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Effect of Rhizobium and Intercropping Systems on Soil Nutrients and Biological Nitrogen Fixation as Influenced by Legumes (*Phaseolus vulgaris and Dolichos lablab*)

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A B S T R A C T

The study was conducted to assess the effect of *Rhizobium* inoculation and intercropping systems on soil nutrients improvement and biological nitrogen fixation as influenced by two legumes (*Phaseolus vulgaris and Dolichos lablab*). To achieve this aim, the field experiments were conducted at Selian Agricultural Research Institute (SARI) for two seasons. The fertility status of the soils and their suitability for cereal/ legumes production at the experimental site was evaluated based on technical indicators of soil fertility. From the soil analytical results, the major soil fertility limitations included low soil organic matter, low total nitrogen and medium available phosphorus for season 1 hence the soils were categorized as of low fertility status and moderately suitable for cereal/ legumes production. This was opposite in season 2 due to legumes biological nitrogen fixation and incorporation of legumes crop residues into the soil as the results of the first season harvest. A randomized complete block design was used in a 3-factorial arrangement with two levels of *Rhizobium* (with and without *rhizobia*), 2 legumes (P. *vulgaris and D. lablab*) and 5 cropping systems. The results showed that *Rhizobium* inoculation was significantly ($P \leq 0.001$) on nodules number per plant; nodules weight (g) and biological nitrogen fixation (kg N ha^{-1}) in season 1 and 2. Based on these findings, it is thus recommended that, *Rhizobium* inoculation is the most profitable biofertilizer for soil fertility restoration.

Introduction

The majority of farmers in sub-Saharan Africa (SSA) lack financial resources to purchase sufficient amount of mineral fertilizers to replace soil nutrients removed through harvested crop products (Jama *et al.*, 2000). This is because price of inorganic fertilizer in SSA is several times higher than world food prices (CIAT-TSBF, 2004). In this case, poor soil fertility has emerged as one of the greatest biophysical constraint to increasing agricultural productivity hence threatening food security in SSA region

(Mugwe *et al.*, 2009). Of all the plant nutrients, nitrogen (N) is the most commonly deficient in soils, contributing to reduced agricultural yields throughout the SSA (Myers, 1988).

In Tanzania soil fertility assessments showed that 77% of agricultural soils have very low (0.01 %) to low (0.15 %) N content (MAFC, 2013). N has been gradually depleted from the soils and now poses serious threats to successful production of food and requires the use of N fertilizer (Kong *et al.*, 2008). This problem can be alleviated by the use of nitrogen fertilizers which on the other hand increases the cost of production to farmers and adversely affect the environment (Gadalla *et al.*, 2010; Gibson *et al.*, 2013). The sustainable way to solve the problem of nitrogen deficiency is to use the environmental friendly biological nitrogen fixation technology in cropping systems (Gibson *et al.*, 2013).

The potential uses of biofertilizers in agriculture play an important role of providing an economically viable level for achieving the ultimate goal to enhance productivity. In a study of *Rhizobial* cross inoculation groups of *Faidherbia albida* and *Acacia nilotica*, *Acacia senegal*, *A. tortilis*, *A. seyal* and *A. melifera*, it was found that the frequency of nodulation and total nitrogen content were maximized when each individual plant species was inoculated with its own isolate of *Rhizobium* (Gadalla *et al.*, 2010).

The nitrogen derived from the air was calculated to be about 79-80% indicating that most legumes fixed more than 70% of its N need from the air (Ahmed *et al.*, 2005). Studies conducted in Australia showed that, legume produced an average of 225 kg N ha-1, and replaced over 60% of the N fertilizer requirement for optimum cotton production (Zablotowicz *et al.*, 2011). The contribution of legume crops on cereal crop yields indicated an increase in yields of crops planted after harvesting of legumes are often equivalent to those expected from application of 30 - 80 kg of fertilizer-N ha^{-1} (Peoples *et al.*, 2009). Legumes, such as common beans (*Phaseolus vulgaris* L.) and dolichos lablab (*D. lablab* L.) have the ability to form a symbiotic relationship with soil bacteria capable of trapping nitrogen gas (N_2) from the atmosphere and converting it into ammonia, which can be used by the plant for growth, development and seed production (Lodwig *et al.*, 2003; Kabahuma, 2013). Atmospheric nitrogen is converted to ammonia by the nitrogenase enzyme in a process known as biological nitrogen fixation (BNF) (Postgate, 1998). The Biological nitrogen fixation (BNF) process offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources (Rahman, 2013).

The capacity of legumes to fix atmospheric nitrogen gives them an advantage over non-leguminous crops when grown on soils low in nitrogen (Postgate, 1998). Dolichos lablab and common bean legumes are capable of producing large quantities of biomass and fix substantial quantities of N for subsequent crops but the benefits they bring to the farming systems in Tanzania are not well documented. Further, *Dolichos lablab* and *Phaseolus vulgaris* are practiced as mono and intercrop without *Rhizobium* inoculation. Therefore, by realizing the benefits of the legume crop-*Rhizobium* symbiosis, it is necessary to quantify the amount of nitrogen fixed by the *Rhizobium* inoculated Dolichos lablab and common bean in maize/legume intercropping systems and its effect into the soil for subsequent crops.

Materials and Methods

Description of the research experimental site

Two field experiments were conducted at SARI farm in northern part of Tanzania (April 2015 to September 2015 and October 2015 to February 2016). SARI lies at Latitude 3º21'50.08" and Longitude 36º38'06.29"E at an elevation of 1390masl with mean annual rainfall of 870mm. The mean maximum temperature ranges from 22ºC to 28ºC whiles the mean minimum temperature ranges from 12ºC to 15ºC respectively.

Soil samples collection and analysis

Following land preparation, but prior to planting, two soil samples at 0 - 20 cm depth were collected from the experimental site in a zigzag mode, pooled, and sub-samples taken for physical and chemical analysis. The soil properties determined included soil pH, electrical conductivity (EC), organic carbon (OC), cation exchangeable capacity (CEC), total nitrogen (TN), available phosphorous, exchangeable bases, plant available zinc, copper, manganese and iron and particle size distribution.

The soil pH was measured electrometrically in 1:2.5 (weight/volume) soil: water suspensions in accordance with the procedure described by Thomas (1996). Organic carbon was determined by the wet digestion (oxidation) method of Walkley-Black (Nelson and Sommers, 1996). Cation exchange capacity (CEC) was determined by the ammonium acetate (CH_3COONH_4) saturation method (Sumner and Miller, 1996), while the leachates from the proceeding steps were used for determination of exchangeable Ca and Mg by atomic absorption spectrophotometer while K and Na were determined by flame photometer. Total nitrogen was determined by the Kjeldah method as described by Okalebo *et al.,* (1993). Available phosphorus was determined by the Olsen method in accordance with the procedure described by Juo (1978). Particle size distribution was determined by the hydrometer method as described by Gee and Bauder (1986) and textural classes of the soils were determined by the United States Department of Agriculture procedure (USDA, 1975). Zn, Cu, Mn and Fe were extracted by DTPA and measured or quantified by atomic absorption spectrophotometer as described by Lindsay and Norvel (1978).

Experimental design and planting

Land preparation involved clearing, ploughing, layout and finally planting. The experimental design followed a randomized complete block design (RCBD) in a 3-factorial arrangement with 4 replications per treatment. The experimental treatments consisted of 2 levels of *Rhizobium* inoculation (with and without *Rhizobium*), 2 legumes (legume 1 being *P. Vulgaris* and legume 2 being *D. Lablab*) and 5 intercropping systems (sole maize or sole legumes, 1 row maize to 1 row legumes (1:1) i.e. 0 m or 0.45 m of legume from maize row, 1 row maize to 2 rows of legumes (1:2) i.e. 0.1 m or 0.2 m of legumes from maize rows). The field plots measured 4 m × 4 m with 5 rows of maize spaced at (0.9 m x 0.5 m) apart and 8 rows of legumes spaced at (0.5 m × 0.2 m). The plots were interspaced by 1 m to allow management of crops. The first season crops were planted on 5th April, 2015 while the second season crops were planted on 14th, November, 2015. Prior to planting phosphate fertilizer as triple superphosphate was applied at the rates of 20 kg P/ha to supply soil P for crops uptake. The fertilizer was uniformly applied

in to the holes and covered with little soil before planting maize or legume seeds to avoid seeds burning. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Maize variety (SEEDCO 503) was obtained from SEEDCO Seed Company in Arusha and Common bean seeds variety (Lyamungo 90) and Dolichos lablab variety (Rongai) were obtained from Selian Agricultural Research Institute-Arusha-Tanzania. Before sowing, the legume seeds were thoroughly mixed with *Rhizobium* inoculants to supply (109cells/gseed), following procedures stipulated by products manufacturer. To avoid contamination, the non-inoculated seeds were planted first followed with the inoculated seeds. Three seeds were planted and thinned to two plants after full plant establishment. Interplant spacing was maintained at 0.5 m throughout for maize and 0.2 m for legumes. The plant density was kept constant on a total plot area basis set at the optimum for sole crops and kept the same in intercrops. The plant population density of maize and legumes were maintained at 44,000 and 200,000 plants per hectare respectively. Weeding and other agronomic practices were done manually using hand hoe at different growth stage of the crop plant.

Data collection

Data collected were plant samples, nodule number per plant, nodule fresh weight and nodule dry weight. Plant samples (maize, common bean and dolichos lablab) collection involved cutting above ground portion of ten plants which were randomly selected at flowering stage from each plot for the determination of shoot percentage nitrogen. Prior to analysis, the fresh plant samples were washed using distilled water and drip dried. Thereafter, the samples were oven dried at 70 ^{o} C to constant weights and ground to a fine powder (0.5 mm sieve) for plant tissue analysis. Total plant nitrogen analysis was determined by Kjeldah method as described by Okalebo *et al.,* (1993). Number of nodules per plant determination involved watering the soil before uprooting the plants and with an aid of a sharpened peg the plants were uprooted and carefully washed by soaking in a half filled bucket. The nodules were carefully detached from the roots and nodule numbers per plant and nodule weight per plant were determined as described by Peoples *et al.,* (1989).

Estimation of N fixation

The technique used to estimate N fixation was the Total Nitrogen Difference (TND) method. This was done by comparing total nitrogen of the legume with that of a non-legume (Murray *et al.,* 2008). The amount of N fixed was calculated by subtracting total nitrogen of the reference crop (maize) from that of the legume (Common bean and Dolichos lablab), and the difference value is assumed as N derived by BNF (N_2 fixed).

Thus, N_2 fixed = Total N in legume -Total N in reference crop

Where,

% Ndfa = ([Total N in legume -Total N in reference crop] / Total N in legume) × 100

% Ndfa is the percentage of N_2 derived from the atmosphere

Total N_2 fixed in plants (kg ha^{-1}) = (Dry matter weight (kg ha^{-1}) × % N in plants)/100

Data analysis

A 3-way ANOVA was used to analyze data collected. The analysis was done using

STATISTICA software program 2010. Fisher's least significant difference was used to compare treatment means (Steel and Torrie, 1980).

Results and Discussion

Changes of physical and chemical properties of the soils and their effect on BNF

The soil textural class of the soils was clay loam (Table 1). It has been reported that soils with high clay loam contents are suitable for maize/ legumes production because of their moderate capacities to retain plant nutrients and soil water (De Datta, 1981). The high clay loam contents in these soils would further allow the moderate percolation of water through the soils, hence encouraging roots respiration and other biological processes in the soils including symbiotic BNF process.

The soils' pH values for the two seasons ranged from 6.45 to 6.80 in season 1 and 2, respectively (Slightly acidic to slightly neutral soil reaction) (Table 1). The optimum soil pH for maize/ legume plants is 6.5 to 7.0 (Landon, 1991). Brockwell *et al.,* (1991) reported a 10^{-3} decrease in the number of soil bacteria in soils with a pH<6 compared to those with a pH>7.0. There is a range of effects of soil pH on *rhizobia*, but relatively few grow and survive well below pH values of 4.5 to 5.0. Although the micro-symbiont appears more pH sensitive than the host partner (Maria de, 2007), acidity also influences both the growth of the legume plant and the infection process.

The percentage total nitrogen in the soils ranged from 0.09 to 0.35% for season 1 and 2 respectively (Table 1). These values are categorised as low to high (Landon, 1991) hence the soils in season 1 were deficient in nitrogen but sufficient in season 2 for plant growth. Pillai (2005) reported that N requirement is categorized as low, medium and high when the percentage total nitrogen values are less than 0.1%, 0.1 – 0.2% and >0.2%, respectively. The low total nitrogen might have been caused by limited use of organic soil amendments, N uptake by plants, leaching and denitrification. The high nitrogen content in soils for season 2 was due to legumes nitrogen fixation and incorporation of first season crop residues in to the soil. High N in the soil inhibits legumes nodule formation and symbiotic nitrogen fixation (Sprent *et al.,* 1988; Weria *et al.,* 2013). However, total nitrogen in soils is not a good index of nitrogen availability as the N in soils occur in complex organic compounds that have to be biochemically transformed to NH_4^+ and NO_3^- that can be taken up by plants.

The organic carbon contents in the soils were 2.42% to 3.99% (Table 1) for season 1 and 2, respectively. These values are rated as low as they are less than 4 % (Landon, 1991). The low percent organic carbon contents translate to low organic matter contents in the soils. Organic matter in soils influence both the physical, chemical and biological properties of soils, such as soil structure, water retention, nutrient contents and retention and micro-biological life and activities in the soils.

The Olsen extractable Phosphorus in the soils ranged from 13.91 to 19.30 mg P kg^{-1} soil (Table 1) for season 1 and 2, respectively. The soils' extractable phosphorus values would be rated as high (Landon, 1991). There are marked differences in *rhizobial* and plant requirements for P with the slow - growing more tolerant to low P than the fast growing *rhizobia* (Weria *et al.,* 2013). Nodules themselves are strong sinks for P and

nodulation and N_2 fixation are strongly influenced by P availability (Leung and Bottomley, 1987). Weria *et al.,* (2013) indicated that when legumes-dependent on symbiotic nitrogen receive an inadequate supply of phosphorus, they may suffer nitrogen deficiency. The observed soil available phosphorus values would not satisfy the phosphate demand by biological nitrogen fixation; hence phosphate application to these soils as inorganic or organic fertilizers aim at raising the P availability status to the critical P concentration range of 15 – 20 mg P kg^{-1} soil is important (Jones *et al.,* 1982; PCARRD, 1978).

The cation exchange capacities (CEC) of the soils for the two seasons ranged from 19.68 to 25.95 $Cmol_{(+)}\,kg^{-1}$ soil for season 1 and 2, respectively (Table 1). These CEC values would be rated as medium, where 5-12.0 $Cmol_{(+)}\ kg^{-1}$ is rated as low, 12.1-25.0 $Cmol_{(+)}\ kg^{-1}$ as medium and 25-40 $Cmol_{(+)}\ kg^{-1}$ as high (Landon, 1991). The medium CEC of the soils is attributed to the nature of the parent materials from which the soils were developed and the type of the layer or amorphous silicate clay minerals in the soils. The high CEC in season 2 was attributed by maize/ legume crop residues incorporated from season 1 harvest. These indicate the presence of sufficient soil cations for BNF process.

The exchangeable bases displaced by NH_4^+ through ion exchange process/ mechanism included calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+) and potassium (K^+).

The exchangeable Ca levels in the two seasons (Table 1) are categorized as very high, that is >2 Cmol Ca kg^{-1} soil (Landon, 1991). A study by Weria *et al.,* (2013) showed that legume plants under N_2-fixing symbiosis, Ca deficiency decreased nitrogen fixation in nodules and also affects attachment of *rhizobia* to root hairs and nodulation. Therefore, the availability of Ca at the study area is not a soil fertility constraint to BNF process.

The exchangeable Mg in the soils (Table 1) are rated as high (>0.5 $Cmol_{(+)}\ kg^{-1}$ soil) according to Landon (1991). However, the availability of Mg of the soils might be reduced by the high Ca: Mg ratio greater than 5:1 due to antagonistic effects of calcium (Landon, 1991).

The exchangeable Na values (Table 1) are rated as very high (Landon, 1991). The legume-*Rhizobium* symbioses and nodule formation on legumes are more sensitive to salt or osmotic stress than are the *rhizobia* (Zahran, 1991). Salt stress inhibits the initial steps of *Rhizobium*-legume symbiosis. A study by Nair *et al.,* (1993) showed little curling or deformation soybean root hairs when inoculated with *Bradyrhizobium japonicum* in the presence of 170 mM NaCl, and nodulation was completely suppressed by 210 mM NaCl. Legume is moderately tolerant to sodic soil conditions (ESP = 20 – 40) hence the crops and BNF process would not be adversely affected by the high exchangeable Na values of the soils.

The exchangeable K in the soils (Table 1) is rated as high (> 0.4 cmol K kg^{-1} soil) according to Landon (1991). It has been reported that soils with large amounts of available K lose some of the K through fixation and those with low amounts have their exchangeable K increased through transformation of the non-available K to available/exchangeable forms under field conditions (Pillai, 2005). Weria *et al.,* (2013) reported a qualitative requirement for K for some *rhizobia* which show restricted growth when K is deficient in the growth medium. Thus the soil K values (1.52 to 2

$cmol_{(+)}$ kg^{-1} soil) are above the minimum levels of (0.07 and 0.2 cmol K kg^{-1} soil) for BNF hence not deficient.

The DTPA extractable Zn, Cu, Mn and Fe in the soils ranged from 1.15 to 1.7, 7.65 to 8.55, 127 to 136.64 and 137 to 143.58 mg kg^{-1} soil, for season 1 and 2 respectively as shown in Table 1. These values are rated as high > 1.0 mg Zn kg^{-1} soil, > 0.75 mg Cu kg^{-1} soil, > 1.5 mg Mn kg^{-1} soil and > 5 mg Fe kg^{-1} soil according to Landon (1991). Adverse effects of heavy metals on nodulation and N_2 fixation of legumes have been reported for clover and chickpea (Rother *et al.,* 1983; Yadav and Shukla, 1983). Giller *et al.*, (1989) suggested two possibilities to explain the mechanism by which the elevated metal concentrations eliminated N_2 fixation: (i) one or more of the metals present might have prevented the formation of N_2-fixing nodules by effective *Rhizobium* strains present in the soil or (ii) the metal contamination might have resulted in elimination of the effective *Rhizobium* strains from the soil. Based on the soils analytical data, the soil fertility limitations with respect to maize/ legume production in SARI farm include low soil organic matter, low total nitrogen and medium soil phosphorus. These limitations can be corrected or addressed through application and incorporation of plant residuals in soils and maize/ legume intercrops.

Effect of *Rhizobium* inoculation and intercropping systems on number of nodules, nodules fresh weight, nodules dry weight and nitrogen fixation of two legumes

The study indicates that *rhizobium* inoculation significantly ($P \leq 0.001$) increased number of nodules by 19.7 and 20.5%, nodules fresh weight increased by 22.6 and 18.7%, nodules dry weight increased by 19.1 and 27.3% while nitrogen fixation increased by 17.7 and 17.5% in season 1 and 2 respectively (table 2) for two legumes (*P. vulgaris* and *D. lablab*). The reason might be due to the synthetic inoculation of *Rhizobium*, which increased the number of bacteria and hence more nodules number, nodules weight and nitrogen fixation per plant (Muhammad, 2011). Further studies by Bambara (2009), Tairo and Ndakidemi (2013) showed that *Rhizobium* inoculation increased number of nodules in both glasshouse and field experiment. The results also revealed that few nodules were observed on uninocculated plots. This is an indicator of the presence of indigenous legumes nodulating bacteria in the experimental soil (Bekere and Hailemariam, 2012). Differences among the legumes in nodule numbers were probably an expression of the interaction between *Rhizobium* strain and host plant. Rennie and Kemp (1984) reported cultivar differences in inoculation caused by the presence of toxic substances in seed coats, or differences in root exudates. The inoculated plants had the highest nodule dry weight and amount of nitrogen which differed significantly from non-inoculated plots. This also indicates that there were native *rhizobia* in the soils where legumes were planted. Although symbiotic nitrogen fixing potential in common bean is considered to be low in comparison with dolichos lablab, the present study also showed increased in nitrogen associated with N fixation suggesting a prospect for improving nodulation by using inoculants. These differences also reflect the sensitivity of the bean-*Rhizobium* symbiosis to many environmental factors that can have either an enhancing or reducing effect on N fixation. Graham and Vance (2003) explained that numerous changes occur in host and bacterial gene expression during infection, nodule development, and function with

approximately 100 host legume and *rhizobial* genes involved. A study by Chemining'wa *et al.,* (2007) on effect of *rhizobia* inoculation and starter-N on nodulation and yield of grain legumes revealed that in most cases, common bean had significantly higher nodule numbers and nodule weight than most of the other legumes. Mukhtar and Nourai (1988) found that high doses of nitrogen reduced nodulation and nitrogenous activity and concluded that the starter doses of 10 kg N/ha improved plant stand and enhanced N_2 fixation and production. Otieno *et al.,* (2007) also reported similar results that *rhizobial* inoculation significantly increases nodule number and dry weight in studied legume species. When dolichos lablab plant is inoculated can fix up to 400 kg N ha $^{-1}$yr $^{-1}$ (Smith and Hume, 1987). Positive and significant correlations of nodule number were observed with nodule weight and nitrogen fixation in both seasons but on contrary, there was no significant correlation between nodules fresh weight and nodule dry weight.

Cropping systems were significant ($P \leq 0.001$) on number of nodules per plant with increase of 4.5 and 5.5%, nodules fresh weight with increase of 12.1 and 9.7%, nodules dry weight with increase of 12.2 and 12.5%, nitrogen fixation with increase of 42.3 and 35.9% in season 1 and 2 (Table 2). The cropping system 1 (sole crop) had the highest nodules number per plant, nodules fresh and dry weight and nitrogen fixation in season 1 and 2. Regardless of inoculants used in cropping systems, the overall increases of the measured parameters were in cropping system 1. On the other hand cropping systems 2, 3, 4 and 5 were close to each other (table 2).

Intercropping legumes with non-leguminous crops result in competition for light, water and nutrients that can affect number of nodules and N_2 fixation negatively. A study by Moses *et al.,* (2013) noted that some nodulated legumes are capable of fixing atmospheric N and therefore will reduce competition for N when cereals are included in an intercropping system. However, it has been shown that when mineral N is depleted in the root zone of the legume component by the non-leguminous intercrops, N fixation of legumes may be promoted (Ashish *et al.,* 2015). Intercropping increase opportunities for N-use complimentarily, reducing the need for fertiliser-N, either by increasing the availability of soil N or by N transfer (Chris van and Christopher, 2000). The present study indicated that, the total amount of N fixed per unit area in intercropped systems is often lower due to decreased legume population densities, and increased competition for light, water and nutrients by the non-legume. An increase in the total amount of N_2 fixed could occur when the intercropped legume uses more effectively limited resources.

The interaction between *Rhizobium* inoculation and cropping systems was significant ($P \leq 0.01$) on nodules dry weights for season 1 and 2 respectively. These results are in line with the studies of Santalla *et al.* (2001) and Anshu (2014) that the nodulation and nitrogenase activity led to increased nodules dry weights of plants inoculated with *Rhizobium.* Dolichos lablab gave higher nodules weight in all cropping systems than common bean with cropping system 1 (sole crop) being dominant.

Intercropping maize and dolichos lablab or common bean with *rhizobium* inoculation enhanced the Biological Nitrogen Fixation (BNF) process which increased N fixation on average by more than 27 kg N ha^{-1} with an improvement of soil nutrients from season 1 to season 2.

Table.1 Some of the chemical and physical properties of the composite soil samples from the research site

	Season 1				Season 2			
Soil Parameters	Soil samples		Mean	Rating [1]	Soil samples		Mean	Rating[1]
	1	2			1	2		
pH (water)	6.40	6.50	6.45	Slightly acidic	6.70	6.90	6.80	Slightly neutral
EC (mS/cm)	0.16	0.16	0.16	Normal	0.18	0.19	0.19	Normal
Organic carbon (%)	2.43	2.41	2.42	Low	3.86	4.12	3.99	Medium
Organic matter (%)	4.23	4.19	4.21	Low	6.72	7.17	6.95	Medium
Total nitrogen (%)	0.09	0.09	0.09	Low	0.28	0.41	0.35	Medium
Extractable P (Olsen, mg kg^{-1})	13.80	14.01	13.91	Medium	19.20	19.40	19.30	High
Exchangeable Ca (cmol kg^{-1})	5.60	5.50	5.55	High	5.90	6.20	6.05	High
Exchangeable Mg (cmol kg^{-1})	4.20	4.20	4.20	High	5.30	5.60	5.45	High
Exchangeable Na (cmol kg^{-1})	5.70	5.80	5.75	High	6.80	7.10	6.95	High
Exchangeable K (cmol kg^{-1})	1.50	1.57	1.54	High	1.90	2.10	2.00	High
CEC (cmol kg^{-1})	19.60	19.75	19.68	Medium	24.70	27.20	25.95	High
Zinc ($mgkg^{-1}$)	1.10	1.20	1.15	High	1.60	1.80	1.70	High
Copper ($mgkg^{-1}$)	7.50	7.80	7.65	High	8.40	8.70	8.55	High
Manganese ($mgkg^{-1}$)	128.00	126.00	127.00	High	133.08	140.20	136.64	High
Iron ($mgkg^{-1}$)	137.00	137.00	137.00	High	141.10	146.30	143.58	High
Particle size distribution								
Sand (%)	40.00	40.00	40.00		39.00	39.50	39.25	
Silt (%)	28.00	28.00	28.00		28.00	28.00	28.00	
Clay (%)	32.00	32.00	32.00		33.00	32.50	32.75	
Textural class	CL	CL	CL		CL	CL	CL	

Note: CL= Clay loam

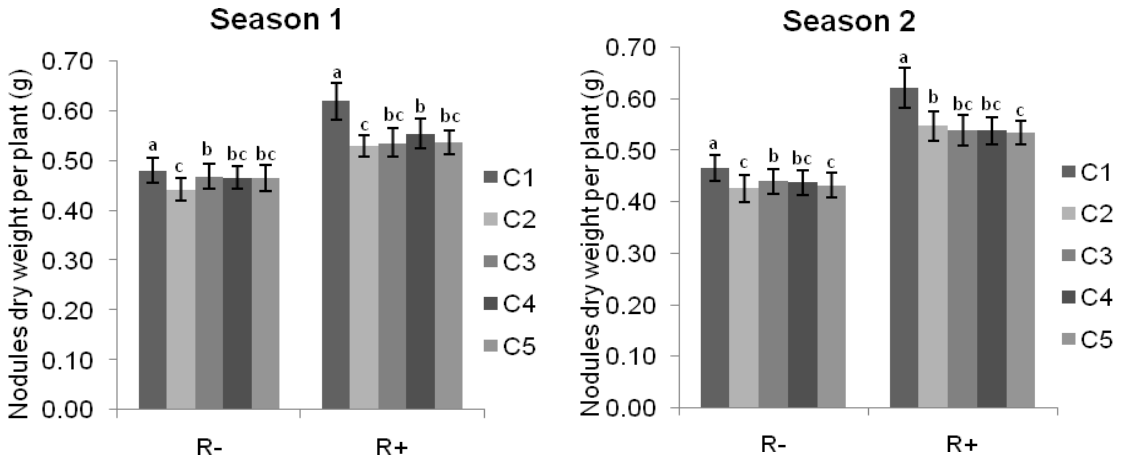
Soil parameters rating was done according to Landon (1991).

Table.2 Effects of Rhizobium inoculation legumes and cropping systems on number of nodules, nodules fresh weight, nodules dry weight and nitrogen fixation of two legumes (P. vulgaris L. and Lablab purpereus L.)

Treatments	Season 1				Season 2			
	No of nodules	Nodule fresh weight (gm)	Nodule dry weight (gm)	N fixation (kg/ha)	No of nodules	Nodule fresh weight (gm)	Nodule dry weight (gm)	N fixation (kg/ha)
Rhizobium								
R-	15.50±0.21b	0.93±0.02b	0.47±0.01b	65.54±2.83b	14.85±0.15b	1.07±0.02b	0.44±0.01b	78.60±3.06b
R+	18.55±0.27a	1.14±0.03a	0.56±0.01a	77.17±2.90a	17.90±0.15a	1.27±0.03a	0.56±0.01a	92.35±3.72a
Legumes								
1	17.55±0.39a	0.91±0.02b	0.45±0.01b	57.32±2.00b	16.50±0.31a	1.04±0.02b	0.43±0.01b	68.09±1.97b
2	16.50±0.26b	1.16±0.02a	0.58±0.01a	85.39±2.02a	15.77±0.24b	1.29±0.02a	0.57±0.01a	102.85±2.49a
Cropping systems								
1	17.25±0.43a	1.11±0.06a	0.55±0.03a	90.08±4.36a	16.94±0.43a	1.24±0.06a	0.54±0.03a	104.95±5.78a
2	17.50±0.76a	0.99±0.04c	0.49±0.02c	69.84±4.69b	16.50±0.43b	1.13±0.04cd	0.49±0.03b	84.20±5.31b
3	16.81±0.49a	1.02±0.04bc	0.50±0.02bc	67.36±3.88ab	16.31±0.45b	1.16±0.04bc	0.49±0.02b	80.96±4.79ab
4	16.81±0.48a	1.04±0.05b	0.51±0.02b	66.21±3.99ab	16.06±0.48bc	1.17±0.05b	0.48±0.02b	80.05±4.92ab
5	16.75±0.53a	1.00±0.04c	0.50±0.02bc	63.29±3.93c	16.06±0.47bc	1.13±0.04d	0.48±0.02b	77.20±4.91c
3-Way ANOVA (F-statistic)								
Rhiz	80.02***	465.05***	196.00***	81.57***	262.66***	366.11***	343.19***	66.44***
Leg	9.48**	699.59***	396.93***	475.42***	22.87***	614.40***	464.82***	424.88***
Cr syst	0.76ns	19.11***	11.11***	55.51***	3.00*	16.01***	13.51***	35.13***

-R: Without Rhizobium, +R; With Rhizoubium, Legume 1: Common bean; Legume 2: D. Lablab; Cropping System 1, 2, 3, 4 and 5 are sole maize, 10cm, 20 cm, 45cm and 0 cm of legumes from maize row respectively; Rhiz; Rhizobium, Leg; Legume, Cr Syst; Cropping Systems. Values presented are means ± SE, n=4. *; **; *** = significant at P≤0.05, P≤0.01, P≤0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter in a column are significantly different from each other at P=0.05 according to Fischer least significance difference (LSD

Fig.1 Interactive effects of Rhizobium and cropping systems on nodules dry weights for season 1 and 2 (R-: Without Rhizobium, R+: With Rhizobium, CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5)



Therefore, it can be concluded from the results that the *Rhizobium* inoculation has positive impact on nodulation, nodules weights and nitrogen fixation. Increased cultivation of legumes intercropping is essential for the regeneration of nutrient-deficient soils and providing needed nutrients to humans and animals. In order to realize sustainable agriculture that is not dependent on mineral N fertilizer, studies on legume-*rhizobia* symbiosis is highly required that will contribute efficient crop legume production which leads to decrease production costs. *Rhizobial* inoculants have to be frequently applied/ used as biofertilizers because of its antagonistic effect on soil nutrients restoration. However there may be situations where N has to be applied, such as to cereal/ legumes intercrops, small quantity has to be applied as a starter dose of which may not affect nitrogen fixation of the legume crop.

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