



Evaluation of Rhizobial Inoculants for Symbiotic Performance of Faba bean (*Vicia faba* L.) in the Arsi Zone, Southeastern Highlands of Ethiopia

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Abstract:

This study was initiated to evaluate the effect of locally isolated Rhizobium inoculants on nodulation and yield of faba bean at Arsi Zone, Ethiopia for two consecutive years. Eight treatments comprising six effective isolates of rhizobia, un-inoculated (negative control), and recommended N-fertilized (18kg N ha⁻¹) were laid out in a randomized complete block design with three replications. The result of the experiment indicated that all inoculation treatments increased nodule number, nodule dry weight, and nodule fresh weight, yield, and yield components over the control check in all experimental sites. The result, however, showed the non-significant ($p < 0.05$) effect of Rhizobium inoculation on 100 seed weight in all Experimental sites as compared to each treatment. Inoculating FB-24 and FB-120 gave the highest mean grain yield (4273.4 kg ha⁻¹ and 4192.2 kg ha⁻¹) respectively. These records were 61 and 58% over the un-inoculated treatment respectively. In all experimental sites, FB-24 and FB-120 inoculation resulted in the highest nodulation and grain yield production as compared to the other treatments. In general, isolates from the Arsi zone showed good performance in all yield and yield parameters Therefore, FB-24 and FB -120 were recommended as candidate isolates for faba bean biofertilizer production in the Arsi Zone of Ethiopia.

Keywords: Rhizobium, inoculants, faba bean, biofertilizer, yield.

INTRODUCTION

One of the main pulse crops produced in Ethiopia's highlands is the faba bean (*Vicia faba* L.) (Fedaku et al., 2019). Ethiopia is second only to China among the major producers of faba beans in the globe (FAO, 2019). The edible seed of the high-value crop known as the faba bean serves as an essential source of protein for Ethiopians who eat a diet high in cereals, especially the less fortunate who cannot purchase animal products. According to estimates, it makes up 15% of the protein consumed, which would help 36% of the undernourished people. The faba bean should be included in a sustainable farming scheme in Ethiopia as a crop to rotate with cereals (Tamene Temesgen et al., 2015). It currently takes up 31% of the nation's 1,863,445 hectares dedicated to growing pulses (CSA, 2019). The crop is important for improving land fertility and providing food for humans and animals (Mulugeta et al., 2019). However, compared to the average yield (3.7 t ha⁻¹) achieved in the world's main faba bean-producing nations, the productivity of the crop in the nation is low (2.12 t ha⁻¹) (FAOSTAT, 2017; CSA, 2019). Currently, intensive agricultural practices confront several difficulties that seriously jeopardize the security of the world's food supply. Chemical fertilizers and pesticides are used extensively to increase crop output to meet the growing global population's nutritional needs (Matthews et al., 2019). Due to excessive use of harmful pesticides and chemical fertilizers, modern agriculture has lost the ability to be sustainable, which has increased cultivation costs, decreased food security and safety, and

ultimately decreased soil fertility (Saritha and Prasad Tollamadugu, 2019). Without allowing any crop or livestock manure residues on farmland, Ethiopia is gradually using more chemical fertilizers. This action results in a reduction in the spread of soil fauna and flora, poor soil structure, low soil nutrient and water holding capacity, and distracted soil microorganisms. The role of biological nitrogen fixation in increasing crop output plays an undeniable role in minimizing these environmental and soil damages brought on by chemical fertilizers, especially in preserving soil health.

Although using rhizobia in inoculating legumes can significantly increase growth and yield productivity and improve soil fertility, low productivity in grain legumes is caused by declining soil fertility and reduced N₂ fixation. There is no doubt that specificity exists between rhizobia strain and the legume variety, and compatibility between the two is essential for successful nodulation and nitrogen fixation (Raja et al., 2013). High yield and land fertility are both significantly impacted by the biological nitrogen fixation of faba beans. Additionally, the N and P that plants can uptake and share with grain and straw have a beneficial residual effect on soil fertility, the quality, and the production of faba bean seed (Klippenstein, 2019). A yearly global total of 175 million tons of biological N₂ fixation is produced by rhizobium symbiosis with legumes (Yadav and Verma, 2014). Microbial inoculants' biological activity aids in recovering nutrients and mobilizing their availability, which improves the overall condition of the soil (Yadav and Sarkar, 2019). A ground-breaking innovation, biofertilizer, promises to have a substantial effect on farmers' crop production and productivity. It is referred to as bio-inoculants that contain living organisms that aid plant roots in the rhizosphere in the availability and absorption of nutrients. Rhizobial inoculants may be able to stop nitrification for an extended length of time while enhancing the soil's fertility (Sun et al., 2020; Fasusi et al., 2021). They are among the essential elements of Integrated Nutrient Management (INM) strategies, which aim to balance the productivity and sustainability of the soil while also maintaining environmental safety, being pollution-free, economically viable, and providing plants with renewable nutrients to supplement synthetic fertilizers in a sustainable production system (Yadav and Sarkar, 2019). De Vives-Peris et al., (2020); Fasusi et al., (2021) have developed microbial inoculants that contain the culture of dormant or live cells of the efficient strains of N-fixing, P-solubilizing/mobilizing, and K-solubilizing. It has become innovative and environmentally friendly to use beneficial microorganisms as bio-fertilizers in sustainable agricultural practices to increase soil fertility and plant development (Bertola et al., 2019; Murgese et al., 2020; Fasusi et al. 2021). Therefore, the goal of this research is to assess how well rhizobial inoculants perform in terms of faba bean grain and biomass yields in the main Arsi zone faba bean growing regions.

MATERIALS AND METHOD

Description of the Research Site

The field evaluation experiment was conducted in three districts of the Arsi zone, Kulumsa, Hitosa, and Bekoji during the 2019 and 2020 crop seasons under rain-fed conditions. The soil was not infused with any previous rhizobia strains nodulating faba beans.

Nodule Sample Collection

The samples were collected in the Arsi Zone's 11 principal faba bean farming regions (Table 1). These districts were picked because they have never received a faba bean rhizobia inoculation and have a history of having a high faba bean yield. The root nodules were taken during the blossoming period. The roots were harvested for their pink nodules. As the rhizobia were isolated and identified, the nodules were collected, placed in vials with a desiccant (silica gel), and wrapped

in 1 centimeter of cotton wool before being transported to the Holleta soil microbiological laboratory at the Holleta agricultural research facility (Somasegaran, P. and Hoben, H. J, 1994). In the Holleta soil microbiological laboratory at the Holleta Agricultural Research Center, sand culture under greenhouse conditions was isolated, characterized, and tested for symbiotic efficacy. The six best native faba bean rhizobia (FB-8, FB-24, FB-120, FB-EAR-5, FB-EAR-15, and FB-EAR-13) were then chosen for field testing. Faba bean (Numan variety) from Kulumsa Agricultural Research Center was utilized.

Table 1: Soil samples and Nodule collection GPS data at different Agro-ecology of Arsi Zone

| No | Sample cod | Location (Kebele) | Woreda (district) | Previous crop | GPS Coordination | | |
|----|------------|-------------------|-------------------|---------------|------------------|--------------|---------------|
| | | | | | Altitude (M) | Latitude (N) | Longitude (E) |
| 1 | FB-120 | Kulu-on station | Tiyo | wheat | 2161 | 08° 00' 989" | 39° 08' 976" |
| 2 | FB - EAR60 | Kulu-on station | Tiyo | wheat | 2176 | 08° 01' 206" | 39° 09' 393" |
| 3 | FB -01 | Lemu Tijo | Shirka | maize | 2695 | 07° 34' 368" | 39° 26' 301" |
| 4 | FB -02 | Lemu Tijo | Shirka | barley | 2820 | 07° 33' 918" | 39° 24' 970" |
| 5 | FB -03 | Bedi-Micael | Lemu bilbilo | barley | 3137 | 07° 31' 138" | 39° 17' 895" |
| 6 | FB -04 | Cheba micael | Lemu bilbilo | barley | 3019 | 07°31' 249" | 39° 17' 018" |
| 7 | FB - EAR05 | Meraro Station | Lemu bilbilo | wheat | 2993 | 07° 24' 423" | 39° 14' 959" |
| 8 | FB - EAR6 | Dawa-bursa | Lemu bilbilo | fallow | 2925 | 07° 26' 672" | 39° 14' 846" |
| 9 | FB - EAR13 | Bekoji-station | Lemu bilbilo | barley | 2811 | 07° 32' 728" | 39° 15' 383" |
| 10 | FB - EAR15 | Lemu dima | Lemu bilbilo | barley | 2683 | 07° 34' 857" | 39° 14' 543" |
| 11 | FB -08 | Ashebeka wolkte | Digelu Tijo | potato | 2478 | 07° 42' 291" | 39° 09' 602" |
| 12 | FB -10 | Haro bilalo | Tiyo | barley | 2551 | 07°52' 624" | 39° 07' 509" |
| 13 | FB -11 | Shorima sherera | Hitosa | wheat | 2342 | 08° 03' 056" | 39° 14' 194" |
| 14 | FB -12 | Gonde shorima | Hitosa | wheat | 2260 | 08° 03' 558" | 39° 12' 529" |
| 15 | FB -17 | Tulu jebi | Lode hitosa | barley | 2473 | 08° 07' 529" | 39° 26' 196" |
| 16 | FB -18 | Efa lode | Lode hitosa | wheat | 2689 | 08° 06' 102" | 39° 29' 602" |
| 17 | FB -19 | Hela wolkite | Diksis | Oat | 2713 | 08° 02' 502" | 39° 33' 814" |
| 18 | FB -20 | Doyo gora | Arsi robe | wheat | 2540 | 07°56' 967" | 39° 34' 966" |
| 19 | FB - EAR21 | A.robe on Station | Arsi robe | wheat | 2437 | 07° 53' 017" | 39° 37' 722" |
| 20 | FB -22 | Tankicha gefersa | Diksis | rapeseed | 2669 | 08° 07' 550" | 39° 32' 525" |
| 21 | FB -23 | Banben | Siere | barley | 2525 | 08° 11' 457" | 39° 31' 623" |
| 22 | FB -24 | Boreno Ogissa | Siere | Oat | 2501 | 08° 14' 378" | 39° 32' 021" |
| 23 | FB -25 | Akiya tulogu | Chole | barley | 2861 | 08° 09' 853" | 39° 54' 196" |

| | | | | | | | |
|----|--------|------------|----------|--------|------|--------------|--------------|
| 24 | FB -26 | Koro gugu | Chole | barley | 3020 | 08° 13' 059" | 39° 54' 970" |
| 25 | FB -27 | Gado abita | Chole | barley | 3047 | 08° 14' 455" | 39° 55' 087" |
| 26 | FB -28 | Rea amba | Abajemma | barley | 2742 | 08°19' 958" | 39° 52' 256" |
| 27 | FB -29 | Jajiro | Abajemma | wheat | 2498 | 08° 23' 578" | 39° 54' 196" |

Experimental Design and Treatments

Six native faba bean rhizobial isolates were chosen and examined at three separate Arsi zone locations: FB-24, FB-8, FB-120, FB-EAR₅, FB-EAR₁₅, and FB-EAR₁₃ (Kulumsa, Bekoji, and Hitosa). The studies were carried out with three replications at a plot size of 4 m x 2.6 m and were done using a randomized full-block design (RCBD). To reduce cross-contamination, the distance between plots and blocks was increased by 0.5 and 1 meters, respectively. Rows and plants were 40 cm and 10 cm apart, respectively. The carrier-based rhizobial inoculants were applied at a rate of 500 g ha⁻¹. Each experimental plot received a base application of 100 kg P ha⁻¹ from TSP at planting time. Urea applied at a positive control received 18 kg N ha⁻¹. Contrarily, the negative control, does not apply any inputs (N and P). The experimental fields and experimental units were managed using the approved agronomic faba bean methods.

Preparation of Rhizobia Inoculants and Seed Dressing

At Holleta Agricultural Research Center, soil microbial laboratory rhizobia isolates were made in carrier-based inoculants. The carrier material for the investigation was 10⁶ micrometer-mesh-sized powdered lignite that had been pH-adjusted and could pass through it. In white polyethylene heat-resistant bags with a partial seal, 50 grams of lignite were placed, and the bags were sterilized at 121 °C for 30 minutes. Then, using the unsealed portion, 10 ml of high-quality broth culture from each rhizobia isolate was inoculated. The broth culture contained more than 10⁹ colony-forming units per milliliter, and it was homogenized in an aseptic environment before being incubated at room temperature for two weeks to cure. Viable cell counts were used to check for contamination and to determine the number of minimum-threshold rhizobia cells (Vincent, 1970). Yellow and opaque plastic bags were used to shield the inoculants from direct sunlight exposure. The recommended rate of faba bean seeds was 200 kg ha⁻¹ weighed, moistened with sticker solution, and dressed carefully with the respective inoculant until all the seeds in plastic bags were uniformly coated. The whole seed dressing procedure was carried out under the shade. The fully-dressed and air-dried seeds were planted and immediately covered with soil.

Soil Sampling and Analysis

Random composite soil samples were taken at a depth of 0–20 cm from each experimental plot before planting and after harvesting. The soil samples were crushed to fit through a 2 mm filter after being air-dried. The pH of the soil was measured in a ratio of 1:2.5. The Walkley and Black (1934) wet digestion method was used to calculate soil organic carbon. The soil's accessible phosphorus content was determined using the Bray-II extraction method, and its total nitrogen content was determined using the Kjeldahl (1883) wet-digestion method.

Data Collection

Nodulation Parameters:

Five randomly chosen faba bean plants from each plot were uprooted at flowering as part of a disruptive sample technique from the border rows for the nodulation investigation. On a screen, roots were gently cleaned with tap water that was slowly flowing, and nodules were then separated and counted. An effective number of nodules: To determine the effective number of nodules, the color of the nodule inside was examined by cutting with a sharp blade; nodules that

were pink to dark red were deemed to be effective, while green nodules were considered to be ineffective. The effective nodules then underwent additional analysis, including nodule number, nodule fresh weight, and nodule dry weight. The average values of the effective nodules from the five plants were used to compute the number of nodules per plant. The collected nodules were uniformly dried in an oven for 65 hours at 75 °C to determine the nodule dry weight per plant. The average of five plants was used to compute the nodule dry weight per plant.

Agronomic Parameters:

Plant height (PH), number of pods per plant (NPPP), number of seeds per pod (NSPP), above-ground biomass yield (BY), hundred seed weight (100SW), and grain yield (Adj. GY) were all recorded for each plot. Grain yields were corrected for 10% moisture content for statistical analysis, and the yield per plot was converted to kg ha⁻¹. The average value of five representative plants per plot was used to calculate the effect of rhizobia isolates on plant height, the number of pods per plant, and the number of seeds per pod.

Statistical Analysis

Using the SAS software package, the measured data were statistically examined for analysis of variance (SAS Institute, 2010). The 5% level of significance LSD mean comparison approach was used to separate the significant treatment means.

RESULT AND DISCUSSION

Soil Analysis Results

According to the soil pH, all sites experienced somewhat acidic soil reactions (H₂O). The bulk of crops can be produced in that range (FAO 2020). The pH of the soil from the test site is almost within the range of productive soils. Tadese (1999) noted that the soil's total nitrogen content and level of organic matter were both low before planting.

Table 1: Soil chemical properties before planting of faba bean

| Soil Properties | Values | | | | | |
|--|----------------|----------------|---------------|---------------|---------------|---------------|
| | Kulumsa (2019) | Kulumsa (2020) | Hitosa (2019) | Hitosa (2020) | Bekoji (2019) | Bekoji (2020) |
| pH (H₂O) | 5.94 | 6.08 | 6.26 | 6.54 | 5.66 | 5.68 |
| Organic matter (OM) (%) | 3.35 | 4.31 | 4.82 | 4.60 | 3.16 | 4.88 |
| Total Nitrogen (TN) (%) | 0.15 | 0.14 | 0.14 | 0.15 | 0.13 | 0.14 |
| Available P (mg kg⁻¹ soil) | 17.47 | 11.33 | 8.68 | 14.89 | 15.81 | 9.06 |

The three sites' soil assessments after the faba bean harvest showed that adding strains considerably ($p \leq 0.05$) increased the soil's available P, total N, and organic matter contents (Table 2). Consequently, it is crucial to apply inoculation with effective strains in the study regions to replace any lost nitrogen that the soil was unable to deliver to the crop. Olsen *et al.* (1954) found a high quantity of phosphorus that was readily available, and the pH of the soil varied somewhat but not significantly in the experimental sites.

Table 2: Mean soil chemical properties as influenced by application of rhizobia strains after harvesting of faba bean.

| Year | Parameters (kulumsa) | | | | Parameters (Bekoji) | | | | Parameters (Hitosa) | | | |
|-------------|----------------------|-------------------|--------------------|------------------|---------------------|-------------------|--------------------|-------------------|---------------------|-------------------|--------------------|-------------------|
| | pH | TN | AvaP | OM | pH | TN | AvaP | OM | pH | TN | AvaP | OM |
| 2019 | 6.6 ^a | 0.16 ^b | 12.29 ^b | 2.6 ^b | 5.70 ^a | 0.18 ^b | 10.98 ^b | 3.79 ^a | 5.96 ^b | 0.17 ^b | 10.77 ^b | 4.22 ^a |

| | | | | | | | | | | | | |
|------------------|------------------|--------------------|--------------------|-------------------|-------------------|--------------------|--------------------|-------------------|-------------------|--------------------|---------------------|--------------------|
| 2020 | 6.7 ^a | 0.20 ^a | 19.86 ^a | 3.6 ^a | 5.89 ^a | 0.23 ^a | 20.94 ^a | 4.86 ^a | 6.84 ^a | 0.20 ^a | 23.34 ^a | 4.47 ^b |
| Mean | 6.6 | 0.18 | 16.08 | 3.16 | 5.81 | 0.21 | 15.96 | 4.32 | 6.40 | 0.18 | 17.06 | 4.34 |
| LSD | 0.14 | 0.04 | 1.25 | 0.37 | 0.12 | 0.04 | 2.57 | 0.53 | 0.09 | 0.02 | 1.45 | 0.46 |
| Treatment | | | | | | | | | | | | |
| C- | 6.5 ^a | 0.13 ^b | 14.82 ^a | 2.84 ^a | 5.71 ^a | 0.14 ^b | 14.19 ^a | 3.79 ^a | 6.68 ^a | 0.13 ^b | 15.58 ^b | 3.86 ^b |
| C+ | 6.6 ^a | 0.17 ^{ab} | 15.59 ^a | 3.13 ^a | 5.74 ^a | 0.17 ^{ab} | 15.60 ^a | 4.27 ^a | 6.70 ^a | 0.19 ^{ab} | 16.54 ^{ab} | 4.18 ^{ab} |
| FB-8 | 6.6 ^a | 0.17 ^{ab} | 16.26 ^a | 3.14 ^a | 5.85 ^a | 0.22 ^a | 16.37 ^a | 4.36 ^a | 6.71 ^a | 0.18 ^{ab} | 16.92 ^{ab} | 4.34 ^{ab} |
| FB-24 | 6.8 ^a | 0.21 ^a | 17.24 ^a | 3.36 ^a | 5.90 ^a | 0.22 ^a | 17.04 ^a | 4.76 ^a | 6.77 ^a | 0.23 ^a | 19.43 ^a | 4.48 ^{ab} |
| FB-120 | 6.8 ^a | 0.21 ^a | 17.25 ^a | 3.24 ^a | 5.82 ^a | 0.22 ^a | 16.66 ^a | 4.56 ^a | 6.74 ^a | 0.23 ^a | 17.62 ^{ab} | 5.06 ^a |
| FB-EAR-5 | 6.8 ^a | 0.17 ^{ab} | 15.94 ^a | 3.22 ^a | 5.81 ^a | 0.18 ^{ab} | 16.24 ^a | 4.52 ^a | 6.72 ^a | 0.17 ^{ab} | 16.63 ^{ab} | 4.29 ^{ab} |
| FB-EAR-15 | 6.7 ^a | 0.17 ^{ab} | 15.05 ^a | 3.03 ^a | 5.80 ^a | 0.17 ^{ab} | 16.03 ^a | 4.20 ^a | 6.70 ^a | 0.16 ^b | 16.37 ^b | 4.12 ^b |
| FB-EAR-13 | 6.7 ^a | 0.17 ^{ab} | 16.44 ^a | 3.30 ^a | 5.79 ^a | 0.17 ^{ab} | 15.52 ^a | 4.11 ^a | 6.71 ^a | 0.18 ^{ab} | 17.37 ^{ab} | 4.41 ^{ab} |
| CV | 3.40 | 5.78 | 3.29 | 3.79 | 3.21 | 5.57 | 3.14 | 6.31 | 2.42 | 2.64 | 4.57 | 5.26 |
| LSD | 0.27 | 0.03 | 2.49 | 0.73 | 0.24 | 0.05 | 5.15 | 1.07 | 0.18 | 0.06 | 2.90 | 0.92 |

Mean values in the same column with different letter(s) are significantly different at a $p \leq 0.05$.

pH = measure of acidity or alkalinity, TN = Total Nitrogen, $AvaP$ = Available Phosphorous, OM = Organic matter

Effects of Rhizobia Isolates on Nodulation

According to the data analysis, there was a significant interaction between Rhizobium inoculation treatments and nodule number (NN), nodule fresh weight (NFW), and nodule dry weight (NDW) at $p \leq 0.05$. Rhizobium inoculation at several experimental sites showed that there were noticeably more nodules per plant. Figure 1a shows that strain inoculation had a substantial ($p \leq 0.05$) impact on nodule number/plant. More nodules were formed overall by inoculation plants than by control plants. When these strains were inoculated alongside the native faba bean Rhizobium strains that are already present in the study area, the results suggested that they would be more competitive and suited. Inoculating the Rhizobium strain with faba bean seed produced more nodules, according to Woldekiros *et al.* (2018). Indeed, research by Gedamu *et al.* (2021), Nagwa *et al.* (2012), and El-Khateeb *et al.* (2012) found that inoculating faba bean with a Rhizobium strain significantly increased the number of nodules. According to Desta *et al.*, (2015), the inoculation of faba bean Rhizobium strains significantly increases the number of nodules per plant. According to the results, FB-24 and FB-120 were the two samples with the most nodules overall. The inoculated rhizobia at the Kulumsa site often produced the most nodules compared to the other two sites instead of (Bekoji and Hitosa). Figure 1b illustrates how the nodule fresh weight (NFW) and nodule dry weight (NDW) increased in comparison to the control. Inoculant FB-24 produced noticeably greater mean nodule fresh and dry weights at all sites, followed by FB-120. Carter *et al.* (1995) hypothesizes that the absence of competent native rhizobia-modifying faba beans in the soil where the experiment was conducted is the reason why the control check had the lowest NN, NFW, and NDW values. The nodulation of all uninoculated faba bean plants in all study locations served as confirmation of the presence of ineffective soil resident rhizobia strain populations across the study sites.

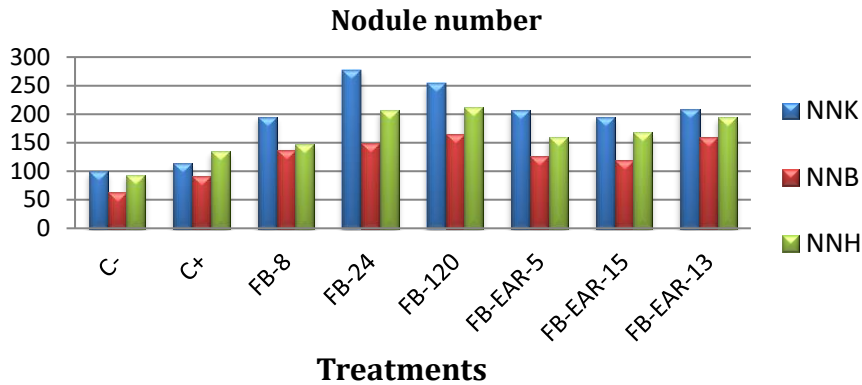


Figure 1a: Effect of rhizobia inoculation on mean nodule number

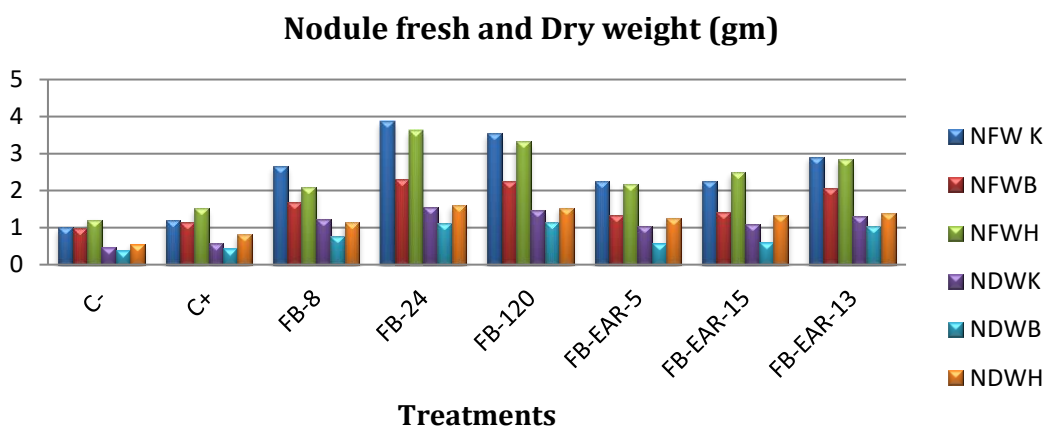


Figure 1b: Effects of rhizobia inoculation on mean nodule fresh and dry weight (gm).

NNK= nodule number Kulumsa, NNB= nodule number Bekoji, NNH= nodule number Hitosa, NFWK= nodule fresh weight Kulumsa, NFWB= nodule fresh weight Bekoji, NFWH= nodule fresh weight Hitosa, NDWK= nodule dry weight Kulumsa, NDWB= nodule dry weight Bekoji, NDWH= nodule dry weight Hitosa

Effect of Inoculation on Yield and Yield Components

The current study found that there were statistically significant differences between the treatments in terms of plant height (PH), number of pods per plant (NPPP), number of seeds per pod (NSPP), above-ground biomass yield (BY), grain yield (Adj.GY), and hundred seed weight (100 SWt) (Table 3,4 and 5). Inoculating with strain FB-24 led to the maximum plant height at the Hitosa experimental site of 160.98 cm, with FB-120 coming in second (158.47 cm). A similar pattern was seen for the quantity of pods per plant as measured by plant height. At all experimental sites, the Rhizobia isolates FB-24 and FB-120 performed best in terms of plant height and the number of pods per plant (Tables 3, 4, and 5).

Nonetheless, in terms of plant height and the quantity of pods produced per plant, there was a statistically significant difference ($p \leq 0.05$) between the treatments. According to research by Bejandi *et al.* (2012), seed inoculation significantly boosts nitrogen uptake, which enhances plant performance and growth with the potential to increase plant height. Faba bean pod production was shown to be significantly impacted by Rhizobium inoculation by Anteneh and Abere (2017), Albayrak *et al.* (2006), and Meena *et al.* (2007).

In terms of plant height, pods per plant, and seed production, the negative control showed the lowest result. At Hitosa, the rhizobia isolate FB-24 and FB-120 had superior NPPP scores of 22.95 and 20.05, respectively. Rhizobium inoculation increased the number of seeds produced per plant, as shown by Asrat and Fassil (2019) and Argaw & Tsigie (2015). In addition, isolate FB-24 was substantially greater for NSPP compared to the other treatments ($p \leq 0.05$). For 100SWt no significant difference ($p \leq 0.05$) was observed among the rest of the treatments except the negative control at all experimental sites. The negative control was the only treatment for which there was no significant change ($p \leq 0.05$) across all the experimental sites.

Table 3: Effect of inoculation on the combined mean of growth and yield parameters at Hitosa

| Parameters | | | | | | |
|------------|----------------------|---------------------|--------------------|--------------------|---------------------|----------------------|
| Year | PH | NPPL | NSPP | 100SWt (gm) | BY (kg/ha) | Adj.GY (kg/ha) |
| 2019 | 141.89 ^b | 16.75 ^b | 3.3 ^a | 93.8 ^b | 6068.6 ^a | 3037.9 ^a |
| 2020 | 162.08 ^a | 22.18 ^a | 3.2 ^a | 108.4 ^a | 6993.7 ^a | 3190.8 ^a |
| Mean | 151.99 | 17.36 | 3.26 | 101.1 | 6531.11 | 3114.35 |
| LSD | 9.01 | 2.18 | 0.15 | 31.13 | 1424.4 | 510.09 |
| Treatment | | | | | | |
| C- | 135.70 ^b | 12.50 ^b | 2.82 ^b | 95.4 ^b | 3866 ^c | 1630.9 ^c |
| C+ | 152.18 ^{ab} | 17.45 ^{ab} | 3.20 ^{ab} | 102.5 ^a | 5883 ^{bc} | 2819.7 ^b |
| FB-8 | 148.51 ^{ab} | 18.30 ^{ab} | 3.18 ^{ab} | 100.1 ^a | 6546 ^{abc} | 3372.2 ^{ab} |
| FB-24 | 160.98 ^a | 22.95 ^a | 3.48 ^a | 104.7 ^a | 8990 ^a | 4273.4 ^a |
| FB-120 | 158.47 ^a | 20.05 ^a | 3.25 ^{ab} | 102.0 ^a | 7490 ^{ab} | 3929.1 ^a |
| FB-EAR-5 | 150.81 ^{ab} | 16.43 ^{ab} | 3.23 ^{ba} | 100.2 ^a | 5830 ^{bc} | 2889.8 ^b |
| FB-EAR-15 | 152.85 ^{ab} | 17.71 ^{ab} | 3.21 ^{ab} | 100.9 ^a | 6251 ^{abc} | 2570.3 ^{bc} |
| FB-EAR-13 | 149.18 ^{ab} | 17.75 ^{ab} | 3.15 ^{ab} | 103.0 ^a | 7391 ^{ab} | 3429.5 ^{ab} |
| CV | 15.01 | 10.63 | 10.07 | 12.68 | 18.44 | 13.85 |
| LSD | 18.03 | 4.36 | 0.31 | 62.26 | 2848.8 | 1020.2 |

Note. Mean values in the same column with different letter(s) are significantly different at a $p \leq 0.05$.

PH- Plant height, NPPL- Number of pods per plant; NSPP- Number of seeds per pod, 100SWt- 100 Seed weight, BY- Total biomass yield, Adj.GY- Adjusted Grain yield

Inoculating faba bean seeds with rhizobial isolates caused a significant change ($p \leq 0.05$) in above-ground biomass yield and adjusted grain yield at all study sites (Tables 3, 4, and 5). The inoculation of FB-24 and FB-120 resulted in the highest BY (9290 kg ha⁻¹) and Adj.GY (4273.4 kg ha⁻¹), which were respectively 8991.8 kg ha⁻¹ and 4192.2 kg ha⁻¹ at Hitosa and Kulumsa district. The negative control, however, had the lowest adjusted grain yields and above-ground biomass yields across all experimental sites. This outcome is consistent with several studies demonstrating that rhizobium inoculation considerably increases faba bean seed output (Anteneh and Abere, 2017 Habtemichial *et al.*, 2007). However, it concurs with the findings of Rugheim and Abdelgani (2012), Yohannes Desta *et al.* (2015), and Sameh *et al.* (2017), who showed that the inoculation of efficient rhizobial strains greatly boosted faba bean biomass and grain yield output. The rhizobial isolate inoculations gave the best results in the experimental year of 2020 as compared to 2019 because of the unstable rainfall conditions that occurred.

Table 4: Effect of inoculation on the combined mean of growth and yield parameters at Kulumsa

| | Parameters | | | | | |
|------------------|----------------------|---------------------|--------------------|-------------------|-----------------------|----------------------|
| Year | PH | NPPL | NSPP | 100SWt (gm) | BY (kg/ha) | Adj.GY (kg/ha) |
| 2019 | 152.46 ^a | 20.03 ^a | 2.97 ^b | 96.4 ^a | 7699.3 ^a | 2696.05 ^b |
| 2020 | 113.66 ^b | 10.45 ^b | 3.15 ^a | 79.1 ^b | 6618.1 ^b | 3117.48 ^a |
| Mean | 133.06 | 15.24 | 3.06 | 87.7 | 7158.68 | 2906.76 |
| LSD | 6.95 | 2.64 | 0.15 | 23.13 | 763.34 | 214.48 |
| Treatment | | | | | | |
| C- | 116.01 ^b | 11.38 ^b | 2.88 ^b | 83.8 ^b | 3966.6 ^d | 1739.8 ^f |
| C+ | 137.16 ^a | 16.01 ^{ab} | 3.08 ^{ab} | 87.3 ^a | 8196.2 ^{ab} | 2868.3 ^{cd} |
| FB-8 | 138.50 ^a | 16.18 ^{ab} | 3.06 ^{ab} | 88.0 ^a | 7291.6 ^{bc} | 3412.1 ^b |
| FB-24 | 141.66 ^a | 19.86 ^a | 3.30 ^a | 88.6 ^a | 8889.0 ^a | 3978.6 ^a |
| FB-120 | 137.31 ^a | 18.05 ^a | 3.22 ^a | 91.6 ^a | 8991.8 ^a | 4192.2 ^a |
| FB-EAR-5 | 129.51 ^{ab} | 14.73 ^{ab} | 2.95 ^b | 85.7 ^a | 6102.3 ^{cd} | 2363.1 ^e |
| FB-EAR-15 | 132.33 ^a | 15.25 ^{ab} | 3.05 ^{ab} | 89.3 ^a | 6989.0 ^{bc} | 2475.9 ^{de} |
| FB-EAR-13 | 132.00 ^a | 17.50 ^a | 3.06 ^{ab} | 87.6 ^a | 7545.1 ^{abc} | 2964.3 ^c |
| CV | 14.42 | 14.68 | 10.2 | 12.22 | 9.02 | 16.24 |
| LSD | 13.91 | 5.29 | 0.31 | 46.27 | 1526.7 | 428.95 |

Note. Mean values in the same column with different letter(s) are significantly different at a $p \leq 0.05$. PH- Plant height, NPPL- Number of pods per plant; NSPP- Number of seeds per pod, 100SWt- 100 Seed weight, BY- Total biomass yield, Adj.GY- Adjusted Grain yield.

Table 5: Effect of inoculation on the combined mean of growth and yield parameters at Bekoji

| | Parameters | | | | | |
|------------------|---------------------|---------------------|-------------------|--------------------|-----------------------|---------------------|
| Year | PH | NPPL | NSPP | 100SWt (gm) | BY (kg/ha) | Adj.GY (kg/ha) |
| 2019 | 101.80 ^a | 10.37 ^b | 2.70 ^b | 98.1 ^a | 6853.2 ^a | 2977.8 ^a |
| 2020 | 120.01 ^b | 13.30 ^a | 3.30 ^a | 102.7 ^a | 6422.3 ^a | 3148.5 ^a |
| Mean | 110.98 | 11.83 | 3.00 | 100.4 | 6637.7 | 3063.12 |
| LSD | 7.51 | 1.27 | 0.13 | 45.90 | 648.7 | 160.48 |
| Treatment | | | | | | |
| C- | 95.40 ^b | 8.78 ^c | 2.91 ^b | 97.4 ^a | 4223.3 ^d | 1646.0 ^e |
| C+ | 113.50 ^a | 12.36 ^{ab} | 2.91 ^b | 99.9 ^a | 5803.0 ^c | 2511.2 ^d |
| FB-8 | 111.35 ^a | 11.35 ^{ab} | 2.98 ^b | 99.4 ^a | 6914.3 ^{abc} | 3417.3 ^c |
| FB-24 | 118.15 ^a | 13.18 ^{ab} | 3.11 ^b | 103.0 ^a | 7438.1 ^a | 3807.2 ^b |
| FB-120 | 117.00 ^a | 13.86 ^a | 3.28 ^a | 104.8 ^a | 8029.4 ^a | 4166.0 ^a |
| FB-EAR-5 | 107.35 ^a | 10.88 ^{bc} | 3.01 ^b | 100.3 ^a | 7188.9 ^{ab} | 3115.6 ^c |
| FB-EAR-15 | 117.35 ^a | 12.68 ^{ab} | 2.98 ^b | 99.3 ^a | 6057.2 ^{bc} | 2549.3 ^d |
| FB-EAR-13 | 109.80 ^a | 11.58 ^{ab} | 3.05 ^b | 99.1 ^a | 7547.8 ^a | 3292.6 ^c |
| CV | 15.7 | 9.10 | 13.72 | 13.86 | 8.27 | 14.43 |
| LSD | 15.02 | 2.54 | 0.26 | 91.803 | 1297.4 | 320.96 |

Note. Mean values in the same column with different letter(s) are significantly different at a $p \leq 0.05$. PH- Plant height, NPPL- Number of pods per plant; NSPP- Number of seeds per pod, 100SWt- 100 Seed weight, BY- Total biomass yield, Adj.GY- Adjusted Grain yield.

CONCLUSION AND RECOMMENDATIONS

Eventually, at all of the experimental sites, the Rhizobium inoculation boosted the output of faba beans. Based on their performance of nodulation and yield production findings, FB-24 and FB-120 from the total of six rhizobial isolates can be candidates for producing biofertilizers for faba beans

in all experimental sites. The selected isolates must go through additional testing utilizing different agroecologies and on different soil types before being commercialized.

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