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Evaluation of Rhizobium Bio-Fertilizer Inoculation on Yield and Yield Components of Faba Bean (Vicia Faba L.) at Jarso District, East Hararghe, Oromia, Ethiopia

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ABSTRACT

The Faba bean is a highly important grain legume grown in the highlands of Ethiopia, yet its production and productivity have been consistently dropping due to soil fertility depletion. In order to increase yield and reduce soil fertility problems, an experiment was conducted to assess the efficacy of Rhizobium strain inoculation on yield and yield components of the Faba bean in Jarso district, Eastern Hararghe, Oromia, Ethiopia. The experiment was conducted over two consecutive 2020-2021 cropping seasons, and consisted of eight treatments: control, recommended NPS, FB 17, FB; EAR 15, FB 18, FB 17 + 50 kg NPS/ha, EAR 15 + 50 kg NPS/ha and FB 18 + 50 kg NPS/ha. The treatments were laid out in a complete randomized block design with three replications, and a Dosha improves the variety of the Faba bean was used for the trial. Results showed that Rhizobium strains inoculation had a significant effect on yield and yield components of the Faba bean, including nodule number. Moreover, the results indicated that the application of Rhizobium strain FB 18 with 50 kg/ha NPS fertilizer can be a viable and beneficial option for Faba bean production in the study area. This is encouraging, and more research should be done to further explore the potential of this approach. It would also be beneficial to isolate indigenous bacteria from the soil to determine if they could be more effective in increasing biomass and yield than the Rhizobium strain FB 18. This could give farmers in the study area an even better option for enhancing their crop production.

KEYWORDS: *Nitrogen Fixation, Rhizobium, Strain, Soil Fertility, Yield*

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INTRODUCTION

Grain legumes are a primary source of amino acids providing about a third of all dietary protein and second to cereals in providing food for humans worldwide (Kudapa *et al*., 2013). They are considered a vital crop for achieving food and nutritional security for both poor producers and consumers (Allito *et al*., 2014). These grain legumes are an important source of dietary protein and daily food supplements for the majority of Ethiopian population.

Faba bean is high- yield pulse crop that's both– economic and ecologic role is very significant; they contain up to 35% of crude protein, approximately 50% of carbohydrate and no more than 15% of crude lipid (Proskina *et al*., 2016). As a grain legume, it provides cheap and quality protein (lysine) supplement (20- 40%) in the cereal- based Ethiopian diet (Nebiyu, 2014). Faba bean is the most important grain legume in the highlands of Ethiopia (1800-3000 m) in terms of area, production, foreign exchange earnings, protein source, soil amelioration and nitrogen provision in cropping systems (Agegnehu, 2018). It occupies 27.34% of the total land area under pulse crops in Ethiopia (1,598,806.51 ha^{-1}) and of the entire Oromia region area under pulse crop production (662,144.90 ha), 32.85% was covered by faba bean (CSA, 2017/18). Net profit ha-1 of faba bean was found to be higher than most other pulses and cereals (Yirga *et al*., 2010). This attributed to the saving of 150-200 kg ha^{-1} of N as well as some 20-50 kg in the subsequent crop. Despite its multifaceted benefits the productivity of faba bean, regional production, 23.64qu/ha (CSA, 2017/18), which is remained low compared to its attainable yield >3t/ha (MoA, 2011).

Soil fertility deficiency is the main constraint factor affecting the production and productivity of Pulses crops mainly Faba bean both in terms of quality and quantity. To improve the productivity of faba bean, alleviation of soil fertility depletion through proper fertilizer management is very important along with other agronomic practices. Integrating organic and inorganic fertilizers for tackling soil fertility depletion and sustainably increasing crop yields is the best alternative to avert soil fertility depletion and increase crop productivity (Mahajan *et al*., 2008, Gete *et al*., 2010). One of the best organic materials for increasing crops yield is biological nitrogen fixation, especially rhizobia-legumes symbiosis is one of the alternative solutions and the promising technologies which play an important role in reducing the consumption of chemical N-fertilizers, increasing soil fertility, decreasing the production cost, and eliminating the undesirable pollution impact of chemical fertilizers in the environment (Upendra *et al*., 2013, Livija *et al*., 2017). Rhizobium inoculants are selected strains of beneficial soil microorganisms cultured in a laboratory and packed in with or without a carrier. They are host-specific, low cost and an environmentally friendly source of nitrogen (EIAR, 2016). Rhizobia inoculants coated on legume seeds before planting enhance growth, yield of legume crops, and provide nitrogen and organic carbon for subsequent or associated crops (Fact sheet, 2016).

Several studies showed that Ethiopian soils harbored symbiotically effective rhizobia, which are tolerant to different stresses such as acidity (Argaw, 2012). Inoculation of Faba bean with effective rhizobia strains can reduce the need of inorganic fertilization to achieve higher crop yield under low soil fertility condition. The inoculation of the legume seed material with active nitrogen fixing bacteria strains before sowing has a significant role for the increase of the legume yield (Woldekiros 2018). Inoculation can improve crop yields in cases where appropriate rhizobia are not present in the soil or the soil contains a significant proportion of ineffective nitrogen-fixing strains. Inoculation of legume seeds with Rhizobium affects soil microbial community and processes, especially in the rhizosphere (Livija *et al*., 2017). Different leguminous crops require specific Rhizobium species for the formation of effective nodules and N2 fixation and the various strains of Rhizobium species differ in their efficiency of N2 fixation (Aserse, *et al*, 2020). The low level of nutrient supply and the lack of effective indigenous Rhizobium populations in soil have limited the faba bean yields (Gorfu, *et al*., 2012). Thus, the crop should be inoculated with the proper Rhizobium species and strains. Therefore, understanding the impact of rhizobia inoculation and contrasting soil rhizobia on nodulation and Nitrogen fixation in faba bean is crucial to optimize the crop yield, particularly under low fertility soil conditions. Therefore, the aim of this study was to evaluate the rhizobium strains (biofertilizer) inoculation on growth and yield components of faba bean (Vicia faba L.) at Jarso woreda, East Hararghe zone of Oromia region.

Materials and Method

Descriptions of Study Areas

The study was conducted in Jarso district, which is one of the woreda in the Oromia Region of Ethiopia. Part of the Misran (East) Hararghe Zone, Jarso is bordered on the south by the Harari Region, on the west by Kombolcha, on the north by the city of Dire Dawa, on the east by the Somali Region, and on the southeast by Gursum. The administrative center of this woreda is Ejersa Goro. Geographically found at Latitude: 9° 24' 59.99" N Longitude: 42° 09' 60.00" E and the altitude of this District ranges from 1050 to 3030 meters above sea level.

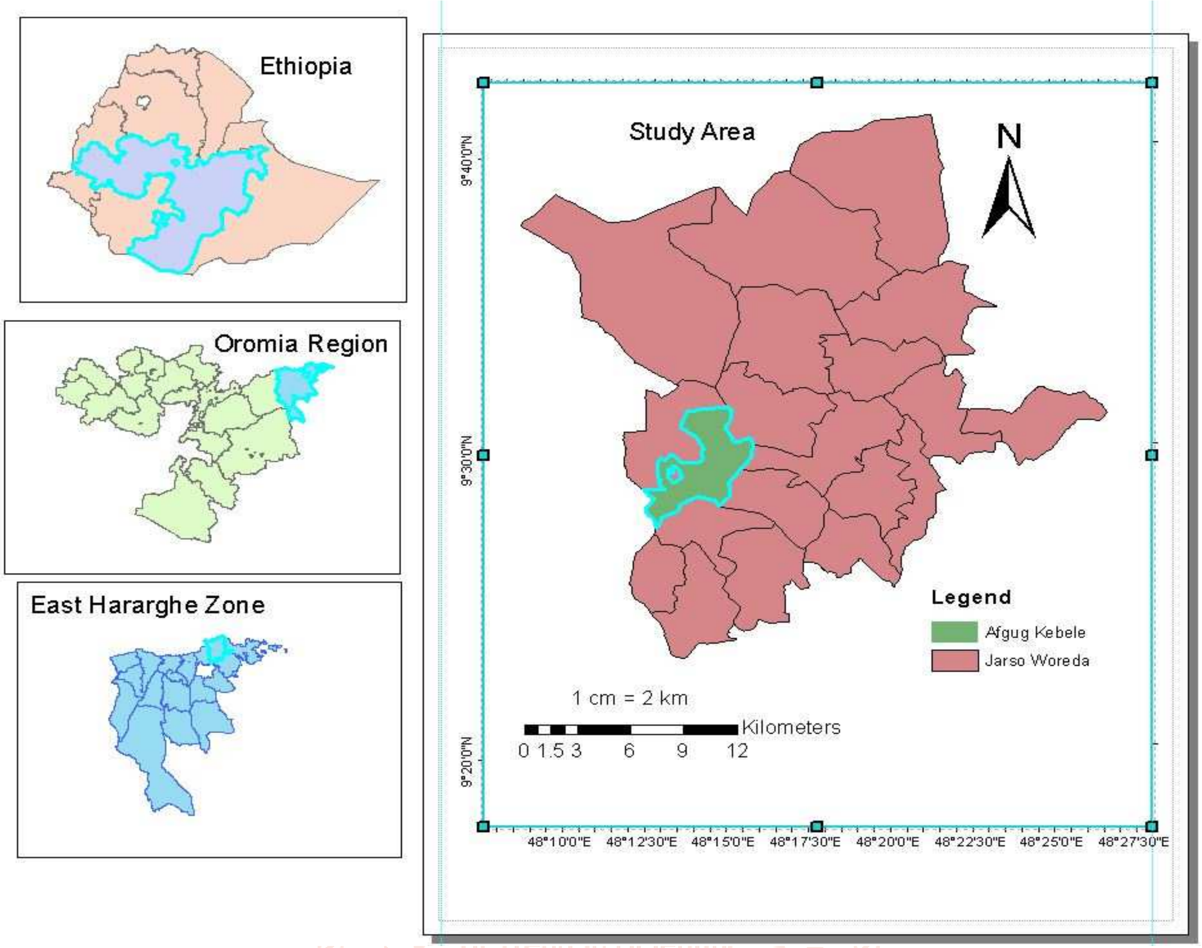

Figure 1. Map of the study area

Experimental Design, Treatments and Procedures

The experimental plots were laid out in a Randomized Completely Block Design (RCBD) with three replications with a plot size of 3m*4m. An improved variety of Faba bean (Dosha) used as testing crop for the experiment.

The treatments were the combination of different type of strain and fertilizer
T1: Absolute control (no fertilizer no strain)
T2: Recommended NP (100kg NPS)
T3: FB; 17
T4: FB; EAR 15
T5: FB 18
T6: FB; 17 + 50 kg NPS
T7: FB; EAR 15+ 50 kg NPS
T8: FB 18+ 50 kg NPS

Treatments were designed based on available strains (bio-fertilizers) solely and with half of recommended NP for economic advantage. TSP was used as a source of P applied in the rows mixed with soil just at time of planting. Improved Faba bean (Dosha) variety with higher yielding during adaptation trial was used for the experiment. The recommended number of seed (134kg/ha) Were soaked in distilled water for six hours and also the seeds were inoculated with Rhizobia strains (FB1018, FB17, EAR 15) which were received from Holetta Agricultural Research Center. During inoculation of rhizobia strains mixed with sugar by addition of some water in order to facilitate adhesion of the strains to the seed. The seed was dressed with mixed strains and planted immediately. All management practices applied uniformly as per research recommendations for faba bean on each plot. All necessary agronomic and soil data collection done at appropriate crop growth stages following recommended procedures. At the 50% flowering stage, five plants from each treatment uprooted and nodule number were recorded. Soil Sampling and Analysis Before planting, soil samples were collected from the experimental field at a depth of 0 to 20 cm in zigzag manner using an auger and the samples were mixed thoroughly to produce one representative composite sample. After harvesting soil sample were taken from each plot at depth of 0 to 20 cm using auger in a Crisscross movement from five spots and mixed up to one sample based on treatments accordingly (Abera *et al*., 2020). The collected soil sample was air-dried, grounded and analyzed for total N, pH, organic carbon (OC), Organic matter, CEC and texture.

Data Collection

Data for yield and yield components were collected as per the procedures mentioned as follows. Five plants from the central rows of each plot were randomly selected for measuring plant height. Then the average values of these plants were recorded as the plant height of the crop. Nodulation assessments were undertaken at the mid (50%) flowering stage by carefully uprooting five plants randomly from each plot. The plants were separated into shoots and roots. The adhering soil was carefully washed from the roots over a metal sieve. The nodules from each plant were picked and spread on the sieve to drain water from their surface. Nodules were counted and their average will be taken for plots as nodule number per plant. At harvesting time number of pods per plant, number of seeds per pod, hundred seed weight and grain yield, was recorded. For hundred seed weighted sun-dried seeds was randomly taken from the seed lots of each plot and then weighed by using sensitive balance.

Data Analysis

The collected data were subjected to analysis of variance (ANOVA) using SAS version 9.1 GLM procedures and Least Significant Difference (LSD) were used to separate means at $p<0.05$ probability levels of significance.

Partial Budget Analysis

Grain yield data were economically evaluated using partial and marginal analysis for the feasibility of fertilizer and strain application. A treatment was considered worthwhile farmers when it's minimum acceptable rate of return (MAR) is 100% (CIMMYT, 1988), which is suggested to be realistic. This enables to make farmer recommendations from marginal analysis.

RESULT AND DISCUSSION

Soil analysis results

Table 1: The soil chemical properties before experiment and after harvest from experimental site

Soil Parameters	Sand (%)	Silt (%)	Clay (%)	Textural Class	pH (H_2O)	TN (%)	OC (%)	Ava. P (mg/kg)	CEC (meq/100g)	EC (Mmhos/cm)
Value	28	23	42	Clay loam	7.3	0.19	0.56	6.7	35.7	0.16

Before application of the experiment, the experimental field characterized for selected soil physical and chemical properties, soil samples were collected from 0-15 cm depth for initial determination of soil textural class, pH, Total Nitrogen, Organic Carbon, Available Phosphorus, Cation exchange capacity and Electrical conductivity parameters (Table 1). The soil of the experimental site has a proportion of 28% sand, 23% silt, and 42% clay; and it classified as clay loam according to the soil triangle texturally. The pH of the experimental site (1:2.5 ratio of soil to water suspension) was 7.3, which implied that the soil of the study site was moderately acidic.

The experimental site has total nitrogen of 0.19% by Keljdal digestion and distillation followed by titration method; which implied that the soil of the experimental site has a medium level of total nitrogen according to Tekalign *et al.,* (1991). The organic carbon of the soil was 0.56%, which rated as very low. The available P content of the experimental site was 6.7 mg kg^{-1} rated as low (Jones, 2003). The CEC of the experimental site was 35.7meq/100g which was rated as high.

Nodule Number: Inoculation of Faba bean seed with Rhizobium strain shows the statistical significance difference on the number of fresh nodules per plant at 5% significance level. The highest fresh nodule number per plant (105.5) was recorded from the treatment FB 18 + 50 kg NPS/ha which was in statistical parity with the other all inoculated and recommended fertilizer except control, the lowest nodule number (51.6) per plant was recorded from control treatment. This result indicated that the inoculation of these strains in the study area could be more effective and lack of competition with the existing indigenous bacteria. Similarly, Kebede and Lele (2022) reported that inoculation of rhizobium strain could be more appropriate and competitive than the existing native strains of Faba bean rhizobia. Desta *et al*., (2015) reported that inoculation of Faba bean rhizobium strains significantly increases nodule number per plant. However, in contrary this work with Endalkachew Fekadu *et al*., (2018) reported that Faba bean plants, which, not inoculated with rhizobium, have a higher number of nodules as compared with plants inoculated and received the same treatment. Gedamu *et al*., (2021) also reported that inoculation of Rhizobium strains may best suited as compared to the existing Faba bean rhizobium strains to bust the nodule number.

Table 2: The mean effect of Rhizobium Strain Inoculation on yield and yield components

No	Treatments	Nodule Number	Plant height(cm)	No of pod/ plant	No of seed/ pod
1	Control	51.6[b]	78.3[c]	10.78[d]	3.2
2	Recommend NP/100 NPS kg/ha	79.67[ab]	86.1[bc]	16.4[bc]	3.06
3	FB 17	85.07[ab]	95.52[b]	16.3[bc]	3
4	FB; EAR 15	81.1[ab]	88.3[b]	20.9[ab]	3.3
5	FB 18	76.6[ab]	89.3[b]	16[c]	3.1
6	FB 17 + 50 kg NPS/ha	79.97[ab]	92.1[b]	19.5[bc]	3
7	EAR 15 + 50 kg NPS/ha	88.67[ab]	89.4[b]	16.5[bc]	3.3
8	FB 18 + 50 kg NPS/ha	105.5[a]	107.3[a]	25.4[a]	3.3
	LSD	37.88	10.42	4.5	NS
	CV (%)	26.7	6.5	15.12	6.36

Plant Height

The results revealed that rhizobium strain inoculation significantly affect the plant height of Faba bean at 5% level of significance. The highest plant height (107.3cm) was recorded from the treatment FB 18 + 50 kg NPS/ha, which have statistical significance difference with the other treatments. The lowest plant height (78.3cm) was recorded from control treatment, which statistical parity with Recommend NP/100 NPS kg/ha treatment. This implies that inoculation of effective rhizobium strain on Faba bean and fertilizer increase the Nitrogen yield of the crops, which increase the height of the plant. This result agreed with the result of Gedamu *et al*. (2021) which affirm that inoculation of rhizobium strains to Faba began supplying additional nitrogen through symbiotic nitrogen fixation and lead to increased plant height. Similarly, (Kebede and Lele 2022) reported that Rhizobium inoculation increases Faba bean growth parameters by increasing nitrogen supply.

The significant increase in plant height in response to the application of Rhizobium inoculation attributed to the increased availability of nitrogen in the soil for uptake by plant roots, which might have sufficiently increased vegetative growth through enhancing cell division and elongation. The increment of plant height due to Rhizobium inoculation might also be due to the sufficient amount of nitrogen fixed by the bacteria which likely resulted in enhanced vegetative growth of the plants (Kutafo and Alemneh. 2020).

Number of Pods per Plant

A Significant effect of rhizobium inoculation was observed on the number of pods per plant at 5% level of significance. The highest pods per plant (25.4) was recorded from the treatments FB 18 + 50 kg NPS/ha which was in statistical parity with treatments FB; EAR 15. The lowest pods per plant (10.78) were observed on the control treatments, which, was statistically in significant with the all treatments. The result indicates that inoculation rhizobium strain significantly increases number of pods per plat with addition of fertilizer at planting time. Similarly, Bezabih *et al*., (2018) reported that number of pods per plant showed significant response to phosphorus fertilization and Rhizobium inoculation. The result is also in conformity with the finding of Birhanu (2021) who reported that the highest number of pods per plant recorded.

Inoculation of faba bean seeds with Rhizobium strains also had a statistically significant effect on the number of seeds per pod compared to un-inoculated treatment (Kebede and Lele., 2022). According to Gedamu *et al*., (2021) the rhizobia strain could significantly increase the number of pods plant.

Number of Seed per pod

The result shows that there no significance effect of rhizobium inoculation the number seed per pod at 5% level of significance. The highest seed per pod (recorded from treatment FB; EAR 15, EAR 15 + 50 kg NPS/ha and FB 18 + 50 kg NPS/ha and the lowest seed per pod (3) recorded from treatment FB 17. The result of the present study agrees with that reported by Zerihun and Abera (2014), who showed that the number of seeds per Faba bean pod was not significantly affected by fertilizer rate and rhizobia inoculation. This finding disagrees with Gedamu *et al*., (2021) who reported that inoculation of rhizobium strains statistically affected number of seeds /pods as compared to the un-inoculated treatment.

Effect of Treatments on Yield Components

No	Treatments	Biomass kg/ha	Yield kg/ha	Hundred seed weight
1	Control	5054[b]	1441.2[c]	71.67b[c]
2	Recommend NP/100 NPS kg/ha	6481[ab]	1902.9[bc]	74.33b[c]
3	FB 17	7050[ab]	2343.1[b]	77.3[ab]
4	FB; EAR 15	6667[ab]	2278.7[b]	73[bc]
5	FB 18	6217[b]	2053.7[b]	71.3[bc]
6	FB 17 + 50 kg NPS/ha	6362[b]	1994.5[b]	68.67[c]
7	EAR 15 + 50 kg NPS/ha	5860[b]	1935.3[bc]	70.33b[c]
8	FB 18 + 50 kg NPS/ha	8579[a]	3016.6[a]	84.67[a]
	LSD	2149.5	536.94	8.625
	CV (%)	18.8	13.36	6.63

Biomass

Analysis of variance revealed that different rhizobium strains significantly affect the biomass of Faba bean at 5% level of significance. Treatment with FB 18 + 50 kg NPS/ha score the highest (8579kg/ha) biomass which was statistical parity with Recommend NP/100 NPS kg/ha. FB 17 and FB; EAR 15 treatments, while the lowest biomass (5054kg/ha) was measured from control treatment. Application of FB1018 of Rhizobium strains and 50 kg NPS/ha improves aboveground biomass production by 40.39% as compared with control treatments. The present result has in line agreement with Kutafo and Alemneh, (2020) observe that application of FB1018 of Rhizobium strains alone improves aboveground biomass production by 35.85% as compared with control treatments.

The biomass yield difference obtained from the inoculation of Faba bean rhizobium strains could be from the additional supply of nitrogen through the remarkable biological nitrogen fixation by the inoculated strains and Rhizobium strains inoculation significantly influenced Faba bean biomass weight un-inoculated treatment (Gedamu *et al*., 2021). Similarly, Kebede and Lele (2022) reported that the Difference in biomass yield obtained from the inoculation of Faba bean Rhizobium strains could be from the additional supply of nitrogen through the notable biological nitrogen fixation by the inoculated strains. Effective Rhizobium nodulation contributes to increased Faba bean growth and yield parameters by supplying nitrogen to plants by fixing it from the atmosphere and converting it into plant-available nutrient forms.

Grain Yield

The analysis of variance results showed that the strain, NPS, and the interaction between the two had a significant effect on the grain yield of faba beans at a 5% level of significance. The highest grain yield (3016.6 kg/ha) was seen when FB 18 and 50 kg of NPS were applied together, and this result was statistically different to all other treatments. On the other hand, the lowest grain yield (1441.2 kg/ha) was seen in the control group and this was statistically similar to the results seen in the Recommend NP/100 NPS kg/ha and EAR 15 + 50 kg NPS/ha treatments. The inoculation of faba bean seeds with Rhizobium strains in combination with phosphorus fertilizer increased grain yield by 52.22%.

This result is in agreement with Kutafo and Alemneh (2020), who reported that the application of FB1018 of Rhizobium strains improved grain yield production by 72.6% from the control. The increase in nitrogen levels due to the fixation of atmospheric nitrogen by Rhizobium strains, as well as the application of fertilizer at planting time, are likely to have caused the observed yield improvements. The results of this research consistently demonstrated that faba bean plants inoculated with rhizobia produced a greater seed yield than those left un-inoculated (Abera *et al*., 2015; Reda *et al*., 2016). Moreover, Gedamu *et al*., (2021) established that rhizobia inoculation yielded a significantly higher weight of faba bean biomass than those not inoculated. This difference in biomass yield may be attributable to the nitrogen supplied by the rhizobia strains through their remarkable ability to biologically fix nitrogen. Furthermore, Rhizobium inoculation of faba bean markedly improved its grain yield when compared to not inoculated seed (Abera *et al*., 2015; Bezabih *et al*., 2018).

Hundreds seed weight

The result clearly showed a marked difference in terms of hundred seed weight. It was observed that strains significantly affected hundred seeds weight at 5% level of significance. FB 18 + 50 kg NPS/ha contributed maximum hundred seed weight (84.67 gram) which was statistical parity with FB 17 treatment. The lowest hundred seed weight (68.67gm) was recorded from FB 17 + 50 kg NPS/ha treatment which was in statistical parity with the rest treatment except FB 17 and FB 18 + 50 kg NPS/ha treatments. The result indicate that rhizobium strain demonstrates in increment of yield which improve the weight of the seed. Similarly, Endalkachew *et al*., (2016) indicated that hundred seed weight was significantly higher in inoculated treatments in the pot experiment. The weight difference gained from this work could be attributed the effect of the grain filling ability of nitrogen through nitrogen biological fixation (Gedamu *et al*., (2021).

Partial Budget Analysis

The partial budget analysis for marginal rate of return showed that Faba bean rhizobium strains gave acceptable marginal rate of return (i.e., MRR greater than 100%). According to CIMMYT (1988) when there are two and more treatments with MRR greater than 100%, the treatment with greater net benefit should selected for recommendation. Therefore, inoculation of rhizobium strain FB 18 with 50 kg NPS per hectare brought the maximum net benefit 177151 Ethiopian Birr per hectare while possessing MRR of greater than 100% and thus it is economically feasible for Jarso district.

SN	Treatment	TVC	adjusted yield	Yield price birr/Kg	Total revenue	Net benefit	Marginal Return
1	Control	1600	1441.2	60	86472	84872	
2	FB18	1920	2053.7	60	123222	121302	11384
3	EAR 15	1940	2278.7	60	136722	134782	67400
4	FB 17	1960	2343.1	60	140586	138626	19220
5	FB 18 + 50 NPS kg/ha	3845	3016.6	6o	180996	177151	2043
6	EAR 15 +50 NPS kg/ha	3865	1925.3	60	115518	111653	
7	FB 17 +50 NPS kg/ha	3885	1994.5	60	119670	115785	206.6
8	RECOM NPS	5450	1902.9	60	114174	108724	

Conclusion and Recommendation

Soil fertility deficiency is the main constraints factor affecting the production and productivity of Pulses crops mainly Faba bean both in terms of quality and quantity. In order to improve the productivity of faba bean, alleviation of soil fertility depletion through proper fertilizer management is very important along with other agronomic practices. The inoculation of the legume seed material with active nitrogen fixing bacteria strains before sowing has a significant role for the increase of the legume yield. Inoculations of rhizobium strain alone and with fertilizer significantly improve yield and yield-related traits of faba bean. As indicated from the result, rhizobium inoculants significantly affected yield and yield components of faba bean at 5% level of significance.

Inoculation of effective rhizobium strain with fertilizer increase the nodule number, plant height, pods per plant, total biomass, grain yield of Faba bean crop. FB 18 strain and applying 50 kg NPS/ha increase biomass and yield 40.39% and 52.22% respectively, as compared to control and the brought the maximum net benefit 177,151 Ethiopian Birr. Thus, FB 18 strain with 50 kg NPS/ha recommended in the study district and similar agro ecological zones.

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