

Effects of Bradyrhizobia Inoculation on Growth, Yield and Yield Components of Cowpea Varieties (Vignaunguiculata(L.)Walp) at Hawassa, Ethiopia

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Abstract
Declining of soil fertility, especially deficiency of nitrogen is one of the major factors adversely affecting crop productionin Ethiopia. Though the problem can be addressed through application of inorganic fertilizers, the unprecedented increase in the cost of fertilizer is increasingly limiting the use of this input. Increasing N-availability through Bradyrhizobium-legume symbiotic N-fixation process is a viable alternative for improving N-nutrition of crops. However, there is a need for identifying effective Bradyrhizobium strains compatible with specific varieties of leguminous crops. Therefore, this study was conducted to investigate the effect of differentBradyrhizobium strains on growth, yield and yield components of Cowpea (Viginaunguiculata(L.)Walp)varieties at Hawassa. A factorial combinations of four treatments [three Bradyrhizobium strains GN100, GN102 and MB140) and a positive control], and five Cowpea varieties (Bole, Black eye bean, TVU, Assabot and Wonder) were laid out in RCBD with three replications. The plot size was a 2.2m x 3 m and cowpea seeds were planted with inter and intra row spacing of 50cm by 20cm respectively. Data on nodule number, nodule volume, nodule dry weight and agronomic parameters like shoot dry weight, leaf area index (LAI), plant height, number of branches, pods, seeds per pod, grain yield, biomass yield, harvest index (HI), 100 seed weight, total plant N and some soil chemical parameters(OC%, P, N&pH) were collected and subjected to ANOVA.Inoculaton with Bradyrhizobiumsignificantly increased the nodulation parameters, Agronomic performance, yield and yield components. Nodule parameters yield and yield components of the five Cowpea varieties inoculated with strain GN102 were significantly superior followed by GN 100 and MB 140. On Black eye bean and Assabot varieties, Strain GN102 increased the grain yield by 14 and 9%, respectively relative to the positive control. Cowpea varieties also varied significantly in their performance with respect to nodule parameters, yield components and total nitrogen content. Accordingly, Black eye bean variety performed best relative to the other four varieties. Bradyhizobia by varieties interaction effects were also significant in terms of yield and yield components. Compared to before planting,strain GN100, GN 102 and MB140 increased soil N by 51.6, 46 and 40%, respectivelyafter planting.Despite the commonly reported non responsiveness of Cowpea for inoculation, the result presented here indicated the potential that exists to increase the yield of the crop through selecting and inoculating with effective Bradirhizobial strain.
Keywords: Biological N-fixation,Bradyrhizobium strainsCowpea, inoculation, Nitrogen, nodulation and Soil fertility decline

1. INTRODUCTION

The tropics have the potential to be the most productive cropping environment in the world with regard to year round sunshine, vast area of land, ambient temperature and day neutral growing period. Where rainfall is sufficient, crops can be grown year round, unlike only in the warm season as in the case in the temperate region. Despite these natural advantages, yield in the tropical cropping system is often pitifully low. The unpredictability of the climate, in particular the timing of the rain, and the lack of nutrient for plant growth in many soils limit crop production in the tropics. Whilst we can do little to modify the climate, we can use various approaches to solve the problem of soil fertility (Giller, 2001).

The majority of tropical African countries are facing two major crises: increasing over exploitation of wood reserves for timber or fuel wood and diminishing soil fertility of cultivated land, resulting in reduced agricultural productivity. These crises are inter related, originating from the high population growth rate and subsequent need for more food production. These have led to greatly shortened rotations, a rapid loss in productivity and need to bring more forested land under cultivation. In general, the problems facing farmers everywhere is often the diminished capacity of their soils to supply the high quantities of nutrients required by crops and a rapid decline once cropped. One of the ways to curb this problem is to increase the biological inputs of nutrients and it is here that biological fixation of atmospheric N has a crucial role to play in increasing the sustainability of yields within the minimal external inputs (Giller, 2001). Feasibility of biological nitrogen fixation in Ethiopia has been well reviewed and documented (Hailemariam and Tsige, 2006). Biological nitrogen fixation contributes more than 170 million tons of fixed nitrogen to the biosphere. Eighty percent of the stable biologically fixed nitrogen is obtained from bacteria symbiotically associated with legumes and certain non-legume plants (Earl and Ausubel, 1993). The contribution of BNF to the N cycle on the other hand can be controlled by manipulating various physical, environmental, nutritional or biological factors (FAO 2010).

Legumes play crucial role in the farming system of the tropics, and particularly in the Sub-Saharan Africa, Their adoption has not yet had the revolutionary impact that it has had in the temperate zones (Tosti and Negri(2002). Although legumes are known to contribute substantial amount of N, this potential is currently still underutilized, particularly in the tropics. Moreover, little information is available on N fixation by legumes in the tropical Africa. Biological nitrogen fixation contributes to productivity both directly and indirectly by contributing to the maintenance or enhancement of soil fertility in the agricultural system by adding N to the soil. Mixed cropping of legumes and cereals is a traditional farming practice in developing countries. Benefits of mixed cropping may arise from increased N and dry matter production

Cowpea (*Vignaunguiculata*) is usually more efficient in N fixation and derives high percentage of its N from the atmosphere (Bown, 1999). Also with the recent need for integration of livestock into the farming system, fodder is becoming more valuable. Its ability to fix atmospheric N would help to improve the fertility of the soil and thus increase the yield of the succeeding crop. Cowpea plant plays a major role in low input crop-livestock production systems. Beside Its ability to fix atmospheric nitrogen and contribute positively towards the nitrogen balance of the soil, it is rich in protein and quality feed for livestock. Hence, Its contribution for small scale farming system is a key factor in sustaining long-term fertility in crop-livestock production especially in developing countries like Ethiopia. Inoculation can be beneficial to the establishment of effective N-fixation on new planting seeds if done properly in areas where a legume of the cross inoculation group has not been grown previously or where N-fixing bacteria in the soil populations have been severely reduced by adverse soil conditions such as drought or soil acidity.

The study was conducted with the following objectives

- ✓ To identify effective Bradyrhizobial strain that result in increased growth, yield and nitrogen content of Cowpea.
- ✓ To identify better responding Cowpea varieties to Bradyrhizobia inoculation under Hawassa condition.
- ✓ To investigate the presence of interaction between *Bradyrhizobium* inoculants and Cowpea varieties.

2. MATERIALS AND METHODS

2.1. Characterization of study area and climatic conditions

The experiment was conducted at the research and farm center of Hawassa University in 2012 cropping season. Hawassa is located 275 km south of Addis Ababa, the capital city of Ethiopia, at 07°03ꞌ North and 38°30ꞌ East at an altitude of 1620 meter above sea level. The data with regard to rainfall, temperature both (maximum and minimum) prevailed during the cropping season as recorded from Hawassa Meteorological Station are presented in Fig 1. It has an average daily temperature of 20.5°C.The area received a maximum monthly rainfall of 155.2 mm during cropping season.

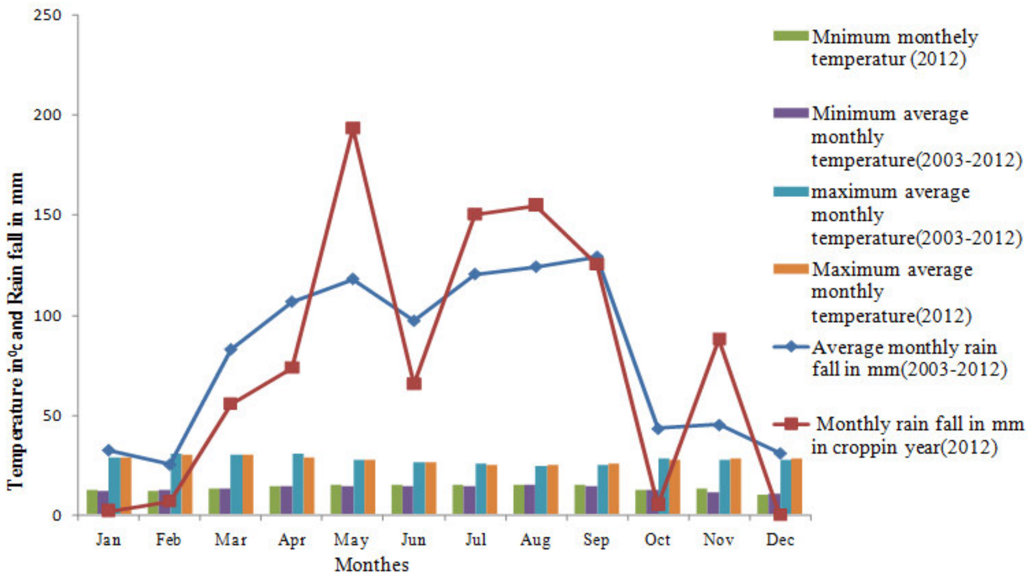

Figure 1: Meteorological data of monthly and average rainfall and temperature during the experimental year (2012) & past ten years (2003-2011) at Hawassa(Source: National Meteorology Agency Hawassa Branch).

The experiment comprised of 20 treatments made up of a factorial combination of two input factors [Three Bradyrhizobial strains (GN100, GN102, MB140) and a positive control (N fertilizer at 100kg/ha)] and five Cowpea varieties, (Bole, Assebot, Black eye bean Wonder and TVU). The three strains were isolated from Ground nut (GN100 and GN102) and Mung bean (MB140) and obtained from NUFU LEGUME project at College of Agriculture, Hawassa University and the five Cowpea varieties, (Bole, Black eye bean,TVU,Wondre and Assabot) were obtained from Mellekassa agriculture center, and inoculated by peat based carrier.

To avoid contamination, the seeds were surface sterilized using diluted sodium hypochloride for one minute and then rinsed with sterilized water five to six times and dried before inoculation of seeds takes place. Then, peat-based inoculants were surface applied for all varieties according treatment by using plastic bag before planting; Peat which protects the strains and maintains viability, is a common carrier. The inoculants carrier was applied to ensure the strain adhere to the seed. To aid adhesion, the inoculants were mixed with a sticker, water containing sugar and uniformly coated on. Only a small amount of liquid (sterilized water) was used to prevent the seeds from getting too wet. The seeds were dried in a cool, shaded location (not exposed to direct sunlight) and planted in the same day. Non inoculated (N fertilizer) seeds were sown first and followed by the inoculated seeds to avoid cross contamination. Moreover, measure was taken to divide the labour into two so that one group planted the non-inoculated plots and the other group planted the inoculated plots.

3. Data Collection

3.1. Soil sampling and analysis

Soils were sampled (from a depth of a depth of 30 cm) at 10 random points in the field and soil analysis was conducted from a composite sample. The soil was sampled following the same procedure, but according to the treatments, after crop harvest and samples from a composite of each treatment was used for analysis of different soil parameters including organic carbon (OC),pH, available p,and soil nitrogen was estimated by alkalinepermanganate oxidation method as outlined by Subbiah and Asija (1956). Available P was determined by Olsen's method as outlined by Jackson (1958), using spectrophotometer (660 nm wave length). Organic carbon was determined by Walkely and Black wet oxidation as described by Jackson (1958). The pH was determined by the electrometric method in a soil suspension with a soil/water ratio of 1:2.5.

3.2. Statistical Analysis

Data collected on various characters or parameters were subjected to Analysis of Variance (ANOVA) using general liner model SAS®, (2002) Software. Mean separations were done using LSD (5%) as following (Gomez and Gomez, 1984). Analysis of variance (ANOVA) was considered to test for significant differences among treatment means.

4. RESULT AND DISCUSSION

4.1. Physico-chemical analysis of experimental site

A. Soil analysis before planting

The laboratory analyses were conducted, (at JIJE soil laboratory) on soil samples collected before planting and after crop harvesting. The analytical results indicated that the textural class of the experimental site soil was sandy loam with a proportion of 26% clay, 26% silt and 48% sand .

Table 1: Selected physico- chemical properties of the experimental soil before planting

Physical properties				**Chemical properties**			
Particle size distribution (%)			Textural class	pH:1.25 H_20	OC (%)	Total soil N (%)	Available p mg/kg
Sand 48	silt 26	clay 26	Sandy loam	7.15	1.25	0.12	48.16

TN=total nitrogen in the soil, OC=organic carbon in the soil,Av.P=available phosphorous in soil

According to Olsen *et al.* (1954) soils can be classified based on their available P contents, with ranges of <5, 5-15, 15-25 and >25 mg kg^{-1}as very low, low, medium and high, respectively. Thus, with respect to available P, the experimental soil rated in high range (>25 mg kg^{-1}). Havlin (1999) indicated that soils having total N contents with ranges of <0.1, 0.1- 0.15, 0.15-0.25, 0.25- 0.5 and >0.5% can be grouped as very low, low, medium, high and very high, respectively. Thus, with respect to total available N, the soils of the experimental area can be rated in a low range (Table2). Most Ethiopian soils, similar to the agricultural soils in other tropical countries, are reported to be generally low in N (Asegelil, 2000). In view of the low level of N in soils and the limitation for crop production, in general, inoculation of legume seeds with *Bradyrhizobia* may increase N fixation and contribute to the replenishment of soil N for better yield in crops. According to Jackson (1958) and Herrera (2005), soil carbon content rated as into very low (< 2%), low (2-4), medium (4-10), high (10-20) and very high (>20%), thus the soil at experimental site has very low carbon content.

The distribution and effectiveness of *Bradyhizobium* strains has been shown to vary with soil pH, temperature, available and organic nitrogen (Ahmed *e.t*al., 1981).Therefore, the soil reaction of the experimental site is optimal for survival and functional symbiosis of Bradyrhizobia*,* which is in accordance to the reported optimal range of 6.5 -7.5 (Somasegran and Hoben, 1995).

4.1. Effect of Bradyrhizobium inoculants and varieties on phonological characteristics of Cowpea

4.1.1.Effect on days emergence and flowering

The Cowpea varieties tested have shown significant variation with respect to days of 50 % emergence anddays of 50% flowering (Table 3). Assabot had taken maximum days to emerge. Paldh (2004) reported that seed size, sowing depth, land preparation and environment influence the germination and emergence of the seedlings. Aikins and Afuakwa (2008) indicated that uniform and complete emergence of vigorous seedlings positively affect the overall output of an annual crop by allowing the establishment of better canopy structure and providing time and spatial advantages to compete with weeds. There was significant variation among strains with respect to days of 50 % flowering. This could be due to the relatively long time required for BNF to begin, which usually takes place between 2-5 weeks after planting .Also, might be attributed to inherent genetic variations in forming effective symbiosis with Bradyrhizobia.

Similarly, also there was a significant difference on days to 50 % flowering among inoculats, maximum number of days recorded on GN100 but the others took similar days and there was also a significant variation on varieties. Assabot recorded maximum and wonder the minimum.

4.1.2. Days to physiological maturity

Days to physiological maturity differed significantly between Cowpea varieties shown in Table 3,Assabot, a late maturing variety had the highest recorded to days of maturity (130), thus indicating that it will have more duration for photosynthate production and dry matter accumulation. Rengel, (2001) hypothesized that such a variety can be nutrient efficient genotype. It may have an increased capacity to exploit the soil (because of its large root surface area, but Bole was the least (111) it may be the early maturing variety.

4.2. Effect on nodulation of varieties

The effect of strains and varieties on the nodulation parameters (nodule number, volume, dry weight), shoot dry weight and leaf area index are shown in Table 3.

Table 2: Effects of *Bradyrhizobium* inoculants and Cowpea varieties on phenology, nodulation (nodule number, volume and dry weight) shoot dry weight and leaf area index at mid flowering

Treatments		**Days 50% emergence**	**Days 50% flowering**	**Days tophysiological maturity**	**Number of nodule**	**Nodule volume (ml)**	**Nodule dry weight (g)**	**Shoot dry weight (g)**	**LAI (%)**
Strains	Urea	10.7	90^{b}	120	11.33^{c}	0.63^{b}	0.039^{b}	24.59^{c}	13.02^{c}
	GN100	10.8	92.9^{a}	119.9	16.53^{a}	1.42^{a}	0.068^{a}	30.64^{ba}	19.3 ^{b}
	GN 102	10.6	90.2^{b}	118.9	16.60^{a}	1.60^{a}	0.073^{a}	32.91^{a}	22.43^{a}
	MB140	10.2	90.7^{b}	119.9	13.53^{b}	0.88^{b}	0.063^{a}	26.41^{bc}	18.62^{b}
LSD (5%)		**NS**	**0.67**	**NS**	**1. 05**	**0.29**	**0.017**	**5.49**	**2.89**
Varieties	Bole	10.16^{b}	90.6^{c}	111.3^{d}	13.83^{b}	1.09^{a}	0.047^{c}	27.14	15.80^{c}
	Black eyebean	11.41^{a}	92.3^{b}	123^{b}	16.00^{a}	1.39 ^{a}	0.093^{a}	31.12	20.14^{a}
	TVU	10.08^{b}	90.9^{c}	121.2^{c}	13.25^{b}	0.79 ^{b}	0.042^{c}	27.53	17.15^{ba}
	Assabot	11.58^{a}	95.5^{a}	130^{a}	15.50^{a}	1.21^{a}	0.072 ^{b}	29.05	18.26^{bac}
	Wonder	9.8^{b}	89.7^{d}	111.8^{d}	13.91^{b}	1.208^{a}	0.053^{b}	28.44	20^{a}
LSD (5%)		**0.67**	**0.75**	**1.66**	**1.17**	**0.324**	**0.018**	**NS**	**3.23**
CV%		**7.74**	**0.99**	**1.68**	**9.76**	**34.45**	**37.12**	**25.92**	**21.31**
Strains *	**varieties**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Mean values within column followed by the same letter are not significantly different ($p \leq 0.05$),NS = non significant at 5% probability level, n=60 (Number of observation for each parameter)

4.2.1. Nodule number

The effect of *Bradyrhizobium*inoculation on number of nodules per plant of Cowpea varieties was highly significant ($P < 0.01$) (Shown in Table 3 and Appendix Table 8). Also, there was a highly significant ($P < 0.01$) difference among strains with respect to nodule number. The effect of strains GN100 and GN102 were statistically similar on nodule number. In this study Black eye bean and Assabot varieties produced the highest number of nodules 16.0 and15.5 respectively. This is in accordance with Thies*et al*., (1991), who showed that inoculation of eight leguminous crops with commercial strains increased the number of nodules per plant.

Nodule formation is very complex processes that readily get disrupted as a result of effects from environment, the Bradyrhizobia itself and/or the genetic determinants. Theis*et al*., (1991) also reported that, nodule formation is controlled and optimized by the plant as the nodules develop only in parts of the root where there is meristematic activity. The lowest numbers of nodules (11) were observed from Bole variety. The result agrees with Solaiman&Rabban (2006), who reported that plant inoculated with *Bradyrhizobium*strains produced significantly higher number of total and effective nodule as compared to that of un- inoculated treatment. Vincent (1970) reported that lack or response to inocula might be attributed to the fact that, the applied inoculum does not become established i.e. failed to survive or to colonize or to compete with local resident in the soil. The results obtained from this study showed the existence of variation in nodule number and size among the varieties in response to inoculation.

Almost all the varieties developed nodules on their roots but nodules in uninoculated plants were white and smaller in size. The formation of nodules on uninoculated plants indicated the presence of native Bradyrhizobia. Thomas (1973) observed that most pasture legumes nodulated naturally; however, the range of variation in number of nodule and size could be attributed to inoculation, plant species and environmental factors. The possibility of the appearance of few nodules and wide range of variation between varieties was also demonstrated by Nutman (1975) who reported that the variation is attributed to the proportional differences in the number of infected root hairs which is host controlled. Many authors also reported that, environmental factors (water stress) have great effect on nodule initiation, structure, development and nitrogenase activity (Sprent, 1972 and Sheoron*et al.,* 1981) through their effect on survival and multiplication.

According to Sprent, (1972) nodulation, nodule color, nitrogenase activity and nitrogen fixation were found to be maximum at field capacity. In this study, though all the species developed nodules on their root, the nodules of inoculated legumes were pink and/or red in color and larger in size. The two Varieties (Black eye bean and Assabot) developed higher number of nodules per plant, a precursor for higher nitrogen fixation in legumes, while most the uninoculated varieties formed white, smaller and fewer nodules per plant. This phenomenon is in agreement with that of Jennings (2004), and Bulter and Evers, (2004) who observed that on legume plants ineffective strains form many small nodules on the legume root but fix little or no nitrogen. Simms and Tailor (2002) also reported that, nodule occupied by effective strain were 2.5 times larger in size than nodules occupied by the native strain in clover. In this study the interaction effects of Bradyrhizibia inoculants and varieties on nodule number were insignificant.

4.2.2. Nodule volume

As shown in Table 3, inoculation with Bradyrhizobial strains significantly increased nodule volume ($P < 0.01$). Strains GN100 and GN 102 were statistically at par and have 125% and 154% more nodule volume over the urea treatment. All the five varieties have Shown a highly significant ($P < 0.01$) difference on nodule volume per plant and highest value was observed for the variety Black eye bean inoculated with the GN100 strain while lower value was recorded on-inoculated (N supplied) TVU variety. However, there was no interaction effect of varieties and strains on nodule volume per plant.

4.2.3. Nodule dry weight

Nodule dry weight differed highly significantly due to inoculation with different Bradyrhizobia strain (Table 3 and Appendix Table 8). Accordingly, strain GN 100, GN102 and MB140 produced higher amount over the plants that received N fertilizer. Generally inoculating with different Bradyrhizobial strains have Shown a highly significant effect on nodule dry weight of the five Cowpea varieties. The highest nodule dry weight was recorded with the variety Black eye bean and Assabot (0.093 and 0.072 g/plant and the lowest from variety Wonder and Bole. The results of this experiment showed great variability in nodule dry weight between the varieties indicating different responses of the varieties for inoculation with Bradyrhizobia strains. This suggests apparent differences in compatibility between the Cowpea varieties and the *Bradrrhizobium*strains. Statistically variety Black eye bean was superior to others. In general inoculated varieties have gained better nodule dry matter accumulation than their respective uninoculated treatments.

According to Yifru (2003), the increased infection of roots by *Bradyrhizobium*due to inoculation, results in higher number and mass of the nodules. Profuse nodulation is important in nitrogen fixation by legumes. However, Sinclair and Vadez (2002) have noted that greater nodulation does not necessarily lead to greater nitrogen fixation. Significant differences in the number and dry weight of nodules among legume varieties of the same species have been reported (Egbe, 2007). The differences in the number and dry weight of nodules observed among Cowpea genotypes used in this study agreed with results of earlier studies (Ayisi*et al.,* 2004; Njoku and Muoneke, 2008), who reported that nodulation parameters varied in plant genotype of Cowpea intercropped with maize and sorghum. The interaction effects due to strains inoculation and varieties were not significant on nodule dry weight.

4.2.4. Nodule color

Nodule effectiveness in response to *Bradyrhizobium*inoculation was also assessed through its effect on nodule color. Inoculated varieties had produced from light red to red /pink colors. As reported by many authors (Adjei and Chambeiss, 2002; Butler and Evers, 2004), legume nodules having dark pink or red centers (due to leghemoglobin presence) are good indicators for effectiveness of the strain in nitrogen fixation. However, with un inoculated (but N fertilized) varieties, especially Black eyed bean and TVU, nodules have shown white and also few of them had green color, which indicated the ineffectiveness of the existing native *Bradyrhizobium* thus justifying the need for inoculation with effective *Bradyrhizobium*strains. Similar results were reported by Kassa (2009), who indicated that in the same location, inoculation of chickpea seed improved nodule number per plant by 60% over control. Moreover, a positive and significant response of legume nodulationto inoculation was also reported by others. (Otieno et al. 2009; Namvar and Sharifi 2011;Verma et al. 2013).In addition, nodules were concentrated at the junction of the main roots and the secondary roots, and varied in size, color and position. In all varieties strain GN102 and GN100 gave the highest number of nodules, volume and dry weight plant-1than MB 140.

4.3. Effect of inoculation and varieties on shoot dry weight

As shown in Table 3 above, there was a significant difference on strains ($P<0.05$) on shoot dry weight, where strain, GN100 and GN102 showed higher value than the others which might be attributed to presence of effective nodules. However, there were no significant differences among Cowpea varieties on shoot dry weight and the interaction effect of both strains and varieties on shoot dry weight was also non significant.

4.4. Effect of inoculation and varieties on leaf area index (LAI) of Cowpea

LAI of the five Cowpea varieties due to treatment with different strains of *Bradyrhizobium*is presented in the Table 3 . Strain GN102 showed statistically higher value than the others, and strain GN100 and MB140 were similar, but significantly better than the N fertlzer. Furthermore, there was significant difference ($P<0.05$), among varieties (Table 3). Highest recorded in Black eye bean but the lowest observed in Bole. Our result agrees with (Panahikord *et al* 2009), who reported nodulation and N-fixation increase vegetative growth of legume plants. Nitrogen is one of the structural components of amino acids and nucleotide and nucleoproteins and is necessary for cell division and cell growth and thus plant growth. Similarly, Thakur and Panwar, (1995) observed that inoculation of Mung bean with *Bradyhizobium*spp. increased plant height, leaf area, photosynthetic rate and dry matter production. Also, Poehlman, (1991) reported that amount of photosynthesis is a function of the total leaf area and the solar radiation intercepted. Therefore, higher LAI is directly proportional to photosynthate production which is triggered by nodulation and N fixation. In agreement to this, the yield components have been positively correlated with LAI.

4.5. Effects of Bradyrhizobial strains and varieties on yield components and total plant N content

The effects of strains and varieties on yield and yield components are shown in Table 4.

Table 3: Effects of *Bradyrhizobium* strains and Cowpea varieties on height, yield and yield components

Treatments		**Plant height**	**Number of Branchs plant^{-1}**	**Number of pod Plant $^{-1}$**	**Number of Seed pod^{-1}**	**Total biomass (t ha^{-1})**	**Grain yield (t ha^{-1})**	**Harvest index**	**100Seed weight (g)**	**Total plant N %**
strains	Urea	62.7^{ba}	9.906^{b}	27.54^{b}	12.54 ^{bc}	5.48^{b}	2.52^{c}	0.468	17.92^{b}	2.93^{c}
	GN100	64.17^{ba}	11.15^{a}	28.06^{b}	14.026 ba	5.8^{ba}	2.75^{ba}	0.495	18.20 ^{b}	3.04^{a}
	GN 102	66.01^{a}	10.18 ^{b}	31.4 ^{a}	14.246 ^{a}	6.07^{a}	2.88^{a}	0.498	18.58^{a}	3.03b^{a}
	MB140	60.65^{b}	10.053^{b}	26.84^{b}	12.333^{c}	5.8^{ba}	2.57^{c}	0.465	18.18^{b}	2.99^{b}
LSD (5%)		**4.143**	**0.966**	**2.73**	**1.581**	**0.36**	**0.2**	**NS**	**0.367**	**0.04**
Varieties	Bole	57.32^{c}	9.86^{b}	25.05^{b}	12.15^{b}	5.68	2.53^{c}	0.460^{b}	18.70 ^{c}	2.95^{cb}
	Black eye bean	73.85^{a}	11.25^{a}	37.52^{a}	15.12^{a}	6.04	2.88 ^{a}	0.518^{a}	26.96 ^{a}	3.08^{a}
	TVU	61.9^{cb}	9.81^{b}	25.37 ^{b}	12.27^{b}	5.5	2.58^{bc}	0.518^{a}	12.19^{d}	2.97^{b}
	Assabot	63.60^{b}	10.5^{ba}	26.88^{b}	14.400 ^{a}	5.9	2.8^{ba}	0.52^{a}	20.77^{b}	3.09^{a}
	Wonder	60.26^{cb}	10.10^{b}	26.85^{b}	12.48 ^{b}	5.77	2.62^{bc}	0.479^{ba}	12.48^{d}	2.92^{c}
LSD (5%)		**4.632**	**1.0802**	**3.05**	**1.768**	**NS**	**0.22**	**0.054**	**0.410**	**0.14**
CV%		**8.84**	**12.66**	**13.0**	**16.10**	**8.54**	**10.10**	**13.67**	**2.72**	**1.8**
Strains*	**varieties**	**NS**	**NS**	**NS**	**NS**	***	***	***	**	**NS**

Mean values with in column followed by the same letter are not significantly different ($p\leq 0.05$),NS = non significant at 5% probability level, n=60 (Number of observation for each parameter

4.5.1.Plant height

As shown in Table 4 above, there was significant difference among inoculants with respect to plant height ($P<0.01$) GN 100 was the best of all others. An increase in plant height of varieties due to strain GN 100 could be attributed to increase in availability of nitrogen and other nutrients. Khalil *et al.* (1989) observed that nitrogen alone or in combination with inoculation gave the maximum plant height in Mung bean. Several other investigators also reported that, inoculation with effective rhizobia has a potential importance to improve growth and yields of the crop not only due to high N fixation activity but also due to the ability of these bacteria to produce antibiotics, growth promoting substances (phytohormones), and the ability to solublize phosphate (Pandey*et al*., 1998). However, the later parameters were not measured in this investigation. Yefru (2003) also observed an increase in plant height of peas in response to inoculation with *Bradyrhizobium leguminosarum.* Similarly, varieties were significantly differed. Variety Black eye bean was the tallest while Bole was the shortest. However here was no significant interaction effect between inoculation and varieties with respect to plant height. The result in Table 4 showed that effect of inoculation on number of branches per plant was statistically significant. In line with this, Black eye bean and Assabot varieties showed highest number of braches per plant and lowest value observed with the variety Wonder. Our results were found to be in good agreement with previous studies. To mention but few, Ibrahim *et al*., (2010), reported that legume seeds inoculated *with Bradyrhizobia* have had increased plant height and fruiting branches. Jatasra and Dahia (1988) also reported, that there was no significant effect on branching, due to increasing level of N fertilizer, and stated that fodder yield was positively correlated with leaf weight, stem weight, plant height and number of branching per plant. Sultana *et al.* (2005) also reported similar results on plant height due to increasing N fertilizer. The interaction effect of varieties and strains was not significantwith respect to branch. Whereas, Branches were positively correlated with yield components (number pods and seeds per pods).

4.5.2. Number of pods per plant

The number of pods per plant was found to be statistically significant (P <0.05) affected by Bradyrhizobial strains. Specially, inoculation with strain GN102 resulted significantly higher number of pods per plant whereas effect of inoculation with GN100, MB140 and the N fertilizer was statistically at par. Cowpea varieties were significantly different (p< 0.01) with respect to number of pods per plant. Where the highest number of pod per plant (41.06) was recorded from the variety Black eye and lowest value (23.03) observed from Bole (Table 4.No interaction effect on variety and strains on number of pod per plant.

4.5.3. Number of seed per pod

Number of seeds per pod varied significantly (P <0.05) due to different strain inoculation (Table 4). Significantly higher number of seeds per pod (14.24) was obtained with GN102 while lower number of seeds per pod (12.33) was noticed with N fertilizer and MB140 strain. Number of seeds per pod differed highly significantly ($P \leq 0.01$) between the varieties. Both Black eye bean and Assabot varieties had higher number of seeds per pod while the others had lower number. Assabot inoculated with GN100 recorded higher number of seeds per pod than variety wonder which is with N fertilizer. This study agrees with the investigation of Wasule*et al.* (2007) who reported that inoculation with *Bradyrhizobium*and phosphate solubilizing microorganism significantly improved soybean growth and its seed size. The interaction effects among varieties and strains were not significant.

4.5.4. Effect of inoculation and varieties on total biomass of Cowpea

Inoculation with Bradyrhizobil strains significantly affected total biomass yield (at P< 0.05) (Table 4 and Appendix Table 8),where, all inoculated treatments (GN102, GN 100 and MB 140) yielded the highest while urea was the least. However Varieties did not differ significantly with respect to total biomass yield (Table 4).Studies by Gyaneshwar*et al., (*1998) reported that inoculation can enhance plant growth by increasing the efficiency of biological N fixation and also by enhancing the availability of trace elements and the production of plant growth promoting substances in the rhizosphere.

As shown in Table 5, the interaction effect of strains and varieties on total biomass (t ha^{-1}) was significantly differed (at P< 0.001). This may be due to symbiotic effectiveness of strain to fix atmospheric N_2varying with different genotype or varied response of cultivars to inoculums.

Table 4: The interaction effect of strains and varieties on total biomass (t ha^{-1})

Strains	**Cowpea Varieties**					
	Bole	Black eyebean	Wonder	Assabot	TVU	**Means**
Urea	5.37^{fgieh}	5.07gih	5.75^{fgeh}	5.83^{fgdec}	5.37^{fghi}	**5.48^{b}**
GN100	4.77i	5.98^{bdec}	5.9^{fbdec}	6.0^{bdec}	6.36^{badc}	**5.8^{ba}**
GN102	6.96a	6.43^{bac}	6.28^{bdac}	5.68^{fgdeh}	5.0^{ih}	**6.07^{a}**
MB140	5.6^{fdegh}	6.6^{ba}	5.15^{fgih}	6.0^{bdec}	5.6^{fdegh}	**5.8^{ba}**
Mean	**5.68**	**6.04**	**5.77**	**5.9**	**5.6**	

Mean values within column followed by the same letter are not significantly different (p>0.05)

4.5.5. Effect on hundred seed weight

The present study indicatedsignificant effect of strain inoculation (p<0.01) with respect to hundred seed weight. Among all strains GN102 recorded higher hundred seed weightand the others strains including urea fertilization were statistically at par. Also, there was statistically significant difference between varieties in hundred seed weight at (p<0.001 (Table 4 and Appendix Table 8). Highest hundred seed weight was recorded from variety Black eye bean and lowest was from Wonder.Also, the interaction effects of both factors were significantly different (p< 0. 01) (Table 6).

Table 5: The interaction effect of strains and varieties on 100 seed weight (g)

	Cowpea varieties					
Strains	**Bole**	**Black eye bean**	**Wonder**	**Assabot**	**TVU**	**Means**
Urea	18.70 ^{fe}	26.26^{b}	12.50 ^{hgi}	20.33^{d}	11.80^{i}	**17.92^{b}**
GN100	17.9^{f}	27.6^{a}	26 ^{hgi}	20.6^{d}	12.63^{hg}	**18.20^{b}**
GN102	19.06^{e}	27.46^{a}	12.30 ^{hgi}	21.700^{c}	12.40^{hgi}	**18.58^{a}**
MB140	19.13^{e}	26.53^{b}	20.46^{d}	20.466^{d}	11.93^{hi}	**18.18^{b}**
Means	**18.70 ^{c}**	**26.96 ^{a}**	**12.48^{d}**	**20.77^{b}**	**12.19^{d}**	

Mean values within a column followed by the same letters are not significantly different (p>0.05)

4.5.6. Effect on grain yield of Cowpea

The effects of inoculation with *Bradyrhizobium* strains and varieties on the grain yield are shown in Table 4.Grain yield differed significantly between varieties, and the highest seed yield was obtained in variety Black eye bean (2.88 t ha^{-1}) and Assabot (2.8 t ha-1). Our result is in accordance with the findings of Bilatu and Biniyam (2011) who had compared seven Cowpea varieties in western Ethiopia, reported 2.9 t ha^{-1} from Black eye bean and also Takim and Uddin, (2010) reported the grain yield of Cowpea 2.0 t ha^{-1} with application of N fertilizer. Similarly, inoculation had a highly significant influence on grain yield. Significantly higher grain yield (2.885 t ha-1) was obtained with GN102 and followed from inoculating with GN100. But there was no difference in yield of MB140 (2.572 t ha^{-1}) and urea (2.527 t ha^{-1}). Namvar*et al...(*2011) reported the usage of 100 kg urea ha^{-1} resulted in the highest biomass production and grain yield of chickpea as compared to N fertilizer. According to this study strain GN102 and GN100 increased grain yield of Black eye bean by 14% and 9% respectively over N fertilizer. one way of improvingN2 fixation in grain legumes is inoculation of the crop seeds with effective strains of rhizobia, despite being mentioned by some as a promiscuous host (Rivas et al. 2007). The yield increase obtained in our investigation might be due to effectiveness of the *bradyrhizobium* inoculants enabled fixation of atmospheric nitrogen. Rani and Kodandaramaiah, (1997) reported that the increase in yield in inoculated treatment might be attributed to increased nodules per plant and nodule dry weight, resulting in higher dry matter accumulation during the growth period and translocation of more photosynthate to the seed. Inoculation with combination of small starter nitrogen fertilizer was also reported by Rashid *et al.* (1999) who indicated increased nodules number resulting in better nitrogen fixation which contributed to higher grain yield. Ashraf *et al.* (2003) showed that seed inoculation with *Bradyrhizobium*strain significantly increased Mung bean seed yield. Peoples and Herridge (1993) hypothesized that high number and effective nodulation is essential for a functioning legume/*Bradyrhizobium*symbiosis. However, this assumption often depends on other factors such as the environment, crop management, choice of micro and macro symbiont and the ability of the plant to support high levels of N fixation. In earlier investigation done by Germew H (2007) at Melkassa research center, the yields Cowpea per hectare without urea application were 2.06 t ha^{-1} and almost all farmers in Ethiopia donot use urea fertilizer for legumes. However, the yields of grain legumes particularly of Cowpea are low, and inoculation with effective *Bradyrhizobium*strain should be considered to increase productivity of small scale farmers in Ethiopia. The interaction effect of strains and varieties were significantly differed with respect to grain yield (*P* <001) as shown (Fig 2) to showthat each variety responded to the inoculants differently. Our result revealed that variety Black eye bean produced highest yield followed by Assabot inoculated with GN 102 and GN 100.

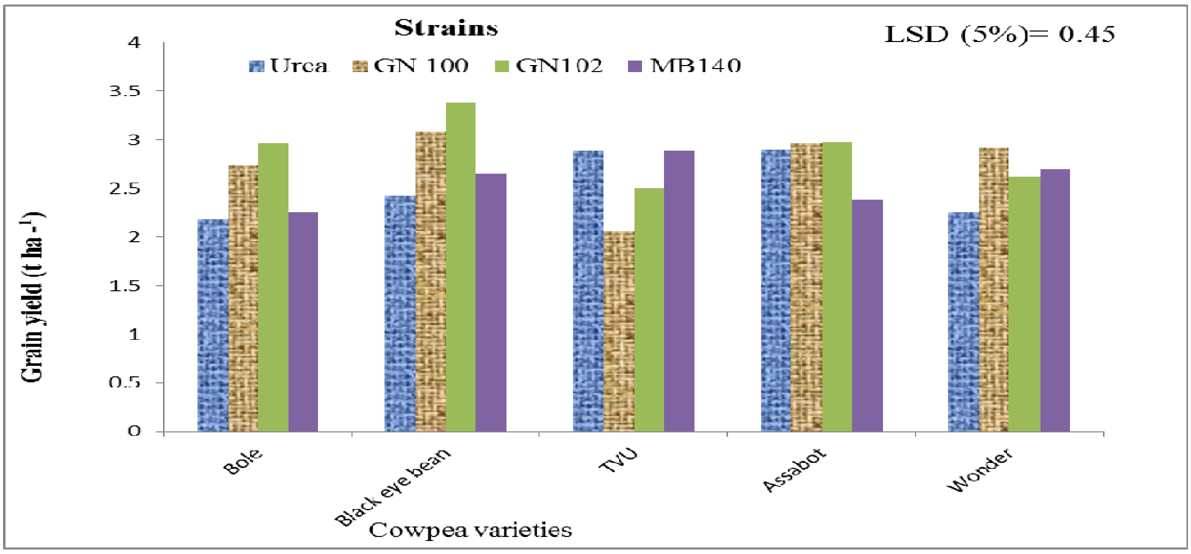

Figure 2: Interaction effects *of Bradyrhizobium* inoculation and Cowpea varieties on grain yield

4.5.7. Effect on harvest indexes of Cowpea

The response of inoculation with *Bradyrhizobium* strains was not significantly differed on harvest indexes (Table 4). However the harvest indices varied significantly at P< 0.005 among Cowpea varieties. Varieties with lower total yields tended to have higher HI and varieties with higher total yields tended to have lower HI. Assabot, Black eye bean and TVU had higher harvest index (70.3 %, 70% &70%) respectively with Wonder having minimum value(64%). The HI indicates the ability of these varieties in translocation dry matter to their seeds and difference in response of the varieties could be attributed to the differences in cultivars under strain inoculated condition. In general, the highest HI was obtained from the high seed yielding varieties, the Black eye bean and Assabot. In general Black eye bean and Assabot were superior while Bole was the least. The result also revealed was significant interaction effect between strains and varieties on harvest indexes (p<0.01) Shown in Fig 3.

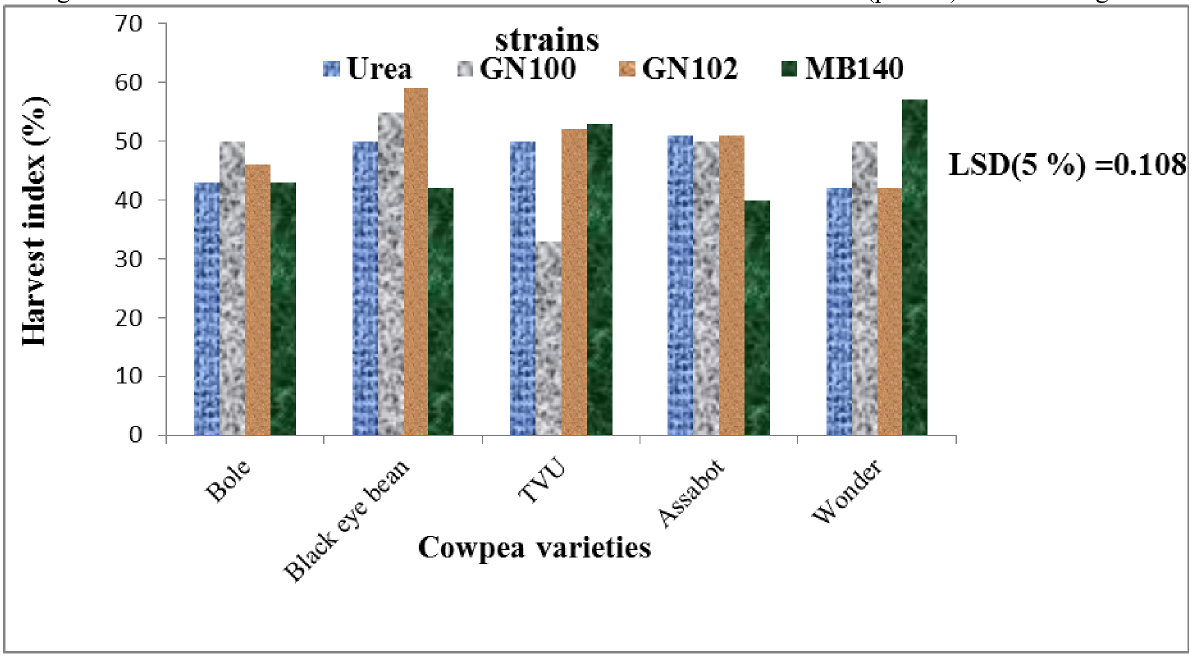

Figure 3: Interaction effects of *Bradrhizobium* strains inoculation and Cowpea varieties on harvest index

4.6. Effect of Bradyrhizobial inoculants on plant total N content

The plant tissue analysis on N content result is shown in Table 4; and there was a significant difference among inoculants on total N contribution. Strains GN 100 and GN102 were superior to the others. Also the five Cowpea varieties were significantly different on total N (P < 0.05). The increased N content of Cowpeais attributed to the inoculation with effective strain which could be responsible for increased availability of nitrogen to fix N_2 from the atmosphere for synthesis of tissue protein of the plants which have been reflected in the increased growth of plants in the form of plant height and yield components. Similar works done by Elsheikh*et..al* (2009) reported that inoculation with *Bradyrhizobium*significantly increased the protein content of both mono cropped and intercropped soybean seeds. Sultana *et al*. (2005) also reported that the increased crude protein content in cowpea forage is due to nodulation in terms of N content Varieties differed significantly, but Black eye bean, Assabot and Bole were similar. This might be attributed to the effectiveness of strains or the genetical potential of the varieties which enhanced maximum exposure of leaves to sun radiation for protracted period. Thornley (2002) observed that, the factor influencing leaf nutrient content also includes the position of leaf tissue which possibly affected photosynthesis, quality of photosynthates and protein partitioning. This agrees with similar findings by Muhammad *et al*. (2006) who reported Cowpea in sole plots more crude protein were obtained than in the intercropped plots. The interaction effect and varieties were not significant on total plant N contribution.

4.7. Effect of Bradyrhizobiumincoculants and varieties on soil chemical properties

Table 6: Effects of *Bradyrhizobium* strains and Cowpea varieties on soil chemical properties

Treatments		pH	Soil TN (%)	OC (%)	Av.pmg/kg
strains	Urea	7.3[b]	0.154[c]	1.64[b]	52.6[b]
	GN100	7.62[a]	0.178[a]	1.75a	57.1[a]
	GN 102	7.53[a]	0.176[a]	1.78[a]	55.5[ba]
	MB140	7.47b[a]	0.168[b]	1.73[b]	55.3[ba]
LSD (5%)		**0.19**	**0.008**	**0.077**	**3.0**
Varieties	Bole	7.5	0.165[b]	1.7[b]	50.8[b]
	Black eye bean	7.58	0.18[a]	1.82[a]	59.3[a]
	TVU	7.38	0.162[b]	1.72[b]	52[b]
	Assabot	7.52	0.175[a]	1.73[ba]	60.7[a]
	Wonder	7.45	0.162[b]	1.65[b]	52.8[a]
LSD (5%)		**NS**	**0.0086**	**0.086**	**3.4**
CV%		**1.88**	**3.28**	**3.22**	**4.03**

pH=soil of hydrogen, TN=total nitrogen in the soil, OC%=organic carbon in the soil and Av.p(mg/kg) = available phosphorous in the soil. Mean values within a column followed by the same letter for all parameters are not significantly different (p≤0.05)

4.7.1. Effect on pH of the soil

The effect of inoculation with *Bradyrhizobium* strains and varieties on soil pH is shown in Table 7, and there was a significant difference among inoculants on soil pH increasment after crop harvest. Where all strains were found to be superior to urea treatment, these agrees with work of Kuykendall *et al*. (2000) who reported that effective inoculants may increase the pH of soil. Fery(1990), reported that Cowpea had the ability to tolerate both acid and alkaline soil conditions and the crop is responsive to favorable growing conditions. FAO (2010) reported that the preferable pH ranges for most crops and productive soils are 6 to 8 and the Cowpea was best fits in slightly acidic to alkaline soil. There was no significant difference found on soil pH among varieties.

4.7.2. Effects on soil total nitrogen

The impact of inoculation with *Bradyrhizobium* strains and varieties on soil nitrogen is presented in Table 7. The effect of inoculation with *Bradyrhizobium* strains on total soil nitrogen to be significantly differed ($P < 0.05$),where train GN100 and GN 102 were the highest and urea the least. Compared to soil N content before planting, these strains GN100, GN 102 and MB140, increased soil N by 51.6, 46 and 40% respectively. This is in agreement with the investigation by Eaglesham and Ayanaba (1987) who reported that if Cowpeais nodulated by effective strains of *Bradyrhizobia,* 90% of the required nitrogen for maximum yield can be obtained from biological nitrogen fixation. Similarly, inoculation with *Bradyrhizobium* strains and varieties has also resulted in a significantly differed on soil nitrogen. Varieties Black eye bean and Assabotperformed better in terms soil fertility for the next season crop than Bole, Wonder and TVU. These may be due to variations in genetical potentials variety and strains to fix N_2 from atmosphere.This study confirms the works of Kassa, ..*et.al* (2009), who found out that Cowpea, contributes to the improvement of soil fertility through fixation of nitrogen (60 - 70 kg N ha^{-1}) to the subsequent crop. Authors like Khan et al. (2003); Gregory (2006) said that there is no single value for below ground N, with variations in published estimates reflecting the influence of species, soil and climate, and other factors affecting the partitioning of dry matter and N between the shoot and the root. Many experimental findings confirm that soil nitrogen levels increased following Cowpea in rotation.

4.7.3. Effects on soil organic carbon

The soil analysis result on organic carbon content result is shown in Table 7; and there was a significant difference among inoculants on organic carbon contribution. And strains GN 100 and GN102 were superior to the others. This line with Tittonell*et al.* (2010), inoculants supplied soil test result showed wide differences between farms for initial organic carbon and after crop harvest of chickpea.Also the five Cowpea varieties showing significant difference with respect to organic carbon ($P < 0.05$). Over all, the organic carbon contribution of inoculation increased carbon content of the soil after planting. The increased organic carbon content of the soil may be due to inoculation and increased availability of nitrogen by the presence of effective strains to fix N_2 from the atmosphere that helps growth and development of the plants which have been reflected in the increased carbon in the soil by plant mineralization.

4.7.4. Effects on soil available phosphorous

With respect to the phosphorous content, the result Shown significant difference among inoculants.The effect of strains GN 100, GN102 and MB140 on soil available phosphorous were the highest and N fertilizerthat of the least. Our results are in accordance with the findings of several other studies (Kassa 2009; Ahmed etal. 2010; Yoseph 2011). Increased supply of nitrogen through BNF and direct supplementation of phosphorus was reported which in turn play important role in soil fertility and enhanced growth for assimilate accumulation, thereby improving the reproductive performance of the plants. This is in conformity with the work of, VanVeen*et al*...(1997) who reported that in addition to P solubilizing, application of bacteria to the soil enhance phosphorus status of soil and plant. We have observed a significant difference among varieties in terms of available soil phosphorous ($P < 0.05$). Where variety Black eye bean, Assabot, and Wonder performed higher but Bole and TUV were the least. The total biomass of Cowpea is of great importance in farm level nutrient cycling due its role as either animal feed or organic matter to be returned to the soil. In this study, total biomass of Cowpea was highly improved by the soil fertility treatments due to the positive effects of nitrogen and phosphorus on vegetative growth, and the synergy of these two nutrients on plant growth.

5. SUMMARY AND CONCLUSION

Ethiopia is among the countries with the highest rates of nutrient loss, mainly due to the low nutrient input, high biomass removal and soil erosion. Especially nitrogen is highly deficient or limiting nutrient almost in all Ethiopian soils and therefore, in the absence of chemical supplies, nitrogen deficiencies are very common and limiting to the crop production of the country. The experiment was comprised of 20 treatments made up of a factorial combination of two input factors [Three Bradyrhizobial strains (GN100, GN102, MB140) and a positive control (N fertilizer at 100kg/ha)] and five Cowpea varieties, (Bole, Assebot, Black eye bean Wonder and TVU). The experimental design was RCBD with three replications and 20 treatment combinations. The three strains were isolated from Ground nut (GN100 and GN102) and Mung bean (MB140) . Results obtained from the experiment indicated that most of the parameters tested significantly responded ($p< 0.05$) to the treatments. For nodulation and growth parameters, inoculants GN100 and GN102 showed significant differences in nodule number, nodule volume, nodule dry weight, plant height and leaf area index. The interaction effect of *Bradyrhizobium*inoculation and Cowpea varieties on total yield, grain yield, harvest indexes and hundred seed weight per plant was highly significant ($P\leq 0.01$). The highest grain yield per plot was observed from inoculated Black eye beanand Assabot with GN102 and GN 100. The results also revealed that, formation of nodules by the varieties was significantly ($P\leq 0.05$) varying under inoculation. Fixation of atmospheric nitrogen by nodulated Cowpea varieties in symbiosis with *Bradyrhizobium*bacteria is thus cheap and useful source of nitrogen, which is contributing maximum food production. Several suitable Cowpea varieties are available in Ethiopia for potential intercropping, relay cropping and rotation which could improve soil fertility, crop yield and roughage quality and make the system more sustainable. However, so far, researches on the contribution of Cowpea varieties to the farming system are given lower emphasis, as compared to haricot bean varieties and their adoption and benefit to the small-scale farmers is very minimal. Inoculation with *Bradyrhizobium* strains was significantly differed on soil nitrogen ($P < 0.05$),strain GN100 and GN 102 were the highest and urea was the least. Compared to soil N content before planting, strain GN100, GN 102 and MB140 increased soil N by 51.6, 46 and 40%, respectively. These may be due to variations in genetical potentials variety and strains to fix N_2 from atmosphere. Increased supply of nitrogen through BNF and the direct supplementation of phosphorus that in turn play important roles in soil fertility and enhanced growth for assimilate accumulation, thereby improving the reproductive performance of the plants. Thus, research on Cowpea varieties improvement and theirnitrogen fixation inputs should continue as a major interest in small holder farmers,Also lack of N fertilizers and economic resources are important constraints for these farmers. Investigations on other agronomic practices which give an appropriate consideration to growth and nitrogen fixation of Cowpea varieties under various locations should also be done. Finally, the present study should be further evaluated under various agro ecological conditions in order to come up with conclusive recommendations to be used by small holder farmers.

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