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The Response of Faba Bean *(Vicia faba L.)* to Different Strains of Rhizobium Biofertilizer (*Rhizobium leguminosarum*) at Horro District, Western Oromia, Ethiopia

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Abstract: Among the major pulse crop produced in Ethiopia, Faba bean is the which widely produced in the highlands and midlands of Ethiopia. In the poor agricultural systems of Ethiopia, many farmers of the country could not use chemical fertilizers in the production of faba bean. In addition, the only using of chemical fertilizer has many side effects on human health, environment and also many small holder farmers in country could not buy and use the chemical fertilizer because of its escalating cost. Therefore instead of chemical fertilizer (urea), farmers can use the cost effective and environmentally sound option biological Nitrogen Fixing (BNF) Rhizobial biofertilizer. This study therefore, conducted to evaluate the effective strain of Rhizobial biofertilizer for the production of faba bean in 2021 at Horro district, Oromia Region, Ethioia. The results from this experiment shown, significantly ($P \leq 0.05$) difference from the treatments on weight of biomass and grain yields. The both maximum Biomass yield (17,966Kg/ha) and grain yield (3587.73Kg/ha) were gained from the treatment of 1Kg/ha of EAL-1017 and 50kg ha^{-1} of NPS. Therefore this experiment gave the conclusion of using Rhizobial biofertilizer strain of FB-EAL 1017 (1Kg/ha) with 50 Kg/ha of chemical fertilizer (NPS) enhances the production of faba bean comparing to other strains at the district.

Keywords: Rhizobial Strains, Biofertilizer, Pulsecrops, Fababean, Rhizobium Leguminasarum

1. Background and Justification

Faba bean *(Vicia faba L.)* is the most popular annual pulse crops at the level of the world. Fababeans are well known to be best performing crops under global warming and climate change scenario because of its unique ability to excel under all most all type of climatic conditions. Faba bean is the major human foods in many country including Ethiopia, China, Egypt, Mediterranean region and parts of Latin America. The fababean (*Vicia faba* L.) is produced. In addition, fababean used to serve ecosystem by offering renewable inputs like Nitrogen to legume plants and soil through its unique activity known as biological N_2 fixation, and a diversification of cropping system. [1] Faba bean is one of the major pulse crops widely produced in the highlands of Ethiopia. It is an annual crop grown by subsistence farmers, during the cool main rainy season (June to September) and used as the source of dietary protein to the majority of population in the country [2]. Faba bean occupies about 28% of the total land area under pulse crops in the country [3]. In the poor agricultural systems of Ethiopia, many farmers of the country could not use chemical fertilizers in the production of fababean and other pulse crops. Traditionally farmers uses pulse crops as restorer of soil fertility subsequently after cereal crops [4]. So to bring the solution to the challenges of sustainable agriculture for Sub-Saharan Africa, farmers have to depend on traditional knowledge to improve management of diverse on-farm resources and integration among various farm enterprises [5].

The escalating costs and environmental impacts of using chemical fertilizer is global concern now a day. Using of alternatives N fertilizers must be urgently sought to bring sustainability in agriculture. Biological nitrogen fixation (BNF), a microbiological process which converts atmospheric nitrogen into a plant-usable form. Nitrogen-fixing systems offer an economically attractive and ecologically sound means of reducing external inputs and improving internal resources. Symbiotic systems such as that of legumes and *Rhizobium* can be a major source of N in most cropping systems [6]. Through Biological nitrogen fixation (BNF), reduction of dinitrogen can be occurred from the air to ammonia by a large number of species of free-living and symbiotic microbes called diazotrophs. BNF presents an inexpensive and environmentally sound, sustainable approach to crop production and constitutes one of the most important Plant Growth Promotion (PGP) scenarios [7]. Rhizosphere soil is top layer soil around plants root which has large diversity of microbial organisms, which caused plant growth promoting activity. The plant growth promoting rhizobacteria (PGPR) colonize roots, enhance root branching, rooting number and increase growth through direct and indirect mechanisms. PGPR modified root architecture by production of phytohormones, siderophores, HCN, Nitrogen fixation and Phosphate solubilization mechanisms. PGPR also modify root functioning, improve plant nutrition and influence the physiology of the whole plant. N-fixers and P-solubilizers play key role in plant growth and yield of various crops. However the PGPR also play very crucial role to maintain the soil fertility and health [8]. Rhizobial bio fertilizer is the mixture of substance which has latent microorganisms, can be applied to seeds, plant surfaces, or soil, and colonize the rhizosphere and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers gives nutrients to legume plants through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth promoting substances. As [9] reported, inoculating of legume crops with latent rhizobial biofertilizers has been approved to enhances the yield and yield components of legumes while maintaining soil health. It is also believed to be eco friendly practices used for improvement of N fixation resulted in increased shoot growth, number of pods and grain yield of faba bean. In Ethiopia, many researches has been done on rhizobiology for more than three decades and significant progresses were also achieved in the country. [10, 11] are the few one among the Studies of rhizobium inoculation and fertilizer treatment on growth and production of pulse crops Ethiopia. However, most of the works had limitations in addressing all agro ecologies of Ethiopia and using the effective strain different edaphic conditions. However, most of the works had limitations in addressing all agro ecologies and selecting the efficient rhizobial isolates under different edaphic conditions. Additionally there were no research conducted in the study area on the evaluation of the effective strains of rhizobial biofertilizer in the production of fababean. The objective of this study was therefore, to evaluate the effect of different strains of Rhizobium biofertilizer in the production of fababean and to select the effective rhizobial strain biofertilizer in the study area to enhance yield and yield components of fababean.

2. Materials and Methods

2.1. Description of the Study Area

2.1.1. Location, Agro Climate and Soil Type of the District

The study was conducted in 2021/2022 at Horro District. The district is located Horro, Guduru Wollagga Zone, western Oromia Regional state, Ethiopia. Shambuu, which is the capital town of the zone and the district it self is located 314 km away from Addis Ababa. It lies at an altitude range of 1800 to 2835 m.a.s.l., latitude 1,042726N to 1,091814N and longitude 270,000E to 316,199 E. The area has one long rainy season extending from March to mid-October with mean annual precipitation of about 1800 mm and the district's mean monthly rainfall is 12.8 to 343.8mm. The mean of monthly temperatures is between 17.2 to 22.90 C^0. Horro district consists of 37.89% highland, 54.75% mid altitude and 7.86% lowland. The season is divided into three: the main rainy season (June-October), dry season (November-February) and short rainy season (March-May). The soil type of the district is nitisols.

2.1.2. Location Map of the Study Area

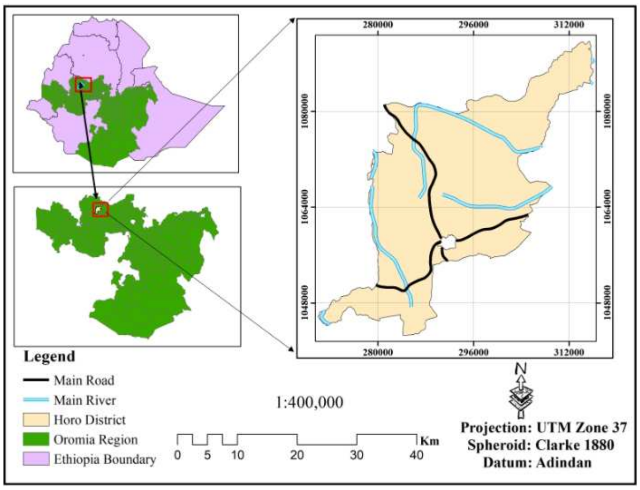

Figure 1. *Location map of Horro District.*

2.2. Site Selection

Sites and farmers were selected in collaboration with key informant farmers, DAs, and the districts' agriculture and natural resource officers. The target sites were selected based on the high potential fababean producing kebele of the district and access of the road and the farm also selected depending on the willingness of the sites owners farmers to to accept the technology. Total 8 farmers were selected from the district and the experiment was conducted on eight farmer's sites.

2.3. Materials and Methodology

The crop used in the experiment was Faba bean Welki variety (EH96049-2) that is well adapted and efficiently perform in the district.

2.3.1. Treatments

The followings are the four *Rhizobium leguminosarum* Strains used as treatment during the conduction of the experiment rhizobial strains.

1. Control (without both chemical fertilizer and bacterial strain).
2. Strain 1 (faba bean strain EAL-1017) + 50 Kg NPS/ha.
3. Strain 2 (faba bean strain EAL-1018) + 50 Kg NPS/ha.
4. Strain 3 (faba bean strain EAL-1035) + 50 Kg NPS/ha.

2.3.2. Experimental Setup

The lands have been prepared using hand hoe (three times ploughing) before planting. The four treatment were replicated three times. The experimental plots have an area of 9 square meter (3*3)m^2 plot size. Treatments has been combined and laid out in randomized complete block design (RCBD) and assigned randomly to the experimental units (plots) within blocks. 1Kg/ha *Rhizobium leguminosarum* strains of biofertilizer has been applied and mixed with the seed under shadow to protect the rhizobium from direct sunlight. The inoculated seed were planted first then the application of 50KgNPS/ha was followed.

2.4. Data Collection Methods

2.4.1. Number Nodule Per Plant

Nodules are found the plant roots and they have been collected by digging of the soil around the plant roots. Among the whole plants in the plots three plants of fababean are randomly selected to be uprooted at their flowering stagefor nodule collection parameter. The soil has been dig out using a hand hoe and the roots of the plant and soils has been excavated using spade. The excavated roots of plants were washed thoroughly by water in washing buckets to make the nodules free from the soil. Then the Nodules were collected in plastic bag for counting. Then the total number of nodules of each three plants in the plot were counted and registered as the number of nodule per plant with the specific treatment.

2.4.2. Plant Height Parameter

The data of plant height was recorded by selecting three plants randomly and measured by using measuring meters at physiological maturity stage. The average of measured height of three plants was recorded as plant height.

2.4.3. The Parameter of Pods Per Plant

To collect the data of pod per plants, five plants were randomly selected and harvested from the rows of each plots. The pods has been collected and counted by separating them from each other. Then the average number of the five plants pod were recorded as number of pods per plant.

2.4.4. Seed Number Per Pod

To collect the data of seeds per pod, the five plants randomly selected and harvested from the rows of each plots had been used by counting the number of grain/seed in the pod. To record the data of seed per pod, the total number of seed on the single plant has been divided by the total number of the pod on the same plant and finally the mean value was recorded as the seed per pod.

2.4.5. The Parameters of Biomass Yield, Grain Yield and Hundred-Grain Weight

To collect the data of above ground dry biomass, five plants were harvested nearest to the ground the maturity stage of the plants from each plots. Then the plants were put in the open air to be dried and finally weighed to measure above ground plant biomass yield. The Grain yield parameter was recorded by measuring the weight of the grain yield of the five plants harvested from each plots and then the weight has been converted to yield per plot and yield per hectare. The grain yield was adjusted to 10% moisture content. Then hundred grain were selected randomly and counted from each treatments and weighed in gram with a digital balance and reported as hundred seed-weight.

2.4.6. The Procedures of Soil Sampling and Analysis

Disturbed surface soil samples has been taken two times before plantation and after harvesting by using an auger at the depth of (0-20cm) from the experimental sites by two way diagonal soil sample taking method. The soil sample has been taken and analyzed to determine the physico chemical properties of the soil. The collected samples were mixed well and dried in an open air then sieved to pass through a 2 mm sieve for the analysis of soil pH, available P, cation exchange capacity (CEC), to determine total N and organic carbon. The soil was pulverized further to pass through a 0.5 mm sieve. The analyses were carried out by the soil laboratory of Nekemte soil research center using standard methods and procedures. Soil pH was determined using a digital pH metre at 1: 2.5 soil to water ratio [12].

The other soil analyzing parameter was organic carbon. OC was analyzed by using the Walkey and Black oxidation method [13]. The texture was tested by using hydrometer method [12]. The Micro-Kjeldhal method was used to estimate the Total N content [14]. The available P was extracted with a 0.5 M $NaHCO_3$ solution [15]. CEC was determined by extracting the soil samples utilizing ammonium acetate (1 M NH_4OAc, pH 7.0) and then by washed again with ethanol (98%) to rinse the left ammonium (NH_4^+) ions. The adsorbed NH_4^+ in the soil was displaced with sodium chloride and then determined by steam distillation and titration using 0.1 M NaOH [16].

2.5. Analysis of the Statistics

The ANOVA analysis were done by using the general linear model (GLM) methods packed in the SAS statistical software version 9.20 (SAS 2008). Least Significance Difference test (LSD) held at 5% probability level and Means were separated using fisher's protected.

3. Result

3.1. The Results of Yield and Yield Related Parameters

The results of combined analysis of variance revealed that there are statistically significant difference between the treatments on the results of plant height, branches per plant, number of pod per plant, grain yield and biomass of fababean with respect to the difference in the treatments.

3.2. Soil Analysis Results

As it is indicated in Table 1, the soil data before planting was taken and analyzed and the results of soil analysis were shown in the table below. The analyzed soil data of after harvesting will be incorporate soon after analysis

Table 1. *The results of Soil analysis the experimental Site.*

Descriptionofthevalues	Range	Mean
pH (H_2O)	5.31-5.87	5.52
EA (H^++$Al^{3+)}$Cmol$_{(+)}$/Kg	0.41-1.34	0.69
Av.P (ppm)	8.53-37.31	27.51
OC (%)	3.37-4.78	4.05
OM (%)	5.8-8.23	6.98
TN (%)	0.29-0.412	0.349
E.Ca^{2+} (cmol$_{(+)}$/Kg)	10.23-18.94	15.15
E.Mg^{2+} (cmol$_{(+)}$/Kg)	3.75-6.76	5.31
CEC (cmol$_{(+)}$/Kg)	24.36-32.64	28.78

PH=power of Hydrogen, EX.Ac=Exchangeable acidity, Av.P=Available Phosphorus, Tot.N=Total Nitrogen, OM=Organicmatter.

3.3. Nodule Number

The greatest numbers of nodules per plant (54.07) in table 2 were observed with the combined application of mineral fertilizer and inoculants of FB EAL -110+50Kg/ha. However the mean of nodulation in the different treatments were rating (range of 32-54). It was insignificant (p>0.05) and not influenced by any treatment. Among the collected nodules from the different treatment about 60-70% nodules were active in the inoculated plants with FB EAL 1017+50Kg/ha. Even there are some nodules were collected from control treatment, about 50-55% are in active. The result is inline with the findings of, [17]. Who reported, the inoculating faba bean has been resulted with high nodulation of effective nodules per plant compared to non-inoculated plants. Also this result in line with [18] who reported The nodule number obtained from the combined effect of (0, uninoculated), (Zn, un inoculated) and (Zn, EAL 17) shows statistically (P ≤ 0.05) lower nodule number compared to other treatments.

3.4. Plant Height and Biomass

In this experiment the results of ANOVA analysis did not show significantly (P ≤ 0.05) difference from the treatments on plant height in Table 2. The highest numbers of plant height (151.30 m) in table 2 were recorded with the treatment combination of the mineral fertilizer 50Kg/ha NPS and inoculants of rhizobial strain FB-EAL-1035. The lower plant height (144.7m) were recorded with control treatment. However the mean of plant height inthe different treatments were rating (Range of 144.7-151), the result is insignificant (p>0.05) and not influenced by any treatment. In this study, The results of ANOVA analysis shown significantly (P ≤ 0.05) difference from the treatmentson weight of biomass. The maximum Biomass yield (17,966Kg/ha) was gained from the combined application of EAL 1017 and 50 kg ha^{-1} NPS, whereas the lowest Biomass yield (11,261Kg/ha) was obtained from the control. This work is in agreed with the study of [19]. Who has shown that inoculation of rhizobial strains with fababean cobined with Chemical fertilizer can significantly increased fababean biomass yield. This results also in line with the finding of [18] who indicated that inoculation of rhizobial strains increases biomass yield of fababean and stated that the difference in the biomass yield of legume plants when inoculated with rhizobial biofertilizer could be from the additional supply of nitrogen to the plants via the biological nitrogen fixation by the inoculated microorganisms.

Table 2. *Combined effect of rhizobial inoculation and NPS fertilizer on faba bean (nodule number plant−1, Plant height and biomass).*

Treatments	Noduleno.	Plantheight	Biomass
Control	35.73	144.70	11261^b
FBEAL-1017+50Kg/haNPS	37.90	150.53	17966^a
FBEAL-1035+50Kg/haNPS	32.33	151.30	12896^{ab}
FBEAL-110+50Kg/haNPS	54.07	149.70	13888^{ab}
Significance	NS	NS	**
LSD (0.05)	23.03	12.84	5082.5
CV	30.57	4.57	19.27

*EAL=Ethiopian Agricultural Legume, Significantat P≤0.05**; LSD: Least significant difference; CV: Coefficient of variation. NS, Non significant;: control: (withoutanyfertilizer),***₌highlysignificant.*

3.5. Pod Per Plant and Seed Per Pod

In this experiment the results of ANOVA analysis did not show significantly (P ≤ 0.05) difference from the treatments on both pod per plant and seed per pod. However the results of ANOVA analysis did not show significantly (P ≤ 0.05), there were difference from the treatments on both pod per plant and seed per pod, the highest numbers of pod $plant^{-1}$ and seed pod^{-1} (17.15 and 2.96) respectively in table 3 were observed with the combined application of the mineral fertilizer and inoculation of FB-EAL-1017+50Kg/ha NPS and the lower pod per plant and seed per pod (16.8 and 2.81) respectively were recorded with control treatment.

Table 3. *The effect of the combination of rhizobial inoculation and NPS fertilizer on faba bean on (Pod $plant^{-1}$, and Seed pod^{-1}).*

Treatments	Podperplant	Seedperpod
Consnitrol	16.85	2.81
FPEAL-1017+50Kg/haNPS	17.15	2.96
FPEAL-1035+50Kg/haNPS	16.81	2.86
FPEAL-110+50Kg/haNPS	16.82	2.82
Significance	NS	NS
LSD (0.05)	0.55	0.182
CV	1.72	3.37

*EAL=Ethiopian Agricultural Legume, Significantat (P≤0.05)**; LSD: Least significant difference; CV: Coefficient of variance, NS: Non significant;: control: (without any fertilizer),***₌ highly significant.*

3.6. Grain Yield and Hundred Seed Weight

Analysis of variance indicated that, faba bean grain yield was highly affected by the treatment of 50Kg/ha NPS and a

Kg/ha FB EAL-1017 rhizobial strains and significantly (P ≤ 0.05) influenced faba bean grain yield. As it has been indicated in above table 4, the result clearly shown the significant difference of different treatments on 100 seed weight and grain yields. The maximum grain yield (3587.73Kg/ha) was recorded with the treatment combination of a Kg/ha EAL-1017 and 50 kg ha^{-1} NPS, while the least grain yield (2612.27Kg/ha) was obtained from the control. The study is in lined with the finding of [19] who shown that combined treatment of chemical fertilizer with inoculation of rhizobial strains significantly increased faba bean grain yield and also in agree with the works of [20] who stated that when rhizobial inoculation applied by combining with P fertilizers it has had a significant influence on nodule number, shoot dry matter, and grain yield of the faba bean. The ANOVA result also shown that combined use of 50 kg ha^{-1} NPS and a Kg ha^{-1} FB EAL-1017 gave statistically significant difference (P ≤ 0.05) on 100 seed weight (0.022Kg) over the control as well as the other strains. This result could in agree with the finding of [21], Who stated that faba bean inoculation with rhizobial strain alone could increase 100 seed weight and the results again in line with the works of [22] which reported that hundred seed weight can be increased by 11.44% when inoculated than un inoculated plants.

Table 4. *The effect of the Combination of rhizobial inoculation and NPS fertilizer on fababean (Grain yields and 100 seed weight).*

Treatments	Grain yield (Kg/ha)	Hundred Seed weight (kg)
Control	2612.27	0.021
FPEAL-1017+50Kg/haNPS	3587.73	0.022
FPEAL-1035+50Kg/haNPS	2976.00	0.021
FPEAL-110+50Kg/haNPS	2810.67	0.020
Significance	***	***
LSD (0.05)	66.036	0.0005
CV	1.17	1.38

EAL=Ethiopian Agricultural Legume, Significantat P≤0.05**; LSD: Least significant difference; CV: Coefficient of variation. NS, Non significant;: control:(without any fertilizer),***=highly significant.

4. Conclusions

In Ethiopia the production of fababean is constrained mainly due to the problems of soil fertility and soil acidity especially in western part of the country. To overcome the problems researches has to dig out many ideas to bring a solution. In this activity different rhizobium strains of Biological Nitrogen Fixers (BNF) are involved to examine the remarkable activity of the different rhizobium strains to increase yield and yield components in the production of fabaean through thier nitrogen fixing activities. Accordingly, field experiment gave different results for the different strains on the yields and yield components of fababean. The hieghst grain yield was obtained from FB EAL-1017 strain combined with (50Kg ha^{-1} of NPS). As indicated from the result it has been concluded as, using the combination of organic, bio fertilizer (a Kg ha^{-1} FB EAL1017) strain and mineral fertilizers (50 Kg ha^{-1} NPS) can significantly enhances the yield of faba bean.

5. Recommendations

These technology is a cheap external source of plant nutritionespecially for smallholder farmers who cannot afford expensive inorganic fertilizers in addition to its environmentally friend. Therefore depending on the result obtained from the experiment, the technology of using rhizobium strains called FB-EAL-1017 biofertilizer combined with 50 Kg ha^{-1} NPS has to be pre scaled, the rhizobium strains called FB-EAL-1017 has to be multiplied and its biofertilizer has to be produced in large numbers of sachets and inoculating of fababean seeds with 1 Kg ha^{-1} of Rhizobium strains called FB-EAL 1017 ($1Kg/ha^{-1}$) with 50 Kg ha^{-1} NPS were recommended at Horro district and the same soil types and agro ecological zones.

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