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Evaluation of best performing indigenous *Rhizobium* inoculants for Chickpea (*Cicer aritenium* L.) production at Ginnir District, Bale Zone, Southeastern Ethiopia

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Effectiveness of indigenous *Rhizobium* strains was selected and evaluated their ability for enhanced nodulation; grain yield and yield component of chickpea (*Cicer aritenium* L.) on farmers' fields at Ebisa and Loboca, of Ginnir District. The experiment experiments were conducted for two consecutive crop-years at field condition from 2016 to 2017 during "*Bona*" cropping seasons. The seven treatments including control (without any fertilizer and inoculation), Inoculation of Strain 110, Strain 004 CP, Strain EAL29 with 36g/plot or 50kg/ha phosphorus were used. Experiment was laid out in a randomized complete-block (RCBD) design with three replications in which each treatment were applied on a plot size of 3m x 2.4m =7m^2, the distance between blocks and within plots are 1.5 m. Test crop chickpea (Ararti vertices) 48.8 g/plot or 65kg/ha, were used. The results this studies indicated that yield and yield component of chickpea (*Cicer arietinum* L.) were significantly influenced by the application of rhizobium inoculation and phosphorus fertilizer. The results show that significantly higher grain yield were obtained under Rhizobium inoculants combined with phosphorus fertilizer (EAL29 + 50 kg ha^{-1} DAP) over the control in which recorded 51.61% higher grain yield (4.7 t ha^{-1}) as compared to control (3.1 t ha^{-1}). From the result of this studies it can be concluded that the effect and interaction of rhizobium, and phosphorus fertilizer (50kgha^{-1} DAP) which contain 9kgha^{-1} N as starter significantly increased yield of chickpea under for the study area.

Keywords: Rhizobium Inoculation; Chickpea; fertilizer DAP; Grain yield

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INTRODUCTION

Pulses, including beans and chickpeas, are one of the most important crops in the world because of their nutritional Oliveira, 2006). chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world after dry beans (*Phaselous vulgaris* L.) and dry peas (*Pisum sativum*) (Parthasarathy et al., 2010). In Ethiopia, chickpea is mainly grown in the central quality. They are rich sources of complex carbohydrates, protein, vitamins and minerals (Costa, Queiroz-Monici, Reis, &, northern and eastern highland areas of the country at an altitude of 1400-2300 m.a.s.l., where annual rainfall ranges between 700 and 2000mm (Anbessa Y, Bejiga G , 2002). Ethiopian soils are deficient of major soil nutrient like nitrogen (N) (Wondewosen Tena *et al*., 2016).The use of *Rhizobium* inoculants is to improve nitrogen *Rhizobium* symbiosis with legumes species is of special importance, producing 50% of 175 million tons of total biological nitrogen fixation annually worldwide (Sarioglu *et al*., 1993). Nitrogen (N) deficiency is frequently a major limiting factor for high yielding crops all over the world (Salvagiotti et al., 2008; Namvar et al., 2011). Nitrogen, a vitally important plant nutrient (Frank *et al*., 2003), is the main factor that limits chickpea production in Ethiopia. When legumes grown in rotation with other crops, under certain environmental conditions, they can improve soil fertility and reduce the incidence of weeds, diseases and pests (Al bayrak et al., 2006).

The *Rhizobium*-legume symbiosis has received most attention as they are widely deployed in agricultural practices for sustainability of crop yield and recovery of soil fertility (Egamberdieva et al. 2015). Chickpea (*Cicer arietinum* L.) is one the common pulse crop grown mainly under rain fed condition in Ginnir district of bale zone of southeastern Ethiopia. Rhizobium technology is efficient in supplying nitrogen to legumes and is a better option for resource poor farmers who cannot afford to purchase expensive inputs and also to improve grain yield of Chickpea. Phosphorus is an essential nutrient both as a part of several key plant structural compounds and as a catalyst in numerous key biochemical reactions in plants. It is also noted especially for its role in capturing and converting the sun’s energy into useful plant compounds (Cordell, Dana & Drangert, Jan-Olof & White, Stuart., 2009). When phosphorus is limited in the soil, the most striking effects are a reduction in leaf expansion and leaf surface area, as well as the number of leaves. The deficiency of phosphorus might also limit nitrogen fixation through its effects on growth and survival of rhizobia, nodule formation, and nodule functioning and host plant growth (Tans,C., Hinsinger,R., Drevon, J.J . , and B.Jaillard., 2001).

Biological nitrogen fixation (BNF) represents a significant potential source of N input in agricultural soils in the country. Generally, it is assumed that a pulse crop well inoculated with the bacteria can fix sufficient quantities of N to eliminate the need for N fertilizer inputs in the cropping year (Walley FL, George WC, perry RM, Patrick MC, Guy P, 2006). Nitrogen (N) deficiency is frequently a major limiting factor for crops production in over the world (Fuzhong, et al., 2008; Aminifard, et al., 2010). Determination of the agronomical response of chickpea crop to Diammonium phosphate (DAP) fertilization and Rhizobium inoculation is very important to maximize yield and economic profitability of chickpea production in a particular environment. Low yield of chickpea in Ethiopia is mainly attributed due to lack of improved package use in chickpea production. Biological nitrogen fixation plays a vital role for sustainable legumes production and important source of N for farmers. Legume seed inoculated with *Rhizobium* shows Better growth, nitrogen fixation, seed yield and also improve nutrient uptake (Mfilinge *et al*., 2014). Biological Nitrogen Fixation (BNF) is an important part of sustainable agriculture (Sessitsch *et al*., 2002) and growers apply rhizobial inoculation often as bio-fertilizers.

Inoculation of seeds with Rhizobium increases nodule number (Salih *et al*., 2015; Tahir *et al*., 2009). According to (Mohamed and Hassan ,2015) reported that inoculated plants produced higher nodule dry weight, grain yield**,** number of filled pods, seed numbers compared to un-inoculated plants. Using high yielding varieties of chickpea together with effective rhizobial strains, starter nitrogen and phosphorus fertilizer sources can enhance its yield and minimize the need for external nitrogenous fertilizer input. Several authors also indicated the positive effect of Rhizobium inoculation and NP fertilizer application in Ethiopia (Angaw and Asfaw, 2006; Amanuel et al., 2000; Ayneabeba et al., 2001). Most of the farmers of the studies area cannot use recommended doses of various fertilizers because of the cost of fertilizer on the others hand soil fertility decline were the major challenge to production and productivity. Low yield productivity of chickpea is one of the main problems in Ginnir district of bale zone of southeastern Ethiopia. To minimizing such problems of low productivity of chickpea the use of improved input is vital role among these selecting efficient, competitive Rhizobium strains, optimum P fertilizer together with nitrogen as starter. It is essential to evaluation and recommending effective Rhizobium strains with optimum rate of P fertilizer together with nitrogen as starter. Therefore these studies were carried out with objective of evaluate DAP fertilizer and new chickpea inoculants on yield and yield component of chickpea.

MATERIALS AND METHODS

Description of the Study Area

Ginir is 519 km away from Addis Ababa, 86 km away from the zonal capital town, Robe. Ginir is located at 07^{o} 15′ N latitude and 40° 66′ E longitude at 1972 m above sea level. The seasonal rainfall is 531 mm and its mean annual minimum and maximum temperatures are 13.4 and 25.5°C, respectively (Wubishet *et al*., 2016). The soil type is *Vertisol.* Ginir experience a mono cropping season (main season) that extends from September to January. The Ginir district is very suitable for the production of cereals but pulse, oil crop and horticultural crops are also produced by farmers. Field experiments were conducted on farmers' from 2016 to 2017 during "*Bona*" cropping seasons on farmers' fields Ebisa and Loboca in Ginnir district bale zone of southeastern Ethiopia. (Figure 1)

METHODOLOGY

Land preparation (ploughing, and leveling) was done based on the recommendation given to the crop. Seed inoculation was performed before sowing using the procedure developed by (Fatima *et al*., 2007). To ensure the sticking of the applied inoculant to the seeds, the required quantity of seed was suspended in 1:1 ratio in 10% sugar solution. The inoculant was gently mixed with dry seeds at the rate of 10 g per kg of seed. Inoculation was done just before sowing under shade to maintain the viability of cells and allow to air dry for a few minutes and then the inoculated seeds were sown at recommended rate and spacing to the respective plots. To avoid contamination, plots with un-inoculated seeds were planted first followed by the inoculated ones. Some treatments (except the negative control) received equal amount of 50 kg DAP ha^{-1} which contain 9 kg of nitrogen as starter.

Treatments and experimental design

Experiment was laid out in a randomized complete-block design (RCBD) in three replications. Each treatment was applied on a plot size 3m x 2.4m =7m^2, the distance between blocks and within plots are 1.5 m. Test crop used was chickpea (Ararti verities) 48.8 g $plot^{-1}$ or 65kg ha^{-1}, with 36g $plot^{-1}$ or 50 kg ha^{-1} starter nitrogen and phosphorus sources from DAP, together with the following strains (Table 1). Inoculants were collected from Holleta Agricultural Research center, National Laboratory and the sole commercial inoculant producer in Ethiopia: Menagesha Biotech Industry PLC, Addis Ababa. (Figure 2)

Data Collected

Different Agronomic and nodulation related data were collected. Number of Nodules: were taken by excavating the roots of plants randomly from four middle rows of each plot at the mid flowering stage of the crop. After five representative samples were taken randomly from the middle row; the numbers of nodules were counted and the mean values of the five plants were recorded as number of nodules per plant. Nodule volumes were immersed in previously measured volume of water in measuring cylinder. The volume of water displaced by nodules was considered as nodule volume (ml). Nodule dry weight were labeled and placed in peter dish. The nodule dry weight per plant was measured after drying the collected nodules in an oven with a temperature of 65^{o}C for 24 hrs until constant weight is attained. Number of seeds per plant: were counted from the non-boarder plots. Above ground biomass yield (kg ha^{-1}): At physiological maturity, plants from central row were manually harvested close to the ground surface. Grain yield (Kg ha^{-1}): It was determined after threshing and adjusting the grain yield at the appropriate moisture level of 10.5%. Finally, yield per plot was converted to tone per hectare basis.

Statistical analysis

The collected data were analyzed following technique valid for (RCB) design using one-way Analysis of Variance (ANOVA) using SAS software. Comparisons of means were performed by the Fisher's Protected LSD test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effects of rhizobial inoculation on nodule number, nodule weight, 1000-seed weight, Biomass, grain yield and nodule volume have been presented in (Table 2). Inoculated plants gave significantly higher nodule number, nodule weight, Biomass and seed yield compared to uninoculated control. The results of the effect of biofertilizer and phosphorus fertilizer on yield and yield components of chickpea (*Cicer aritenium* L.) were summarized (Table 2.) in which most of the agronomic and nodules were significantly difference. The effective indigenous rhizobial strains, its combination with phosphorus fertilizer which contain 9 kg ha^{-1} N as starter were brought significantly better agronomic parameter such as, Nodule of chickpeas such as Plant Height , Number of Seed per Plant , Biomass Yield , Grain yield ,Thousand kernel weight, Number of nodule per plant , Nodule volume , Nodule fresh weight , Nodule dray weight the results indicated(Table 2) in which most of the parameter collected were significantly affected.

The results of study showed that plant growth and nodulation of chickpea were affected by rhizobial strains which stimulated the plant growth. According (Berger *et al*., 2006) growth potential of chickpea depends on the rhizobia association and plant genotype which together influencing the symbiotic performance. This study indicated that response of inoculation with *Rhizobium* could promote pant growth, particularly root growth, as

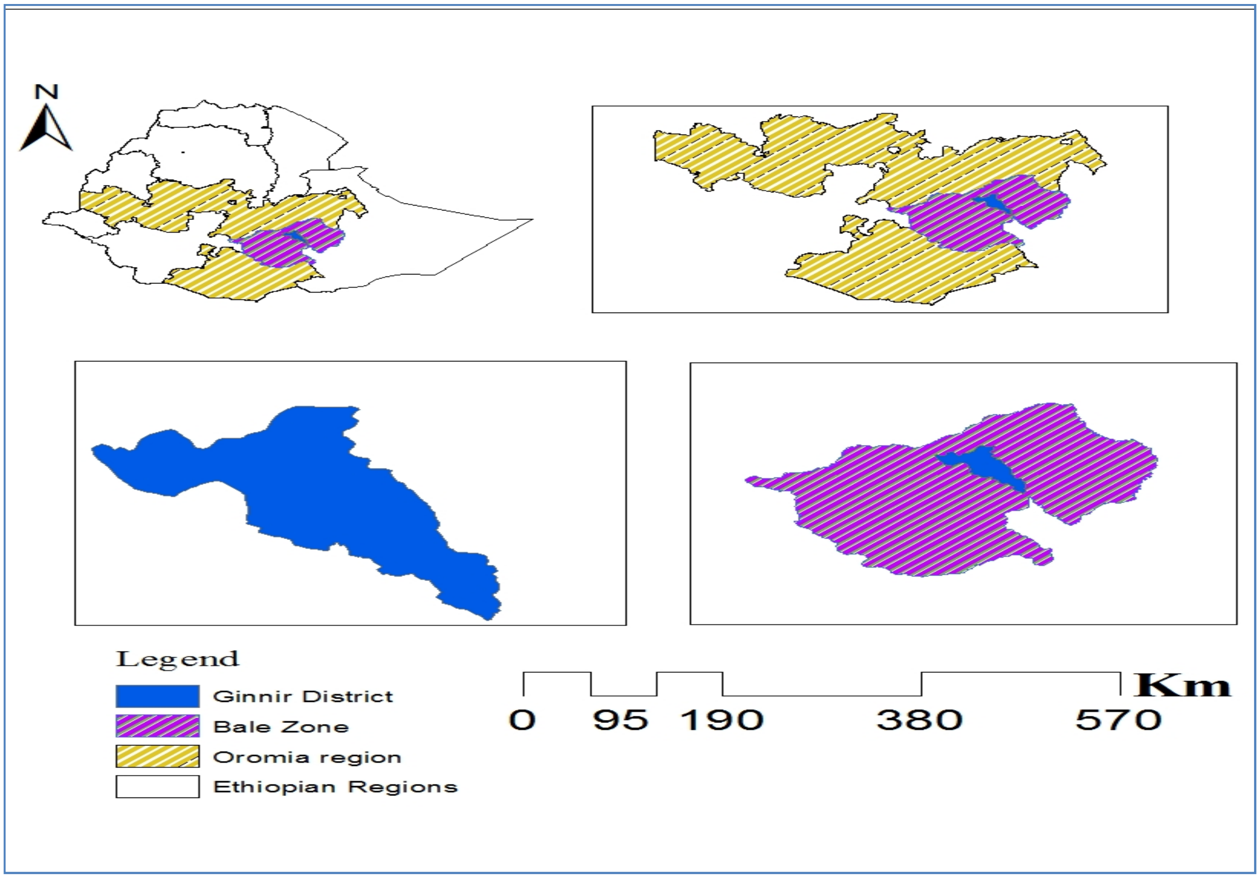

Figure 1. Map of the Study area

Table 1:- List of Treatments use for chickpea production

Treatments	Full name of treatments
T_0	Control (uninoculated)
T_1	110 CP
T_2	004 CP
T_3	EAL29
T_4	CP110 + 50 kg DAP ha^{-1}
T_5	004 CP + 50 kg DAP ha^{-1}
T_6	EAL29 + 50 kg DAP ha^{-1}

well as benefit root uptake from soil in which affected the yield and yield component of chickpea. This study also agree with the finding (Fatima *et al*., 2008) who reported that inoculation of chickpea with rhizobia increased plant growth, ground dry matter, number of pods, seed yield, and nitrogen fixation under various climatic conditions. Similar study has been reported that phosphorus may play a crucial role in increasing nutrient uptake in legumes grown in different soils (Nyoki, Daniel and Patrick A Ndakidemi, 2014a).

The effect of phosphorus fertilizer and rhizobial inoculation on nodulation, grain yield, and yield component of chickpea under rain fed field experiments were indicated (Tables 2). The results obtained from the analysis of variance indicated that phosphorus fertilization and rhizobial inoculation had significant effects on all yield and nodulation studied except on plant height and thousand kernel weight. This study indicated that the significantly higher grain yield were obtained under Rhizobium inoculants combined with phosphorus fertilizer (EAL29 + 50 kg ha^{-1} DAP) over the control in which recorded 51.61% higher grain yield (4.7 t ha^{-1}) as compared to control (3.1 t ha^{-1}). The result this study revealed that, rhizobial inoculants combined with

Figure 2. Field layout of chickpea experiment at Ginnir district

Table 2. The effect of indigenous rhizobium inoculants on yield and yield components of chickpea during "*Bona*" cropping seasons of 2016- 2017 at Ginnir District in the mid altitude area of Bale zone, southeastern Ethiopia

Treatments	PH(cm)	NSPP	BY(t ha^{-1})	Gy(t ha^{-1})	TKW	NNPP	NV(ml)	NFW(g)	NDW(g)
T0 = Control (un inoculated)	62.0	49.6 ^{c}	6.4 ^{c}	3.1 ^{d}	224.7	35.2^{d}	1.8^{c}	2.1^{d}	0.7^{c}
T1 = 110 CP	61.4	59.6 ^{bc}	6.6 ^{bc}	3.9 ^{bc}	231.4	65.9^{b}	2.4^{bc}	2.8^{c}	1.4^{b}
T2 = 004CP	62.0	69.3 ^{bac}	7.0 ^{bc}	3.6 ^{dc}	236.2	67.2^{b}	2.8^{bc}	3.3^{b}	1.5^{ba}
T3 = EAL29	60.2	72.8 ^{ba}	7.6 ^{bac}	4.3 ^{ba}	233.1	45.1^{cbd}	2.3^{bc}	3.2^{cb}	1.4^{ba}
T4 = 110 CP + 50 kg ha^{-1} DAP	61.6	76.4 ^{ba}	6.8 ^{bc}	3.6 ^{dc}	245.2	51.8^{cbd}	3^{bc}	3.2^{b}	1.5^{ba}
T5= 004CP + 50kg ha^{-1} DAP	63.2	70.5 ^{ba}	7.9 ^{ba}	4.0 ^{bc}	245.4	56.5^{cb}	3.3^{b}	3.4^{b}	1.3^{b}
T6 = EAL29 + 50 kg ha^{-1} DAP	63.3	82.1^{a}	8.7 ^{a}	4.7 ^{a}	246.5	93^{a}	4.8^{a}	3.8^{a}	1.7^{a}
Mean	*62.0*	*68.6*	*7.3*	*3.9*	238.9	59.2	2.9	3.1	1.4
CV	9.3	36.7	23.8	17.2	8.1	26. 9	39.2	11.0	17.8
LSD	NS	20.5	1.4	0.5	NS	18.7	1.3	0.4	0.3

Where: Plant Height (PH), Number of Seed per Plant(NSPP) , Biomass Yield (BY), Grain yield (GY),Thousand kernel weight (TWK), Number of nodule per plant (NNPP), Nodule volume (NV), Nodule fresh weight (NFW), Nodule dray weight (NDW), gram(g), milliliter(ml), Non significant difference(NS), Coefficient of variation (CV), Least Significant Difference(LSD).

phosphorus fertilizer using efficient rhizobial inoculants under rain fed conditions improve yield and yield component in the study area. This result agree with (Birhanu and Pant , 2012), who reported that chickpea inoculation gave higher nodule number, nodule dry weight, and biological yield compared to uninoculated plants in Shoa-Robit, Ethiopia. Similar study (Kyei-Boahen *et al.,* 2005), who reported that soil generally increased seed yield over the uninoculated control but the magnitude varied over different seasons depending on the prevailing climatic condition. Increase in grain yield of chickpea with effective *Rhizobium* inoculation has also been reported (Romdhane *et al*., 2007).

Seed inoculation of chickpea with *Rhizobium* strains EAL29 together with 50 kg ha^{-1} DAP was found more significant nodulation, biomass production and grain yield over uninoculated control. This study strongly agree with (Rudresh *et al*., 2005) who has been reported that inoculation of seed with *Rhizobium* has been enhanced nodulation, growth and yields response of legumes. The result indicated that nodule number and nodule dry weight were responsible for significant increase in yield of grain due to high rate of atmospheric nitrogen fixation and its effects on yield related parameter. This results agreed with the finding of (Kyei-Boahen *et al*., 2002) who found that the positive correlation between number of nodule per plant and weight of effective nodules and yield of chickpea. The different study in Ethiopia indicate inoculation plus Phosphorus fertilizer application increased the nodulation and N2 fixation of faba bean (Habtegebrial and Singh, 2006; Habtegebrial *et al*., 2007). Starter N application also increased the yield and nodulation of common bean in eastern part of Ethiopia (Anteneh and Daniel, 2016; Daba and Haile, 2000).

Number of nodules per plant were significantly diffidence (P<0.05) in which the highest value were counted under EAL29 + 50 kg ha^{-1} DAP (93), while the lowest values were found for the control (35.2). Fresh weight of nodules indicated that the highest value the highest value were recorded under EAL29 + 50 kg ha^{-1} DAP (3.8g), while the lowest values were found for the control (2.1g). The result (Table 2) indicated that Dry weight of nodules was significantly affected. Similarly, different authors reported that the dry weight of nodules increased with Rhizobium inoculation (Kantar et al., 2003; Rokhzadi and Toashih, 2011).

Nodule volume is one of the parameters supports the performance of nodules in accordance with their ability to fix atmospheric nitrogen. The highest mean values of nodule volume were recorded under EAL29 + 50 kg ha^{-1}(4.8 ml) while the lost value was recored under the control. This study agree with (Alemu, 2009; Varin *et al*., 2010 and Workneh *et al*., 2012) who found that Rhizobium inoculation with S and Zn application increase nodule volume of legume crops. The symbiotic process between legume roots and bacteria, phosphate has received considerable attention due to the dramatic effects observed in low-phosphate soils when P fertilizer is applied to nodulated legumes, including *Phaseolus vulgaris* L. (Zaman Allah et al. 2006; Abdi et al. 2014). The contribution of phosphorus in plants inoculated has a significant effect on the nitrogen content and the increase was more than 48% compared to control plants (Hmissi et al. 2015). Rhizobium plus phosphate-solubilizing bacteria significantly improved crop yield components (Meena, et al. 2001). This studies showed that Rhizobium inoculation and supplementation of phosphorus had positive significant effects on all parameters measured except plant height and thousand kernel weight which were statistical no significant difference. Therefore, limited N and P problem through Rhizobium inoculants and phosphorus supplementation is the best option in promoting legume yield in highly depleted soils.

SUMMARY AND CONCLUSION

Rhizobium CP EAL29 + 50kg ha^{-1} DAP Cp41, chickpea indigenous rhizobial strain, proved superior in almost all parameters (grain yield, yield component, nodulation,) as compared to the other inoculants and the control(uninoculanted), thus indicating inoculation of chickpea with this particular strain is advantageous in the study area. This study shows that in locations where rhizobial strains effectiveness to the target crop are not available, inoculation with appropriate rhizobial inoculants enhance grain yield of the crop. The percent increase in grain yield of chickpea over the control was observed to be significantly high in plant inoculated with rhizobia strain and phosphate fertilizer which is 51.61% over the control. Seed inoculation by Rhizobium CP EAL29 + 50kg ha^{-1} DAP produced the highest biomass yield (4.7 t ha-1). It can be concluded that from the study, chickpea crop yield can be improved through proper Rhizobium inoculation and phosphorus fertilizer application together with the starter nitrogen in the limited environmental conditions of Ginnir district of Bale lowland. Therefore, more extension efforts need to be done to popularize this cheap and eco friendly technology among resource poor farming community of the area. From this result, Seed inoculation (CP EAL29 + 50kg ha^{-1} DAP) recommended to improving productivity of chickpea.

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