

Bio-Organic Amendments Enhanced Growth, Nodulation, and Nutrient Uptake of Faba Bean in Acidic Soils of Sidama Region, Ethiopia

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Abstract

Faba bean (*Vicia faba* L.) production and productivity in the highlands of Ethiopia are considerably low due to acidic soil conditions. Enhancing soil fertility and increasing nutrient accessibility through optimized agronomic practices is essential for improving productivity. Hence, this study was conducted under controlled conditions in a lath house to examine the effects of coffee husk biochar and *Rhizobium* inoculation on the agrosymbiotic and nutrient uptake of various faba bean varieties. Four varieties (Local, Doshu, Gebelcho, and Numan), four inoculation treatments (uninoculated and inoculated with strains FB-EAR-15, TAL-1035, and EAL-110), and three rates of biochar (0, 5, and 10 t ha⁻¹) were tested on acidic soils collected from the Goriche and Hagere Selam districts. The treatments were arranged in a completely randomized factorial design with three replications. The findings indicated that the varieties, inoculation, and biochar significantly influenced ($p < 0.05$) the majority of the parameters measured. Furthermore, the three-way interaction among these treatments markedly influenced nodule dry weight. Notably, the Gebelch variety combined with strain EAL-110 and 10 t ha⁻¹ of biochar led to a 22.3% increase in nodule dry weight compared to the Local variety that did not receive inoculation or biochar. The use of the Doshu variety, strain EAL-110, along with a biochar application rate of 10 t ha⁻¹, hugely improved the growth, nodulation, and nutrient uptake of faba beans in acidic soil. Therefore, it is critical to evaluate these agronomic practices in real field conditions in Ethiopia to enhance the productivity of faba beans in these areas.

Key words: Biochar, Coffee husk, Faba bean, Nodules, Rhizobium

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INTRODUCTION

Among legumes, faba bean (*Vicia faba* L.), commonly known as broad bean, is one of the oldest cultivated crops globally (Mínguez & Rubiales, 2021). Its production is estimated at approximately 4.8 million tons per year, with China being the largest producer (1.8 million tons per year), followed by Ethiopia, Australia, and the United Kingdom with 0.9, 0.4, and 0.3 million tons per year, respectively (FAOSTAT, 2021). Being a legume, the faba bean fixes atmospheric nitrogen (N) through symbiotic association with rhizobia. It contributes to sustainable agriculture by maintaining and improving soil fertility, which plays a key role in crop rotation (Ghorbi et al., 2023).

Ethiopia is considered a significant center of secondary diversity for faba beans, which thrive in highland regions at altitudes ranging from 1800 to 3000 meters above sea level. It is one of the leading legumes in the country, valued for its ecological and nutritional benefits. However, the average yield across the nation is unfortunately low. This is primarily due to the fragmentation of arable land and a decline in soil fertility, which results from agricultural practices that often involve minimal or no fertilization (Kermah et al., 2018). Furthermore, soil acidity plays a crucial role in limiting production in legume farming (Fekadu et al., 2018; Tadele et al., 2019). It can significantly impact the availability of essential nutrients, hinder root development, and reduce overall plant health, ultimately leading to lower crop yields (Warke &

Wakgari, 2024). As a result, smallholder farmers typically achieve an average crop yield of no more than 2.1 t ha⁻¹ (CSA, 2022).

A range of agricultural strategies has been suggested to address the challenges associated with acidic soils and enhance crop yields (MoA, 2020). These strategies encompass the cultivation of acid-tolerant crops, the application of lime, and the use of organic fertilizers (Tusar et al., 2023). Among these options, the combination of lime and organic fertilizers is frequently considered the most effective method (Enesi et al., 2023). However, the high costs and limited availability of these organic fertilizers and lime have sparked increased interest in the use of locally sourced, cost-effective organic materials and mineral fertilizers in a coordinated approach (Golla, 2019). It has been suggested that organic materials such as crop residues, manure, compost, and biochar could be viable alternatives to lime for remediating acidic soils (Tazebew et al., 2024).

The use of effective rhizobial strains has been shown to enhance various growth parameters in soybean varieties. For instance, Beruk et al. (2024) highlighted improvements in nodulation, dry biomass production, and plant height. Similarly, Samago et al. (2018) demonstrated that inoculating common beans with effective rhizobia strains led to significant increases in plant height, shoot dry weight, the number of nodules, and the dry weight of those nodules. Additionally, Kebede (2021) found that inoculating soybeans with rhizobial strains significantly increased root dry weight. Furthermore, Allito et al. (2020) found that inoculating faba beans with rhizobia enhanced nutrient availability, emphasizing the positive effects of microbial inoculants on soil health and fertility.

Adding biochar to soil offers a strategy for sequestering carbon, managing waste, immobilizing pollutants, enhancing fertility, and reducing nitrous oxide (N₂O) emissions from degraded soils (Jeffery et al., 2015; Afshar & Mofatteh, 2024). Numerous studies have demonstrated the beneficial effects of biochar on acidic soils (Zhang et al., 2019; Premalatha et al., 2023). The mechanism of soil fertility improvement

by biochar can be explained by reduced soil acidity (Bedassa, 2020; Nepal et al., 2023; Bekchanova et al., 2024), improved soil structure and nutrient transport (Kapoor et al., 2022), and increased water and nutrient retention (Alkharabsheh et al., 2021; Yadav et al., 2023; Khan et al., 2024). Given the benefits of these agronomic practices, this study investigated the effect of coffee husk biochar and *Rhizobium* inoculation on faba bean agrosymbiotic performance and nutrient uptake in acidic soils from two districts in the region. It also aims to provide insights into sustainable agricultural practices that can increase crop yields and improve soil health in acidic environments. The findings could be extremely beneficial to farmers in the region, providing practical solutions to increase productivity while promoting environmental sustainability.

MATERIALS AND METHODS

Description of Soil Collection Sites

The pot experiment was conducted from July 10 to September 7, 2021, in the lath house at Hawassa University College of Agriculture. Soil samples were collected from the Hagera Selam and Goriche districts in the Sidama Region of Ethiopia. Hagera Selam is located approximately 350 kilometers south of Addis Ababa, at coordinates 38°29'17" E and 6°31'42" N, with an elevation of 2,749 meters above sea level (m.a.s.l.). In comparison, Goriche is located 317 km from Addis Ababa and 44 km from Hawassa, the regional capital, at a latitude of 38°35'22" E and a longitude of 06°50'39" N, with an elevation of 2,813 m.a.s.l. Both areas experience bimodal rainfall patterns, featuring long rainy seasons and receiving over 1,500 millimeters of precipitation annually (Tadesse et al., 2025).

Sources of Biochar, Faba Bean and *Rhizobium* Strains

Coffee husks intended for biochar production were sourced from coffee processing facilities located in the Dale district of the Sidama region, an area known for its rich coffee cultivation. These husks, which are the outer shells of coffee beans, are typically considered agricultural waste but have gained attention for their potential use in biochar production due to their high carbon content and ability to improve soil health. *Rhizobium* strains (FB-EAR-15, TAL-1035, and EAL-110), were

obtained from the Holeta Agricultural Research Center in Ethiopia. The performance of these strains for cultivating faba beans in Ethiopia has been well-documented in field conditions (Mitiku

& Mnalku, 2019; Tadesse et al., 2025). The details of the faba bean varieties utilized in the experiment are presented in Table 1.

Table 1. Description of faba bean varieties tested in this experiment

Varieties	Seed source	Year of release	Days to physiological maturity	Altitude	Yield (quintal ha ⁻¹) on	
					Research	Farmers field
Dosha	Holeta ARC	2009	120-165	2050-2800	25-62	23-39
Gebelcho	Holeta ARC	2006	103-167	1900-3000	25-44	20-30
Numan	Kulumsa ARC	2016	137-148	1800-3000	36	22-38
Framer's variety				1900- 3000	22	12

ARC = Agricultural Research Center

Biochar Preparation

Biochar was produced at the Akaki Metal Engineering Company in Addis Ababa using a controlled low-oxygen process in an electric furnace pyrolysis reactor. This method optimizes the conversion of organic materials into biochar, a stable carbon form that improves soil quality and sequesters carbon. The production began with selecting shell material as feedstock, which was then heated to 500°C for three hours (Dume et al., 2015). This high temperature facilitates the thermal decomposition of organic material into biochar while minimizing volatile compound release. The low-oxygen environment prevents complete combustion, promoting carbon-rich biochar formation.

Treatments, Experimental Design, and Procedures

The factors studied were four faba bean varieties [Local, Dosha, Gebelcho, and Numan], four *Rhizobium leguminosarum* biovar *viciae* strains [uninoculated and inoculated with strains FB-EAR-15, TAL-1035, and EAL-110], and three biochar rates from coffee husks [0, 5, and 10 t ha⁻¹]. Treatments were arranged in a factorial combination using a completely randomized design with three replicates. Plastic pots with a base diameter of 19.2 cm, a top diameter of 23 cm, and a height of 19.5 cm were used for growing faba

beans. The biochar-soil mixtures incorporated two different rates of biochar: 5 t ha⁻¹ (equivalent to 6.1 g of biochar pot⁻¹) and 10 t ha⁻¹ (12.2 g of biochar pot⁻¹). Each mixture, along with a control soil containing no biochar, was allocated three kilograms and placed into experimental pots, with three replicates for each treatment. These pots were then placed in a controlled environment within a lath house and regularly irrigated to maintain optimal soil moisture. After a two-week incubation period, the seeds of each faba bean variety were inoculated with specific *Rhizobium* strains using a peat-based inoculant, following the recommended application rate of 10 g per kg of seed. *Rhizobial* inoculation was conducted in a shaded area to maintain the viability of the strains. Subsequently, four seeds from each variety and treatment were planted in separate pots. Once germination took place, the seedlings were thinned to keep two healthy plants per pot. Throughout the growing season, the pots were watered to ensure optimal moisture levels for the developing seedlings.

Physicochemical Analysis of the Soil and Biochar

Soil sampling and analysis

The soil sampling and analysis were performed before the implementation of the treatments. To evaluate the initial values at each experimental site, representative random soil samples were collected

from a depth of 0 to 20 cm, following the standard soil sampling protocol. After physically homogenizing the samples, two representative composite subsamples were prepared from each site for physicochemical analysis. The collected soil samples were air-dried at room temperature and sieved through a 2 mm mesh wire for selected physicochemical analyses. The sample soil particle size distribution was determined using the hydrometer method (Dewis & Freitas, 1970). The soil pH was measured using a digital pH meter from the supernatant suspension of 1:2.5 H₂O, as described by Van Reeuwijk (2002). The bulk density of the soil was measured using undisturbed soil samples. The total nitrogen (N) content was determined using the Kjeldahl method, while the measurement of soil organic carbon (OC) followed the protocols established by Walkley & Black (1934). The available P was assessed using the Bray-II method (Bray & Kurtz, 1945). The exchangeable bases, along with the cation exchange capacity (CEC), were evaluated by saturating the soil with neutral 1 M ammonium acetate. The ammonium ions (NH₄⁺) adsorbed on the surface were displaced by applying a 1 M potassium chloride (KCl) solution. Following this, the CEC of the sample was estimated using the Kjeldahl distillation method (Polemio & Rhoades, 1977). Exchangeable Ca²⁺ and Mg²⁺ in ammonium acetate were measured by an atomic absorption spectrophotometer (AAS), and exchangeable K⁺ and Na⁺ were measured by a flame photometer (Anderson & Ingram, 1994). The exchangeable acidity was determined by saturating the soil samples with potassium chloride solution and titrating them with sodium hydroxide, as described by McLean (1965).

Biochar Sampling and Analysis

The coffee husk biochar sample underwent analysis for chemical properties, including pH, total N, OC, available P, exchangeable bases and CEC. The pH was measured in distilled water at a 1:10 biochar: water mass ratio after shaking for 30 minutes (ASTM, 2009). The OC was determined using the Walkley-Black method, and the total N was determined using the Kjeldahl method (Sanvong & Nathewet, 2014). Available P was determined using the Olsen extraction method (Olsen & Sommers, 1982). The exchangeable bases and CEC were

measured at pH 7.0 after displacement using the 1 N ammonium acetate method and then estimated by titration and distillation of the ammonium that was displaced by sodium (Gaskin et al., 2008).

Data Collection

Growth, Photosynthesis and Nodulation Parameters

Plant height was measured at the mid-flowering stage during nodulation and shoot biomass assessments, while chlorophyll content (greenness) was measured on the 36th day before flowering, just at mid-flowering, and at the 56th day after planting by taking three leaves (youngest fully expanded leaves) per plant using a chlorophyll meter (Model Minolta SPAD 502). Nodulation assessment was conducted at the mid-flowering stage (50%) to prevent the detachment of roots along with nodules during the uprooting process, utilizing the third replicated pot that housed three plants. Given the interlocking nature of the roots and the challenges associated with separating nodules from individual plants without causing damage, we carefully uprooted all three plants simultaneously. The plants were then carefully divided into shoots, roots, and nodules. The soil that clung to the roots was gently shaken off. The nodules from each plant were picked and spread on the sieve to wash and drain water from their surface. Then, they were counted, and their average was taken for pots as nodule number plant⁻¹. These nodules were oven-dried at 70°C for 48 hours for the determination of their dry weight. The shoot dry matter of the plants was determined at the mid-flowering stage from plants sampled for nodulation. The plant sample was placed in labeled perforated paper bags and oven-dried at 70°C for 48 hours to determine the shoot dry matter yield.

Nutrient Concentration and Uptake Analysis

Shoots and roots of harvested faba bean plants were oven-dried at 70°C until a constant weight was recorded. The oven-dried samples were then ground in a simple grinder with a stainless-steel blade and stored in polyethylene bags for analysis. The %N in the sample was estimated through the Kjeldahl method; 5 mL of an aliquot was taken into the distillation flask, and 10 mL of 40% sodium hydroxide (NaOH) was added to it. The flask was then connected to the distillation setup with a 100-

mL conical flask containing 25 mL of boric acid solution. The distillate was cooled for a few minutes and titrated with 0.01 N standard sulphuric acid. Then, the %N was calculated as follows:

$$\% N = \frac{(T \times N \times 1.4)}{\text{wt of sample}}$$

Where T = volume of acid used for titration (mL), N = normality of acid = 0.01 N, and sample weight = 1 g.

$$N \text{ uptake} = \%N \text{ concentration in shoot} \times \text{shoot dry matter (g plant}^{-1}\text{)}$$

The concentration of P in the shoot sample was determined according to Ryan et al. (2001). Plant tissue samples (1.0 g) were heated in porcelain crucibles at 500°C for 5 hours. The ash was transferred into a 200-ml Erlenmeyer flask with 20 ml of 20% HNO₃, and the sample was filtered through a No. 1 filter paper into a 100-ml volumetric flask. The contents were then washed until 90 ml of the filtrate was collected. The filtrates were analyzed with a spectrophotometer at 460 nm.

$$P \text{ uptake} = \%P \text{ concentration in shoot} \times \text{shoot dry matter (g plant}^{-1}\text{)}$$

Data Analysis

The normality of the data was checked in advance using the Shapiro–Wilk normality test. The homogeneity of variance between the two soil sites was tested using Bartlett's test, and the results were found to be valid for conducting a combined analysis. The combined analysis of variance was performed to assess the variation among treatments (*Rhizobium* strains, faba bean varieties, biochar rates, and interactions among all four factors) using the general linear model (GLM) of the Statistical Analysis System (SAS) software version 9.4. Mean separation was performed using Duncan's Multiple Range Test (DMRT) at a 5% probability level. For the sake of this manuscript, only a three-way interaction of variety, *Rhizobium*, and biochar was considered. Correlation analysis was performed using Pearson's simple correlation coefficients for the intended parameters.

RESULTS AND DISCUSSION

Physicochemical properties of soils and biochar

Table 2 details the physicochemical properties of the soils and the biochar utilized in this experiment. The result indicates that the textural class of the soil taken from Gorche was clay loam with a pH of 5.2, while the soil from Hager Salam was clay with a pH value of 5.0, categorized as strongly acidic (Tadesse et al., 1991). The results showed that the soil from both sites was acidic and needed some kind of reclamation for the nutrients to be available for the plant. The OC contents of the sites were medium (2.1%) and low (1.4%) for Gorche and Hagere Selam, respectively (Debele, 1982). The total N and available P contents of the sites were low (Ethio, SIS, 2014), while the exchangeable bases were categorized as medium and low (Debele, 1982). The CEC of the soils from both sites falls between the ranges of medium (Hazelton & Murphy, 2016). However, the available Fe (40.6 and 43.2 mg kg⁻¹) and Mn (44.4 and 50.8 mg kg⁻¹) contents of the soils were more than the optimal requirements of the plants (Lindsay & Norvell, 1978). On the other hand, the pH value and OC content of the biochar were 10.5 and 21.1%, respectively (Table 2). Overall, the results showed that biochar had superior chemical properties to soils from both sites. Furthermore, biochar demonstrated a higher CEC, which measures the soil's ability to hold positively charged ions. This property is important for soil health because it helps retain essential nutrients and reduces leaching, promoting sustainable agricultural practices. The alkaline nature of biochar also contributed to an increase in soil pH, which can be beneficial in acidic soils and help to create a better environment for plant growth.

Table 2. Physicochemical properties of soils and biochar

Sites	Soil parameters																
	Particle size distribution (%)			Textural class	pH (H ₂ O)	Bulk density (g cm ⁻³)	Total N	Organic C (%)	Available P	Exchangeable cations							
										Fe	Mn	Zn	Ca	Mg	K	Na	CEC
	Clay	Silt	Sand							(mg kg ⁻¹)			(cmol _c kg ⁻¹)				
Goriche	41.2	15.0	43.8	Clay loam	5.2	1.23	0.15	2.1	4.91	40.6	44.4	1.5	6.3	1.4	0.7	1.2	20.5
Hager Salam	37.2	25.0	37.8	Clay	5.0	1.22	0.12	1.4	3.43	43.2	50.8	2.0	5.2	1.2	0.4	1.2	19.5
Biochar parameters																	
	-	-	-	-	10.5	-	0.54	21.1	14.3	16.2	0.8	7.2	52.5	9.8	2.5	4.2	52.5

Effect of Treatments on Growth, Photosynthesis and Nodulation

Plant Height and Chlorophyll Content

The averaged data from the two sites revealed that plant height and chlorophyll content were significantly ($p < 0.05$) affected by varieties and *Rhizobium* inoculation. However, biochar rates did not affect plant height or chlorophyll content of the faba bean (Table 3). The Numan and Dosha varieties demonstrated the tallest plant heights and the highest chlorophyll content, respectively. This indicates that these two varieties possess superior traits that may contribute to their overall vigor and photosynthetic efficiency. Conversely, the Local variety had the shortest plant heights and the lowest chlorophyll content. These differences in plant height and chlorophyll levels among the different varieties can likely be attributed to genetic variations (Zhu et al., 2024). Additionally, Theeuwens et al. (2022) emphasized that genetic factors greatly influence plant morphology and physiological traits, such as growth rates and chlorophyll production.

Likewise, the tallest plant height and highest chlorophyll content were recorded from plants

inoculated with strains FB-EAR-15 and EAL-110, indicating their unique growth-enhancing properties. Plants treated with the FB-1035 strain also showed higher leaf chlorophyll content, though less than the others. In contrast, uninoculated plants had the lowest height and chlorophyll levels, emphasizing the role of these beneficial bacteria in plant health. The improved growth observed in strains EAR-15 and EAL-110 inoculated plants can likely be attributed to enhanced access to N, a critical nutrient for plant development (Adissie et al., 2018; Samago et al., 2018). *Rhizobium* bacteria are known for their ability to fix atmospheric N, converting it into a form that plants can readily absorb and utilize. This increased N availability may not only support greater overall growth but could also positively influence other growth-related traits, such as total leaf chlorophyll content. Chlorophyll is essential for photosynthesis, and higher levels of this pigment can lead to improved energy capture and utilization, further contributing to the plant's vigor and productivity (Fathi, 2022). In line with these results, Kandil & Özdamar (2023) reported higher chlorophyll content in plants in response to *Rhizobium* inoculation.

Table 3. Growth, chlorophyll content, and nodulation as affected by main effects of faba bean varieties, inoculation and biochar application

Treatments	Plant height (cm)	Chlorophyll content (%)	Nodule number (plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)
<u>Varities</u>					
Dosha	70.1 ^b	37.7 ^a	103.8 ^a	5.8 ^a	4.6 ^a
Gebelecho	69.3 ^b	37.4 ^{ab}	99.6 ^b	5.6 ^{ab}	4.3 ^b
Numan	72.7 ^a	37.6 ^{ab}	102.5 ^a	5.7 ^{ab}	4.3 ^b
Local	68.2 ^b	37.0 ^b	98.5 ^b	5.22 ^b	3.9 ^c
LSD	2.0	0.63	3.96	0.46	0.22
<u>Rhizobium</u>					
EAL-110	71.9 ^a	37.8 ^a	104.5 ^a	5.9 ^a	4.5 ^a
FB-EAR-15	71.5 ^a	38.1 ^a	104.7 ^a	5.8 ^a	4.4 ^a
FB-1035	68.5 ^b	37.2 ^{ab}	99.1 ^b	5.4 ^b	4.2 ^a
Un inoculated	68.0 ^b	36.6 ^b	87.3 ^b	5.2 ^c	3.9 ^b
LSD	2.4	0.99	4.8	0.17	0.23
<u>Biochar (t ha⁻¹)</u>					
0	63.6	36.0	90.75 ^c	4.8 ^c	3.8 ^c
5	70.7	37.7	102.0 ^b	5.7 ^b	4.3 ^b
10	75.9	38.7	111.5 ^a	6.3 ^a	4.85 ^a
LSD_{0.05%}	NS	NS	2.84	0.29	0.39
CV (%)	10.1	3.75	6.72	6.1	9.1

Values followed by dissimilar letters in the column are statistically significant; NS = non-significant.

Nodule number and dry weights

The averaged data from the two sites revealed that nodule number, shoot and root dry weights were significantly ($p < 0.05$) affected by varieties, *Rhizobium* inoculation and biochar applications (Table 3). Likewise, the three-way interaction effect was significant on nodule dry weight of the faba bean (Table 4). Among the varieties, Dosha and Numan produced more nodules and shoot biomass compared to Gebelecho and the local varieties (Table 3). This marked increase in nodule formation indicates a more effective symbiotic relationship with N_2 -fixing bacteria, allowing these varieties to utilize atmospheric N more efficiently. As a result, this enhanced nodulation ability plays a crucial role in their overall biomass production. This observation is consistent with findings from several researchers who have reported notable differences among the legume varieties studied. For example, Biruk et al. (2024) identified similar trends in their research, highlighting that certain legume varieties consistently perform better in nodule formation and biomass growth. Similarly, Tadewos et al. (2022) supported these conclusions, stressing the importance of selecting appropriate legume varieties to improve soil health and boost crop yields in agricultural practices. In addition, the ability of legumes to form nodules and produce biomass is largely influenced by a combination of soil characteristics and biological factors, including levels of available P, soil pH, type and vigor of legumes, rhizobial population, and their effectiveness (Gopalakrishnan et al., 2015). Moreover, different legume varieties exhibit varying capacities for nodule formation and biomass generation, which can be attributed to their genetic makeup and adaptability to specific environmental conditions (Yohannes et al., 2024). The results revealed that plants inoculated with *Rhizobium* strains FB-EAR-15 and EAL-110 exhibited a greater number of nodules and higher biomass yields compared to those inoculated with strain FB-1035 or the uninoculated control (Table 3). The formation of more nodules is indicative of a more effective symbiotic relationship, as these nodules are the sites where N_2 fixation occurs. Consequently, the legumes that formed more nodules were able to access more fixed N, which is essential for their growth and development. The enhanced growth and dry matter accumulation

observed in the plants inoculated with the more effective *Rhizobium* strains can primarily be attributed to their ability to supply fixed N directly to the legumes. This N is crucial for various physiological processes, including protein synthesis, enzyme function, and overall plant metabolism. As noted by Allito et al. (2020), the presence of compatible rhizobial strains not only boosts N_2 fixation but also contributes to the overall health and productivity of legume crops. Accordingly, the compatibility between legume species and the particular rhizobial strains present in the soil is vital for the success of their symbiotic association. When the right rhizobia are present, they significantly enhance legumes' ability to absorb N from the atmosphere, thus fostering their overall growth and biomass accumulation.

The use of biochar resulted in higher nodule formation and biomass than the control treatments (Table 3). This increase in the number of nodules can likely be attributed to the rise in soil pH, which creates a more conducive environment for beneficial soil bacteria. These bacteria play a crucial role in the N_2 -fixing process, which is essential for the growth and development of leguminous plants. The elevated pH levels not only promote the proliferation of these beneficial microorganisms but also boost enzyme activity, which is vital for various biochemical processes in the soil. Furthermore, biochar contributes to the availability of essential nutrients, such as N, P, and K that support overall plant growth and health. Supporting this observation, Sisay & Abebawe (2021) found that faba beans grown in acidic soils showed the highest nodule counts when 20 t ha^{-1} of biochar was applied along with the recommended P. This finding underscores the importance of biochar in improving soil conditions, particularly in environments where soil acidity can limit plant performance. The synergistic effect of biochar and P not only enhances nodule formation but also promotes better nutrient uptake, leading to healthier and more productive plants. Additionally, Iijima et al. (2015) reported that the application of biochar led to improved growth, increased nodulation, and enhanced yields in bean crops. Their research highlights the multifaceted benefits of biochar, which include not only the physical and chemical improvements to soil structure but also the

biological enhancements that facilitate plant growth. The increased nodulation observed in their studies suggests that biochar may help create a more favorable habitat for rhizobia, the N₂-fixing bacteria that form symbiotic relationships with leguminous plants.

The interaction between variety, inoculation, and biochar application revealed that the Gebelcho variety had the highest nodule dry weight when inoculated with strain EAL-110 and treated with 10 t ha⁻¹ of biochar. However, when neither biochar application nor inoculation was used, the local variety showed the lowest nodule dry weight (Table 4). The average dry weight of nodules did not show marked differences among the three strains and various biochar rates. However, there were 29.8%, 24.2%, and 21.7% increments of the dry weight for the Gebelcho, Numan, and Dosha varieties, respectively, when inoculated with EAL-110 and treated with 10 t ha⁻¹ of biochar, compared to the uninoculated control without biochar. This finding emphasizes the challenges faced by rhizobial species in acidic soils, which can decrease their viability and functionality, disrupting their

symbiotic relationships with leguminous plants and hindering N₂ fixation. Acidic conditions can lead to nutrient deficiencies and decreased microbial activity. Conversely, biochar enhances soil structure, increases water retention, and boosts nutrient availability. Additionally, it stabilizes soil pH, creating a more favorable environment for the growth of rhizobia. These improvements encourage the proliferation and activity of *Rhizobium* species, strengthening their symbiotic relationship with leguminous plants, which is crucial for effective N₂ fixation and enhanced nutrient uptake. The observed differences confirm that nodulation depends on the interactions of the legume variety, the *Rhizobium* strain, and the biophysical environment of the soil (Giller et al., 2013). The present result was consistent with the findings of Allito et al. (2020), who reported a significant interaction of host varieties with rhizobial strains and location on symbiotic traits of faba bean varieties. Moreover, to optimize the benefits of symbiotic N₂ fixation, the inoculant strain must be efficient and match the desired variety in a growing agroecological zone (Beltayef et al., 2018).

Table 4. Three-way interaction effect of variety, inoculation and biochar on the nodule dry weight of faba bean

Varieties	<i>Rhizobium</i>	Nodule dry weight (mg plant ⁻¹)		
		Biochar (t ha ⁻¹)		
		0	5	10
Dosha	EAL-110	209.9 c-m	216.0 a-h	221.3 a-c
	FB-EAR-15	202.5 j-o	207.6 d-n	213.1 b-k
	FB-1035	199.8 l-p	211.9 b-l	217.7 a-e
	Uninoculated	207.47d-n	207.1 e-n	219.6 a-d
Gebelcho	EAL-110	201.2 k-p	217.2 a-f	225.8 a
	FB-EAR-15	203.5 i-o	212.3 b-k	216.4 a-g
	FB-1035	198.6 m-p	209.6 c-m	217.9 a-d
	Uninoculated	196.9 n-p	202.7 j-o	205.2 f-n
Numan	EAL-110	198.2 m-p	207.2 e-n	223.0 ab
	FB-EAR-15	196.4 n-p	215.4 a-i	220.1 a-c
	FB-1035	198.6 m-p	202.9 j-o	215.9 a-i
	Uninoculated	199.0 m-p	203.7 h-o	204.1 g-n
Local	EAL-110	189.4 pq	207.3 d-n	213.6 a-j
	FB-EAR-15	181.6 qr	191.3 o-q	209.6 c-m
	FB-1035	181.4 qr	207.4 d-n	211.6 b-l
	Uninoculated	175.5 r	206.3 e-n	207.7 d-n
LSD _{0.05%}			12.4	
CV (%)			9.9	

Values followed by dissimilar letters in the column are statistically significant.

Effect of Treatments on Nutrient Concentration and Uptake

The combined data from the two sites revealed significant differences in nutrient concentration and uptake between the different faba bean varieties. The Dosha variety had the highest N and P concentrations, as well as the greatest nutrient uptake. The Gebelecho and Numan varieties closely followed, displaying impressive nutrient levels as well. In contrast, the local variety had the lowest concentrations and uptake of these nutrients (Table 5). The variations observed among different faba bean varieties provide an important foundation for the development of new cultivars with higher mineral content. In line with these findings, Etemadi et al. (2018) noticed differences in nutrient concentration and absorption among faba bean varieties. Similarly, Hacisalihoglu (2024) identified a group of faba beans with superior nutrient composition, which could be beneficial for research focused on genetic improvement and tackling future global food security issues.

The inoculation of *Rhizobium* significantly increased N and P concentrations, as well as improved faba bean uptake compared to the control treatment (Table 5). This marked increase in nutrient levels and absorption indicates that the *Rhizobium* strain used in this study has greater competitive advantages and effectiveness than the native rhizobia typically found in the soil. The increased N levels can be attributed to the symbiotic relationship formed between *Rhizobium* bacteria and the faba bean roots, which aids in converting atmospheric N into a form readily available for plant utilization. Similarly, the increase in P levels is vital, as P is an essential nutrient that plays a key role in various physiological processes, including energy transfer, photosynthesis, and the synthesis of nucleic acids. The improved P uptake observed in the faba beans treated with *Rhizobium* indicates that the introduced strain may also enhance the plant's ability to access this critical nutrient, potentially through mechanisms such as the secretion of root exudates that solubilize P or the formation of more extensive root systems (Janati et al., 2021). In line with this finding, Beyene & Tuji (2024) reported that inoculation resulted in enhanced plant growth and consequently increased

biological activity associated with the roots of the host plant due to increased root exudation and organic matter decomposition, which in turn improved nutrient availability. Likewise, Khoso et al. (2023) found that plant growth-promoting rhizobacteria influences the overall physiology of plants, leading to improved nutrient absorption and enhanced root activity.

The interaction between variety, inoculation, and biochar application revealed that the Gebelcho variety had the highest nodule dry weight when inoculated with strain EAL-110 and treated with 10 t ha⁻¹ of biochar. However, when neither biochar application nor inoculation was used, the local variety showed the lowest nodule dry weight (Table 4). The average dry weight of nodules did not show marked differences among the three strains and various biochar rates. However, there were 29.8%, 24.2%, and 21.7% increments of the dry weight for the Gebelcho, Numan, and Dosha varieties, respectively, when inoculated with EAL-110 and treated with 10 t ha⁻¹ of biochar, compared to the uninoculated control without biochar. The application of biochar has been shown to remarkably enhance the concentrations and uptake of N and P compared to control treatments (Table 4). The increased N levels and plant uptake can be attributed to a variety of factors, including improved nodulation and root growth in faba beans following the addition of biochar to the soil. Improved nodulation is critical because it strengthens the symbiotic relationship between faba beans and N₂-fixing bacteria, resulting in greater N availability for the plants. Similarly, the increase in P uptake can be largely attributed to changes in soil pH caused by biochar's liming effects. When biochar is incorporated into the soil, the pH increases, allowing phosphate ions to be released more easily. In many soils, P is bound to Al and Fe, making it less available to plants. However, the higher pH levels caused by biochar application help to liberate these phosphate ions, increasing their availability for plant uptake. Furthermore, biochar not only provides essential nutrients but also helps faba beans absorb P more effectively. Its porous structure enhances soil aeration and water retention, promoting root growth and nutrient uptake. It is also high in organic carbon and minerals, which

help to replenish the soil's nutrient levels. This enrichment not only increases the availability of nutrients for plant uptake, but it also promotes

improved nutritional status and plant growth (Egamberdieva et al., 2021).

Table 5. Nutrient concentrations and uptake as affected by main effects of faba bean varieties, inoculation and biochar application

Treatments	N concentration (%)	N-uptake (mg g ⁻¹)	P concentration (%)	P-uptake (mg g ⁻¹)
<i>Varieties</i>				
Dosha	4.3 ^a	25.7 ^a	0.17 ^a	10.0 ^a
Gebelecho	4.3 ^a	24.6 ^a	0.17 ^{ab}	9.5 ^{ab}
Numan	4.2 ^a	24.6 ^a	0.17 ^{ab}	9.5 ^{ab}
Local	3.9 ^b	21.04 ^b	0.16 ^b	8.6 ^b
LSD	0.19	2.26	0.005	0.96
<i>Rhizobium</i>				
EAL-110	4.6 ^a	27.4 ^a	0.17 ^a	10.1 ^{ab}
FB-EAR-15	4.4 ^b	25.7 ^b	0.17 ^a	10.3 ^a
FB-1035	4.1 ^c	22.7 ^c	0.16 ^{ab}	9.00 ^{bc}
Uninoculated	3.7 ^d	19.8 ^d	0.15 ^b	8.2 ^c
LSD	0.19	1.28	0.01	1.2
<i>Biochar (t ha⁻¹)</i>				
0	3.6 ^c	17.4 ^c	0.18 ^a	11.3 ^a
5	4.3 ^b	24.6 ^b	0.17 ^b	9.6 ^b
10	4.7 ^a	29.7 ^a	0.15 ^c	11.3 ^a
LSD_{0.05%}	0.29	2.19	0.01	1.1
CV (%)	8.4	10.3	6.74	9.7

Values followed by dissimilar letters in the column are statistically significant; NS = non-significant.

Correlation Analysis

A significant positive correlation was found between shoot dry matter and various factors, including chlorophyll content ($r = 0.49$), nodule number ($r = 0.78$), root dry weight ($r = 0.72$), N and P concentration ($r = 0.78$ and $r = 0.65$), and uptake ($r = 0.93$ and $r = 0.94$), respectively (Table 6). This suggests that faba bean varieties with higher shoot dry weight would have better growth, nodulation, and higher nutrient concentration and uptake, which in turn increases productivity. In addition, the positive relationship between shoot dry weight and root nodulation indicates improved N₂ fixation, which, in turn, leads to higher crop growth and dry matter production. In line with this finding, Oono & Denison (2010) suggested that shoot dry weight and nodulation were good indicators of symbiotic N₂ fixation efficiency in legumes. Moreover, Mothapo et al. (2013) indicated that plants that formed more nodules produced higher shoot and root dry matter. The positive association between

shoot dry weight and leaf chlorophyll content ($r = 0.49$) further suggests enhanced rates of photosynthetic and carbohydrate accumulation in faba beans through improved plant N content. The stronger association of shoot dry weight with root dry weight could be due to the stronger association of root dry weight with that of N and P concentration that ultimately enhances the growth and development of below- and aboveground parts of the faba bean plant.

Table 6. Correlation (r) between shoot dry weight and nodulation, growth, and nutrient concentration and uptake of faba bean varieties

Parameters	Pearson's correlation coefficient (r)	P value
Shoot dry weight versus		
chlorophyll content	0.49	**
nodule number	0.78	***
root dry weight	0.73	***
N concentration	0.78	***
P concentration	0.65	***
N uptake	0.93	***
P uptake	0.94	***

N = nitrogen, P = phosphorous, **, ***, significant at the $p \leq 0.01$ and $p \leq 0.001$ levels, respectively.

CONCLUSIONS

Soil acidity presents a significant challenge for the successful cultivation of faba beans, impacting factors such as plant growth, nodulation, and nutrient absorption. This research demonstrates that incorporating coffee husk biochar and inoculating faba bean seeds with different strains of rhizobial bacteria resulted in notable improvements in the soil's physicochemical properties. These improvements led to better nutrient uptake, increased growth rates, and enhanced nodulation in faba bean plants. Thus, the strategic use of efficient inoculants and biochar has been critical in increasing the agrosymbiotic and nutrient use efficiency of faba bean varieties. Nevertheless, it is essential to conduct field tests on these amendments before offering reliable recommendations to our farmers, thereby assisting them in boosting production and ensuring their household food security.

Data Availability

The data used to support the results of this study are included within the manuscript and any further information is available from the corresponding author upon request.

Disclosure statement

The authors declare that they have no conflicts of interest.

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