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# Determination of the Rate of Rhizobial Biofertilizers for Faba Bean (V. faba), in the Major Growing Areas of Arsi Zone

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**Abstract**
Different rates and types of isolates of rhizobia and their benefit have been presumed beneficial in regard to soil resource amendment and increase yield and yield parameters. The objective of this study was to assess the effect of different rates of bio-fertilizer. The treatments were consisted of six different rates (600, 750, 900, 1050, 1150 and 1250gm ha-1) of rhizobia (strain FB-1017) and recommended rates of balanced fertilizer laid out in randomized complete block design with three replications. The field experiment was conducted at four potential districts of Arsi zone, during the main cropping seasons of 2017/18 and 2018/19. Application of different rates of biofertilizer had effects an evident from significant difference ($p<0.05$) in agronomic and yield traits. In the first year(2017/18) the effects of treatments were not apparently significant ($p<0.05$) at Kulumsa but, at Limu Dima-1 rate 1025 gm/ha gave significantly 46%(1372kg/ha more better than recommended fertilizer rate ,other rates such as 750 gm $ha^{-1}$( at Kulumsa),900 gm $ha^{-1}$ (at Bekoji Negesso) and 1150 gm $ha^{-1}$ (at Gora Silingo ) performed poor and significant effect of treatment RCR was observed rather more pronounced which recorded 31.2%(798 kg/ha) more better than the 900 gm /ha rate(at Bekoji Negesso),24.8%(941kg $ha^{-1}$) than 725gm/ha (at Kulumsa) and 36%(1622kg $ha^{-1}$) than rate 1150 gm/ha (at Gora Silingo).With respect to harvest index and plant height all locations except Gora Silingo and Bekoji Negesso, were not significantly affected(($p<0.05$) by treatments. The year 2017/18 had better productivity in both grain yield and biomass than year 2018/19 and patterns of significant enhancement in yield by increased rate have been observed which generally consistent trend was observed by rate 1025 gm $ha^{-1}$ but more and more locations need to be assessed in order that the fluctuating environment and unknown rhizosphere would bring about important information.
**Keywords**: Arsi, Biological Nitrogen Fixation, FB-1017, Faba Bean
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## Introduction

Faba bean is a crop of high economic value with its edible seed serving as an important protein complement in the cereal based Ethiopian diet, particularly for the poor who cannot afford animal protein(Tamene Temesgen *et al*., 2015), that accounts for approximately 15 percent of protein intake which according to FAO (2014) would supplement the undernourished 36% of population. In Ethiopia, faba bean is also a suitable rotation crop with cereals and should be a component of a sustainable farming system(Tamene Temesgen *et al*., 2015).Furthermore, supply an important added value to the crop by fixing atmospheric nitrogen in symbiosis with soil bacteria, thus, reducing costs by less fertilizer use and minimizing impact on the environment by natural soil maintenance (IFPRI, 2010; Alghamdi *et al*., 2012). However its production is hampered by poor soil fertility, in that regard an alternative source of nutrient is required. Bio remedy to the dwindling production will be required when the indigenous micro flora are incapable of supporting adequate $N_2$ fixation.

Modern agriculture lost its sustainability owing to excess use of chemical fertilizers and harmful pesticides further leading to higher cost of cultivation, declined food security and safety, and finally the reduction in soil fertility(Saritha and Prasad Tollamadugu, 2019).

Biological nitrogen fixation is a central life supporting process that provides most of the fixed nitrogen needed to sustain life. Animals, including humans, rely on plants to supply a great deal of the energy and nitrogenous compounds required for survival. Plants are likewise dependent upon the availability of nitrogenous compounds produced from atmospheric $N_2$ either commercially or biologically by microbes. In this way, nitrogen fixation assumes significant importance in agriculture because good crop yields depend on an adequate supply of fixed nitrogen by which the biological process contributes about 65% of the total annual yield of fixed nitrogen(Fisher and Newton, 2002).

The purpose of this study , therefore, is designed with the objective increasing the yield and yield related parameters of faba bean by different rates of selected rhizobial strains The result will serve as base line data for future endeavor of utilizing biological nitrogen fixing system of faba bean to increase productivity into low-input agriculture besides to the thriving mono cropping agricultural system of the zone and utilizing and supplementing the currently adopted rates of few strains of rhizobia and generally in the region.

 

**Material and Methods**

**Study sites**

The study was undertaken for two years in four districts of two potential Faba bean production weredas namely Tiyo &Limu-Bilbilo (Figure 1) The soils are classified as follows: at Bekoji a haplic Nitisol, and at Kulumsa an intergrade between a haplic Nitisol and a luvic Phaeozem(Amanuel Gorfu *et al.*, 2000) where Wheat is dominantly produced with mean annual rain fall 823 mm and 1020 mm respectively of Kulumsa and Bekoji.

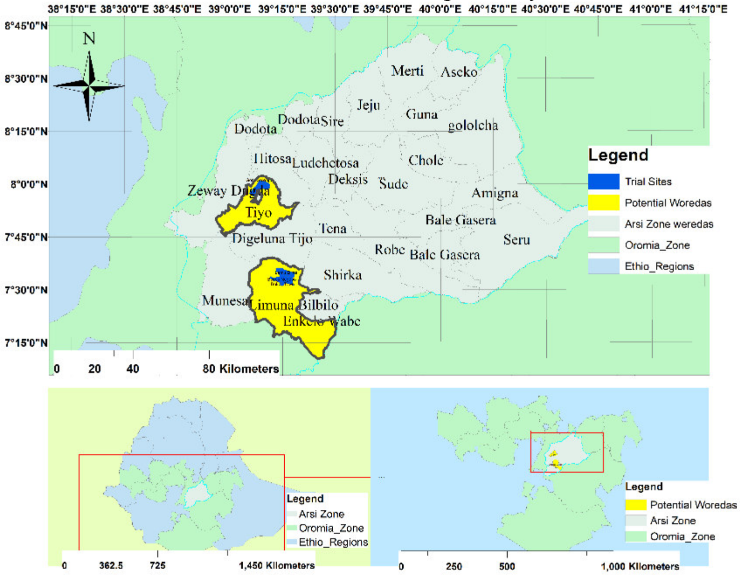


Figure 1 Map of Study area

Table 1 Soil chemical property (2018/19)

| Sites | pH(1:2.5 soil water ration) | Available P | % N | %OC | %OM |
|---|---|---|---|---|---|
| Bekoji Negesso | 5.16 | 0.50 | 0.21 | 3.02 | 5.21 |
| Kulumsa | 5.17 | 2.99 | 0.12 | 1.90 | 3.27 |
| Gora Silingo | 4.74 | 11.26 | 0.20 | 3.31 | 5.71 |

**Experimental Design,**

The Field trial were designed as Randomized Complete Block Design(RCBD) with three replication for which six treatments of different rates (600gm $ha^{-1}$,750gm $ha^{-1}$,900gm $ha^{-1}$,1050 gm $ha^{-1}$,1100gm $ha^{-1}$ and 1250gm $ha^{-1}$) of strain FB-1017 and two control treatments with null and one with balanced inorganic fertilizer.

 

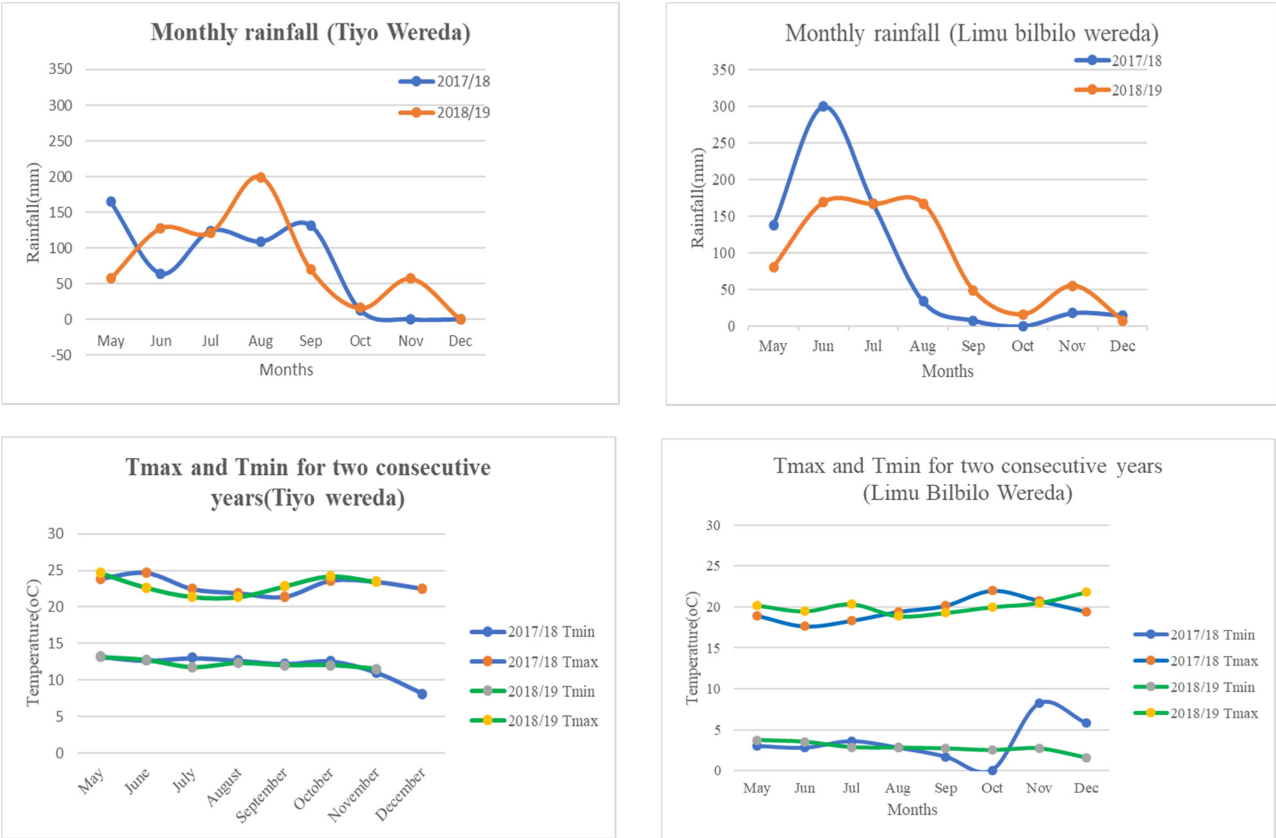


Figure 2 Rainfall and temperature patterns of districts during 2017/18 and 2018/19 growing seasons (**Source:** kulumsa Agricultural Research Center meteorological stations)

**Sources of seeds and Rhizobium strain**

Faba bean variety Dosha was supplied by Highland pulse research of Kulumsa Agricultural Research Center, Ethiopia. Variety was selected based on their yield, their maturity time and recentness of year of release. Strain of Rhizbium spp. (FB-1017) was obtained from Holetta Agricultural Research Center.

**Planting and Agronomic Practices**

Field experiments were carried out in the two successive years of growing seasons, 2017/18 and 2018/19 at four locations in Arsi zones where Faba bean production is at potential and monoculture production system is dominant. Faba bean seeds were sown in the rate of 100 kg seeds ha−1 and were cultivated in strips. Each block (4m× 24.5 m) consisted of eight plots. Each plot area was 10.4$m^2$ and consisted of 10 rows, spaced 0.4m apart. An additional eleventh row was placed in each plot and served as a border, and was not involved in calculations. Each strip was spaced apart by 1m apart to prevent bacterial migrations. Weeds, insects, and fungal pathogens were controlled by chemical spray applications, as required, at rates according to manufacturers' recommendations. At harvest, yield was determined by the manual mechanical harvesting of the entire plot.

**Statistical Analysis**

The data were subjected to analysis of variance(ANOVA) and statistically significant treatment means were separated and Least Significant Difference (LSD) test was used to separate treatment means at a probability level of 0.05 (SAS, 2009;R Core Team, 2019)

**Yield and Yield Components**

Yield and yield attributes of faba bean were recorded. Plant height (PH),Pods per plant (PPP),seeds per pod(SPP),Total grain yield (GY) per hectare adjusted to 10% moisture content, biological yield (TBY) per hectare and harvest index (HI) ,number of tillers per plant were determined.

**Results and Discussions**

Analysis in chemical soil nutrient indicated that the soils of the highland were more acidic but better in both nitrogen, phosphorus and organic matter content but special case for Gora silingo which is more acidic but had higher phosphorus (Table 1)Our result indicated that Inoculation of faba bean by different rates of biofertilizer had effect at different districts (Table 2 to Table 5).

Elucidating treatments differed with respect to years, in the first year(2017/18) the effects of treatments on grain yield were not apparently significant at Kulumsa and Limu Dima-2 but, different rates significantly($p<0.05$) affected Limu Dima-1 which rate 1025 gm/ha gave significantly, 46.8%(1332.9kg/ha) more in grain yield than recommended fertilizer rate and the same trend was followed for biomass yield in that Kulumsa (18495.97 kg $ha^{-1}$) which recorded twice as much the other two locations (Limu Dima-1= 8638.72 kg $ha^{-1}$,Limu Dima-2=7451.67kg $ha^{-1}$).

The tillering rate was also greater(2.69 $plant^{-1}$) at Kulumsa than Limu Dima-1(1.39 plant $^{-1}$),and Limu Dima-2(1.16) but treatment effects was not observed at Limu Dima-2 and Kulumsa, but at Limu Dima-1 all treatments did not respond except rate 1150 gm $ha^{-1}$ which was the least(1 $plant^{-1}$).

In the second year (2018/19) output in biomass yield and grain yield from the three locations were observed that the treatments had similar effects except rate 750 gm $ha^{-1}$ at Kulumsa,900 gm $ha^{-1}$ at Bekoji Negesso and 1150 gm $ha^{-1}$ at Gora Silingo which performed poor and significant effect($p<0.05$) of treatment RCR was observed rather more pronounced which recorded 31.2%(798 kg/ha) more better than the 900 gm /ha rate(Bekoji Negesso) , 24.8%(941kg $ha^{-1}$) than 725gm/ha (at Kulumsa) and 36%(1622kg $ha^{-1}$) than rate 1150 gm/ha (Gora Silingo) the trend for biomass yield was also similar to that of grain yield.

During the first year the mean plant height recorded was Kulumsa(159.8 cm) followed by Limu Dima-1(154.8cm ) and Limu Dima-2 (143cm) and it was observed that in both years treatments had no effect at Kulumsa with respect to plant height and harvest index and the drier season with early mature might have attributed to low harvest index ,TKW and HI are relatively greater at highland, tiller and plant height were better at the mid altitude ,despite the fact that the cultivar used(Dosha) released for both high and mid altitudes it was observed that it performed better at mid land(Kulumsa and Gora Silingo). Further reasons can be pointed out that, soil moisture, relatively low precipitation and higher temperature (Figure 2) during floral initiation when nodule initiation is maximum, acid soil, water logging, even though Faba bean has relatively shallow roots, thus the crop may suffer from drought stress in soils that dry quickly but seems to be tolerant to short period of water-logging(Etemadi *et al*., 2019) and competition, nutrient, such as phosphorous and combined nitrogen (NO3-, NH4+, and urea)has been demonstrated to influence symbiotic $N_2$ fixation from the initial bidirectional signal exchange between symbionts through to nodule senescence in combination to other factors such as water supply which the relationship between soil moisture and nodulation salinity and the proportion of root hairs containing infection threads is reduced by about 30% (Zhang and Smith, 2002) might contribute however, the observed performances were not as such consistent across locations. Inorganic Nitrogen is required by legume plants during the ‘nitrogen hunger period’ for their nodule development, shoot and root growth before the onset of $N_2$-fixation. The success of legume grain crops is dependent on their capacity to form effective nitrogen-fixing symbioses with root-nodule bacteria(Youseif *et al*., 2017) it was mentioned in other legumes that high N affect N fixation but interestingly, faba bean differs from many other legumes and maintains its N-fixing capabilities, even if soils are relatively rich in N(Etemadi *et al*., 2019). However, many soils may not have adequate amounts of native rhizobia in terms of number, quality, or effectiveness to enhance biological nitrogen fixation. Root lateral expansion and early biomass in relation to seed mass were the major traits influencing soil N uptake regardless of the level of soil N availability.

Faba bean was separated from other species having a higher nodule biomass, a higher $N_2$ fixation and a lower seed reserve depletion able to simultaneously fix $N_2$ and take up soil N(Dayoub *et al*., 2017). Although rhizobium bacteria remain alive for 3–5 years in soils(Etemadi *et al*., 2019), it is recommended to inoculate the seeds every year for achieving maximum N fixation and the study by Maj *et al*. (2010) demonstrated a plausible approach of isolating and characterizing flavonoid-responsive field isolates that could be further developed into relevant legume inoculants. Another reason that needs to be sought is tillage methods that affect many soil characteristics such as aeration, structure, temperature and water use, all of which affect the microbial composition, nitrogen fixation and nodulation. And it was identified that conservation tillage typically enhances nodulation and nitrogen fixation, through increased soil moisture retention and soil temperature, and increased soil microbial biomass. However, conservation tillage can lead to a reduction in soil pH and increased soil compaction, which can reduce nodulation(Torabian *et al*., 2019).

The general observation that increased rates of bio-fertilizer (Figure 3, Figure 4) is more or less aligned with in increment in almost all parameters was apparent in Arsi soils where N is limiting, and while Nitrogen is not limiting the crop yields could be strongly improved by means of competitive and viable strains inoculation but fertilization might not help. From our results found that response to different rates of strains was greater or comparably better than the full N fertilization that faba bean inoculation could effectively reduce the need of applied inorganic N- fertilizers while achieving higher grain yield, this apparent comparative advantage and alternative to the use of inorganic N fertilizer also un economical use , that was also confirmed by different authors in similar districts (Amare Tadesse et al., 2017;Wendesen Melak *et al*., 2019).

 

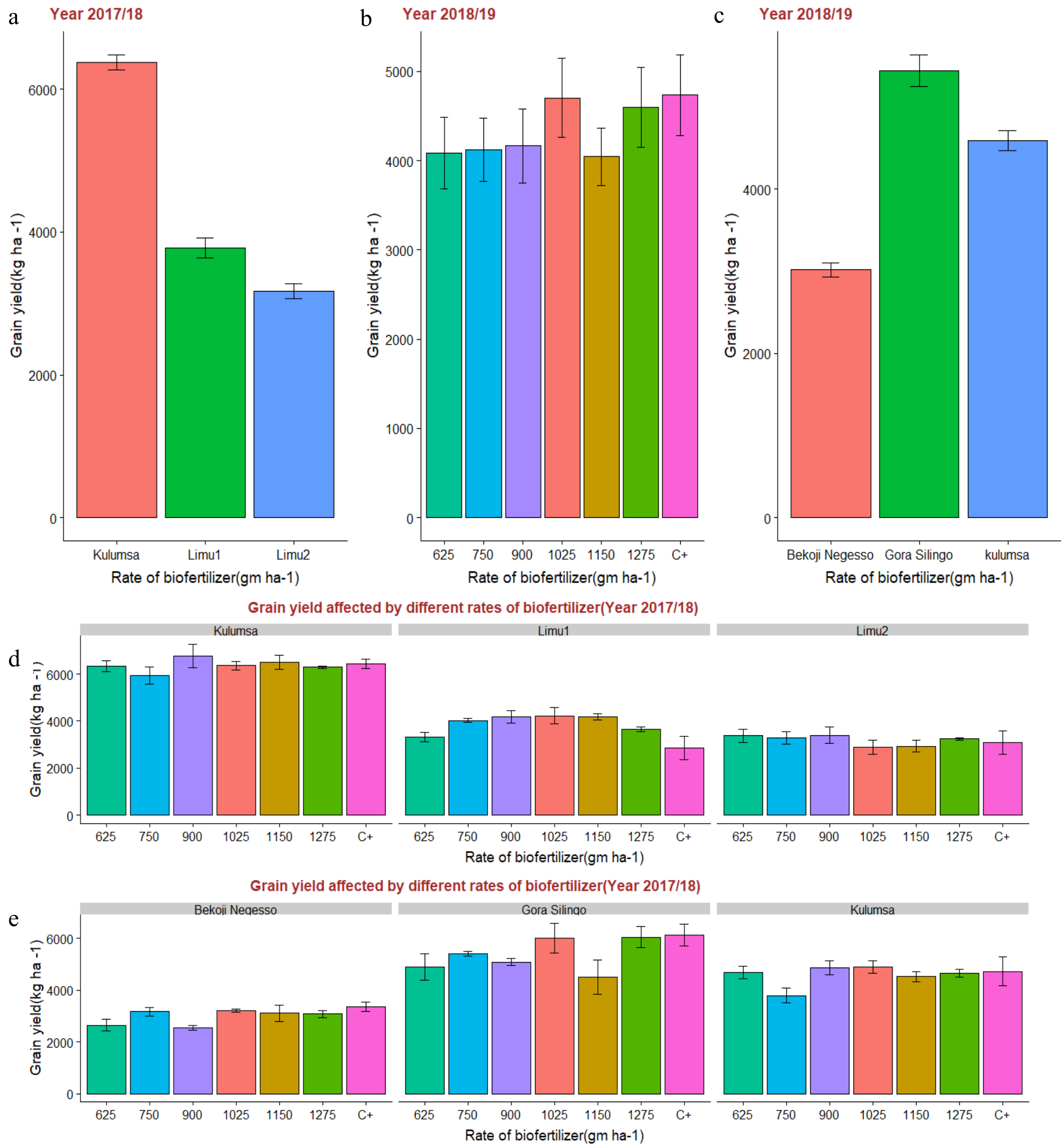


Figure 3 Grain yield affected by different rates of bio-fertilizer (FB-1017) and effects in different district(a-e)

 

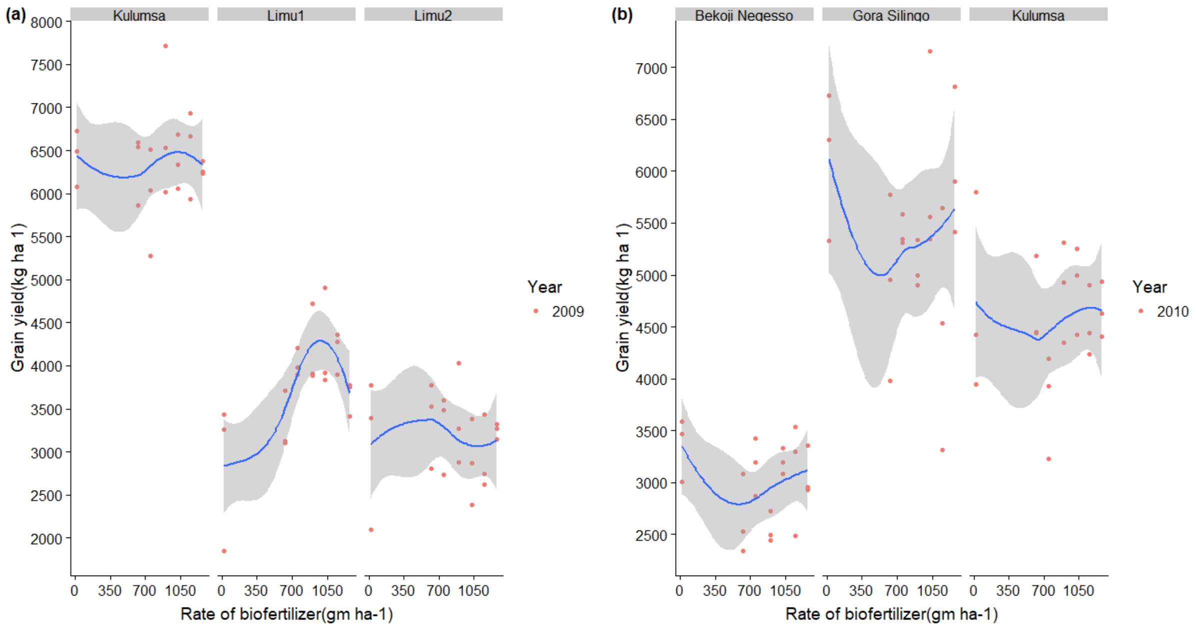


Figure 4 General observed trend of grain yield affected by different rates of bio-fertilizer (FB-1017)

Table 2 Total biomass yield, grain yield and tiller per plant of faba bean var Dosha along different rates of bio-fertilizer (FB-1017) at two potential districts at year 2017/18

| Treatments | TBY(kg ha$^{-1}$) | | | GY(kg ha$^{-1}$) | | | Tiller(plant$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Limu Dima 2 | Limu Dima 1 | Kulumsa | Limu Dima 2 | Limu Dima 1 | Kulumsa | Limu Dima 2 | Limu Dima 1 | Kulumsa |
| 625 | 7648.82a | 7665.05bc | 18419.06a | 3366.45a | 3315.80bc | 6334.81a | 1.07a | 1.60a | 3.27a |
| 750 | 8017.87a | 9394.41ab | 17164.07a | 3274.02a | 4026.39ab | 5939.99a | 1.13a | 1.60a | 2.53a |
| 900 | 8501.60a | 9914.25a | 20338.34a | 3392.97a | 4173.53ab | 6754.25a | 1.20a | 1.20ab | 2.40a |
| 1025 | 6786.57a | 9317.42ab | 17616.01a | 2878.40a | 4217.45a | 6363.32a | 1.33a | 1.33ab | 2.53a |
| 1150 | 6563.11a | 9332.15ab | 19066.86a | 2933.70a | 4177.99ab | 6507.99a | 0.93a | 1.00b | 2.80a |
| 1275 | 7782.83a | 8342.67abc | 17591.29a | 3242.85a | 3648.09abc | 6287.88a | 1.13a | 1.53a | 2.87a |
| C+ | 6860.90a | 6505.10c | 19276.15a | 3087.89a | 2845.05c | 6433.34a | 1.33a | 1.47a | 2.47a |
| **Mean** | **7451.67** | **8638.72** | **18495.97** | **3168.04** | **3772.04** | **6374.51** | **1.16** | **1.39** | **2.69** |
| **CV (%)** | **19.58** | **12.56** | **13.10** | **17.21** | **12.86** | **7.93** | **20.34** | **18.65** | **19.06** |
| **LSD(0.05)** | **2595.69** | **1929.97** | **4311.33** | **970.07** | **863.00** | **899.99** | **0.42** | **0.46** | **0.91** |

Table 3 Total al biomass yield and grain yield of faba bean var Dosha along different rates of biofertilizer (FB-1017) at four potential districts at year 2018/19

| Treatments | TBY(kg ha$^{-1}$) | | | GY(kg ha$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | Bekoji Negesso | Kulumsa | Gora silingo | Bekoji Negesso | Kulumsa | Gora silingo |
| 625 | 6343.29ab | 13247.08ab | 11577.53a | 2650.71bc | 4690.30ab | 4901.69ab |
| 750 | 6871.36ab | 11960.70b | 10775.05a | 3163.03ab | 3784.81b | 5416.50ab |
| 900 | 5995.16b | 14525.31ab | 10957.29a | 2555.63c | 4861.10a | 5079.52ab |
| 1025 | 7072.30ab | 13262.59ab | 12617.41a | 3203.80ab | 4888.21a | 6018.85a |
| 1150 | 6973.43ab | 13514.75ab | 9864.34a | 3108.25abc | 4526.07ab | 4499.35b |
| 1275 | 6836.51ab | 16130.34a | 12677.24a | 3083.12abc | 4658.34ab | 6044.52a |
| C+ | 7588.94a | 13521.61ab | 11365.29a | 3353.97a | 4726.07ab | 6121.78a |
| **Mean** | **6811.57** | **13737.48** | **11404.88** | **3016.93** | **4590.70** | **5440.34** |
| **CV(%)** | **11.94** | **15.59** | **18.39** | **10.98** | **11.94** | **14.58** |
| **LSD(0.05)** | **1447.19** | **3779.43** | **3699.85** | **589.53** | **967.03** | **1399.45** |

*Means followed by the same letter within a column are not significantly different at the P=0.05 level using LSD test(GY=grain yield, TBY=Biomass yield, HI=harvest index)*

Table 4 Thousand kernel weight, Harvest index and plant height of faba bean var Dosha along different rates of bio-fertilizer (FB-1017) at two potential districts at year 2017/18

| Treatments | TKW | | | HI (%) | | | PH (cm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Limu Dima 2 | Limu Dima 1 | Kulumsa | Limu Dima 2 | Limu Dima 1 | Kulumsa | Limu Dima 2 | Limu Dima 1 | Kulumsa |
| 625 | 798.10ab | 770.79a | 726.81a | 49.45a | 48.04a | 36.57a | 141.33a | 153.00a | 157.33a |
| 750 | 784.02ab | 765.81a | 722.91a | 45.66a | 47.59a | 36.97a | 147.00a | 155.00a | 163.67a |
| 900 | 816.96a | 776.81a | 708.03a | 44.60a | 46.15a | 34.99a | 148.67a | 155.33a | 162.33a |
| 1025 | 781.67ab | 779.66a | 701.69a | 47.28a | 47.61a | 38.59a | 138.00a | 154.00a | 150.67a |
| 1150 | 756.48b | 784.77a | 703.93a | 50.15a | 48.25a | 36.45a | 141.67a | 158.33a | 154.67a |
| 1275 | 752.86b | 767.05a | 728.35a | 47.29a | 48.01a | 38.61a | 144.67a | 162.67a | 162.33a |
| C+ | 797.82ab | 772.95a | 700.27a | 50.03a | 48.34a | 35.51a | 140.00a | 145.33a | 167.67a |
| **Mean** | **783.99** | **773.98** | **713.14** | **47.78** | **47.71** | **36.81** | **143.05** | **154.81** | **159.81** |
| **CV(%)** | **3.81** | **2.05** | **2.92** | **7.42** | **6.89** | **12.03** | **5.44** | **7.48** | **6.55** |
| **LSD(0.05)** | **52.66** | **28.19** | **30.04** | **6.25** | **5.85** | **7.88** | **13.85** | **20.60** | **18.63** |

Table 5 Thousand seed weight and Harvest index and plant height of faba bean var Dosha along different rates of biofertilizer(FB-1017) at four potential districts at year 2018/19

| Treatments | TKW | HI (%) | | | PH (cm) | | |
|---|---|---|---|---|---|---|---|
| | Bekoji Negesso | Bekoji Negesso | Kulumsa | Gora silingo | Bekoji Negesso | Kulumsa | Gora silingo |
| 625 | 857.20a | 51.96a | 32.53a | 41.01b | 100.00ab | 155.67a | 166.00a |
| 750 | 800.76a | 55.55a | 30.73a | 50.14a | 100.67ab | 159.67a | 152.67ab |
| 900 | 754.67a | 52.20a | 30.73a | 43.95ab | 92.00b | 155.67a | 154.00ab |
| 1025 | 739.04a | 55.01a | 34.51a | 44.96ab | 103.33a | 160.33a | 159.33ab |
| 1150 | 914.09a | 53.46a | 32.78a | 43.66ab | 98.67ab | 158.67a | 150.67ab |
| 1275 | 768.76a | 54.44a | 26.13a | 43.53ab | 103.00a | 160.33a | 164.00ab |
| C+ | 759.60a | 54.04a | 32.94a | 47.80a | 103.00a | 149.00a | 142.67b |
| **Mean** | **799.16** | **53.80** | **31.48** | **45.01** | **100.09** | **157.04** | **155.62** |
| **CV(%)** | **17.87** | **4.38** | **20.44** | **6.70** | **5.27** | **6.54** | **8.47** |
| **LSD(0.05)** | **239.86** | **4.18** | **11.35** | **8.45** | **9.38** | **17.87** | **23.26** |

*Means followed by the same letter within a column are not significantly different at the P=0.05 level using LSD test(TKW=thousand kernel weight,HI=harvest index,PH=plant height)*

**Conclusions and recommendations**

Different strains for biological nitrogen fixation and rates have been utilized in decades and still being tried to help reconciling the soil fertility, productivity concerns and feeding millions. In this study it was observed that crop cultivars as well as acid tolerant, adapted to high and fluctuating microclimates ought to be concerned when rhizobia rates are studied, the study demonstrated that the strains soil interaction had caused the greater extent of difference in agronomic and yield parameters with relative increments in rates, in so doing the recurring issues of global climate change, population growth, dwindling soil fertility and loss of productivity the need for intervention of crop diversification amendments will be plausible.

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