

Research Article

Effect of Bio-fertilizers on Growth Performance and Nodulation of Faba Bean (*Vicia faba* L.) on Nitisols of Anded District, North-western Ethiopia

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Abstract

The increased dependency of applying excessive synthetic input of chemical fertilizers not only increased farmers cost of production but also has caused several environmental problems related to greenhouse effect, soil deterioration, and air and water pollution. Hence, the inoculation of bio-fertilizers could be a desirable alternative as they make the soil more sustainable and environmental-friendly; indeed, the growing crops using bio-fertilizers are worthy for human health. A field experiment was conducted to evaluate the effect of bio-fertilizers on nodulation and growth of faba bean under limed and unlimed soil conditions. The treatments includes four types of strains [(BF-17, BF-04, BF-1018 and BF-1035), Control, 21kg ha^{-1} and 150 kg ha^{-1} urea under limed and unlimed condition) was arranged in split plot design with three replications. The growth and nodulation parameters were collected following standard data collection procedure and analyzed using SAS software 9.1.3 version. The study revealed that the highest shoots height (97.73cm) and root length (26.27cm) was recorded from plots treated with FB-17 and FB-04 strains under limed condition. Similarly, maximum value of shoot dry weight (24.87gm) and root dry weight (4.57gm) was measured from plots treated with FB-1018 strain under limed condition. Moreover, inoculation of fababean seeds with FB-17 strain under limed condition produced the highest nodule dry weight (0.4gm) and plant height (141.0cm) and in case of nodule number, the maximum number was recorded from plots treated with FB-04 strain together with lime. While in all measured parameters, the lowest value was recorded from the control plots. Therefore, for better growth and nodulation of fababean, it is recommended to inoculate seeds either FB-04 or FB-17 strains under limed soil condition in acidic areas.

Keywords

Acidity, Growth, Nodulation and Strains

1. Introduction

Faba bean (*Vicia faba* L.) is among cool season food legume crops based on area of production and foreign exchange earnings. In Ethiopia faba bean production accounts for over 3.45% of cultivated land with average national productivity

of 2.1t ha^{-1} [4]. It has also a great contribution for sustainable soil fertility management due to its ability to fix atmospheric nitrogen (N) in a form available to plants [28]. Therefore, it is a very valuable legume crop that contributes to the sus-

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Received: 14 June 2024; Accepted: 5 July 2024; Published: 6 August 2024



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tainability and diversification of cropping systems through improving soil health, decreasing disease, insect pest and weed buildup [17]. It is also grown for green manure production or as a rotation crop with cereals. However, the crop shows stunted in growth and inefficiency in nodulation due to the use of intensive agricultural system that has been mostly accomplished due to the use of synthetic chemical fertilizers.

An increased dependence of modern agriculture especially using an excessive, imbalanced, and steady synthetic input of chemical fertilizers has caused deterioration of soil quality. Furthermore, excessive supply of chemical fertilizers to soil than used by the crops gets stored in plants and often causes potential losses (by leaching and acidification) and environmental pollution problems by emissions of greenhouse gases like nitrous oxide (N_2O) from fertilizer production and application [23]. Because of the above-mentioned drawbacks of chemical fertilizers, it is essential to reduce the consumption of chemical fertilizers in agriculture without having any adverse effect on crop production by the incorporation and usage of harmless, renewable inputs of fertilizers. The most suitable alternatives for chemical fertilizers are bio-fertilizers that include living organisms.

Biological fixation of atmospheric nitrogen in legume-Rhizobium association is well known eco-friendly, environmentally safe and cost effective practice used for the improvement of N_2 fixation resulted in increased shoot growth, number of pods and seed yield of faba bean [28]. However, several biotic and abiotic factors contributed to stunted growth and poor nodulation of which poor soil fertility, acidity of the soil in high rain fall areas and low existence of effective indigenous rhizobium population are the most important constraints [11]. To have a successful establishment, inoculated strains must be able to survive in a health soil because the better survival rate and soil persistence of Rhizobium enhanced the possibility of effective nodulation and nitrogen fixation.

Among abiotic constraints, soil acidity is one that globally known to reduce nodulation and growth of faba bean [9, 22]. Research finding demonstrated that a commercial strain of *Bradyrhizobium japonicum* increased soybean yield in Ethiopia, as reported by [16], although the effectiveness of the strain was inconsistent across locations. Hence, despite the potential to improve yield and soil fertility, biological N_2 fixation by soybean in areas with acid soil is greatly constrained. The inconsistent results of inoculation with strain may therefore be related to soil acidity. Improving the soil through amelioration of soil acidity by the addition of only lime is expensive and a more cost-effective approach rather it needs integration with inoculation of suitable acid-tolerant strains [14].

Acid soils can be productive through application of lime and use of biological strains in different parts of the world because liming causes a significant increase soil pH and, thus, a change in microbial biomass in the soil system [19], mi-

crobial dynamic and diversity on N mineralization. However, the contribution of lime with different rhizobium strains on nodulation and growth of faba bean at Anded district had not been investigated in which faba bean was one of the potential legume crops. Therefore, this experiment was implemented to investigate the effect of integrated use of rhizobium strains with lime and their interaction on growth and nodulation of faba bean.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted on Nitisols of Debre Markose Agricultural Research Center Anded district, north-western Ethiopia during main cropping season. Its elevation is 2493 meters above sea level (masl). The experimental site is geographically located at $10^{\circ} 16'.5''$ N latitude and $037^{\circ}46'.47.2''$ E longitude. The total annual rainfall of the area ranges from 1040 to 1559mm. Mean annual maximum temperature is $31^{\circ}C$ and the minimum being $11.2^{\circ}C$.

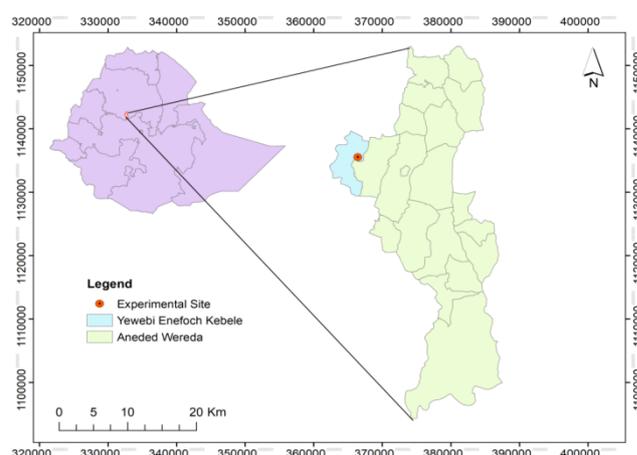


Figure 1. Study map of the experimental area.

2.2. Soil Sampling and Analysis

Prior to conducting the experiment, representative composite soil samples were collected from experimental site at 0-20cm depth and analyzed following standard laboratory procedures. The collected soil samples were air-dried and ground to pass through a 2mm sieve mesh size. Soil pH was determined (1:2.5 soil to water ratio) using a glass electrode attached to a digital pH meter [26], soil texture was determined by hydrometer method [6]. Walkley and Black method was used for organic carbon determination, and wet oxidation Kjeldahl method was used to determine Total N [15]. Exchangeable acidity was determined through titration with a standard NaOH solution based on the procedure described by [21]. Available P was determined using Olsen extraction

method [24]. The cations exchange capacity (CEC) of the soil was determined following ammonium acetate extraction (at pH=7).

2.3. Experimental Design and Procedure

The experiment consists of 14 treatments (Control, four types of strains [FB-17, FB-04, FB-1018 and FB-1035], 21kg ha⁻¹ and 150 kg ha⁻¹ urea under limed an unlimed soil condition) arranged in split plot design with three replication. The amount of lime applied was determined based on the exchangeable acidity, mass per 0.15m furrow slice and bulk density of the soil, considering the amount of lime needed to neutralize the acidity of the soil up to the permissible level for crop growth.

The total experimental area consists of 42 treatments accommodating ten rows plot⁻¹ and gross plot area was 3m*4m (12m²). The plots were kept with 0.1m and 0.4m spacing between plants and each row respectively. High yielding and popularly accepted Tumisa variety was used as a test crop. Planting was done early June according to farmer's local planting calendar where the seeds were put on a hill and covered with thin soil on ridges made. The thick slurry of the inoculant was mixed gently with dry seed so that all the seeds received a thin coating of the inoculant. All inoculations were done just before planting under shade to maintain the viability of bacterial cells. The inoculated and uninoculated seeds were then planted separately. Seeds were covered immediately with soil after sowing to avoid the death of bacterial cells due to direct sun light radiation. Triple super phosphate (46% P₂O₅), used as a source of P was applied to all plots uniformly during sowing time, while N in the form of urea was applied in splits to avoid losses through leaching, i.e. half during sowing time and the remaining half rate was applied a month after planting. All cultural practices (hoeing, weeding) were done uniformly for all treatments, as per the recommendation for faba bean production in the area.

2.4. Data Collection and Analysis

2.4.1. Growth and Nodulation Parameters

Five representative plants were selected randomly from the second border rows of each plot when the plant attains 50% flowering stage for recording growth and nodulation parameters. Then the whole plant was carefully uprooted using a spade to obtain intact roots and nodules to record nodulation parameters and dry weight of plants. Uprooting was done by exposing the whole-root system to avoid loss of nodules. The adhering soil was removed by washing the roots with intact nodules gently with clean water. Shoots were then cut from the roots at the collar, and partitioned into root and shoot. Similarly, five representative plants from each plot were used to record number of nodules per plant and nodule dry weight.

2.4.2. Statistical Analysis

The measured data were subjected to statistical analysis using SAS 9.1.3 version software [27] and the interpretation was made following the procedure of [12]. Least Significance Difference (LSD) at 5% probability level was used to assess differences among treatment means when significant differences were observed by the analysis of variance.

3. Results and Discussion

3.1. Physico - Chemical Properties of the Soil

The experimental soil was highly acidic in reaction (pH = 4.90) according to the rating done by Tekalign [29] and clay in textural class with clay (46%), silt (16%) and sand (38%). Total N and CEC of the soil, before planting were found to be 0.19% and 17.80 cmol (+)/kg which was rated as medium, according to [29, 13] respectively. The organic carbon was 2.14 and rated as medium according to [29]. The value of available phosphorous and carbon to nitrogen ratio (C: N) were found 7.33 ppm and 11.26% and rated as low, according to [29, 18] respectively. The lower nutrient level and other chemical properties indicated that the experimental soil had some limitations with regard to its use for crop production.

3.2. Shoot Height

The data on shoot height showed that, as compared to the control plots, inoculation of faba bean seeds combined with lime produce significant improvement (Table 1). The highest shoot height (97.73cm and 97.60cm) was recorded from plots treated with FB-17 and FB-04 strains under limed soil conditions respectively, while lowest shoot height (46.93cm) was recorded from control plots. This improvement in shoot height was equivalent to 109.59% over the negative control and 35.60% over the positive control. The superior performance in shoot height might be due to a synergistic effect between the two factors (strains and lime) which in turn favored height increments by minimizing the effect of soil acidity. This enhancement of shoot height could be attributed to the fact that rhizobia may augment plant growth by providing products of N₂ fixation and lime through making the neutral and make P available. In addition, the inoculated bio-fertilizers (strains) enhance plant growth by synthesizing growth-promoting chemicals. This result was in consistent with the finding of [1] who reported that fababean inoculation showed pronounced improvement in shoot height compared with negative control (untreated) plots.

3.3. Root Length

The ANOVA result showed that there was a positive interaction effect on fababean root length due to integrated

application of bio inoculants with lime. The maximum root length (26.33cm) was recorded from plots treated with FB-04 strain along with lime which was statically at par with inoculation of FB-17 strain together with lime i.e. (26.27cm) while the lowest root length (15.8cm) was recorded from the control plots indicating when the soil is acidic P, which is the master key for root development becomes deficient unless the soil is treated. The maximum value recorded from combined use of bio inoculant with lime might be due to the availability of P via liming because phosphorus nutrition benefits the plants by producing deeper and abundant roots in the soil system. Moreover, the significant increases in root length with the integration of lime and strain inoculation could attributed to the general improvement of the soil environment in terms of decreased acidity, increased availability of plant nutrients (especially P) and microbial establishment that in turn increased downward growth of roots, and thereby increased over all fababean vegetative performance.

In addition, liming acidic soils increase the available P through inactivation or precipitation of exchangeable and soluble Al and Fe hydroxides, increases soil pH, available P, exchangeable cations and percent base saturation, and enhancing the density and length of root hairs for P uptake [30] which is in line with the present result. Hence, toxicity arising from excess soluble Al, Fe and Mn is corrected through liming so that root growth is promoted, and uptake of nutrients is improved. Liming also stimulates microbial activities and enhances fixation and mineralization of N, thereby legumes benefit highly from liming as Al toxicity and acidity suppresses microbial activities and nutrient cycling [20].

3.4. Shoot Dry Weight

The ANOVA result indicates inoculating fababean seed with FB -1018 strain coupled with lime recorded highest

shoot dry weight (24.87gm) compared to un-inoculated plots that gave lowest shoot dry weight (2.87gm). The increase in shoot dry weight due to integrated use of lime and strains to the soil probably creates more favorable physicochemical conditions in the soil, such as reduced aluminum toxicity and increased nutrients availability, which ultimately enhances faba bean growth because application of bio inoculants along with lime provide growth-regulating substances and improves the physicochemical and microbial properties of soils.

Furthermore, there is reduction of Al toxicity, which restricts roots growth and creates difficulty in accessing nutrients and water from a longer distance in the soil. Finally, application of lime along with strains could improve root nutrient uptake of the plant through promoting its growth. The current result was in agreement with the finding of [7] who reported that inoculation of bacterial Rhizobium strain with lime brought significant aboveground biomass yield on faba bean.

3.5. Root Dry Weight

The ANOVA result showed that the highest root dry weight (4.57gm) value was recorded from inoculation of fababean seeds with FB-1018 strain coupled with lime while the lowest root weight (1.23gm) was measured from the control plots. The highest value of root dry weight recorded from the inoculated coupled with limed plots suggests that fababean responded to strains and lime and influenced their root growth and development. Moreover, results of this study indicate that application lime for root growth and development due to availability of P and rhizobium inoculation for nodulation collectively enhances nodulation, plant growth and development. The current result was in line with [3] who observed that both inoculation and P fertilizer application significantly influenced root dry matter of soybean.

Table 1. Effects of strains and lime on shoot height, root length, shoots weight and root weight of faba bean.

Rhizobium inoculants	Shoot height (cm)		Root Length (cm)		Shoot dry weight (gm)		Root dry weight (gm)	
	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed
FB-17	97.73a	81.00cd	26.27a	21.00cd	19.53b	12.87cde	4.40ab	3.33b-d
FB-04	97.60a	85.80bc	26.33a	21.07cd	19.53b	14.20cd	4.2abc	3.43a-d
FB-1018	95.80ab	85.60bc	24.27abc	22.47a-d	24.87a	14.40c	4.57a	3.07cd
FB-1035	87.7abc	80.23cd	22.30cd	21.53cd	16.13bc	13.17cde	3.17cd	2.53de
Control	62.27e	46.93f	22.20cd	15.80e	6.73fg	2.87h	1.67ef	1.23f
21 kgha ⁻¹ urea	68.47e	61.47e	23.20abc	18.93de	9.47efg	6.27gh	2.27def	2.33def
150 kgha ⁻¹ urea	82.40cd	72.07de	23.80abc	20.53cd	10.57de	10.33ef	2.37de	3.03cd
Mean	84.57	73.30	24.05	20.19	15.26	10.59	3.24	2.71
LSD (0.05)	10.93		3.99		3.83		1.20	

Rhizobium inoculants	Shoot height (cm)		Root Length (cm)		Shoot dry weight (gm)		Root dry weight (gm)	
	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed
CV (%)	8.27		10.78		17.73		24.13	

Means followed the same letter within a column are not significantly different from each other at $P < 0.05$.

3.6. Nodule Number

The ANOVA result showed that there was highly significant difference ($P < 0.01$) among treatments on number of effective nodules plant^{-1} due to inoculation of strains coupled with lime compared to uninoculated (control) plots. The highest number of nodules plant^{-1} (139.00) was recorded from plots treated with inoculation of FB-04 strain coupled with lime while the lowest nodule number plant^{-1} (9.00) was recorded from the control plots. The highest nodule number plant^{-1} produced from inoculation of strains along with lime might be due to the effectiveness of the inoculated strains when the soil of the experimental site is treated with lime. On the other hand, inoculation of strains might be effective when the indigenous microbial population in the soil is insufficient; the N content of the soil is low and when the soil acidic level is optimum for survival of microorganisms. The current result is in consistence with the finding of [5] who reported that increasing of nodule number when the seeds treated by rhizobium strain indicated that the native rhizobium population density nodulating the legume might not be adequate and this in turn brought response of the plant to inoculation of the rhizobium strains. Similarly, Otieno [25] reported that rhizobium inoculation significantly increase the number of nodules plant^{-1} .

In addition, inoculation of strains may be best suited and competed in the study area compared to the existing native fababean Rhizobium strains. The current result was in line with finding of [31] who reported that inoculation of Rhizobium strain with fababean seed gave higher nodules. Correspondingly, [8, 10] findings confirmed that inoculating of Rhizobium strain to fababean significantly increased nodule number significantly compared to uninoculated seeds.

3.7. Nodule Dry Weight

The analysis of variance result revealed that inoculation of fababean seeds under limed condition significantly increases

nodule dry weigh. The highest dry nodule weight (0.4gm) was recorded from plots treated by FB-17 strain along with lime while the lowest value was recorded from control plots indicating when the soil is acidic in reaction almost no nodulation is expected. The highest nodule dry weight recorded with the strains FB-17 and the lowest nodule dry weight recorded from the control might be the result of high and low nodules number respectively. In addition, the highest nodule dry weight recorded from plots treated with FB-17 strain coupled with lime might be the result of effective biological N_2 fixation as affected by effective compatibility of this strain. This finding was in conformity with [5] who reported that nodule number and nodule dry weight was positively correlated.

3.8. Plant Height

Based on the ANOVA result, the value of plant height ranges in between 15.00 to 141.93cm where the maximum value was recorded from plots treated with FB-17 strain under limed soil condition while lowest value was recorded from the control plots. The highest plant height obtained from rhizobial inoculation together with lime might be due to the maximum provisions of nitrogen from the soil through biological N_2 fixation as compared to control plots. However, the lowest plant height from the control plots might be due to the higher acidity problem of the soil that hinders nutrients availability and performance of rhizobium in the soil. The current result was in line with finding of [2] who confirmed that seed inoculation significantly increase N uptake and thereby plant vegetative growth.

In general, separate application of strain and lime alone was not adequate to increase the growth and nodulation of fababean significantly when compared with integration of lime and bio inoculants in acidic soil conditions. This indicates that the synergistic effect of agricultural lime along with effective strains was significant in ameliorating soil acidity and increasing overall economy of legume crops as we observe from the current result.

Table 2. Interaction effect of strains and lime on plant height, nodule number and nodule weight of faba bean.

Rhizobium inoculants	Nodule dry weight (gm)		Nodule number		Plant height (cm)	
	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed
FB-17	0.40a	0.20a-d	116.87abc	92.40c	141.93a	104.07cd
FB-04	0.33ab	0.27a-d	139.0a	106.53abc	134.47ab	113.47bcd
FB-1018	0.37ab	0.21a-d	134.67ab	99.20bc	140.33ab	117.33abc
FB-1035	0.23a-d	0.30a-d	98.40bc	98.60bc	133.13ab	116.93bc
Control	0.03d	0.03d	21.07de	9.60e	88.93de	15.00f
21 kg ha^{-1} urea	0.10b-d	0.03d	33.73de	21.73de	96.93cde	74.20e
150 kg ha^{-1} urea	0.07cd	0.07cd	52.00d	22.13de	119.13abc	88.80de
Mean	0.22	0.16	85.11	64.31	122.12	87.83
LSD (0.05)	0.27		39.23		26.94	
CV (%)	36.33		21.40		15.34	

Means followed by similar letter within a column are not significantly different from each other at $P < 0.05$.

4. Conclusion

According to the results obtained, we can conclude that growth and nodulation of faba bean have been improved through the inoculation of rhizobial strains coupled with lime when the soil is acidic in reaction. Among the treatments observed, FB-17 and FB-04 strains showed superior performance compared to the remaining treatments. Hence, these rhizobial isolates are the best candidate for the development of commercial rhizobial inoculants of faba bean in acid prone faba bean growing areas.

In addition, inoculation of rhizobium together with lime increased nodulation of faba bean with better response. For better health of the soil and reducing cost of production, it is better to use seeds treated by bacteria but further study should be done by considering other soil and crop factors. Therefore, for the current result application of lime together with appropriate rhizobium strains could be a suitable combination, from an economic benefit, to ameliorate soil acidity and improve soil fertility for sustainable fababeen production with improved productivity in Anded district. However, to draw a conclusive recommendation, the study has to be repeated over several seasons as lime and bio inoculants have long-term effects on improving soil properties.

Author Contributions

Habetamu Getinet is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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