, GY-grain yield, GW-grain weight. Mean values followed by the same letters in each column and treatment showed no significant difference by DMRT (p = 0.05). *, **, ***,denotes significance at p = 0.05, 0.01, 0.001,respectively, and NS denotes no significant difference.

A significantly higher nodule number was recorded by lentil variety Alemaya as compared to the Teshale variety under pot experimentation. Significantly higher plant height, and 100-grain weight results were also recorded for Teshale under field and pot experimentation. No significant differences in grain yields were observed between the two varieties. No significant difference in the treatment×variety interaction was observed in the field experiment for all traits studied. Under pot experimentation, however, there was a significant difference in the strain × variety interaction on plant height. The high biomass production capacity of Teshale variety makes it a desirable cultivar in view of soil fertility maintenance or for feed purpose.

Total N uptake and seed protein content: *Rhizobium* inoculation and N fertilizer application significantly ($p < 0.001$) increased total N uptake and seed protein content compared to the control treatments in both the field and pot experiments (Table 5). When grown in the pot experiment, total N uptake was increased by 160, 129, 115, and 100% over the uninoculated control, by inoculation with Lt29, N fertilizer application, Lt87 inoculation, and Lt5 inoculation, respectively. Similarly, during the field experiment, total N uptake was increased by 80, 64, 55, and 37% over the uninoculated control, by inoculation with Lt29, N fertilizer application, Lt87 inoculation, and Lt5 inoculation, respectively. During the pot experiment the highest seed protein concentration was recorded in plants inoculated with Lt29 (26.8%), followed by Lt5 (26.3%). Similarly, in the field experiment the maximum seed protein content (29.5%) was recorded in plants inoculated with Lt29, followed by the N fertilizer treatment (29.2%). This finding is in agreement with a previous reports [31,32] that inoculation with *Rhizobium* species increased the protein and N content of beans.

N derived from the atmosphere: *Rhizobium* inoculation of lentil showed great variation in %Ndfa and quantity of fixed N in the seed (Table 5). Under pot experimentation the mean %Ndfa varied from 81.5 to 72.5%, induced by indigenous isolate Lt29 and the LtNSTC inoculant, respectively. Similarly, under field conditions, the highest Ndfa (65.7%) was recorded in plants inoculated with Lt29, whereas LtNSTC inoculant resulted in the lowest Ndfa (55.4%). Seed inoculation significantly influenced the amount of seed N fixed, which increased from 0.37 (g/pot) in plants treated with the LtNSTC inoculant, to 0.66 (g/pot) in those treated with Lt29 under pot experimentation. Similarly, under field conditions, inoculation with LtSK and Lt29 increased the amount of seed N fixed from 16.9 to 31.2 kg·ha^{-1}, respectively. Percent Ndfa and total N fixed did not differ significantly between varieties. Others have reported similar findings on rain fed inoculated lentil [33].

Table 5. Effect of *Rhizobium* inoculation and N fertilizer application on total N uptake (TNU), seed protein concentration (SPC), percentage N derived from the atmosphere (%Ndfa) for the seed, and amount of seed N fixed for lentil cultivars.

	Pot Experiment				**Field Experiment, 2012/13**			
Descriptions	**TNU (g/pot)**	**SPC (%)**	**Ndfa (%)**	**N_2fixed (g/pot)**	**TNU (kg/ha)**	**SPC (%)**	**Ndfa (%)**	**N_2fixed (kg/ha)**
Treatment								
Control	0.51^{e}	18.6^{b}	-	-	35.7^{d}	25.2^{d}	-	-
Nitrogen	1.17^{ab}	24.5^{a}	-	-	58.6^{ab}	29.2^{a}	-	-
LT05	1.02^{bcd}	26.3^{a}	78.1^{ab}	0.56^{ab}	55.2^{abc}	28.6^{ab}	62.1^{ab}	26.0^{ab}
LT29	1.33^{a}	26.8^{a}	81.5^{a}	0.66^{a}	64.3^{a}	29.5^{a}	65.7^{a}	31.2^{a}
LT87	1.10^{abc}	24.8^{a}	77.0^{abc}	0.53^{ab}	49.0^{bcd}	27.4^{bc}	59.1^{bc}	21.5^{bc}
LT136	0.91^{cd}	25.6^{a}	73.9^{bc}	0.43^{bc}	44.7^{cd}	27.3^{bc}	56.9^{c}	18.9^{c}
LtSK	0.81^{d}	24.7^{a}	73.9^{bc}	0.39^{c}	41.0^{d}	26.8^{c}	56.5^{c}	16.9^{c}
LtNSTC	0.80^{d}	25.1^{a}	72.5^{c}	0.37^{c}	42.3^{cd}	26.9^{bc}	55.4^{c}	17.6^{c}
Variety								
Alemaya	0.95	26.4^{a}	76.2	0.50	45.9	28.0^{a}	58.7	20.8
Teshale	0.96	22.6^{b}	76.1	0.48	51.7	27.2^{b}	59.8	23.2
Treatment	***	***	**	***	***	***	***	***
Variety	NS	***	NS	NS	NS	*	NS	NS
Treatment XVariety	NS	*	NS	NS	NS	NS	NS	NS
CV	20.8	7.2	4.7	21.8	22.2	4.8	6.5	24.8

Note: Mean values followed by the same letters in each column and treatment showed no significant difference by DMRT ($p = 0.05$). *, **, ***,denotes significance at $p = 0.05$, 0.01, 0.001, respectively, and NS denotes no significant difference.

This study demonstrated that inoculation with effective rhizobial strains increased the grain yield and associated yield components, and fixed N of lentil relative to an unfertilized and uninoculated treatments. Aside from N_2 fixing efficiency, the adaptability of *Rhizobium* strains for survival in a soil environment may be an important second-order criterion for selecting effective commercial strains. The adaptability of inoculants can be related to the ecological conditions of the area from which they were isolated [34]. Others [35,36] found indigenous *Rhizobium* strains to be more highly effective symbiotic N fixers for the uptake of nutrient content and grain yield than introduced commercial inoculants. The current result was also similar with a previous report [37] that evaluations of rhizobial strains isolated from Ethiopian soils revealed higher rates of N fixation on African clovers, but that highly effective strains were not found among introduced commercial rhizobial strains. Recently several reports from different countries demonstrated that indigenous rhizobia inoculation improve growth, seed yield, Nfixation and also nutrient up take of legumes [38–43].

The results of our study indicated that lentil yield can be improved through proper *Rhizobium* inoculation and N application. Grain yield differences were not recorded between the two varieties. This indicated that the two varieties are suitable for a reduced input production system due to successful nodulation characteristics under rain fed conditions. Inoculation by different rhizobial strains had a pronounced effect on grain yield, yield components, nodulation, total N uptake, grain protein content, %Ndfa for the seed, and amount of seed N fixed as compared to non-inoculated treatments. Indigenous *Rhizobium* strain Lt29 was found to have a more significant effect on most of the studied parameters, followed by Lt5 and Lt87. These results indicated that the indigenous lentil rhizobial strains used in this study are better adapted to the soil environment and survived in adequate numbers as compared to LtNSTC and LtSK commercial inoculants. Lt29 and Lt5 are very competitive and influence the lentil yield in the same extent as the N fertilizer treatment. These two strains not only increased the lentil yields but also enhance the seed protein content and total seed N. This is particularly important in that these two strains could be used for inoculation to replace fertilizer application of lentil in Southern Ethiopia. This indigenous strain could be further studied on a wider range of soils to evaluate the likelihood of its successful incorporation into the existing cropping system.

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