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Performance of Rhizobial Inoculants on Yield and Yield Components of Faba Bean in Southern Ethiopia

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Abstract

Biological fixation of atmospheric nitrogen by legumes is a known way to recycle nitrogen into a plant-available form. The efficiency of nitrogen fixation depends on the legume genotype and requires a host-specific rhizobia strain for nodule formation and yield enhancement. Therefore, the field experiment was conducted to evaluate the effectiveness of rhizobial inoculants on yield and yield components of faba bean under rainy conditions during two consecutive main growing seasons. Experiments consisted of a control, 121 kg NPS ha^{-1}, FB 04, FB 1018 and FB 1035, each strain treated separately with 60 kg ha^{-1} NPS and TSP, and designed in a three replicate randomized complete block design. Rhizobium inoculation showed a highly significant ($p \leq 0.05$) effect on yield and yield attributes compared to un-inoculated plants. Over the years, the results showed that the inoculated plants gave a significant increase ($p \leq 0.05$) in nodule number and a benefit in grain yield compared to the un-inoculated plants. The highest yield (5.87 ton ha^{-1}) was recorded with FB 1018 inoculated together with 60 kg ha^{-1} TSP compared to those without inoculation which gave (2.48 ton ha^{-1}). All tested rhizobia inoculates together with TSP fertilizer showed better nodule formation to increase yield of faba bean and therefore recommended for the study area and similar agro-ecologies.

Keywords

Faba bean, Fertilizer, Inoculation, Rhizobium strains, Yield Impact

Introduction

The faba bean (*Vicia faba* L.) is an important grain of the legume family and is grown for food and forage in many countries [1]. It is the main food and feeds legume due to the high nutritional value of its seeds, which are rich in protein and starch [2]. The faba bean plays an important role in fixing atmospheric nitrogen in the form available for the plants. Biological fixation of atmospheric nitrogen in the legume is known ecological practice to improve N-cycling resulted in higher shoot growth, higher number of pods and higher bean yield [3]. Yadav and Verma reported that nitrogen fixation by legumes accounts for 50% of the 175 million tons of total biological N2 fixation worldwide annually [4]. However, nitrogen fixation depends on the legume genotype, the Rhizobium strain and their interactions with the biophysical environment and Rhizobium symbiosis nodule formation [5]. Therefore, the amount of fixed nitrogen varies with cultivar of legumes [6] and the effectiveness of associated microsymbionts [7]. The report of Ouma et al. [8] also confirmed that host-specific Rhizobium strains are better adapted to local soil environmental conditions. To have successful establishment, the rhizobium strain must be able to survive in the soil environment, as the best survival rate and persistence in soil of Rhizobium improves the possibility of effective nodulation and nitrogen fixation [9]. Otherwise, the low-efficiency Rhizobium strains can compete and gain an advantage over the efficient Rhizobium strains used for inoculation [10]. Of course, the soil can support certain native rhizobia that form ineffective nodules; however, effective nodulation is highly dependent on the competitiveness of the inoculants [11]. Inoculation of faba beans with a host-specific rhizobial strain that is effective and appropriate is critical to enhance symbiotic nitrogen fixation and productivity [12]. Inoculation affects the microbial community by increasing the population of desired rhizobial strains in the rhizosphere [3]. The symbiotic performance of nodulation is largely determined by the abundance of effective rhizobia strains and their competitiveness [13]. Thus, inoculation

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with effective host-specific rhizobial strains is required for effective nodulation and nitrogen fixation [14]. Hence, the present study was initiated to identify the rhizobia strains with the best performance in faba bean for nodulation and better yields for two consecutive main growing seasons under rainy conditions in Gummer district Guraghe Zone, Southern Ethiopia.

Materials and Methods

Description of study area

A field experiment was conducted for two years (2019 and 2020) in consecutive main growing seasons under rainy conditions in Gummer, Guraghe Zone, Southern Nations Nationalities and Ethiopian Peoples Regional State. The test site is at 8°01'56.2" N and 38°01'58.3" E and at an altitude of 2767 m above sea level. The area receives a bimodal rainfall with a mean annual rainfall of 1,200 mm. Precipitation is spread over the short rainy season (March to April) and the main rainy season (June to September). Mixed farming is the dominant economic activity in study area.

Experimental design and treatments

The experiment was established in a randomized complete block design with three replicates. Eight treatment levels were (control, 121 kg NPS ha^{-1}, FB 04, FB 1018 and FB 1035, each strain treated separately with 60 kg ha^{-1} NPS and TSP fertilizer). The size of the plot was 3 × 3 m (9 m^2), the improved variety of faba bean (Dosha) was used for the trials at spacing of 0.1 and 0.4 m distance between plants and rows respectively and the distance between plots and blocks was 1 and 1 m, respectively. Inoculants were obtained from the soil microbiology laboratory from the Holeta Agricultural Research Center, the seeds were dipped in warm water to anchor themselves to the Rhizobium strains. The sugar suspension was used as an adhesive for the carrier-based inoculants so that the inoculums adhere to and coat the seeds. The inoculated seeds were allowed to air dry for a few minutes and planted in the shade immediately after drying. The un-inoculated treatments were sown before the starts of inoculation thoroughly avoid cross-contamination. Nitrogen and phosphorus fertilizers were applied at sowing by sowing faba bean seeds in a row. Furrows were made between each plot to reduce movement of bacteria and leaching/addition of nutrients from one plot to another or from the external environment.

Data collection procedures

Nodulation: Sampling for nodulation was performed by digging up the roots of five randomly selected plants in each plot at the mid-flowering stage of faba beans by destructive sampling from border rows. A hoe was used to dig up the root surrounding the soil and the spade was used to excavate at a depth of about 20 cm, which is about the root depth of faba beans and the radius extends about 12 cm from the central stem, which contains the entire root system of the faba bean. The excavated soil was washed off the roots with a washing bottle. Nodules from the crown region and lateral roots were then removed from the roots and were collected in a plastic bag for counting. The total number of nodules was counted, on these five sample plants, considering the intensity of pink color (visual observation) nodules with pink color are regarded as nodules number.

Plant height: Five plants were randomly selected from the middle rows to measure their height at physiological maturity with a tape measure. The average height of five plants was taken from each plot and considered the plant height.

Number of pods per plant: Five plants were randomly selected from harvestable rows from each plot. The pods were collected and counted separately from each plant and their average was taken and reported as the number of pods per plant.

Number of grains per pod: After counting the pods from each of the five randomly selected non-border plants, the grains were separated from the pods to obtain the number of grains per plant. For each plant, the number of grains per pod was calculated by dividing the total number of grains per plant by the number of pods per plant.

Biomass yield and grain yield: At physiological maturity, plants from 5 rows were harvested manually near the soil surface. The harvested plant was sun-dried and weighed to determine above-ground plant biomass yield. The grain yield of each plot after threshing was also determined. Finally, the yield per plot was converted into grain yield per hectare.

Soil physico-chemical analysis: Composite soil samples were analyzed for bulk density, particle size distribution, pH, organic carbon, cation exchange capacity, total nitrogen and available P of the representative soil before planting. The bulk density of the soil was estimated by the core method to a depth of 30 cm and calculated as ρb = Ms Vt where ρb is the bulk density of the soil (g/cm^3), Ms = mass of dry soil (g) and Vt = total volume of Soil sample (cm^3) [15]. The pH of the soil was determined using the potentiometric method in a soil: Water ratio of 1: 2. [16]. Cation exchange capacity was determined using the 1 M ammonium acetate method at pH 7 [17], while organic carbon was determined using the dichromate oxidation method [18], and total nitrogen using the micro-Kjeldhal method [19], and available P was analyzed using the Olsen method [20]. The grain size distribution of the soil was determined using the hydrometer method [21] (Table 1).

Statistical analysis: Collected data were subjected to analysis of variance (ANOVA) variance using SAS 9.4 software packages and mean separation using LSD at a probability level of 5%.

Result and Discussion

Effect of Rhizobium inoculation on grain yield

Mean over the years showed that rhizobium inoculation significantly ($P < 0.05$) affected faba bean grain yield at this site. Statistically, the highest yields were recorded on inoculated plants compared to un-inoculated plants. As shown in Table 2, the maximum grain yield (5.875 ton ha^{-1}) was obtained from the inoculation of FB 1018 followed by FB 1035 and FB 04 yielding 5.29 and 5.078 ton ha^{-1} respectively together with

Table 1: Chemical and physical properties of the soil before sowing.

pH	BD	%OC	%TN	AP	CEC	Textural class			
						% sand	% clay	% silt	texture
5.9	0.99	1.1	0.094	1.28	41.2	70	14	16	Sandy loam

Table 2: Combined mean of biomass and grain yield affected by inoculation of rhizobium strain.

Treatments	Combined Mean of Biomass (ton ha^{-1})	Combined Mean of Grain Yield (ton ha^{-1})
T1: Control	5.758d	2.48c
T2: 121 kg ha^{-1} NPS	11.462ab	5.635a
T3: 60 kg ha^{-1} NPS+ FB 04	9.452bc	4.375b
T4: 60 kg ha^{-1} NPS + FB 1035	8.962c	4.406b
T5: 60 kg ha^{-1} NPS + FB 1018	10.05abc	5.035a
T6: FB 04+60 kg ha^{-1} TSP	11.518ab	5.293a
T7: FB 1035+60 kg ha^{-1} TSP	10.558abc	5.078ab
T8: FB 1018+60 kg ha^{-1} TSP	11.868a	5.875a
Mean	9.95	4.77
LSD (0.05)	2.384	1.123
CV (%)	20.4	20.1

LSD (0.05%): Least Significant Difference at 5% level; CV: Coefficient of Variation; Means in a column followed by the same letters are not significantly different at 5% level of significance.

60 kg ha^{-1} TSP, while the lowest yield of grain was obtained from the non-inoculated (2.48 ton ha^{-1}). The increase in yield of the inoculated plants could be effective nodules formation of the rhizobium strains thereby improve nitrogen supply through biological fixation. This study is consistent with the result of [22] who reported that inoculation of rhizobia strains significantly increased faba bean yield. Desta et al. [23] also confirmed that application of effective rhizobia strains alone and/or in combination with zinc significantly increases faba bean yield. The report by Youseif et al. [24] also shows that application of effective strains increases faba bean grain yield by up to 44-47%.

Effect of inoculation on above ground biomass

Inoculation of Rhizobium strains affected biomass yield significantly ($P \leq 0.05$). From (Table 2) shows that those with FB 1018, FB 1035 and FB 04 together with 60 kg ha^{-1} TSP inoculated plant had the highest biomass compared to the un-inoculated ones, which statistically gave the lowest biomass yield. Effective rhizobium nodulation contributes to increased faba bean growth and yield parameters by supplying nitrogen to plants by fixing atmospheric nitrogen and converting it into plant-available nutrients. This result is consistent with the finding of [25] who reported that inoculation of bacterial rhizobia strains resulted in significant above ground biomass in faba beans. Gedamu et al. also showed that inoculation of rhizobia strains significantly affected the weight of faba bean biomass compared to without treatment inoculate [26]. The difference in biomass yield obtained from inoculation of faba bean is that Rhizobium strains could be due to the additional supply of nitrogen through the remarkable biological nitrogen fixation by the inoculated strains.

Effect of inoculation on the number of nodules

Inoculation with Rhizobium showed a significant increase in the number of nodules per plant. Table 3 shows that seed inoculation significantly affected the number of nodules/plant. A greater *($P \leq 0.05$)* number of nodules were obtained from all inoculated plants compared to the un-inoculated plants, since inoculation with effective rhizobia inoculate improves nodulation, this result indicated that the inoculation of these strains in the study area could be more appropriate and competitive than the existing native strains of faba bean rhizobia. [27] reported that inoculation of the Rhizobium strain with faba bean seeds produced highest nodules. Likewise, the results of [26] and [28] confirmed that inoculation of Rhizobium strains in faba beans significantly increased the number of nodules. Desta et al. [23] also reported that inoculation of rhizobia from faba bean significantly increases the number of nodules/plant.

Effect of inoculation on the number of pods Plant-1

As indicated in Table 3, inoculation with Rhizobium strains had a statistical effect on the number of seeds/pods compared to un-inoculated treatment. The number of pods/plants was affected by inoculation of all Rhizobium strains FB04, FB1035 and FB1018 increase in growth parameters of faba bean. Woldekiros et al. [27] reported that the number of pods per plant was significantly ($P < 0.05$) affected by inoculation of rhizobia. According to Desta et al. [23] and Gedamu et al.

Table 3: Combined mean of growth and yield parameters of faba bean affected by inoculation.

Treatments	Nodule number	Plant height(cm)	Pod plant^{-1}	Seed plant^{-1}
T1: Control	69.6d	90b	14.5b	33b
T2: 121 kg ha^{-1} NPS	89.4c	111a	27.5a	49a
T3: 60 g ha^{-1} NPS + FB 04	109c	110a	25.3a	47a
T4: 60 kg ha^{-1} NPS + FB 1035	118c	112a	29a	49a
T5: 60 kg ha^{-1} NPS + FB 1018	137a	110a	30.5a	51a
T6: FB 04+60 kg ha^{-1} TSP	121bc	105a	29.8a	49a
T7: FB 1035+60 kg ha^{-1} TSP	121bc	121a	29.5a	50a
T8: FB 1018+60 kg ha^{-1} TSP	135ab	112a	31.3a	55a
Mean	112	107	27.5	48
LSD (0.05)	15.5	12.2	5.62	9.53
CV (%)	11.8	9.7	17.4	16.9

LSD (0.05%): Least Significant Difference at 5% level; CV: Coefficient of Variation; Means in a column followed by the same letters are not significantly different at 5% level of significance.

[26], the rhizobia strain alone could significantly increase the number of pods/plants. This study disagrees with Zerihun and Abera [29] who showed that the number of seeds per faba bean pod was not significantly affected by fertilizer rate and rhizobia inoculation.

Effect of inoculation on plant height

The combined mean result in Table 3 showed that inoculation of seeds with Rhizobium increases plant height. Rhizobium inoculation increases faba bean growth parameters by increasing nitrogen supply. Bejandi et al. confirmed that seed inoculation significantly increases nitrogen uptake, thus improving plant growth and yield, and possibly increasing the potential of plants to produce more height [30].

Conclusion and Recommendation

Rhizobium inoculation significantly affected all parameters of the faba bean and improved grain yield. The inoculated plants gave the greatest yield benefit compared to the un-inoculated ones. All tested rhizobia strains together with TSP fertilizer performed better in ecologically competent and symbiotically effective in nodule formation and yield increase, therefore recommended for the study area and similar agro-ecologies.

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Conflicts of Interest

Authors declare that there are no conflicts of interest regarding the publication of this paper.

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