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Evaluation of Bradyrhizobium Strain Rates for Growth, Nodulation and Yield of Soybean (*Glycine Max* L.) at Seka District, Jimma Zone, Southwestern Ethiopia

Habetamu Getinet[1*] and Obsa Atinafu[2]
[1]Ethiopian Institute of Agricultural Research, Debre Markos Agricultural Research Center, P.O. Box, 357, Debre Markos, Ethiopia.
[2]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, P.O. Box, 92, Jimma, Ethiopia.
[*]Corresponding author email id: habtamugetinet12@gmail.com
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Abstract **– Rhizobial strains have contributed to enhance nitrogen fixation thereby soil fertility and yield of legumes crops when they are inoculated to seeds with sufficient quantity. Therefore a field experiment was conducted at Jimma Agricultural Research Center (JARC) to determine appropriate rate of Bradyrhizobium japonicum strains on nodulation and seed yield of soybean in south western Ethiopia during 2017/18 main cropping season. Six rates of strains (125, 250, 500, 625, 750 and 900 gm ha^{-1}), one control (un-inoculated) and one 18 kgha^{-1} N were used. The experiment was designed in a randomized complete block design (RCBD) replicated three times giving a total of 24 plots. Nodulation parameters (nodule number and dry nodule weight,) was highly ($P<0.01$) affected due to inoculation and plant height, seed number plant^{-1}, seed yield and above ground biomass was significantly ($P < 0.05$) affected while pod height, pod number and hundred seed weight showed non- significant difference ($P>0.05$) due to bio inoculation of varying Bradyrhizobium strains. Accordingly, the highest seed yield (2027.78 kg ha^{-1}) was obtained from control (uninoculated) plots corresponding 7.40% yield advantages compared with the minimum strain rate which was 1877.78 kg ha^{-1}. The results clearly suggested that proper application of effective strains along with optimum rate affect nodulation and yield attributes of soybean.**

Keywords **– Nodulation, Seed Yield, Strain.**

I. Introduction

Rhizobia are soil inhabiting bacteria that establish specific symbiotic relationships with leguminous plants resulting in the formation of nitrogen-fixing nodules in plant roots (Peix *et al.*, 2015). Rhizobium inoculation is a significant technology for the manipulation of rhizobia for improving crop productivity and soil fertility. Soybean rhizobium inoculation is the process of applying rhizobium inoculants to the soybean seed before planting in order to increase the nitrogen fixation and nodulation of the soybean roots. Inoculating soybean provides adequate number of bacteria in the soybean root zone, so that effective nodulation will take place. This biological nitrogen fixation offers an economically attractive and ecologically sound means of improving soybean yield, reducing external N inputs and enhancing the quality of soil, which consequently reduce the dependence on mineral fertilizers that could be costly and unavailable to smallholder farmers. This is achieved when effective and compatible strain is inoculated to the soil/or seed corresponding sufficient quantity of strain.

Nitrogen is the most abundant element on the atmosphere however; still it is the most limiting element for plant growth and yield in most ecosystems. Despite its abundance in the atmosphere as a gas, it is not found in available form and cannot be utilized directly by plants. In such situation buildup of bacterial population is an alternative means because these bacteria can fix nitrogen gas (N_2) from the atmosphere into ammonia, which is assimilated by the plant and supports growth particularly where nitrogen availability in soils is limiting.

Legumes are the second most important plant family, only after graminiae, in agronomical importance and offer the advantage of requiring low or null supply of nitrogen fertilizers when nodulated by either natural or inoculated population of effective rhizobia (Rodriguez-Navarro *et al.,* 2011). In fact, the advantages of inoculating legume crops with high quality rhizobial inoculants over the use of chemical N-fertilizers are numerous and include economical as well as environmental benefits (Curatti and Rubio, 2014). However, rhizobial inoculants often lack efficacy because of the competition problem, a phenomenon in which inoculated rhizobia fail to nodulate legumes because indigenous rhizobia populations are better adapted for root infection and, consequently, for nodule occupancy (Friesen, 2012). Practically, the degree of settlement and persistence of an inoculant strain generally decreases when increasing densities of native rhizobial populations. For this reason, in order to be used as inoculants for legumes, rhizobial strains should be not only highly efficient in nitrogen fixation but also highly competitive for nodulation (Rodriguez-Navarro *et al.,* 2011).

Currently chemical fertilizers which are required to raise crop production levels are too expensive and most smallholder farmers cannot afford them, even not accessible at need time. Nitrogen has been considered one of the best crop-input investments that a farmer can make in terms of return on dollars spent (Pikul *et al.,* 2005); however, N is the most expensive nutrient for growing grain crops (Stanger and Lauer, 2008). The application of mineral fertilizer as sole soil fertility management method under intensive continuous cropping is also no longer feasible due to scarcity, high cost (Akinrinde and Okeleye, 2005) where available and the numerous side effects on the soil (Anetor and Akinrinde, 2006). Hoekenge *et al.* (2003) reported that continuous use of ammonia fertilizers under intensive agriculture is capable of further acidifying the soil. Adesemoye *et al*. (2010) suggested that for agricultural production to keep pace with the growing global population the use of chemical fertilizers will continue and proper management techniques must be designed and implemented against the pollution potential of fertilizers to achieve sustainability. Increasing cost of production, storage and transportation of nitrogen fertilizers have stimulated biological nitrogen fixing systems (Cheema and Ahmad, 2000). One possible solution for sustainable crop production that use legume break supplemented with chemical fertilizers because proper interventions in soil fertility management must generate cropping systems that are productive, sustainable and economically attractive for small holder subsistence farmers.

Production of soybean crop is increasingly practiced by smallholder farmers in western part of Ethiopia but it productivity remains low due to soil nutrient depletion and insufficiency of beneficial microorganisms in the soil system. Both productivity and N_2-fixing abilities of legume crops can be enhanced when the Rhizobium population in the soil is at optimum level. Therefor major drawbacks associated with microbial bio fertilizers that need immediate attention through further research as well as proper planning include their plant specificity and continuous refining of the existing rates of strains. Thus the experiment was implemented to determine and to refine existing rates of Bradyrhizobium strains for nodulation and yield of soybean.

II. MATERIALS AND METHODS

2.1. *Description of the Study Area*

The experiment was conducted at Jimma Agricultural Research Center Southwestern Ethiopia under rain fed conditions during main cropping season to evaluate the effect of bio fertilizer rate on nodulation and seed yield of soybean. The experimental site is geographically located at 07° 40′.157″ N latitude and 036°46′.999″ E longitude and an altitude of 1771m above sea level. The average annual maximum and minimum air temperature ranges from 11.8°C to 27.2°C and, the area receives adequate amount of rainfall, 1198 mm per annum. The major reference soil type in the area dominated by and is sandy clay loam and clay loam in texture, which is poor to medium in organic matter content and deficient in nutrient contents because of the long cropping history without replenishment of nutrients.

2.2. *Experimental Procedure*

Clarck 63K soybean variety was used as a test crop in the experiment which consists four levels of Bradyrhizobium strains (125, 250, 500, 625,750 and 900 gm ha^{-1}), one negative control and one positive control (18 kgha^{-1} starter N) were used. It was laid out in a completely randomized block design (RCBD) with three replications, having a total of 24 treatments accommodating seven rows and net plot area was 3m *4m (12m^2). Planting was done early according to farmer's local planting calendar where two seeds per hill were drilled in a row and the seeds were covered with thin soil on ridges made. The experimental plots were kept with 0.05m and 0.60m spacing between plants and each row respectively. To ensure that all the applied inoculum stick to the seed, the required quantity of strain was suspended in 1:1 ratio in a 10% sugar solution. The thick slurry of the inoculant was mixed gently with dry seed so that all the seeds received a thin coating of the inoculant. All inoculations were done just before planting under shade to maintain the viability of bacterial cells. The inoculated and un-inoculated seeds were then planted separately at a spacing of 0.05m between plants and 0.60m between rows making seven rows per plot. Seeds were covered immediately with soil after sowing to avoid the death of bacterial cells due to direct sun light radiation. Harvesting was done when the crop attains physiological maturity by leaving the outer most rows on both sides of each plot to avoid border effects.

2.3. *Soil Fertilization and Sowing*

Soybean seed was selected based on size and healthiness by physical observation. Each strain was applied based on the treatment set up using the slurry method as described by (Somasegaran and Hoben, 1994). In order to ensure that all the applied inoculum stick to the seed, the required quantity of strain was suspended in 1:1 10% sugar solution. The sugar slurry was gently mixed with dry seed and then with carrierbased inoculant so that all the seeds received a thin coating of the inoculant. Then the strain was mixed thoroughly with seeds. For each inoculation, separate plastic bag was used and care was taken to avoid contact among each strain during sowing. The seed was allowed to shaded air dry for a few minutes and then sown at 60 kg ha^{-1} seed rate.

2.4. *Soil Sampling and Analysis*

A representative composite soil sample 0 to 20cm depth was taken using auger before treatment application. The working soil sample was air-dried, ground using a pestle and a mortar and allowed to pass through a 2 mm sieve and analyzed for soil particle size, soil pH, organic carbon (OC), total nitrogen (TN), available phosphorus (Av.P) and cation exchange capacity (CEC) using standard laboratory procedures at Jimma Agricultural Research Center (JARC) soil laboratory. The determination of particle size distribution was carried out by the Bouyoucos hydrometer method as described by (Bouyoucos, 1962). Total N was determined by Kjeldahl digestion, distillation and titration method (Bremner, 1982). Soil pH was determined (1:2.5 soil: water ratio) according to (Okalebo *et al.,* 2002). Available P was determined using Bray II extraction method as described by Bray and Kurtz (1945). Soil OC was determined by reducing potassium dichromate by OC compound and determined by reduction of potassium dichromate by oxidation-reduction titration with ferrous ammonium sulfate and OC was determined by the method of Nelsen and Sommers (1982). Cation Exchange Capacity (CEC) was determined by leaching the soil with neutral 1 N ammonium acetate (at pH = 7) (Van Reeuwijk, 2002).

2.5. *Data Collection*

Representative plants was randomly selected to determine nodulation parameters at 50% flowering stage and yield attributes of soybean at harvesting stage. Five plants from the harvestable rows of each plot were randomly selected and uprooted with ball of surrounding soils using a spade so as to obtain intact roots and nodules. Shoots were then cut from the roots at the collar, and partitioned into root and shoot. The adhering soil on the roots was carefully removed, and the roots with intact nodules were washed gently using clean water. The nodules on the roots were picked and counted. Total sum of the nodules from five sampled plants plot^{-1} was averaged to obtain number of nodules plant^{-1}. All nodules from the five plant plot^{-1} were pooled and oven dried at 70 °C for 48 hr. and the average nodule weight plant^{-1} was estimated.

At physiological maturity the number of pods per plant was counted from five randomly selected plants of the middle rows at the time of harvesting from each plot and their averages were recorded. To estimate the number of seeds per pod the total number of seeds per pod was counted in each plot during harvesting from five randomly selected plants. Seed yield of soybean (kg ha^{-1}) was measured from each plot and converted into hectare base. Similar to seed yield, biological yield from the net plot area was harvested at physiological maturity, after gained constant weight the dried above ground biomass was weighed and converted into hectare base. Hundred seed weight (gm) was estimated by counting representative seeds using seed counting machine and its weight was measured using sensitive balance and adjusted to 10% standard moisture content of legumes. Finally, the average value was taken as hundred seed weight.

2.6. *Data Analysis*

The collected data were subjected to Analysis of Variance (ANOVA) using 9.0 computer software versions (SAS, 2010). Mean separations and comparisons were done using LSD procedure at 5% level of significance.

III. Results and Discussion

Table 1. Soil Physico-chemical properties of experimental site before planting.

Soil Properties	Value	Rating	References
pH (1:2.5 H_2O)	5.60	Moderately acidic	Tekalign Tadesse (1991)
Soil BD (g cm^{-3})	1.25	Optimum	Hunt and Gilkes (1992)
CEC (cmol (+) Kg^{-1})	16.06	Medium	Hazelton and Murphy (2007)
Total N (%)	0.19	High	Tekalign Tadesse (1991)
Avail. P (mg kg^{-1})	8.18	Low	Landon (1991)
OC (%)	1.84	Medium	Charman and Roper (2007)

Soil Properties	Value	Rating	References
OM (%)	3.17	Medium	Nelson and Sommers (1982)
Soil Textural Class	Clay loam	Ideal	Onwueme and Sinha (1991)

Nodule Number:

All rates of the strain evaluated in the experiment produced nodules, however not in equal amount. Thus, the ANOVA table showed that there was highly significant difference (P<0.01) observed among treatments on number of effective nodules per plant due to various rates of biological strain compared in each strains but less in number compared with positive control (application of 18 kgha^{-1} starter N). Accordingly the highest numbers of nodules per plant (74.67) were obtained from application nitrogen at rate of 18 kg ha^{-1} while the lowest nodule number (53.5) was recorded from inoculation of seeds with lowest strain rate (125g ha^{-1}) (Figure 1). The plot which is treated with 18 kg ha^{-1} starter nitrogen also produced the highest nodule dry weight plant^{-1} (6.78 g) followed by inoculation of seeds at rate of 500g ha^{-1} strain which is (6.35 g) nodule dry weight plant^{-1}, while the lowest number of nodules (51.0) and nodule dry weight (3.9g) plant^{-1} was recorded from inoculation of the highest rate of strain. Application of N at a rate of 18 kg ha^{-1} increased nodule number by 31.70% and 42.48 % nodule dry weight by 26.10 compared with the maximum strain rate used.

The highest nodule number produced from chemical fertilization (uninoculated) plot compared with the added inoculant might be due to the presence of sufficient rhizobial population in the soil of the experimental site which is more effective and competent than exotic rhizobia strain. In addition, it might be due to planting of legume crops two or three years before where there is buildup of microbial population in the experimental site even though we haven't assed before inoculated. Microbial population of soils usually lacks Bradyrhizobium japonicum strains unless soybean or other specified host is grown on them for at least five or more years. It is therefore important to inoculate seeds with relevant strains of bacteria before sowing especially if the crop is to be grown for the first time on the land. The result is in agreement with the finding of (Kutcher *et al.*, 2002) who reported that inoculation responses are associated primarily with the first planting of a legume in soil having no prior history of the crop. In any case, to get the maximum benefit out of inoculation there is a need to follow correct and careful inoculation procedures, cropping history and microbial population of any experimental sites.

Mugnai and Karubiu (2012) stated that presence of high numbers of indigenous rhizobia may have limited nodule formation by introduced strains. Moreover, the presence of availability soil nitrogen also reduced the extent of nodulation. The process of nodulation may be promoted by relatively low levels of available nitrate or ammonia, but higher concentrations of N almost always depress nodulation. Similarly, Zahran (1999) also reported that high level of soil nitrogen, applied or residual is a consequence to reduce nodulation and N-fixation. High soil N can result in a reduction in the number of nodules that form (Diouf *et al.*, 2008). This indicates the presence of indigenous rhizobia strains in the field due to a long history of planting of soybean in those fields. The larger populations without inoculation indicated that the indigenous rhizobia strains were more competitive than the inoculants ones.

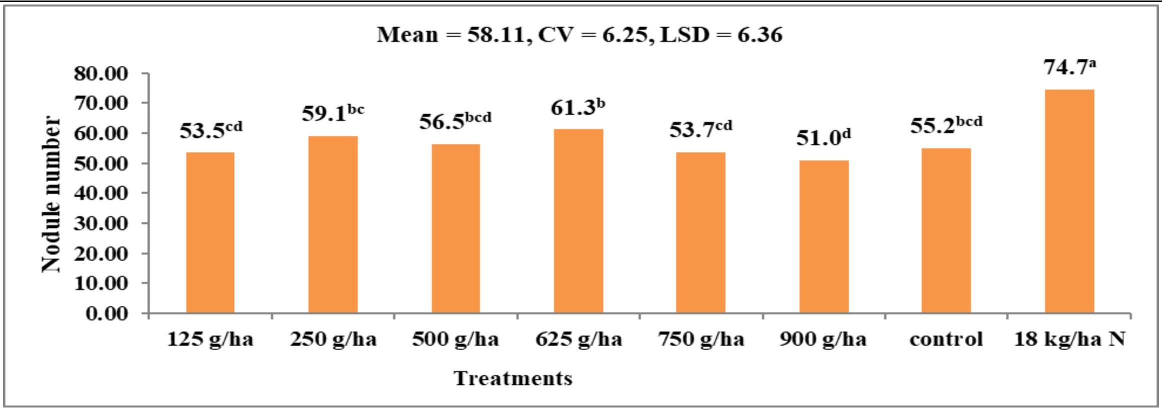

Fig. 1. Mean value of nodule number as influenced by bradyrhizobium strains.

Nodule Dry Weight:

Similar to nodule number, the highest nodule dry weight (6.80g) per plant was produced from plots treated with nitrogen at a rate of 18 kg ha^{-1}. Optimum growth of leguminous plants including soybean is usually dependent on symbiotic relationships with N_2-fixing bacteria. Therefore, inoculation soybean seed with rhizobia where farm history showed no similar crops before was very crucial for economically feasible and sustainable production of soybean. The higher figures recorded in the non-inoculated treatment comparatively to the inoculated plots could probably be due to the existence of active indigenous nodulating bacteria in the experimental soil as reported by Bekere and Hailemariam (2012). However, in this study, we did not assess the initial rhizobia populations in the soil.

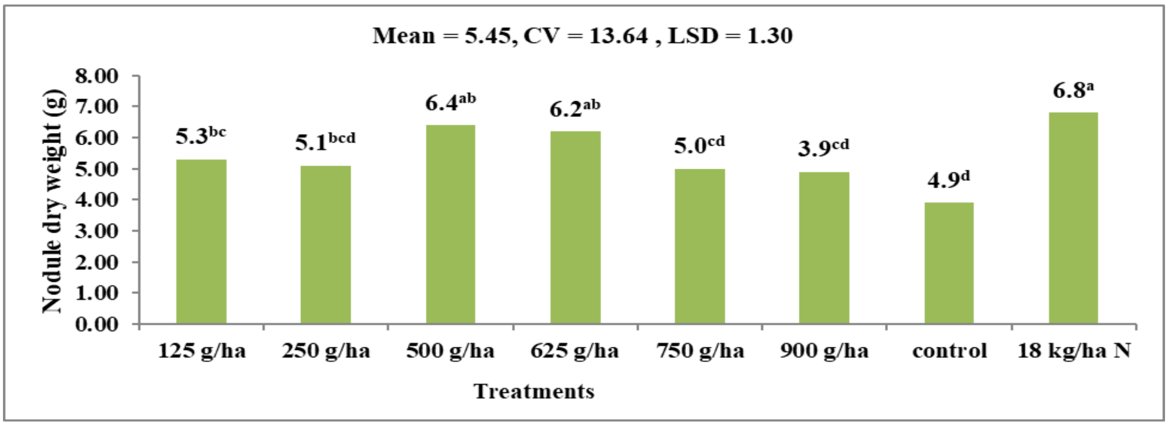

Fig. 2. Mean value of nodule dry weight as influenced by bradyrhizobium strains.

Effects of Inoculation on Plant Height:

Mean plant height doesn't showed significant variation among treatments due to inoculation of seeds with various rates of bradyrhizobium strains. The non-significant effect of inoculation could probably be due to the competitiveness of the indigenous rhizobia in the soils at the experimental sites which outcompeted the inoculated rhizobium isolate, even though the native rhizobia population in the experimental fields was not assessed. The nature of soil rhizobial populations may affect the N_2 fixation potential of legume crops when the number of available invasive rhizobia may be insufficient to nodulate the host adequately and when the average effectiveness of the population may be inadequate to support the host's fixed-N_2 requirements. When both the above conditions are present, we might reasonably expect that successful inoculation with an effective

Rhizobium isolate would enhance unless no fixation carried out. The current finding agreed with (Rudresh *et al.*, 2005) who suggest that native rhizobia capable of nodulating the host were present in soil used for the experiment and they competed with inoculated isolates in the inoculated treatments but contradicted the findings of Amani (2007) and Caliskan *et al.* (2007) who reported that plant height increases with application of inoculants in combination with mineral nitrogen fertilizer.

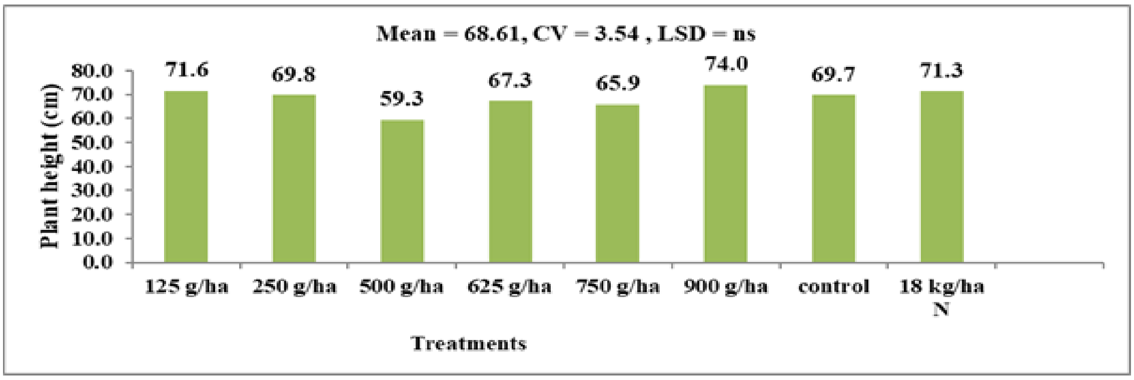

Fig. 3. Mean value of plant height as influenced by bradyrhizobium strains.

Number of Pods Plant^{-1}:

The ANOVA table showed that number of pods plant^{-1} was highly significantly affected ($P < 0.01$) due to inoculation of strains in different rates. Inoculation of bio fertilizer at a rate of 900 g ha^{-1} followed by 750 g ha^{-1} produced the highest number of pods per plant (34.7 and 33.9) which produced 16.43% and 14.45% more number of pods per plant respectively as compared to uninoculated seeds. Application of sufficient strains plays an important role in physiological and developmental processes in plant life and the favorable effect of these strains might accelerate the growth processes, which ultimately resulted in increased yield and quality. In general, when the number of viable rhizobia inoculated per seed increases, the number of pods per plant formed is influenced by the treatment given by Bradyrhizobium strain thereby nodulation and yield are improved. This proves that bacteria that work with root nodules affect plants in forming pods. The current result is supported by Abdel-Fattah *et al.*, 2011 and Bhuiyan *et al.*, 2008) who researched that inoculating mung bean and soybean with Bradyrhizobium significantly increased pod number and pod weight.

Number of Seeds Pod^{-1}:

Analysis of variance showed that inoculation of seeds with strain significantly affects ($p<0.05$) seed number per pod. The maximum number of seeds pod^{-1} (2.70) was obtained from inoculation of seeds with 500 gha^{-1} strains which produced 11.11 % and 14.81% seed advantages over uninoculated seeds and use of the lowest rate of strain respectively. Increasing levels of strain application from 125 to 500g ha^{-1} showed an increased seed number of soybean. In situations where inoculation is required, it clearly showed that when the number of rhizobia applied per seed increased, nodulation and yield increased. Inoculation of efficient nitrogen-fixing symbioses most directly related to effective nodule formation for enhanced plant growth. However, the population density, effectiveness in forming nodules, competitive ability and concentration of available soil N are the major factors that determine the degree of inoculation response as reported by (Singleton and Tavares 1986). Thus, advantages of inoculation of legume crops can be an important practice if the farmer’s field is low in N availability or if the soybean cultivar has large N demand (Kubota *et al.*, 2008).

Seed Yield:

Analysis of variance showed that there was significant effect observed on seed yield of soybean due to inoculation of seeds in different rates. The maximum seed yield (2027.78 $kgha^{-1}$) was obtained from the control (uninoculated) plots, which produced 7.40% yield advantages compared with the lowest bradyrhizobium strain rate used. In comparing only bradyrhizobium strain rates, as increasing the inoculation rates from 125 to 900 g ha^{-1} there was an increased in seed yield of soybean where the maximum seed yield (1877.80 kg ha^{-1}) was recorded from the maximum rate of bio inoculant while the lowest seed yield (1877.78 $kgha^{-1}$) was obtained from plots inoculated with 125 gha^{-1} strain. The maximum yield produced from uninoculated plots showed that the soil of the experimental site contains effective and competent indigenous microbial population and sufficient quantity of nitrogen because nitrogen fixation is sound when nutrient availability in the soil is low. One pathway to improvement is to identify native rhizobia with superior symbiotic and competitive abilities and to use them in large doses within inoculants, building upon the biodiversity of indigenous rhizobial populations. The result is in agreement with the finding of (Panchali, 2011) who reported that forming effective N_2 fixing symbioses between legumes and their N_2 fixing bacteria is developed whenever there is low nutrient availability (most probably nitrogen) which can affect the development of the plant. Unless this condition is avail, the most effective rhizobium strains cannot form effective association in nodulation and N fixation with the host plant.

In areas where soils lack appropriate rhizobia, the response of soybean to inoculation is strong when the rhizobia are introduced into a new environment (Van Kessel and Hartley, 2000). Soils with insufficient provision of inorganic N presumptively have a yield advantage to crops which have been inoculated in such soils. Most researchers find out the effect of fertilizer nitrogen on soybean growth and N_2 fixation concluded that fertilization reduces N fixation through a reduction in the number, weight and activity of nodules. According to Thies *et al.* (1991) the response of legume to inoculation is hugely based upon the number of indigenous rhizobia which is existed in the soil before, the management practice and the availability of soil nitrogen.

Biomass Yield:

The ANOVA table showed that above ground biomass of soybean was a significantly affected ($p <0.05$) by increasing rate of microbial strains from 125 to 900 gha^{-1}. The highest value of biomass yield (5922.20 kg ha^{-1}) was recorded from inoculation of strains at rate of 900gha^{-1}, while the lowest biomass yield was obtained from inoculation of seeds with minimum rates which is (125gha^{-1}). Several researches have shown significant increase of rhizobia inoculation on plant biomass, nodulation and grain yield. According to Dorivar *et al*, (2009) the use of rhizobia inoculants positively influenced soybean grain yield by an average of 130 kg ha1 and that nitrogen accumulation, plant biomass and grain N were also increased in soybean with the use of inoculants on soybean seed.

Table 2. Effects of Bradyrhizobium strain on soybean pod number, seed number, seed yield and biomass.

Treatments (Bio Inoculant Rates (gha^{-1}))	PN	SN	SY ($kgha^{-1}$)	BY ($Kgha^{-1}$)
T_1 = 125	33.10^{ab}	2.40^{bc}	1877.80^{b}	4577.80^{d}
T_2 = 250	32.80^{ab}	2.40^{bc}	1905.60^{b}	4855.20^{bcd}

T_3 = 500	30.70[ab]	2.70[a]	1905.60[b]	4750.00[cd]
T_4 = 625	33.10[ab]	2.40[bc]	1911.10[b]	5072.20[bcd]
T_5 = 750	33.90[a]	2.40[bc]	1938.90[ab]	5377.80[abc]
T_6 = 900	34.70[a]	2.30[c]	1977.80[ab]	5922.20[a]
T_7 = -ve control	29.00[b]	2.40[bc]	2027.80[a]	5416.70[ab]
T_8 = 18 kg ha^{-1} N	30.70[ab]	2.50[b]	1911.10[b]	5183.30[bcd]
LSD (0.05)	4.73	0.16	115.04	663.97
CV (%)	8.38	3.78	3.40	7.37

Where, PN = Pod number, SN = Seed number, SY = Seed yield and BY = Biomass yield.

IV. Conclusion

Based on the results obtained we can conclude that in modern-day agricultural practices to ensure sustainability of agricultural production, incorporating bio fertilizers in cropping system is an important component in terms of a viable alternative of chemical fertilizers that are associated with various environmental hazards. Bio fertilizers can fix and make available atmospheric nitrogen in soil and root nodules, solubilize phosphate (from insoluble forms like tri-calcium, iron, and aluminum phosphates) into available forms, sift phosphates from soil layers, produce hormones and antimetabolites to uphold root growth, and decompose organic matter for soil mineralization. This causes increased harvest yields, enhanced soil structure (by influencing the aggregation of the soil particles for better water relation) and increasing root development).

However, an increased demand and awareness among farmers and planters about the use of bio fertilizers can pave the way for new entrepreneurs to get into bio fertilizer manufacturing, which also requires encouragement as well as support from the governments. Bio fertilizer technology, which is an inalterable part of sustainable agriculture, has to be appropriate for the social and infrastructural situations of the users, economically feasible and viable, renewable, applicable by all farmers equally, stable in long-term perspective, acceptable by different societal segments, adaptable to existing local conditions and various cultural patterns of society, practically implementable, and productive.

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AUTHOR'S PROFILE

First Author
Habetamu Getinet, Ethiopian Institute of Agricultural Research, Debre Markos Agricultural Research Center, P.O. Box, 357, Debre Markos, Ethiopia.

Second Author
Obsa Atinafu, Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, P.O. Box, 92, Jimma, Ethiopia.