Original Research Article||

Growth, nodulation and yield response of field-grown faba bean (*Vicia faba* L.) to *Rhizobium* inoculation in Tocha District, Southern Ethiopia

Aynalem Kutafo[1]* and Yehuala Alemneh[1]

[1]*Southern Agricultural Research Institute (SARI), P.O. Box 06, Hawassa, Ethiopia*

Abstract

Inoculation with effective and crop specific *Rhizobial* strain of bacteria is necessary to improve symbiotic nitrogen fixation and optimize faba bean productivity. Field experiment was conducted at Tocha district, southern Ethiopia during 2017-2019 main rainy seasons to evaluate the effects of Rhizobium strain on the yield and yield components of faba bean. The experiment consisted of 7 treatments: control, FB1018, FB1035, FB04, 50 kg ha^{-1} TSP + FB1018, 50 kg ha^{-1} TSP + FB1034 and 50 kg ha^{-1} TSP + FB04. A starter N-dose (9 kg $N{\cdot}ha^{-1}$) was applied with different Rhizobium inoculants except for the control. Soils of the experimental plot before planting was silt loam in texture, moderately acidic in pH with high levels of organic carbon, total nitrogen and cation exchange capacity, and medium content of phosphorous. The experiment was laid out in Randomized Complete Block Design with three replications. Inoculation of FB1018 strain significantly ($p < 0.05$) increased the plant height (cm), number of pods per plant, above-ground biomass (kg), and grain yield (kg) as compared to the control. The maximum grain yield (3354.2 kg ha^{-1}) and minimum (1942.7 kg ha^{-1}) were obtained from the application of FB1018 strains and control treatment, respectively. Therefore, application of FB1018 strain with 9 kg $N{\cdot}ha^{-1}$ can be recommended to improve Faba bean yield at Tocha distric and other areas with similar agro ecology and soil conditions.

Key words: Faba bean, Fertilizer, *Rhizobium* strain, Yield

***Corresponding author's address:** Aynalem Kutafo, Email: aynuaa71@gmail.com
Coauthor: Yehuala Alemneh; email: alemnehyehuala@gmail.com

INTRODUCTION

Faba beans is one of the legume crops capable of fixing nitrogen in an endosymbiosis association with root nodule bacteria. Ethiopia is one of the primary centers of diversification for faba beans that are produced in many regions of Ethiopia. The crop occupies about 26.9% of the total land area under pulse crops in the country (CSA, 2016). It is the most commonly cultivated pulse crops in Southern Nations Nationalities and Peoples Region (SNNPR). About 63,279.03 ha of land was covered with the crop and 104,076.6 tones yield was produced with average productivity of 1.65 tons ha^{-1} in 2015/16 cropping seasons (CSA, 2016). About 8,312 ha of land was covered with the crop in the study area and 13,239.1 tons of grain yield was produced with average productivity of 1.59 quintals per ha in 2015/16 cropping season (CSA, 2016).

Despite its multifaceted benefits, the productivity of faba bean, both national and regional, 18.93 and 16.39 quintal ha^{-1}, respectively, remained low compared to its attainable yield, which is >2 t /ha^{-1} (MoA, 2011; CSA, 2015). Biological nitrogen fixation, especially rhizobia-legumes symbiosis, is one of the alternative solutions and the promising technologies which play an important role in decreasing the consumption of inorganic N-fertilizers, enhancing soil fertility, and reducing the production cost, (Peoples et al., 1995). Nitrogen (N_2) fixed by nodulated legumes (pulses and oilseeds) is estimated to contribute 21.45-million-ton N annually to global agricultural systems (Herridge et al., 2008).

External seed inoculation of rhizobia is also one of sustainable and environment friendly practices to increase the nitrogen fixation potential of the crops since there might be low population of effective indigenous rhizobia or due to maximum competitions with non-effective ones (Tolera et al., 2009). However, the information on contribution of different *Rhizobium* strain alone and with fertilizer on yield and yield components of faba bean in Tocha district is limited. Therefore, the objective of this study was to identify optimum performing *Rhizobium* strains on yield and yield components of faba bean in the study area.

MATERIALS AND METHODS

Description of the study duration and area

The experiment was conducted from 2017 to 2019 main cropping season on farmers' field in Tocha district, Dawro Zone, Southern Ethiopia. The district is located at 499 km south of Addis Ababa, 247 km west of Hawassa and 39 km of Tarcha which is the principal city of Dawro Zone. The district has been receiving 600 - 2300 mm mean annual rain fall with a minimum and maximum temperatures of 19ºC and 28ºC, respectively over the experimentation periods. Mixed farming system is a dominant agricultural activity in the study area.

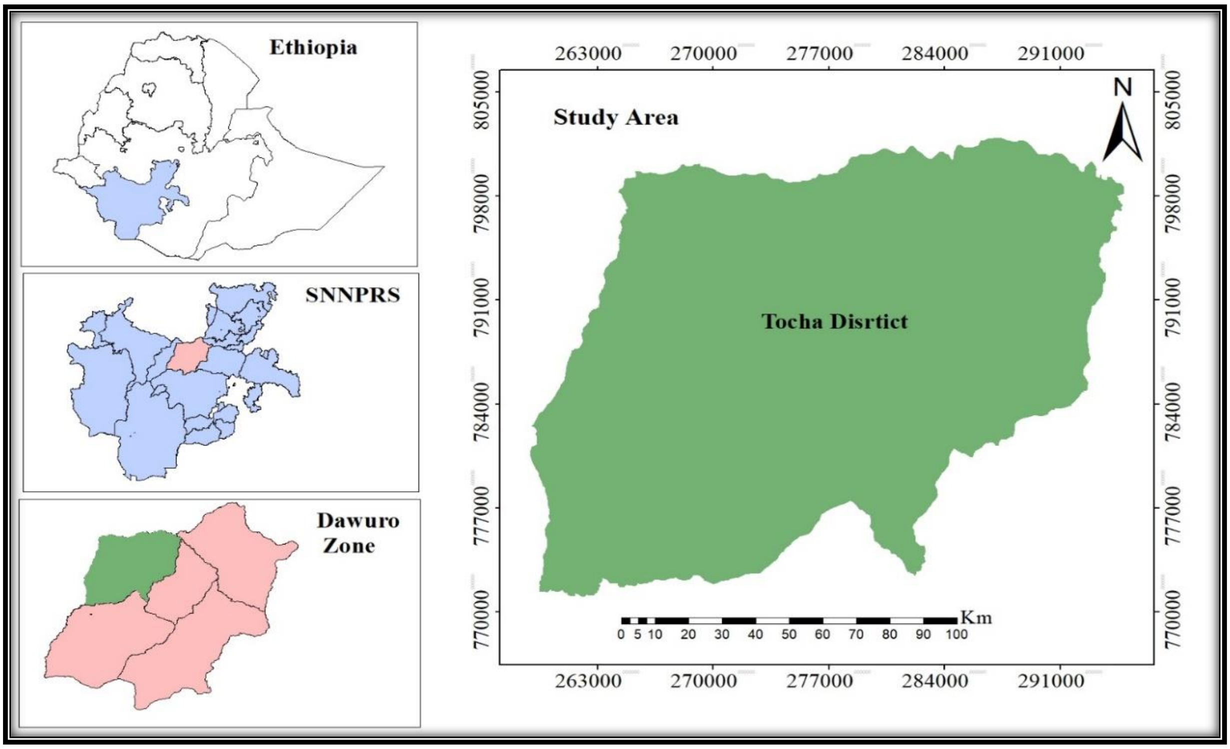

Figure 1. Map of the study area

Source: Authors - Extraction from ArcGIS Map 10.7.1

Soil Sampling and Analysis

Composite surface soil samples (0–30 cm depth) were collected from experimental area by zigzag sampling method before planting for determination of selected physicochemical properties of the soil. The collected samples were air dried at room temperature and were ground to pass through a 2 mm sieve for most parameters and through 0.5 mm sieve to determine total nitrogen and organic carbon. Soil particle size distribution was determined by hydrometer method (Bouyoucus, 1951). Soil pH was determined by potentiometric method at soil:water ratio of 1:2.5 (Van Reewijk, 1992). Cation exchange capacity was determined by 1 M ammonium acetate method at pH 7 (Chapman, 1965). Organic carbon was determined by the dichromate oxidation method (Walkley and Black, 1934). Total nitrogen was determined by the conversion of organic carbon and available P was analyzed by Olsen method (Olsen *et al.*, 1954).

Treatments, Design and Procedures

Faba bean seeds were surface sterilized by mercuric chloride (0.1%) for 2 minutes and rinsed with and soaked in distilled water for a minute. For inoculation with peat-based inoculants, seeds were moistened in sugar solution (48%) only to those inoculated treatments before application to get a thin uniform coating of inoculums on seeds immediately before sowing. The treatments consisted of 1) control (without fertilizer and strain), 2) FB1018, 3) FB1034, 4) FB04, 5) 50 kg ha^{-1} TSP + FB1018, 6) 50 $kgha^{-1}$ TSP + FB1034, and 7) 50 $kgha^{-1}$ TSP + FB04. A starter N-dose (9 kg N ha^{-1}) was applied in combination with the *Rhizobium* strains except for the control and the treatments were laid out in randomized complete block design (RCBD) with three replications.: The plot size was 4 m by 4 m with 1 m spacing between plots and blocks, with the seeds planted 10 cm and 40 cm spacing between plants and rows, respectively. TSP fertilizer (rate) was applied at the planting time and all other management practices were also applied uniformly as per site recommendations starting from field preparation to harvesting.

Data Collection and Measurement

Plant height (cm) from the ground level to the tip of 5 randomly taken plants at physiological maturity were taken. Total number of nodules was determined by counting from five plants from each net plot (2.4 m by 2.4 m) area at 50% flowering. Nodules from the plant roots were removed carefully. The nodules were separated from the soil by washing and the total numbers of nodules are determined by counting. Number of pods per plant was determined by counting from 5 randomly taken representative samples from each net plot (2.4 m by 2.4 m) area at harvest. Total above-ground biomass (kg ha^{-1}) was also taken after harvesting from aboveground plant parts from each net plot. Grain yield (kg ha^{-1}) was determined after threshing the seeds from each net plot. The seed yield was adjusted to a 12.5% moisture level and converted to kg ha^{-1}.

Statistical Analysis

SAS statistical software (SAS, 2007) was used for analysis of variance and mean separation among treatment was done by least significant differences (LSD) at $p<0.05$ probability level.

RESULTS AND DISCUSSION

Physicochemical Properties of the Soil

The analyzed soil results showed that soil particle size distribution of the experimental site was in proportions of 39, 54 and 7 % of sand, silt, and clay, respectively (Table 1). Based on USDA (1998) soil textural classification, the textural class of the soil in the experimental are is silt loam, which is suitable for faba bean production. The soil pH of the experimental field before planting showed that the pH value of the soil was 5.62. According to Jones (2003), the rating of soil pH was moderately acidic. The pH of the soils in the study area was within the satisfactory range for faba bean production as well as for most agricultural crops (Landon, 1991). The OC and TN content of the experimental site were 3.54 and 0.18%, respectively, which is rated as under high range (Tekalign 1991). The high TN contents indicated that the soil of the experimental site was sufficient in available N to support proper growth and development of agricultural crop production.

The analysis result also shows that available P content of the experimental site was 13.97 ppm, which made it rated as medium (Jones, 2003), indicating that the soil has probable yield responses to P application (Olsen et al., 1954). Based on the analysis, the cation exchange capacity (CEC) of the experimental site soil was 35.4 meq/100 g (Table 1). According to Landon (1991) rating, the CEC soil of the study site was under high class and adequate for crop production. Furthermore, such high CEC value provides the soil with maximum buffering capacity so that one can apply the required amount of fertilizer dosage without any immediate negative effects on the soils.

Table 1. Some physical and chemical properties of soil of experimental site before planting

Soil Properties	Values	rating	Reference
% sand	39		
% silt	54		
% clay	7		
textural class		silt loam	
Soil pH	5.62	Moderately acidic	Jones, 2003
Organic carbon (%)	3.54	very high	Tekalign, 1991
Phosphorus (ppm)	13.97	medium	Jones, 2003
Total nitrogen (%)	0.18	high	Tekalign, 1991
Available potassium (ppm)	59.55	low	Jones, 2003
Cation Exchange Capacity (meq/100g)	35.4	high	Landon, 1991

Growth Parameters

Plant Height

The plant height of faba bean was significantly ($p< 0.05$) affected by the application of different types of *Rhizobium* strains alone and with TSP fertilizer (Table 2). The maximum plant height (131.67 cm) and the minimum plant height (111.6 cm) were recorded from the treatment FB 1018 and control, respectively (Table 2). The lower plant heights obtained at the control treatments might be due to retarded growth caused by the imbalance in nutrient availability.

The significant increase in plant height in response to the application of *Rhizobium* inoculation might be 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. Similarly, Sameh et al., (2017) reported that plant height of faba bean was significantly increased upon inoculation by all tested strains as compared with uninoculated treatments (control). 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. In conformity with this result, Nyoki and Ndakidemi (2014) reported that *Rhizobium* inoculation in cowpea significantly improved the plant height measured at four, six and eight weeks after planting relative to the control treatment under both green house and field experiments.

Total Number of Nodules

The result showed that the total number of nodules per plant of faba bean was non-significantly ($p< 0.05$) affected by the *Rhizobium* strains alone and with TSP fertilizer. The maximum number of nodules per plant (29) was recorded from the treatment FB 1018 while the minimum number (21) was recorded from control treatment (Table 2). The result is also in conformity with the finding of Abebe and Tolera (2014), who reported that inoculation of *Rhizobium* strain did not significantly increase nodules per plant in contrast with uninoculated seeds on faba bean. Numerically, *Rhizobium* inoculation increased number of nodules per plant compared to un-inoculated treatment (control) which could be due to the fact that inoculated bacteria strain had good nodulation inducing capacity.

Table 2. Yield and yield component of faba bean as affected by *Rhizobium* strains

Treatments	NNo	Plant Height (cm)	Pod Number	AGB kg ha^{-1}	Seed Yield kg ha^{-1}
Control	21	111.6^{b}	12.8^{c}	4968.8^{b}	1942.7^{c}
FB1018	29	131.67^{a}	16.2^{a}	6750^{a}	3354.2^{a}
FB1034	20	126.3^{ab}	13.87^{bc}	5687.5^{ab}	2520.8^{b}
FB 04	23	125.93^{ab}	16.47^{a}	6270.8^{a}	2604.2^{b}
50kg ha^{-1} TSP + FB1018	26	128.53^{a}	15.83^{ab}	6304.2^{a}	2814.2^{ab}
50kg ha^{-1} TSP + FB1034	24	127.47^{a}	17.6^{a}	6125^{a}	2614.6^{b}
50kg ha^{-1} TSP + FB04	28	128.37^{a}	16.3^{a}	6322.9^{a}	2708.3^{b}
CV	21.2	6.9	8.1	12.1	10.4
LSD	NS	15.49	2.23	1299.6	479.31

Means in the table followed by the same letter(s) are not significantly different 5% level of significance, LSD (0.05) =Least significant difference at 5%; and CV (%) =Coefficient of variation; AGB = above ground biomass yield kg ha^{-1}; NNo= number of nodules per plant

Yield and Yield Components

Number of Pods per Plant

Significant ($p<0.05$) effects of *Rhizobium* strains alone and with TSP fertilizer were observed on the number of total pods per plant (Table 2). The highest number of total pods per plant (17.6) was recorded at an application rate of 50 kgha^{-1} TSP + FB1034, whereas, the lowest number of total pods (12.8) was obtained from the control (Table 2). The result is also in conformity with the finding of Birhanu (2021) who reported that the highest number of pods per plant (15) was recorded from the combination of 240 g ha^{-1} NPSZnB and 500 g ha^{-1} of *Rhizobium*; while, the lowest number of pods per plant of faba bean was recorded from the control plots. Similarly, Bezabih et al. (2018) reported that number of pods per plant showed significant response to phosphorus fertilization and *Rhizobium* inoculation. This result was also in agreement with the study by Çiğdem Kucuk (2011) who indicated that inoculation had given significantly higher number of pods per plant for common bean over the control (no fertilizer and no inoculant).

Total Above-ground Biomass

It was observed that the different types of *Rhizobium* strains alone and with TSP fertilizer had a significant ($p<0.05$) influence on the aboveground biomass production (Table 2). The study showed that the maximum aboveground biomass of 6750 kg ha^{-1} was obtained from plots receiving FB1038 of *Rhizobium* strains alone whereas the minimum above-ground biomass of 4968.8 kg ha^{-1} was obtained from plots received no treatments (control). Application of FB1018 of *Rhizobium* strains alone improves aboveground biomass production by 35.85% as compared with control treatments. The resort from the current trial was in line with the result of Sameh et al., (2017), who reported that Faba bean plants inoculated with *Rhizobium* strains recorded the maximum root dry weight (2.53 g/plant) and shoot dry weight (14.9 g/plant) with significant increases over the control.

Grain Yield

It was revealed that the application of *Rhizobium* strains alone and with TSP fertilizer brought significant ($p<0.05$) effect on grain yield increments (Table 2). The result showed that the maximum grain yield (3354 kg ha^{-1}) was recorded from the application of FB1018 *Rhizobium* strains and the minimum grain yield (1943 kg ha^{-1}) was obtained from control treatments (Table 2). Application of FB1018 of *Rhizobium* strains alone improved grain yield production by 72.6% from the control. The observed yield improvements with *Rhizobium* inoculation might be due to the increased N as result of atmospheric nitrogen fixation by effective strain application. In line with this result, Abebe and Tolera (2014) reported that inoculation significantly increased grain yield in faba bean. Sameh et al., (2017) observed that significantly higher grain yield (4.36 ton ha^{-1}) was obtained from inoculated seed as compared to control. Nyoki and Ndakidemi (2014) also reported that *Rhizobium* inoculation significantly improved the yield and yield components such as number of pods per plant, number of seeds per pod, number of seeds per plant, and seed yield over the control. Similarly, Abera and Tadele (2016) also reported the maximum grain yield (2416 kg ha^{-1}) from seed inoculation with *Rhizobium* strain (HB-129). Likewise, Bezabih et al., (2018) observed that *Rhizobium* inoculation independently increased the seed yield as compared to uninoculated seed, which might be due to the fact that inoculation of seeds with *Rhizobium* enhances nitrogen uptake (Bejandi *et al.*, 2012) and thereby plant growth and performance increases.

In general, inoculation gave significantly higher grain yield for the faba bean crops in the experimental site. Maximum yield with the *Rhizobium* inoculation could be due to the sufficient availability of nutrients in the soil and better nodulation under the influence of inoculation resulting in better growth and development, which might be attributed to enhanced allocation of photosynthates towards the economic parts.

Relationship between Faba Bean Yield and Yield Components

The result indicated that the parameters such as grain yield, above-ground biomass, number of pods per plant, plant height, and number of nodules per plant were positively and significantly correlated to each other but number of pods per plant are non-significantly correlated with number of nodules per plant. The comparable investigation was reported by (Paponov *et al.*, 1996) in which they reported that, highly significant ($p<0.01$) and positive correlation between nodule number, fresh weight, and grain yield of fenugreek.

Table 3. Pearson's simple correlation coefficient (r) of relevant parameters of faba bean

	NodN	PH	PodN	AGB	GY
NodN	1				
PH	0.57**	1			
PodN	0.32NS	0.54*	1		
AGB	0.72***	0.75***	0.45*	1	
GY	0.66***	0.69***	0.41*	0.78***	1

*** very high significant, ** highly significant, * significant, NS= none significant, NodN = Nodule number per plant, PH= Plant height, PodN= Pod number per plant, AGB= Above Ground Biomass yield per hectare, and GY= grain yield per hectare

CONCLUSION AND RECOMMENDATION

The analysis of soil samples collected before planting indicated that the experimental site had insufficient amount of soil fertility status for faba bean crop production. The inoculation of faba bean with *Rhizobium* strains alone and with TSP fertilizer revealed a significant improvement of the plant height, number of pod per plant, above-ground biomass, and grain yield as compared to the control Application of FB1018 Resulted in the highest grain yield. These results indicated that inoculation of faba bean with effective *Rhizobium* strains can reduce use of inorganic fertilization and improve productivity of crop yield. The use of FB1018 strain with 9 kg/ha starter N can be recommended for soils of the study area and similar agro-ecology.

Acknowledgements

The authors thank Southern Agricultural Research Institute for funding the experiment and Areka Agricultural Research Center for monitoring and evaluating the activities. All the technical and field assistants of Natural Resource Management divisions are also acknowledged for unreserved effort during executionof the experiment.

REFERENCES

Abebe Zerihun and Tolera Abera 2014. Yield Response of Faba bean to Fertilizer Rate, *Rhizobium* Inoculation and Lime Rate at Gedo Highland, Western Ethiopia. *Global Journal of Crop, Soil Science and Plant Breeding*, 2(1): 134-139. [Scholar Google]

Abera Habete and Tadele Buraka 2016. Effect of *Rhizobium* inoculation and Nitrogen fertilization on nodulation and Yield response of common bean at Boloso Sore, Southern Ethiopia. *Journal of Biology, Agriculture and Healthcare*, 6 (13): 72-75. [Scholar Google]

Bejandi, T.K., Sharifii, R.S., Sedghi, M., and A. Namvar 2012. Effects of plant density, *Rhizobium* inoculation and micro elements on nodulation, chlorophyll content and yield of chickpea (*Cicer arietinum* L.) *Annals of Biological Research*, 3:951-958. [Scholar Google]

Bezabih Woldekiros, Walelign Worku and Girma Abera 2018. Response of Faba bean (Vicia faba L.) to *Rhizobium* Inoculation, Phosphorus and Potassium Fertilizers Application at Alicho Wuriro Highland, Ethiopia. *Acad. Res. J. Agri. Sci. Res*, 6(6): 343-350. [Scholar Google]

Birhanu Gebeyehu 2021. Effects of *Rhizobium* Inoculation and Blended Fertilizer Rate on Yield and Yield Components of Faba Bean (Vicia faba L.) in Dangila District, Northwestern Ethiopia. *International Journal of Research in Agricultural Sciences*, 8(1): 55-63. [Scholar Google]

Bouyoucos, J. 1962. Hydrometer method improved for making particle size analysis of soil. *Agronomy Journals*, 54:464-465. [Scholar Google]

Chapman, H.D. 1965. Cation exchange capacity by ammonium saturation. Pp. 891-901. *In*: C. A. Black, L. E. Ensminger and F. E. Clark (Eds.). *Method of Soil Analysis. American Society of Agronomy*. Madison Wisconsin, USA. [Scholar Google]

Çiğdem Küçük 2011. Inoculation with *Rhizobium* spp. in kidney bean (*Phaseolus vulgaris* L.) varieties. *Agriculture*, 98:49–56. [Scholar Google]

CSA (Central Statistical Agency) 2015. The Federal Democratic Republic of Ethiopia Central Statistical Agency. Agricultural sample survey, volume I, report on area and production of major crops (private peasant holdings, 2014/15 meher season), statistical bulletin 578, Addis Ababa, Ethiopia.

CSA (Central Statistical Agency) 2016. Area and production of major crops private peasant holdings, Meher season, in 2015/2016. Central Statistical Authority (CSA), Addis Ababa, Ethiopia.

Herridge, D.F.; Peoples, M.B.; Boddey, R.M. 2008. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil*, *311*: 1–18. [Scholar Google]

Jones J.B. 2003. Agronomic Handbook: Management of Crops, Soils, and Their Fertility. CRC Press LLC, Boca Raton, FL, USA. [Scholar Google]

Landon, J.R. 1991. Booker tropical soil manual Hand Book for soil survey and agricultural land evaluation in the tropical and sub tropics. Long man scientific and technical, ESSXEX, New York.

MoA (Ministry of Agriculture) 2011. Animal and plant health regulation directorate. Crop variety register. Issue No. 14. Addis Ababa, Ethiopia. pp. 71-73.

Nyoki, D. and Ndakidemi, P.A. 2014. Effects of *Brady Rhizobium japonicum* inoculation and supplementation with phosphorus on macronutrients uptake in cowpea (*Vigna unguiculata* L.) Walp. *American Journal of Plant Sciences*, 5: 442-451. [Scholar Google]

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US. Dep. Agric., Circ.939, USA. [Scholar Google]

Paponov, I., Aufhammer, W., Kaul, H. P., Ehmele, F.P. 1996. Nitrogen efficiency components of winter cereals. European Journal of Agronomy, 5: 115–124. [Scholar Google]

Peoples, M.B.; Herridge, D.F.; Ladha, J.K. 1995. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? *Plant Soil*, *174*: 3–28. [Scholar Google]

Sameh H. Youseif, Fayrouz H. Abd El-Megeed and Saleh A. Saleh 2017. Improvement of Faba Bean Yield Using *Rhizobium*/Agrobacterium Inoculant in Low-Fertility Sandy Soil. *Article in Agronomy*, DOI: 10.3390/agronomy7010002. [Scholar Google]

SAS (Statistical Analysis Systems) 2007. SAS/STAT user‟s guide Version 9.1 Cary NC:SAS Institute Inc. USA

Tekalign Tadesse 1991. Soil, plant, water, fertilizer, animal manure and compost analysis. Working Document No. 13. International Livestock Research Center for Africa, Addis Ababa, Ethiopia. [Scholar Google]

Tolera A, Daba F and Zerihun A 2009. A review of organic and Biological Soil Fertility Management integrated with NP on Crops Yield and Soil Fertility Improvement in High and Mid Altitude Areas of Western oromiya, Ethiopia. Improved natural Resource management Technologies for Food Security, Poverty Reduction and Sustainable Development. Ethiopian Society of Soil Science, March 22-27, 2009, EIAR, Addis Ababa, Ethiopia

Van Reewijk, L.P. 1992. *Procedures for Soil Analysis (3rd Edition).* International Soil Reference Center, Wageningen, Netherlands, 371p. [Scholar Google]

Walkley, A. and Black, I. 1934. An examination of Degt jareff methods for determining soil Organic matter and a proposed modification of the chromic acid titration method. Wiley-Intern Science Publication, 37: 29-38. New York, USA. [Scholar Google]