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ORIGINAL ARTICLE

Effectiveness of *Rhizobium* strains on faba bean (*Vicia fabae* L.) at Gumer district, highland area of Southern Ethiopia

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The biological fixation of Nitrogen by legumes is one of the well-known ways of fixing atmospheric nitrogen to plant-available form. However, effectiveness in nitrogen fixation depends on the genotype of legumes and requires host specific *Rhizobium* strain for effective nodulation to boost the productivity of legumes. Thus, the field experiment was conducted to evaluate the effectiveness of three *Rhizobium* inoculants of faba bean (FB 1018, FB 1035, and FB 04) laid in randomized complete block design with three replications from June 2019 to December 2020. Inoculation of faba bean revealed a highly significant ($p \leq 0.05$) effect on all parameters compared to the un-inoculated plants. Over years results showed that inoculated plants gave a significant increase in nodule number against the un-inoculated plants. Correspondingly, all the plants that treated with *Rhizobium* inoculums gave grain yield advantage over the control. The highest yield (5.875 ton/ha) was recorded from inoculates of FB 1018+60kg/ha TSP compared to the un-inoculated that gave 2.48 t/ha. *Rhizobium* strains undertaken for evaluation behave better in ecologically competent and symbiotically effective in nodulation for increased yield and thus recommended for the study area and similar agro-ecologies.

Keywords: Faba bean, Fertilizer, *Rhizobium* strain, Yield.

Introduction

Faba bean (*Vicia faba* L.) is a major grain belonging to the legume family and widely cultivated in many countries for source of dietary and feed purposes (Sillero, J.C, 2010). It accounts major food and feed legumes because of the high nutritional value of its seeds, which are rich in protein and starch (Duc et al. 2010). China has been the main leading country of faba bean production, followed by Ethiopia, Egypt, Italy, and Morocco. According to CSA (2018), faba bean production in Ethiopia accounts for over 3.45% of cultivated land with average national productivity of 2.1 tons ha^{-1}. Faba bean plays a major role by fixing atmospheric nitrogen to plant-available form (Siczek and Lipiec, 2016). Biological fixation of atmospheric nitrogen in legume-*Rhizobium* is well known eco-friendly practice used for the improvement of N fixation resulted in increased shoot growth, number of pods, and grain yield of faba bean (Siczek and Lipiec, 2016). Yadav and Verma, 2014 reported that the fixation of nitrogen by legumes accounts for 50% of 175 million tons of total biological N_2 fixation annually globally. However, the fixation of nitrogen depends on the genotype of legume, *Rhizobium* strain, and the interactions of these with the bio-physical environment and symbiosis nodulation of *Rhizobium* (Giller, et al. 2013). Therefore, the fixed amount of nitrogen varies with legume species and/or variety (Abdul-Aziz, 2013) and effectiveness of partner microsymbiont (Argaw, S. 2012).The report of Ouma et al., 2016 also confirmed the host-specific rhizobia strains of common bean and soybean adapted better to the local soil and environmental condition. To have a successful establishment, inoculants strain must be able to survive in soil environment because the better survival rate and soil persistence of *Rhizobium* enhanced the possibility of effective nodulation and nitrogen fixation (Knezevic-vukcevic, 2011). If not, poorly efficient *Rhizobium* strains may outcompete and gain an advantage over effective *Rhizobium* strains used for inoculation (Fujita et al. 2014). Although soil may harbor certain ineffective nodule forming native rhizobia, however effective nodule formation largely depends upon the competitiveness of inoculants strain (Laguerre et al., 2007). As a result inoculation with host-specific effective *Rhizobium* strains species is required for effective nodulation and nitrogen fixation (Goss, et al., 2002). In this scenario, inoculating faba bean with effective and appropriate rhizobial strain is crucial to improve symbiotic nitrogen fixation and to boost its productivity (McKenzi et al., 2001). Inoculation affects the microbial community by increasing desired rhizobia strain population in the rhizosphere (Siczek and Lipiec, 2016). Symbiotic performance of nodulation is highly governed by the abundance of effective rhizobia strain and its competitiveness (Laguerre et al., 2003). Thus, the present study has been initiated to identify best performing *Rhizobium* strains on faba bean for nodulation and increased yields for two consecutive years under rain fed conditions at Gumer District South Eastern Ethiopia.

Materials and Methods

Description of study area

A field experiment was carried out consecutive main cropping seasons for two years (2019 and 2020) under rain fed conditions at

Gumer, Guraghe Zone, Southern Nations Nationalities and Peoples' Regional State of Ethiopia. Experimental site is situated at 8°01'56.2"N and 38°01'58.3"E, and at altitude of 2767 m.a.s.l with temperature of min 7.5% and max 20%. The area receives a bimodal rainfall with an annual average rainfall of 1200 mm. Rainfall is distributed between the short rainfall season (March to April) and the main rainy season (June to September).Mixed crop-livestock farming is the dominant economic activity in the rural areas. The geographical location of the study area is highlighted at (Fig. 1).

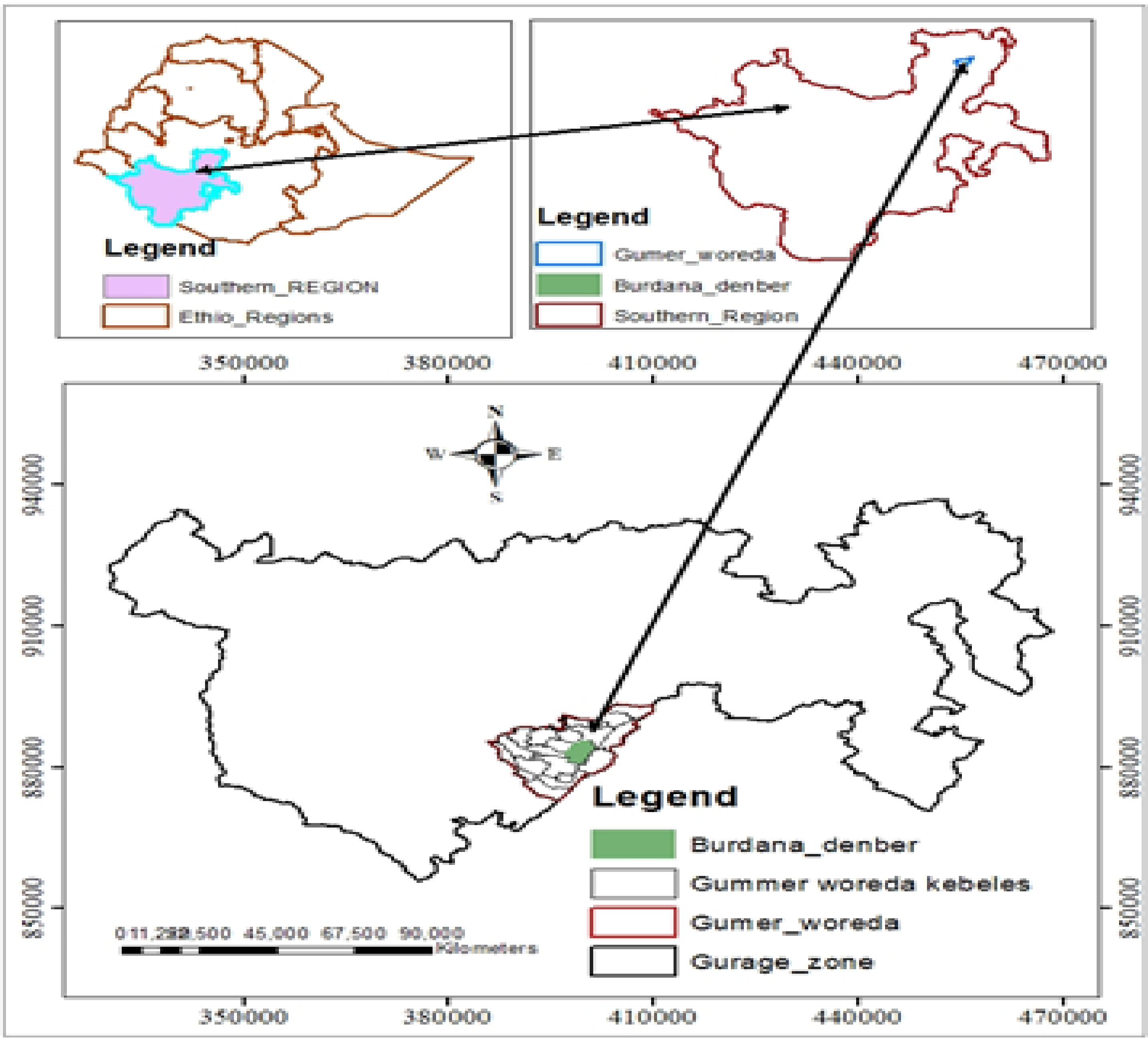

Fig. 1. Location map of the study area.

Experimental design and treatments

The experiment was laid out in randomized complete block design with three replications. Eight levels of treatments were (T1:Control, T2:121 kg/ha NPS, T3:60 kg/ha NPS+FB1018, T4: 60 kg/ha NPS+FB 1035, T5: 60 kg/ha NPS+FB04, T6: FB1018+TSP 60 kg/ha, T7: FB1035+TSP 60 kg/ha, T8: FB04+TSP 60 kg/ha). The plot size was 3 × 3 m (9 m^2) and improved Faba bean variety (Dosha) was used for experimentation at the spacing of 40 and 10 cm between rows and plats respectively. Inorganic fertilizer (NPS and 60 kg/ha P in the form of TSP) were applied at planting by drilling with faba bean seeds in the row. Carrier-based inoculants of faba bean were obtained from the Soil Microbiology Laboratory of Holeta Agricultural Research Centre (HARC). Seeds were immersed in warm water to be anchored *Rhizobium* stains. The sugar slurry was used as a sticker for carrier-based inoculants so that the inoculums sticking and coating to the seeds. The inoculated seeds allowed to air dry for a few minutes and planted immediately after drying in shade. Un-inoculated treatments were sown before the commencement of inoculation to avoid cross-contamination thoroughly.

Physical and chemical soil characteristics

Before commencement of the experiment, the experimental field was characterized for selected soil physical and chemical properties, soil samples were collected from 0-15 cm depth for initial determination of soil fertility parameters. The soil samples were analyzed for pH, available, exchangeable acidity P, % N, and % OM (Table 1).

Table 1. Chemical and physical properties of soil before planting.

pH	EA	BD	% OC	% TN	AP	CEC	Textural class			
							% sand	% clay	% silt	texture

5.9	2.69	0.99	1.1	0.094	1.28	41.2	70	14	16	Sandy loam

Agronomic data collection

The yield and yield attributed components: plant height, Nodule Number, number of pods per plant, number of seed per plant, above-ground biomass, and grain yield were collected and subjected to analysis of variance (ANOVA). The grain yield was determined from each experimental plot and adjusted to constant moisture levels of between 16.1% and 18%.

Statistical analysis

Data collected from the crop was subjected to analysis of variance using SAS software packages and mean separation was done using LSD (Gomez and Gomez, 1984) at a 5% probability level.

Results and Discussion

Effect of *Rhizobium* inoculation on grain yields

Over years mean showed that *Rhizobium* inoculation significantly (P<0.05) affected faba bean grain yield at this location. The highest yields were recorded from inoculated plants compared to un-inoculated. As it is shown in Table 2, among inoculated plant with FB 1018+60 kg/ha TSP, FB 1035+60 kg/ha TSP and F04+60 kg/ha TSP, numerically the maximum grain yield (5.875 ton ha^{-1}) was obtained from the inoculation of FB 1018+60 kg/ha TSP followed by FB 1035+60 kg/ha TSP and that gave 5.29 and 5.078 ton ha^{-1} respectively whereas statistically the lowest grain yield was obtained from the un inoculated.

This study agrees with the finding of Rugheim and Abdelgani (2012) who reported that inoculation of rhizobial strains significantly increased bean grain yield. Desta et al. (2015) was also confirmed that application of effective *Rhizobium* strain alone and/ or in combination with zinc significantly increased grain yield of faba bean. Reported of Youseif et al. (2017) also show that the application of effective strains increases the grain yield of faba bean up to 44-47%. Combined mean grain yields affected by inoculation of *Rhizobium* strain highlighted at Table 2.

Effect of inoculation on biomass yield

Rhizobium strains inoculation **s**ignificantly (*P* ≤ 0.05) affected biomass yield. From Table 2 indicates inoculated plant with FB 1018+60 kg/ha TSP, FB 1035+60 kg/ha TSP and FB 04+60 kg/ha TSP recorded highest biomass compared to un-inoculated that gave lowest biomass yield statistically. This result is in agreement with the finding of El Azeem et al., (2007) who reported inoculation of bacterial *Rhizobium* strain brought significant aboveground biomass on faba bean. Gedamu et al., (2021) also showed that *Rhizobium* strains inoculation significantly influenced faba bean biomass weight un-inoculated treatment. 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.

Table 2. Combined mean of biomass and grain yield affected by inoculation of *Rhizobium* strain.

Treatments	Combined mean of Biomass t/ha	Combined mean of Grain Yield t/ha
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.

Effect of inoculation on nodule number

Rhizobium inoculation showed asignificantly increase number of the nodules per plant. Table 3 shows that inoculation of strains significantly (P ≤ 0.05) affected nodule number/plant. A higher nodule number was obtained from all inoculated plants compared to uninoculated. This result revealed that inoculation of those strains may be best suited and competed in the study area compared to the existing native faba bean *Rhizobium* strains. Woldekros et al., 2018 reported that inoculation of *Rhizobium* strain with faba bean seed gave higher nodules. Correspondingly, Gedamu et al., 2021 and El-Khateeb et al., 2012 findings confirmed that inoculating of *Rhizobium* strain to faba bean significantly increased nodule number. Desta et al., 2015 also reported that inoculation of faba bean *Rhizobium* strains significantly increases nodule number/plant.

Effect of inoculation on number of pods plant-1

As it is indicated in Table 3, inoculation of *Rhizobium* strains statistically affected number of seeds/pods as compared to the un-inoculated treatment. The number of pods/plants was affected by the inoculation of all FB04, FB1035, and FB1018 *Rhizobium* strains. This increment might is attributed to the increment of faba bean growth parameters like plant height. Woldekiros et al. 2018 reported that the number of pods per plant was significant ($p<0.001$) affected by *Rhizobium* inoculation. According to Gedamu et al. and Desta et al., 2015, rhizobial strain alone could significantly increase the number of pods/plants. This study is in disagreement with Zerihun and Abera, 2014 showed that the number of seeds per pod of faba bean was not significantly affected due to fertilizer rate, *Rhizobium* inoculation, and lime rate.

Effect of inoculation on number of seed/plant

Rhizobium inoculation independently increased the seed yield as compared to un-inoculated seed (Table 3). This may be because inoculation of seeds with *Rhizobium* increases nitrogen uptake (Bejandi et al., 2012), and thereby plant growth and performance were enhanced. *Rhizobium* inoculations can increase the potential of plants to produce more plant height. Gedamu et al., 2021 reported that inoculation of seed gave significantly higher seed per plant. However, this finding contradicts with finding of Zerihun and Abera (2014) who indicated that inoculation of rhizobial strain did not impose a significant difference over the un-inoculated one. Woldekiros et al., 2018 also reported that the number of seeds per pod did not vary significantly ($p>0.05$) between inoculated and un-inoculated.

Effect of inoculation on plant height

Combined mean result in Table 3 revealed that inoculation of seeds with *Rhizobium* increase plant height. Bejandi et al., 2012 confirmed that seed inoculation significantly increase nitrogen uptake and thereby plant growth and performance enhanced with possibly increase the potential of plants to produce more plant height.

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 kg 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.

Conclusion

Rhizobium inoculation significantly affected all parameters of faba bean and improved grain yield. Inoculating legumes with host-specific competitive and effective *Rhizobium* is crucial to boost productivity. Inoculated plants gave the highest yield advantage compared to un-inoculated. All Strains along with 60 kg/ha TSP better in ecologically competent and symbiotically effective rhizobia in nodulation and thus, recommended for the study area and similar agro-ecologies in the region.

Conflicts of Interest

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

Authors' Contributions

All authors significantly contributed technical support and directly involved to experiment during entire period of investigation.

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