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Evaluation of Best Performing Indigenous Rhizobium Inoculant for Growth and Yield of Soybean (Glycine Max L.) at Kersa District South-Western Ethiopia

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ABSTRACT

Integrated application of starter inorganic NP fertilizer and inoculation of bio-inoculant exhibits various effects on growth parameters, yield and yield components of soybean. A field experiment was conducted on Nitisols of Kersa district Southwestern Ethiopia during 2016/17 cropping season to investigate the effect of bio-fertilizer inoculation and starter NP on growth and yield of soybean. The experiment was laid out in RCBD with seven treatments replicated three times on farmer's field. The treatments were (control, MAR-1495 strain, SB-12 strain, SB-6B1 strain, MAR-1495 strain + 50 kg ha-1 DAP, SB-12 strain + 50 kg ha-1 DAP and SB-6B1 strain + 50 kg ha-1 DAP). The collected data was subjected to ANOVA using SAS 9.3 software. LSD test was used to separate means at 5% level of significant (P < 0.05). Soybean grain yield and Biomass yield, were highly affected by integrated use of indigenous bioinoculant with starter mineral NP fertilizer. The highest pod length (22.60cm), number of pods (48.60), grain yield (2071.30 kg ha-1), biomass yield (8303.60 kg ha-1) and harvest index (27.91%) were obtained from application of 9 N + 10 P kg ha-1 combined with MAR-1495 strain, corresponding to 40.86% yield advantage over control while the lowest grain yield (1225.30 kg ha-1) and biomass yield (5900.20kg ha-1) were recorded from control. The results clearly suggested that application of starter NP along with bio-inoculant strain affect growth and yield of soybean and improve grain quality as well as benefit on soil quality.

Keywords: *Indigenous bio-inoculant, Soybean, starter mineral fertilizer*

INTRODUCTION

Soybean (*Glycine max* (L.) *Merrill)* is an economically important legume crop, which grows in the tropical, subtropical and temperate climatic condition and is well - known as an important source of protein in human diet and animal ration; containing substantial amounts of all the essential amino acids, oil, minerals and vitamins (Tefera, 2010). The balanced combination of protein, fat and carbohydrates of soybean products were serve as a valuable food, feed and bio-feed stocks of crops (Gardner and Pyne, 2003). Many other products with a soybean basis are also directly used for human consumption (soymilk, soy yogurt, snacks, soya sauce, protein extract and concentrates) (Collombet, 2013). It improves soil fertility by fixing atmospheric nitrogen (N) and its oil is increasingly being used for biodiesel (Acikgoz *et al.*, 2009). Inherently poor or nutrient depleted soils are characterized by low soil organic matter, available phosphorus and total nitrogen (FAO, 2005). Agricultural practices that augment or conserve these nutrient stocks are therefore required for sustainable soybean production.

In Ethiopia, soybean is an important food crop widely produced in high rainfall areas, in west and southwestern parts including the study area and recently integrated into the cropping systems and serves as a cash crop for smallholder farmers of the area (Nigussie et al., 2009). It has the capacity to fix atmospheric N_2 in association with rhizobia. In this symbiosis, they partly supply their own N needs and provide some nutrients left over to succeeding crops through decomposition of their nodule, roots and biomass (Chianu et al., 2011). Thus, biological N_2 fixation offers an economically attractive and ecologically sound means of improving crop yield, reducing external N inputs and enhancing the quality of soil resources, which consequently reduce the dependence on mineral fertilizers that could be costly and unavailable to smallholder farmers as soybean hold promise in this regard. Solomon et al. (2012) reported that legumes including soybean could obtain between 50 and 80% of their N requirements through N_2 fixation Sanginga (2002), however, reported that the current promiscuous soybean genotypes could not meet all their demand for growth and seed development only by N_2 fixation rather they need integrated with starter mineral fertilizer. Moreover, populations of Bradyrhizobia strains are seldom available in soils where soybean crop has not been previously grown; therefore, nodulation of soybean may require specific species of Rhizobium sources for effective N_2 fixation through optimization of management factors including application of starter mineral nutrient sources (Abaidoo et al., 2007).

Nitrogen and P are two important soil nutrients affecting the N_2-fxing process of legume crops including soybean (Wall et al., 2000). The majority of the soils in southwestern part of Ethiopian soil are deficient in both N and P, which are the major factor resulting in decreased crop plant yields and recognized as a major problem to continue cereal cropping in soils of Ethiopia (Zeleke et al., 2010). Nitrogen is considered the most limiting nutrient for plant growth in most soils of Ethiopia (Tadesse, 2001). Phosphorus is also an essential major nutrient for the development of plants as it stimulates early development promotes healthy growth of seedlings and enhances the formation of nodules.

Now a days, there is vast scope for soybean production due to high nutritional quality, more production and short duration (90-110 days), tolerate long dry spell and being leguminous crop

helps in improving the fertility and productivity of soil. However, prices of mineral fertilizers are currently increasing and farmers are facing severe problem on availability of chemical fertilizers for soybean production, therefore it is necessary to reduce the cost of fertilizers by using indigenous strains to increase yield of legume crops. However, biofertilizer cannot fully replace chemical fertilizers, but certainly are capable of reducing their input. Seed inoculation with effective *Rhizobium* strain is therefore optional to ensure adequate nodulation and N_2 fixation for maximum growth and yield of soybean. Growers generally use chemical fertilizers fully to increase soybean production. However, it gives hazardous effect as soil and water pollution. Use of compatible biofertilizer in combination with optimum chemical fertilizers is an attractive and environmental safety method of soybean production. Therefore, to enhance soybean yield should linked to integrated soil fertility management system (Vanlauwe et al., 2010). Because integrated soil fertility management has greater prospects in improving soil fertility status and achieving high crop yield because of the combination of mineral fertilizers and inoculants; which could synergistically improve the yield and quality of soil. However, no information is available on the complementary use of bioinoculant strains with starter mineral inputs particularly in the southwestern Ethiopia. Thus, the overall objective of this study was (i) to evaluate the influence of bio-fertilizer source on soybean growth and yield, (ii) to evaluate the combined effect of starter mineral fertilizer (NP) and to identify effective strain on growth and yield of soybean and (iii) to assess the effect of complementary use of mineral fertilizer and bioinoculant source on soybean growth and yield

MATERIALS AND METHODS

Description of the Study Area

The study was conducted at Kersa district Jimma Zone Southwestern Ethiopia under rain fed conditions during 2016/2017 cropping season. The site was located at about 318 km far from Addis Ababa and 28 km East from Jimma Town. Geographically it is located at 7° 40′ 0″ N latitude and 36° 50′ 0″ E longitude with an altitude of 1740 masl. The average annual maximum and minimum air temperatures were 28.8 ^{0}C and 11.8 ^{0}C, respectively and the area receives adequate amount of rainfall, ranging from 1,200 to 2,800 mm per annum. According to Van Ranst et al. (2011), the major reference soil groups in the area is dominated by Nitisols, Acrisols, Ferralsols, Vertisols and Plano sols.

Treatment Set up and Experimental Procedure

The experiment was conducted on farmers' fields during 2016/17 main rainy season. The experiment consists of seven treatments that includes (control, MAR-1495 strain, SB-12 strain, SB-6B1 strain, MAR-1495 strain + 50 kg ha^{-1} DAP, SB-12 strain + 50 kg ha^{-1} DAP and SB-6B1 strain + 50 kg ha^{-1} DAP). It was laid out in a completely randomized block design (RCBD) in three replications, with a total of 21 treatments accommodating eight rows for the experiment and net plot area was 3.6m x 3.5m = 12.6m^2. Planting was done early June, 2016 based on farmers local planting calendar where two seeds per hill was drilled on rides made with 0.05 m and 0.60m spacing between plants and each row respectively and covered with soil and thinned to one seed per hill two weeks later, which accommodates a total of plant population levels 333333 per hectare. Planting was done starting from the un-inoculated plots followed by the inoculated plots to avoid contamination. Harvesting was done at physiological maturity by leaving the outer most rows on both sides of each plot to avoid border effects.

Crop Management

The field was prepared on the farmer's field by removing all unwanted materials. Before sowing the crop and the field plowed with oxen, four times to make a fine seedbed. The source of N and P was DAP. The rate of starter dose of N fertilizer at 9 N + 10 P kg ha^{-1} was set based on recommendation given by (Solomon et al., 2012). Under research sites, 100 kg DAP and 50 kg urea per hectare was used for soybean [(*Glycine max* L.) Merrill] around Ilubabor and Jimma area (Getachew et al., 1987). Soybean seed was selected based on size and healthiness by physical observation. Then the seeds were weighed and surface sterilized by soaking them first with 70% (v/v) ethanol for 10 seconds and 4% (v/v) sodium hypochlorite (NaOCl) solution for five minutes and late washed five times with sterilized water as indicated in Somasegaran and Hoben (1994). Each strain was applied at the rate of 5 gm powder inocula per 1 kg of seed using the slurry method as described by Woomer et al. (1994). In order to ensure that all the applied inoculum stick to the seed, the required quantity of inoculants was suspended in 1:1 10% sugar solution. The sugar slurry was gently mixed with dry seed and then with carrier-based 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 contamination of the inoculated and uninoculated seeds. The seed was allowed to shaded air dry for a few minutes and were then sown at 60 kg ha^{-1} seed rate. Plots with uninoculated seeds were planted first to avoid contamination.

Soil Sampling and Analysis

A composite soil sample was collected in a diagonal pattern from 0-20cm soil depth before treatment application. Uniform slices and volumes of soils were obtained in each sample by vertical insertion using auger. The sample was air-dried, ground using a pestle and a mortar and allowed to pass through a 2 mm sieve. Working samples were prepared and analyzed for 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. Total N in the soil was determined by the Kjeldahl digestion, distillation and titration method (Bremner, 1982). Soil pH was determined (1:2.5 soil: water suspension) according to Okalebo et al., 2002). Available P was determined using Bray II extraction method as described by Bray and Kurtz (1945) and extraction with 0.5M $NaHCO_3$ (Olsen et al., 1954). Organic carbon content of the soil was determined by reduction of potassium dichromate by organic carbon compound and determined by reduction of potassium dichromate by oxidation-reduction titration with ferrous ammonium sulfate and organic carbon was determined by the method of Nelsen and Sommers (1982). Particle size distribution was determined by hydrometer method using particles; less than 2 mm diameter (FAO, 2008) and texture of the soil was determined by sedimentation method (Hesse, 1971). Phosphorus in the extracts was determined with atomic absorption spectro photometer calorimetrically according to the molybdenum blue color method described by Murphy and Riley, (1962).

Agronomic Data Collection

Growth Parameter

Plant Height

Plant height was measured using taprool in centimeter from the ground level to the top of the plant at physiological maturity from five randomly selected plants from each plot.

Yield and Yield Components

Number of Pods per Plant

It was counted from five randomly selected plants of the middle rows at the time of harvesting from each plot by visual observation and their averages were recorded.

Number of Seeds per Pod

Number of seeds per pod was determined as the total number of seeds per pod in each plot at the time of harvesting from five randomly selected plants.

Grain yield (kg ha-1): It was measured from each plot and converted into hectare bas

Biomass yield (kg ha-1): Plants from the net plot area was harvested at physiological maturity, after gained constant and the dried straw was weighed weigh.

Harvest Index: It was expressed as the ratio of economic yield per plant to the total above ground biomass calculated as follow;

$$HI(\%) = \frac{\text{GY kg / ha}}{\text{TBY kg / ha}} X100$$

Where, HI = harvest index, GY = Grain yield (at 10% moisture base), TBY =Total biomass yield

Statistical Analysis

The collected data were summarized and statistically analyzed using the analysis of variance (ANOVA) procedure for RCBD using SAS 9.3 version software (SAS, 2012). Treatment means that differed significantly was separated using LSD procedure at 5% level of significance. Correlation coefficient was determined for parameters using the same software to determine relations between yield and yield contributing characters.

Results and Discussion

Table1. *Soil Physico - Chemical properties of experimental site before planting in 2016/17*

Soil Properties	Value	Rating	References
pH (1:2.5 H_2O)	5.4	Moderate acidic	Tekalign Mamo (1991)
Soil BD (g cm^{-3})	1.25	Optimum	Hunt and Gilkes (1992)
CEC (cmol(+) Kg^{-1})	16.06	Medium	Hazelton and Murphy (2016)
Total N (%)	0.18	Low	Tekalign Mamo (1991)
Avail. P (mg kg^{-1})	8.18	Low	Landon (1991)
OC (%)	2.76	Medium	Charman and Roper (2007)
OM (%)	4.75	Medium	Nelson and Sommers (1982)
Sand (%)	60.5	Ideal	Onwueme and Sinha (1991)
Silt (%)	5.0		
Clay (%)	34.5		
Soil Textural Class	Sandy C. loam		

Plant Height

Analysis of variance showed that Rhizobium inoculation, with starter NP had a highly significant effect (P<0.01) on plant height. Accordingly, the maximum plant height (86.93cm) was recorded from plots treated with 9+10 kg ha^{-1} NP starter mineral fertilizer with MAR-1495 inoculation while the shortest plant height (73.73cm) was obtained from control plots. This result indicates that integrated use of starter mineral nutrient with bioinoculant increase plant height that might be synergetic effects among themselves which has more contribution to the increase in soybean plant height. Therefore, the variation in plant height might be because of inoculating the soil via strain and thus it has the capacity to release fixed N, and increases available nitrogen that can be available to the plant, which directly increased above ground biomass in general, and plant height in particular. Moreover, P improves the growth and development of the crop, as it increases the nutrient uptake efficiency of plants and further improves the growth of other parts. The result is in line with the finding of Shahid et al. (2009), who observed significant improvement in plant height of soybean by P-fertilization.

Pod Height

The maximum pod height (22.60cm) was recorded at combined application of 9+10kg ha^{-1} NP starter mineral fertilizer with MAR-1495 strain, while the minimum pod height (16.53cm) was recorded from the control. This is because as mineral fertilizers (NP) and bioinoculant was applied integratelly increases the pod heights of plant for their role in the development of inter node and growth facilitation. If pod height decrease, it invites loss of yields during harvesting due to nearer to the soil and dwarf of the plant. The results agree with previous finding of Ogunlela et al., (2012) who reported that supplying NP at optimum rate resulted in maximum pod height compared to the control. Pods too close to the soil surface increase harvest losses since some combine harvester heads are unable to pick up the first pods (Caliskan et al., 2007).

Number of Pods per Plant

The interaction of bio-fertilizer inoculation with starter mineral fertilizer showed highly significant effect ($P < 0.01$) on number of pods per plant (Table 2). The maximum pod number per plant (48.60) was recorded from plots treated with 9 +10 kg ha^{-1} NP and MAR-1495 inoculant while the lowest value (29.80) was recorded from control. The addition of starter inorganic N and P is vital for improving food legumes production in degraded soils and hence increases 38.68% pod number over control indicating positive interaction of inorganic NP fertilizer and biofertilizer, which directly affect in increasing number of pods per plant. It is therefore suggested that optimum soil environment is created by supplying the required nutrients at the right time with the required quantity coupled with the application of rhizobium inoculant strain, especially when the soil has low indigenous rhizobia population and fertility due to many years of soil degradation to increase soybean yield potential which is agreed with (Hansen et al., 1995).

Number of Seeds per Pod

The ANOVA result showed that the variations in number of seeds per pod due to NP starter fertilizer in combination with Rhizobium strain were non-significant. Mathematically the highest seed number per pod (3) was recorded while the lowest value was (2.28). Most probably, the number of seeds per pod may vary due to genotype differences however; seeds per pod was less affected by external factors like fertilization when a single genotype is considered.

Grain Yield

Data regarding on grain yield showed there were a highly significant effects ($P < 0.01$) due to combined application of mineral fertilizer and bio-fertilizer. The maximum grain yield (2071.30 kg ha^{-1}) was recorded from combination of 9+10 kg ha^{-1} NP with MAR-1495 bioinoculant, while the minimum grain yield (1225.30 kg ha^{-1}) was recorded from control. Integrated use of MAR-1495 strain with starter mineral fertilizer increased grain yield by about 40.86% and 13.95% compared to control and use of bio-fertilizer alone, respectively. On the other hand, inoculation of MAR-1495 strain increased grain yield by 9.12 % and 5.72% over SB-12 and SB-6B1 strain, respectively, which might be less effectiveness and competitiveness of the later indigenous rhizobia even both of them received similar NP rate.

Rhizobium produced grain yield, which was significantly different from that of the control plot that supports the fact that inoculation alone may not be enough to achieve optimum growth and yield of soybean in the study area. The fact that starter mineral fertilizer resulted in an increase in harvest index over the control and inoculation is an indication that supplementing NP is essential to effectively optimize its potential yield; hence, the plant was able to partition effectively the absorbed nutrient resulting in significant vegetative growth and grain yield. The findings of this study can be attributed to the fact that starter NP (9 +10 kg ha^{-1}) enhanced establishment of a good rooting system, before the commencement of nodule formation which was further supported by top dressing, thus emphasizing the need for N. Furthermore, inoculating with the right strain of rhizobial also improve the potential of the introduced strain to withstand competition from the indigenous strain provided a suitable environment is available for the inoculants to carry out the symbiotic processes. Optimum starter NP supply throughout the growing season is important for high yield as supported by Salvagiotti et al., (2009). Osborne and Riedell (2006) also reported that adding 16 kg ha^{-1} N as banded starter fertilizer had a 6% yield increase over the non-treated plot. This result is in agreement with Dorivar et al. (2009) who reported that a positive response of rhizobia inoculation to nodulation and grain yield of soybean.

Biomass yield

The analysis of variance showed that application of biofertilizer and starter NP integratelly has highly significant effect ($P < 0.01$) on above ground biomass. Accordingly, the highest biomass yield (8303.60 kg ha^{-1}) was obtained from application of 9+10 kg ha^{-1} NP combined with MAR-1495 strain. The lowest biomass yield (5900.20 kg ha^{-1}) was obtained from control plots. From this result, it was observed that above ground biomass yield has a direct positive relationship with the total grain yield of the crop. Because combination of mineral fertilizer with bio-inoculant gave the highest biomass yield, as well as grain yield ha^{-1} and the lowest biomass yield was associated with the lowest grain yield. This result probably due to addition of N from atmosphere through nitrogen fixation, that became available to plants and thus contributes to above ground biomass yield.

Inoculation of soybean seeds with compatible rhizobial had discernible effect on total pods weight, grain yield thereby increases total biomass whenever it is supplemented with optimum starter NP fertilizer but when nitrate was applied above starter amount, the above parameters reduced besides high N applied did not improve the productivity of food legume crops including soybean as reported by Namav et al. (2013).

Harvest index

The maximum harvest index (27.91 and 27.48 %) was recorded from application 9+10 kg ha^{-1} NP combined with MAR- 1495 rhizobial followed by 9+10 kg ha^{-1} NP with SB-6B1 strain while the minimum harvest index (17.82%) was recorded from the control. This is because harvest index was increased by integrated application of mineral and bio-fertilizers, which might be due to the presence of synergistic effect. These results agree with Malik et al. (2006) who reported that nitrogen application has a positive effect on harvest index of soybean.

Table2. *Effects of bio-inoculant on soybean growth and yield contributing parameters at Kersa in 2016/17*

Treatments	PH (cm)	PL (cm)	NPP	NSP	GY (kg ha^{-1})	BY (kg ha^{-1})	HI (%)
Control	73.73c	16.53c	29.80d	2.87	1225.30c	5900.20e	17.82c
MAR-1495 strain	78.93b	19.93abc	39.07bc	2.87	1782.80ab	7104.70cd	25.60ab
SB-12 strain	79.20b	20.40ab	41.27b	3.00	1649.30b	7057.50cd	23.63b
SB-6B1 strain	78.27b	21.60ab	35.07c	3.00	1730.70b	6271.40de	25.02ab
MAR 1495strain+50kgha^{-1} DAP	80.53b	22.60a	48.60a	2.93	2071.30a	8303.60a	27.91a
SB-12 strain + 50 kg ha^{-1} DAP	86.93a	21.88ab	41.13b	3.00	1882.40ab	8008.80ab	23.61b
SB-6B1 strain + 50 kg ha^{-1} DAP	80.40b	18.60bc	41.00b	2.93	1952.80ab	7348.40bc	27.48a
LSD (0.05)	3.57	3.58	4.03	NS	338.49	875.42	2.92
CV (%)	2.52	9.95	5.75	3.68	10.83	6.89	6.73

Where, PH = Plant height, Pl = Pod length, NPP = Number of pods per plant, NSP = Number of seeds per pod, GY = Grain yield, BY = Biomass yield, HI = Harvest index. Different small letters denote significant difference between treatments.

Correlation Analysis

Correlation analysis between growth and yield related parameters is presented in (Table 3). Crop yield is a cumulative interaction effect of all the dependent and independent characters in an experiment. This is because it has a positive relationship with those parameters and contributes a great growth performances and yield parameter for the plants. Plant height was significantly ($P < 0.05$) and positively correlated with number of pods ($r = 0.45$), number of seeds ($r = 0.52$), grain yield ($r = 0.59$) and highly significantly with biomass yield ($r = 0.61$) indicating they have direct relationship on the chlorophyll and photoassmillate for plant growth performance and yield. Number of pods was highly significantly ($P < 0.01$) and positively correlated with grain yield ($r = 0.76$) and biomass yield ($r = 0.72$). Grain yield was highly significant ($P < 0.01$) and positively correlated with biomass yield ($r = 0.64$) and harvest index ($r = 0.57$). Biomass yield was highly significant ($P < 0.01$) and positively correlated with grain yield ($r = 0.64$)

Table3. *Pearson Correlation Coefficients between growth and yield component of soybean*

Variables	PH	PL	NPP	NSP	GY	BY	HI
Ph	1.00						
PL	0.32^{ns}	1.00					
NP	0.45*	0.20^{ns}	1.00				
NS	0.52*	0.18^{ns}	0.15^{ns}	1.00			
GY	0.59*	0.14^{ns}	0.76**	0.27^{ns}	1.00		
BY	0.61**	0.04^{ns}	0.72**	0.24^{ns}	0.64**	1.00	
HI	0.18^{ns}	0.45^{ns}	0.32^{ns}	0.09^{ns}	0.57**	0.06^{ns}	1.00

Where, PH = Plant height, Pl = Pod length, NPP = Number pods per plant, NSP = Number of seeds per pod, GY = Grain yield, BY = Biomass yield, HI = Harvest index, ** = *significant at P < 0.01, * = significant at P < 0.05, ns = non-significant*

CONCLUSION

Now a day, enhancing soybean crop yield is a mandatory practice through integrated soil fertility management because it has greater prospects in improving soil fertility status and achieving high crop yield via combination of mineral fertilizers and inoculants; which could synergistically improves the overall properties of the soil. Based on the result it is possible to conclude that application of starter NP fertilizer in combination with indigenous inoculant is an imperative to enhance soybean production in the study area. Using effective and compatible rhizobial strain is an imperative to boost soybean yield thereby soil quality. Nitrogen and Phosphorus application at the rate of 9 and 10 kg ha^{-1} in combination with rhizobial strain increased the availability and uptake of nutrients thereby growth and yield of soybean. The most beneficial effect of NP with inoculation on N_2 fixation, plant growth and yield can understood given the fact that the experimental soil was severely deficient in N, P as well as rhizobium strain, which can be considered a major limiting factors for quality soybean production at Jimma area. Hence, the integrated application of *Rhizobium* strain with NP could be a viable strategy to improve the yield and quality of soybean, in soils containing suboptimal P and N nutrients. Starter NP (9+10 $kgha^{-1}$) is essential for soybean establishment before full nodule commencement. Therefore, proper fertilization programs including starter NP integrated with inoculation of rhizobium strain should implemented to improve the productivity of food legumes and thereby increase total food production, enhance the supply of good quality proteins and to amend soil quality in Ethiopia.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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