ORIGINAL ARTICLE

The contribution of rhizobia and arbuscular mycorrhizal fungi co-inoculation on growth and yield of haricot bean (*Phaseolus volgaris)*

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ABSTRACT
The disastrous environmental quality effects of indiscriminate use of commercial fertilizers drive research into natural fertilizer sources and bio-stimulants. As a result, this study aimed to look into the effects of interactions between microbial symbionts Rhizobia(R) and arbuscular mycorrhizal fungi (AMF) on haricot bean growth and biomass output. The haricot bean variety 'Hawassa Dume' was inoculated with a mixture of AMF and indigenous and commercial Rhizobia. The experiment was carried out for 90 days. Inoculation of haricot bean seedlings with the R+AMF resulted in a considerable increase in nodulation (45.0±2.3 in IR and 46.0±7.8 in MBI) compared to un-inoculated plants. In the greenhouse trial, dual inoculation of AMF and rhizobia boosted different plant growth parameters: shoot length 12cm in IR and 12.1cm in MBI; shoot dry weight 2.9g in IR and 3.0g in MBI; root dry weight 0.5g in IR and 0.5g in MBI; and number of nodules/plant 4.6 in IR and 4.6 in MBI. The field testing yielded nearly identical results. Similarly, improved nitrogen uptake (0.72% for MBI and 0.56% for IR) and better phosphorus uptake 0.33% and 0.26% for IR and MBI, respectively, were observed compared to single inoculation. Finally, the interactions among the components of the tripartite symbiotic relationship, Rhizobia-AMF-Haricot bean, have boosted plant growth and biomass output. As a result, it is advised that efficient rhizobia and AMF inoculants be produced and distributed to conventional and small-holder agricultural systems, along with more research activities on the efficiency of bio-fertilizers.

Keywords: Arbuscular mycorrhizal fungi, co-inoculation, Nitrogen, *Phaseolus vulgaris*, Phosphorus, Rhizobia, Symbiosis.

INTRODUCTION

The intensive use of chemical fertilizers in agriculture leads to disastrous consequences on the environmental quality prompting the search for environment-friendly natural sources of fertilizers (Hartman et al., 2011). One of the important strategies to increase agricultural production and to improve and sustain soil properties is the development and use of efficient inoculant bio-fertilizers (Mortimer et al., 2008). One of such inoculant bio-fertilizers is arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhiza fungi (AMF), found in higher plants, and the plant roots they associate with form an endosymbiotic interaction. The hyphae of the AMF grow towards the inner cortical cells of the plant root where they differentiate into highly branched structures, the arbuscules and circular storage organs called vesicles (George et al., 1995). In AM symbiosis, the fungus also forms hyphae outside the plant and these provide a connection between the soil and the inner part of the plant and hence facilitate the uptake of nutrients from much larger volume of soil than possible by the plant root itself (Gianinazzi-Pearson, 1996).

AM fungi are found in many soils worldwide, and they form association with more than 80% of all terrestrial plant roots (Smith and Read, 2008). AM fungi are major contributors to plant nutrition, promoting mostly phosphorus uptake and other ions such as zinc, cooper and nitrogen. Moreover, it improves soil structure and protects plants from pathogenic fungi and nematodes (Smith et al., 2003). In return, the AMF derive benefits from the host plant readily synthesized organic carbon compounds

In addition to AMF, the rhizobia-legume endosymbiosis is another environment-friendly source of bio-fertilizer. In contrast to AMF which form non-specific endosymbiosis with large number of plants, only plants within the family Leguminosae (Fabceae) and few other plant families form endosymbiosis with Rhizobia (Franche et al., 2009). Rhizobia are a collection of taxonomically diverse group of bacteria that induce root or stem nodule typically in specific species of leguminous plants irrespective of their nitrogen fixation ability (Giller, 2001). Currently rhizobia include more than 98species of bacteria in 13 genera (Weir, 2016). Among them, the genera *Azorhizobium, Bradyrhizobium, Rhizobium and Sinorhizobium* are notable examples. The rhizobia-legume symbiosis typically forms a completely new organ, the root nodule. In these nodules the bacteria are hosted intracellular and they find the ideal environment to reduce atmospheric nitrogen into ammonia, a source of nitrogen that the plant can use (Mylona et al., 1995).

Nodule formation of rhizobia is a complex process because it requires a continuous signal exchange between the plant and the bacteria (Soto et al., 2006). In this symbiotic association, plant provides an energy source and ecological niche for the bacteria and in return bacteria provide a source of fixed nitrogen to the plant (Perret et al., 2000). The associations of legume with rhizobium spp. have extremely important agronomical value. During their association further nodulation is suppressed in other parts of the root system by a long distance signal exchange, which means the nodulation is auto regulated (Okakira and Kawaguchi, 2006).

Most legumes have two types of microbial symbionts; mycorrhizal fungi and nitrogen fixing bacteria establishing triple association, capable of supplying N and P to the plants (Silveira and Cardoso, 2004). Dual inoculation with both rhizobia and AM fungi results in a tripartite mutualistic symbiosis and increases plant growth to a greater extent than inoculation with only one (Chalk et al., 2006). Murat *et al.,* (2011) reported that inoculation of AMF and rhizobia increased the plants' yield, root colonization and nutrient content. Thus, the plants benefit more from AM fungi and Rhizobium dual symbiotic association than single symbiotic association or no-inoculation.

One of the most important legume crops in Ethiopia is haricot beans. Its production is mostly dependent on application of chemical fertilizers. However, despite their importance in increasing pulse productivity, chemical fertilizers leave dangerous side effects for humans and the environment. As a result, it is advised that bio-fertilizers, which are less expensive and environmentally benign, be used to grow legumes. Though sporadic tests on the use of bio-fertilizers in the production of various crops have been conducted in Ethiopia, there needs to be more data on their dual inoculation with AMF and rhizobium in the production of pulses. Therefore, this study aimed to ascertain how co-inoculation of arbuscular mycorrhizal fungi and Rhizobia affect the growth and yield of haricot bean under greenhouse and field conditions.

MATERIALS AND METHODS

Description of the study area

Hawassa University is located in the Ethiopian rift valley, 275 km South of Addis Ababa and lies between 7^0 3′1.35″N latitude and 38^0 29′43.81″E longitudes with an altitude of about 1736 meters above sea level. The greenhouse and the Hawassa University research village where the research was conducted are located at the eastern corner of the main campus.

Treatments and experimental design

Experiments in plastic pots (2 kg soil carrying capacity) and in the field were carried out in 2019 in Hawassa University greenhouse and research village. The treatments were set in a randomized complete block design (RCBD) for the field experiments and completely randomized design (CRD) in the greenhouse with three replications. Treatments were set for indigenous AMF and Rhizobia compared to standard commercial

rhizobium obtained from Menagesha Biotechnology Industry (MBI) of Ethiopia. Surface sterilized seeds of haricot bean were sown in a 3M X 2M quadrants in the field and inoculated with rhizobia and/or AMF inoculum according to the different treatments in the greenhouse. Control treatments were not inoculated neither with the rhizobia nor AMF. After germination, the seedlings, both in the field and in the greenhouse were thinned and were allowed to grow for 90 days. Throughout the growing season, the plants were monitored daily, weeds were cleared and plants watered as necessary. Detail description of the treatments is presented in Table 1.

Table 1. Treatment arrangements

Treatments	Description
Hb+R	Inoculated with Rhizobium alone
Hb+AMF	Inoculated with AMF alone
Hb+NPK	Treated with NPK fertilizer only
Hb+R+NPK	Inoculated with Rhizobium and treated with NPK fertilizer
Hb+AMF+NPK	Inoculated with AMF and treated with NPK fertilizer
Hb+R+AMF+NPK	Inoculated with Rhizobium plus AMF and treated with NPK fertilizer
Hb + R + AMF	Inoculated with Rhizobium plus AMF
C	Note inoculated & with no NPK

The NPK used in this treatment was (50, 25 and 25) which is 3.57 × 10^{-6} Kg N/pot and 1.78 × 10^{-6} Kg P/pot.

Preparation of AMF inoculum

Mixture of *Gigaspora rosea and Rhizophugus clarus* morphospecies previously isolated (Beyene et al., 2016) by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) from the rhizospheric soil of agroforestry shade trees and crops were multiplied in the roots of Sorghum (*Sorghum bicolor)* plants. The plants were allowed to grow for three months, after which the substrate containing root fragments, mycelium and spores were collected air dried and used as a crude inoculum. Healthy haricot bean seeds were surface sterilized with 0.3% hypochlorite solution for 30 seconds and washed with sterile distilled water (Utobo et al., 2011). Then the seeds were germinated in the mixture of sterilized soil under dark condition. The seedlings were allowed to grow for 8-10 days. Seedlings of uniform height were transplanted in plastic pots containing 2 kg oven sterilized mixture of sand and soil in the ratio 1:1. Seedlings were inoculated with mycorrhizal inoculum containing sorghum root fragments, mycelium and spores (100 g per pot). The treatments were replicated three times.

Isolation, authentication and inoculation of indigenous Rhizobia

Soil and seed sample collection

Analysis of soil physicochemical properties

Collection of soil and seed samples are the first steps in trapping and identification of rhizobia isolates. Using an auger, 20 soil samples each of 3.5kg were collected from a cultivated land without previous leguminous plant production from 10-15 cm depth. Soil samples were collected from 10 villages of Shalla, Wolaita Sodo and Boricha areas with conducive temperature, soil type and altitude to grow haricot bean (Table 1) and were separated into two parts; 3kg for trap culturing and 500g for the analysis of soil physicochemical properties.

Table 2. Geographical locations from where soil samples were collected for Rhizobia isolation

Sampling site	Longitude	Latitude	Altitude (m a.s.l
Shalla, Bekele Daya farm	38⁰25′ 20.8″	7 ⁰17′ 24.3″	1671
Halaba Zone, Gedeba Kebele(FTC)	38⁰8′ 41.6″	7⁰18′ 24.7″	1782
Halaba, 1st Chorko Kebele	38⁰5′ 56.4″	7⁰ 21′ 0.6″	1787
Damot Gale, Hagaza Doge Kebele	37⁰ 52′ 22.2″	6⁰ 58′ 28.7″	1971
Damot gale, Ade Koisha Kebele	37⁰52′ 57.3″	6⁰ 59′ 55.1″	1894
Damot Gale, Chocha Kebele	37⁰ 50′ 43.1″	6⁰ 56′ 05.0″	2076
Damot Gale, Fate Kebele	37⁰ 50′ 14.0″	60 55′18.5″	2104
Damot Woide, Mayo Kole Kebele	37⁰ 51′ 16.4″	6⁰ 53′ 18.2″	2092
Damot Woide, Kindo Koyo kebele	37⁰ 52′ 51.2″	6⁰ 53′ 43.3″	1993
Boricha, Shello Abore Kebele	38⁰ 14′ 01.8″	6⁰ 56′ 45.9″	1840

A 500 g of each soil sample collected was analyzed for its physicochemical properties. The soil samples were air-dried at room temperature for two weeks, grounded, homogenized and passed through a 2mm sieve and preserved at 4°C for analysis of soil physicochemical properties. Soil analysis were undertaken at Debrezeite Agricultural Research Center following standard procedures and methods: Soil textural fractions were analyzed following the hydrometric method after removing organic matter

using H_2O_2 and dispersing the soils with sodium hexameta-phosphate (Black et al., 1965). Soil pH was determined by potentiometric methods using 1:2.5 soil: water ratio. Soil organic carbon (SOC) was determined by the Walkley-Black oxidation method (Schnitzer, 1982). Total nitrogen (TN) was determined using the Kjeldahl distillation method (Bremner and Mulvaney, 1982), and available phosphorous (AP) was determined using Olsen's extraction method (UV/visible Spectrometer, Lambda EZ 201) (Olsen and Dean, 1965). Available potassium (Av. K) was determined by Sodium Acetate (flame photometer) method (Jones, 2001). The exchangeable bases (Ca^{2+} and Mg^{2+}) were measured by atomic absorption spectrophotometer (NOV AA 400) after extraction with ammonium acetate at pH_7 (Black et al., 1965).

Haricot bean trap culturing

Healthy seeds of *Phaseolus vulgaris* with the same size were sterilized by rinsing with 95% alcohol for 10 seconds, followed by 3% Sodium hypochlorite for 4 minutes, then sterilant drained off. After that seeds were rinsed with eight changes of sterile water. Trap culture with haricot bean (10 soil samples) was set in Hawassa University greenhouse according to (Solomon and Fassil, 2014).

Trapping of nodules

Nodulation was induced by 'plant trap' method in green house condition as described by Vincent (1970). The soil samples were filled in 2 kg capacity plastic pots that had been surface sterilized (using 70% alcohol for 5sec.). Similar sized seeds of haricot bean were also surface sterilized with 70% ethanol for 5 seconds and with 3 % (v/v) solution of sodium hypo-chlorate for 3-minutes, and washed thoroughly with five changes of sterile distilled water. Then six seeds were sown in each pot under greenhouse conditions. After germination the seedlings were thinned down to two/pot. Thereby plants were watered every two days for 45 days. After 45 days the pink and undamaged nodules were collected at flowering stage of the plants. Then, the collected nodules were preserved on Silica-gel until analyzed (Agah et al., 2016).

Preparation of culture media

To isolate rhizobia Yeast Extract Mannitol Agar (YEMA) (Vincent, 1970) with the following composition was prepared: Mannitol (10 g/l), K_2HP0_4 (0.5 g/l), $MgS0_4$ $.7H_20$ (0.2 g/l), NaCl (0.1 g/l), Yeast Extract (0.5 g/l), Agar (15 g/l), Distilled Water (1000 ml) and pH (7.0±0.1)(Somasegaran and Hoben, 1994). Finally, the media was autoclaved at 121^0C for 15 minutes.

Isolation of Rhizobia from nodules

Collected nodules were surface sterilized with 95% ethanol for 10 seconds, and transferred to 3% (v/v) solution of sodium hypo-chlorate for 3-4 minutes. The surface sterilized nodules were then rinsed in five changes of sterile distilled water to completely rinse the sterilizing chemicals (Lupwayi and Haque, 1994). Then nodules from each pot were transferred into different sterile Petri-dishes and crushed with alcohol flamed sterile glass rod in a drop of normal saline solution (0.85% NaCl) inside a laminar air flow hood. Then after 0.1ml (loopful) of the suspensions were streaked on plate containing Yeast Extract Mannitol Agar (YEMA) +0.2% CR indicator and incubated at 28 ± 2^0C from 3-5 days.

Purification and preservation of isolates

After 3-5 days of growth, single dome-shaped colonies were picked with sterile inoculating loop and streaked on sterile YEMA plates and incubated at 28±2^0C. The purity and uniformity of colony types were carefully examined through repeated re-streaking and a single well isolated colony was picked and transferred to YEMA slant containing 0.3% (W/V) $CaCO_3$ in a culture tube and incubated at 28±2^0C. When sufficient growth was observed, the culture was transferred to be preserved at 4^0C for future use (Vincent, 1970). The isolated native strains were then characterized on the basis of morphological and physiological/biochemical characters according to Jordan, (1984).

Authentication of isolated Rhizobial strains

Sterile growth plastic pots filled with acid-washed sterile sand (river sand was washed with sulfuric acid (38%; 5 L/20 kg sand) to reduce organic matter that could be an N source for the plants (Lupwayi and Haque, 1994). The sand was then rinsed with tap water until its pH was neutral, autoclaved and was used to test the ability of the isolated rhizobia strains to form nodules on the homologous legume species from which it was originally obtained as stipulated by Somasegaran and Hoben (2012). To activate the rhizobia, a loop full of material from a preserved slant was transferred aseptically to a 100 ml flask containing 60ml of YEM broth and the flasks were placed on a rotary shaker until the liquid is quite cloudy. One surface sterilized seed (as described above) is placed in a small polyethylene pots containing washed and sterilized sand in triplicate. Then 1 ml of the broth culture was pipetted over the seed. The pots then were placed in the green house.

The authentication was done by following Koch's postulates whereby *Phaseolus vulgaris* grown in nitrogen free media were inoculated with pure rhizobia strains (rhizobia strains isolated from *Phaseolus vulgaris* grown in sampled soils) under sterile conditions (Hassen et al., 2014) while other *Phaseolus vulgaris* were grown for 45 days in rhizobia free media and remain non-inoculated as a control. Strains which managed to nodulate *Phaseolus vulgaris* grown in sterile media were proved to

be rhizobia and those failed to nodulate considered as non-rhizobia for *Phaseolus vulgaris*.

Evaluation of symbiotic effectiveness of new Rhizobia strains

Symbiotic effectiveness of the new rhizobia strains were evaluated through greenhouse experiments conducted at Hawassa University greenhouse, whereby sterile seeds of *P. vulgaris* with the same size were grown in rhizobia free media and inoculated with isolated rhizobia strains. The autoclaved river sand was used as sterile media. Number of nodules per plant, shoot Length, Stem Girth, Shoot Dry Weight, Branch per plant and Leaves per plant were used for testing the effectiveness of the isolated strain in nitrogen fixation. The symbiotic effectiveness testing involves preparation of sterile media, preparation of isolated strains, seed sterilization, planting, and taking measurements of the above growth parameters.

i) Preparation of sterile media

The river sand was collected and all debris in it was removed, washed, dried and autoclaved at 121^{0}C for 30 minutes, so as to kill all pathogens. Then autoclaved sand was left for 24 hours to allow it to cool. After cooling, the sterile sand was put in 1/5kg plastic cups/pots ready for growing the *P. vulgaris.*

ii) Preparation of isolated strains for inoculation

The authenticated strains of indigenous rhizobia were re-grown on plates containing Yeast Extract Mannitol Agar (YEMA) with Congo red (CR) and incubated at 28^{0}C. After three days, by using an inoculation loop, rhizobia strains in the same amount for each strain, were taken from the plates and inoculated in 100 ml sterile beakers containing 60 ml of Yeast Extract Mannitol Broth (YEMB). The inoculated beakers of YEMB were covered with autoclaved foil paper and kept in a shaker incubator for 72 hours at 28^{0}C and 120 rpm (rotation per minute) until broth color changed from colorless to the milky indicating presence of rhizobia strains. Then 1 ml of YEMB containing rhizobia were applied in 1/5 kg plastic cups containing sterile sand and germinating seeds of *P. vulgaris*.

iii) Seed sterilization and planting

Seed of *P. vulgaris* with the same size were sterilized by rinsing with 95% alcohol for 10 seconds, followed by 3% Sodium hypochlorite for 4 minutes, then sterilant drained off. After that seeds were rinsed with eight changes of sterile water. After that seeds were submerged in sterile water left in the refrigerator at 4°C for 4 hours in order to allow seeds to imbibe. After 4 hours, the seeds were rinsed with three changes of sterile water and left at room temperature for 24hours, then sown in pots containing sterilized sand. Then some pots were inoculated with the isolated *rhizobia* strains, while some was inoculated with standard rhizobia inoculants "MBI" as positive control and other were left non- inoculated as negative control. After 5 weeks, growth parameters such as number of nodules, stem girth, number of leaves, number of branches and shoot length were measured and compared as described by Mhango et al. (2013).

Assessments of symbiotic effectiveness

The plants were harvested 5 weeks after inoculation. The shoots were collected by cutting the plants at the level of the sand. Then shoots from each growth unit were placed in paper bags and dried at 70 ^{0}C for 48 hours as described by Somasegaran and Hoben (1994) and their dry weight was determined. The roots and adhering sand were dislodged in to a coarse sieve (0.76mm) and were washed with a gentle tap water and observed for nodules. The nodules were collected, and counted. The relative effectiveness of isolates in accumulating plant shoot dry matter was calculated as described in Somasegaren and Hoben (1994) as follows:

$$SE = \frac{\text{Inoculants plant D.M. X 100}}{\text{N-fertilized plant D.M.}}$$

Where, D.M. = dry matter, S.E. = symbiotic effectiveness

The rate of nitrogen fixing effectiveness is evaluated as: Highly effective > 85%, Effective 55-85%, Lowly effective 35-54% and Ineffective <35%.

Morphological and biochemical studies of the bacteria

Morphological study was done on the basis of shape, colour, size, elevation, margin and texture of colonies of Rhizobia on YEMA (Vincent, 1970). Then cultures were struck on YEMA and incubated at 30°C. After 4 days for rhizobia the shape, colour, size, elevation, margin and texture were noted. Finally, selected biochemical tests were done to characterize the isolated rhizobia.

Gram's staining test was done to study gram reaction and staining was carried out according to standard Gram's procedure (Somasegaran and Hoben, 1994). Four different reagents in the order of Gentian violet, Gram's iodine, alcohol distaining reagents, and safranin were used for classifying bacteria based on their gram staining result. In catalase test a clean glass slide was taken and a drop of rhizobial culture suspension was placed. Few drops of hydrogen peroxide were added to the culture. The evolution of air bubbles form the suspension indicates the positive results.

For triple sugar iron agar test, iron agar medium was poured into the sterile test tubes and allowed to solidify. The rhizobial cultures were inoculated into the tubes and incubated at 28± 2°C for 24 hr the result were noted. While for the methyl red tests, 5 ml broth of the Methyl Red Voges Proskauer was prepared and poured into sterile test tubes. The rhizobial isolates were inoculated separately into the tubes and incubated at 28± 2°C for two days. After the incubation period, 5 ml

of methyl red indicator was added to the each test tube. Red coloration of the broth designates the positive result; however turning of methyl red to yellow is a negative result.

For citrate utilization test Simon's citrate agar medium was decanted into the sterile test tubes. The Rhizobial isolates were inoculated separately into the test tubes and incubated at 28±2°C for 4 days. After the incubation period, the green color turned to blue indicates the positive results, **and for starch hydrolysis test, s**tarch agar medium was transferred into the sterile petri plates and allowed to solidify. The rhizobial cultures were incubated into the Petri plates separately and incubated at 28± 2°C for 4 days. After the incubation period, 5 ml of iodine solution was added and observed a clear zone of hydrolysis surrounding the growth of the organisms is indicates the positive results.

Plant and soil sampling procedures

Plants were harvested by removing them completely from the field and greenhouse pots. Plants were divided into fractions. From the greenhouse before dry matter determination of roots, 0.5mg fine root segments (1 cm in length) below the upper 2 cm of the roots was sampled to estimate root colonization by AMF and stored in a 50% ethanol solution until analyzed. Soil samples from each pot which is inoculated with AMF were collected during harvesting, after the plants had been removed and the representative sample was put in a plastic bag separately. Then the soil samples were dried at room temperature for 15 days and preserved at 4 °C until analyzed.

Mycorrhizal colonization and spore density

The roots were cleared with a 10% KOH solution in a water bath at 90^0_C for 1hr and stained with a 0.05% trypan blue solution. Percent root colonization was estimated using a magnified intersection method, hairline graticule was inserted into eyepiece acting as the line of intersection with each root at X200 magnification under the compound light microscope (McGonigle et al., 1990).

To study the spore density in the pot experiments 50cm3 soil samples were collected (as in 3.5 above) and mixed in a two liter capacity beaker containing 1.5 liter of water. The soil in the water was agitated by stirring vigorously by hand and left to settle down. The suspension was then be sequentially sieved with sieves having mesh size of 500, 100 & 50µm in diameter, following the wet sieving and decanting method (Gerdemann and Nicolson, 1963). The last pellet (50 µm) was suspended in 60% sucrose solution and was thoroughly mixed and centrifuged at 2000rpm for 1 minute and the spores were rinsed carefully with tap water and transferred into plastic petri-dishes. The AMF spores and sporocarps of each sample were counted under 4x stereomicroscope. The spore density was expressed as the numbers of spores and sporocarps per 50g of dry substrate (soil plus sand mixture from the pot experiment).

Rhizobial infection

On two harvests, two plants were taken from each pot, shaken free of superficial soil, and all nodules present were carefully removed. The numbers of nodules per plant were counted independently and mean value was calculated for the two harvests.

Nutrient content

Haricot bean shoot samples were dried at 105 °C for 24 hr and ground to determine nutrient content. Plant tissue analyses were undertaken at Debrezeite Agricultural Research Center following standard procedures. The concentration of nitrogen in the samples was determined using an element analyzer based on the Dumas principle (**LECO** CN). For the plant tissue concentration of other elements, dried sample (500mg) was digested in a tri-acid (HNO_3 + $HClO_4$ + H_2SO_4) mixture (Nabrzyski and Gajewska, 1998). K content in the digest was measured using atomic absorption spectrometry (Varian SpektrAA 300) (Beaty and Kerber, 1993). The P content in the plant tissues was analyzed by the vanadomolybdate method after the wet digestion followed by photometry (Varian DMS 200) (Cavell, 1955).

Statistical analysis

Data analysis for comparison of all growth parameters (plant height, stem diameter, root and shoot dry matter yield, etc) among treatments and control plants were carried out using the SPSS 20.0 version for Windows software package. Mycorrhizal dependency (MD) was calculated according to Plenchette et al. (1983) as follows: MD (%) = [(M-NM)/M] X 100; where: M is the total dry biomass of mycorrhizal plant; NM is the total dry biomass of non-mycorrhizal plant. The chemical and microbiological (root colonization, spore density and number of nodules) data were analyzed by one-way analysis of variance and treatment means was compared using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Soil aanalysis

The soil physicochemical properties are presented in Table 3. The soil analysis results show that, pH values ranged between 6.1±0.4c to 7.7±0.4. Organic carbon was above critical levels (0.40%) and ranged from moderate (0.7±0.1) in soil sample from DGH to high (4.9±4.2) in HG and (4.9±0.1) in DWK sampling sites. So, there is a moderate to high level of organic matter in the study area. Total nitrogen was ranged from lowest (0.1%) in

SB to 4.9% in HG. Phosphorus concentration was low in all soil samples ranging from lowest (1.9±0.2mg/kg) in DGF to (6.7±0.2 mg/kg) in DGcho soil samples. Soil texture was clay-loam throughout, except SB and BSA of which their soil texture was clay. The results of the analyzed soil components are presented in Table 3.

Rhizobia strains

A total of 10 bacterial strains were isolated from the nodules of *Phaseolus vulgaris* grown in soils collected from sampling sites in Shalla, Halaba, Wolaita Sodo and Boricha Woredas of Southern Ethiopia. All isolated strains were fast growers having taken 2-5 days to grow in Yeast Extract Mannitol Agar (YEMA) after inoculation. All strains were authenticated and have been proved to be rhizobia by inducing nodulation after inoculating in *P. vulgaris* grown in nitrogen free media. These findings are consistent with Simon et al. (2014), who isolated indigenous rhizobia from haricot bean and chickpea nodules and found colony characteristics similar to those found in this study. These findings provided a promising indicator for the development of haricot bean inoculants, as all of the isolated strains demonstrated the ability to induce nodulation on haricot bean roots. Morphological and biochemical test results of representative examples from haricot bean are shown in tables 4 and 5 below.

Table 3. Physicochemical properties of soils collected from sampling sites in Halaba, Wolaita Sodo and Boricha districts (Woredas)

Parameters	**SB**	**HG**	**Hcho**	DGH	DGA	DGcho	DGF	DWM	DWK	BSA
pH	6.8±0.9^{g}	6.8±4.2^{a}	6.2±0.7^{d}	6.6±0.2^{e}	6.2±0.5^{d}	6.7±0.7^{f}	6.2±0.6^{d}	6.1±0.4^{c}	6.0±0.1^{f}	7.7±0.4^{d}
OC (%)	1.0±0.2^{ab}	4.9±4.2^{d}	1.4±0.5^{ab}	0.7±0.1^{a}	2.2±0.0^{bc}	1.9±0.3^{b}	2.5±0.1^{c}	1.9±0.2^{b}	4.9±0.1^{e}	2.1±0.1^{b}
TN(%)	0.1±0.0^{a}	4.9±4.2^{d}	0.2±0.3^{b}	0.1±0.0^{a}	0.2±0.3^{b}	0.2±0.0^{b}	0.3±0.0^{c}	0.3±0.0^{c}	0.2±0.1^{a}	0.2±0.1^{a}
P(mg/kg)	2.7±1.2^{b}	4.9±0.9^{bc}	5.6±2.9^{c}	4.4±0.2^{bc}	4.6±0.1^{bc}	6.7±0.2^{de}	1.9±0.2^{a}	5.4±0.2^{c}	6.2±0.1^{f}	4.4±0.2^{c}
Mg cmol(+)/kg	5.2±.8^{cd}	5.5±0.4^{cd}	4.27±2.1^{b}	3.6±0.3^{ab}	3.9±0.1^{ab}	5.1±0.3^{cd}	3.3±0.1^{a}	4.8±0.0^{bc}	3.3±0.0^{d}	3.6±0.3^{bc}
Ca cmol(+)/kg	2.3±0.1^{bc}	2.4±0.2^{bc}	3.3±0.0^{c}	2.2±0.0^{bc}	3.4±0.1^{c}	2.5±0.1^{bc}	1.5±0.1^{ab}	1.2±0.1^{a}	2.1±0.1^{c}	3.3±0.1^{bc}
K cmol(+)/kg	3.2±0.2^{bc}	2.4±0.2^{ab}	3.4±0.1^{bc}	3.3±0.0^{bc}	4.4±0.1^{cd}	3.3±0.4^{bc}	2.4±0.1^{ab}	2.1±0.1^{a}	2.9±0.4^{cd}	4.1±0.1^{c}
Texture	SCL	CL	CL	CL	CL	CL	CL	CL	CL	SCL

**SB=Shalla Bekele Daya Kebele;HG= Halaba Zone Gedeba K(FTC); Hcho= Halaba 1stChoroko Kebe; DGH= Damot Gale Hagaza Doge K; DGA=Damot gale Ade Koisha Kebele; DGcho= Damot Gale Chocha Kebele; DGF= Damot Gale Fate Kebele; DWM= Damot Woide Mayo Kole Kebele; DWK= Damot Woide Kindo Koyo; BSA= Boricha Shello Abore Kebele; SCL=silt clay loam; CL= clay loam*

Table 4. Morphological Characteristics of the IR 4hb Indigenous Rhizobia Strains from Haricot bean (representative)

Morphological characteristics								
Shape	Color	Opacity	Surface	Texture	Congo red absorption	Elevation	Margin	Size
circular	milky white	translucent	smooth	mucoid	not absorbing	raised	entire	medium

** IR- indigenous rhizobia; hb- haricot bean*

Table 5. Biochemical characteristics of IR 4hb rhizobial isolates

Bio-chemical characteristics						
Gram stain	**Catalase**	**Oxidase**	**TSI**	**Methyl Red**	**Citrate**	**Starch Utilization**
-Ve	+++	+Ve	ALK/AB	+Ve	+Ve	-Ve

** IR- indigenous rhizobia; hb- haricot bean*

Symbiotic effectiveness of the isolated indigenous rhizobia strains

The rhizobia strains showed significant influence on various growth parameters. They have shown a significant ($p<0.05$) influence on the average number of nodules per plant. Besides, the average shoot dry mass, stem girth and number of branches/plant were significantly different at $p < 0.05$. Isolates didn't show statistical differences ($p <0.05$) in the average number of leaves/plant except the positive control and MBI (Table 6). Amongst the plant growth parameters which showed highly significant differences due to inoculation of the isolated strains in *P. vulgaris* are the number of nodules and shoot length.

The mean number of root nodules per plant ranged from no nodules observed in non-inoculated pots (control) to 96.5 nodules per plant in pots inoculated with HbIR4 as shown in Table 6. The lowest mean shoot length was 25.3cm observed in non-inoculated pots while the highest was 45.4cm. The indigenous rhizobia isolates which performed better next to the MBI in shoot length, dry matter yield and number of nodules per plant were HbIR4 (Tables 6). These findings are consistent with Simon et al. (2014), who discovered that some indigenous isolates had a higher ability to form

effective nodules in the roots of haricot bean and chickpea than commercial/standard strains. Also, unlike Sharma and Kumawat (2011), who demonstrated that nodule number is not an appropriate measure of effectiveness in rhizobia-legume symbiosis, this study demonstrated that nodule can be the best measure of rhizobia symbiotic effectiveness because all highly nodulated plants in this study showed higher shoot length and shoot dry mass. The shoot of the inoculated haricot bean was longer than the shoot of the non-inoculated haricot bean. According to their morphological and biochemical characteristics of the isolated strains HbIR4 isolates that showed better performance in symbiotic effectiveness test were selected for further inoculation trials.

Table 6. Influence of isolated indigenous rhizobia on growth parameters of Haricot bean

Isolated Indigenous rhizobia/control	Growth parameters (Mean ± SEM)					
	SL(cm)	SG(mm)	SDW(g)	NN	BPP	LPP
HbIR1	37.9±0.4^{e}	2.7±0.3^{ab}	1.3±0.0^{d}	50.3±1.1^{h}	1.5±0.2^{d}	14.3±0.8^{e}
HbIR2	31.1±0.6^{cd}	3.6±0.0^{d}	0.8±0.0^{b}	26.4±0.5^{d}	0.7±0.1^{a}	12.5±0.4^{c}
HbIR3	40.4±0.0^{ef}	3.7±0.5^{de}	0.8±0.1^{b}	87±0.5^{j}	2.0±0.5^{f}	12.0±4.0^{b}
HbIR4	45.4±0.9^{g}	3.8±0.1^{f}	1.4±0.1^{e}	96.5±0.5^{k}	2.4±0.4^{g}	12.5±0.2c
HbIR5	39.5±0.5^{ef}	3.4±0.0^{c}	1.3±0.0^{d}	62.1±0.9^{i}	1.1±0.1^{c}	10.7±3.5^{a}
HbIR6	36.5±0.1^{de}	3.8±0.01^{f}	0.8±0.0^{b}	40.8±0.3^{g}	1.8±0.3^{e}	12.8±0.3^{cd}
HbIR7	34.5±0.5^{d}	3.0±0.2^{b}	1.2±0.0^{cd}	22.8±0.3^{c}	1.5±0.5^{d}	13.8±0.8^{de}
HbIR8	35.9±0.4^{de}	3.8±0.3^{f}	0.9±0.0^{bc}	37.7±0.7^{f}	1.3±0.1^{cd}	12.7±0.4^{cd}
HbIR9	29.6±0.7^{c}	3.8±0.1^{f}	1.0±0.1^{c}	15.5±0.9^{b}	0.8±0.0^{ab}	12.8±0.1^{cd}
HbIR10	38.9±0.0^{ef}	3.6±0.0^{d}	1.1±0.0^{c}	38±0.1^{f}	1.1±0.2^{c}	12.0±4.0^{b}
Negative control	25.3±0.3^{b}	2.6±0.1^{a}	0.6±0.0^{a}	0.0±0.0^{a}	1.3±0.0^{cd}	13.2±0.0^{d}
Positive control	22.3 ±19.3^{a}	3.6±0.0^{d}	1.1 ±0.0^{c}	0.0 ±0.0^{a}	1.3 ±0.0^{cd}	12.5 ±0.0^{c}
MBI	41.9 ±0.1^{f}	3.7 ±0.0^{de}	1.5 ±0.0^{ef}	32.5 ±0.1^{e}	2.8 ±0.3^{h}	15.0 ±3.0^{f}

** SL-shoot length; SG-Stem girth; SDW-shoot dry weight; NN-number of nodules; BPP- branches per plant; LPP-leaves per plant. Similar letters in columns show not significant difference between treatments at a 0.05.*

Effect of sole and co-inoculation of AMF and Rhizobia on growth and biomass yield of Haricot bean in the greenhouse

The greenhouse experiment on inoculation of mixture of indigenous arbuscular mycorrhizal fungi (AMF) and indigenous rhizobia (HbIR4) in haricot bean showed that sole rhizobial inoculation increased stem girth (3.4±0.0), shoot dry weight (1.5±0.1), number of pods plant^{-1} (3.3±0.6), over sole mycorrhizal inoculation treatment and sole mycorrhizal inoculation was better in shoot length (10.5±0.1), number of branches plant^{-1} (6.0±0.0) and root dry weight (0.4±.1) as compared with the sole rhizobial inoculation and the control (Table 6).

When the NPK fertilizer use efficiency was tested, in almost all cases Plants treated with sole NPK fertilizers performed significantly lower than those treated with NPK + R and NPK + AMF application, showing that bio-inoculation increases fertilizer use efficiency and reduces application of inorganic fertilizers with residual effects in the environment (Fig 1 a & b).

As to what concerns co-inoculation treatment of haricot bean with R + M + F treatments was recorded better (SL, 12.5±0.5 over R+M 12±0.5; **SG,** 3.9±0 over R+M 3.7±0.0; SDW, 3.10±0.1 over R+M 2.9±0.1 and RDW, 0.6±0.03 over R+M 0.5±0.05) increase in all growth parameters except the root nodules (R+M+F, 38.0±1.7 over R+M, 45.0±2.3) for which better root nodule number was recorded when compared with R + M treatments. In these trials all control treatments were not nodulated as the soil substrate used was sterilized (Fig 1 a & b).

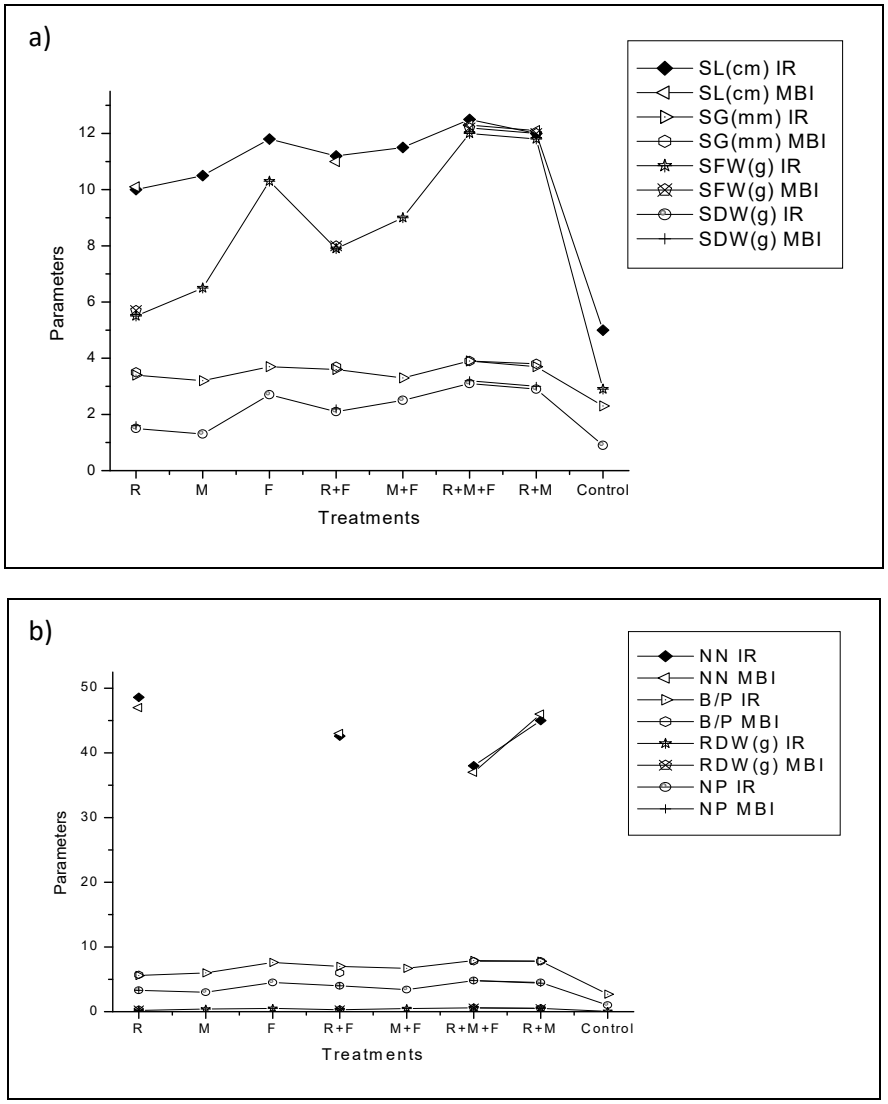


Figure 1 (a, b). Growth parameters: SL-shoot height; SG-stem girth; SFW-shoot fresh weight; SDW- shoot dry weight; NN-number of nodules; B/P-branches plant-1; RDW- root dry weight; NP-number of pods.

The above findings demonstrate that inoculating plants with effective Rhizobia in combination with AM fungi had a greater effect on plant growth, nodulation, and N_2 fixation. Several studies have described numerous significant findings in the synergistic interaction between AMF and symbiotic N_2-fixing bacteria (Kathiresan and Selvam, 2006).

Co-inoculation of a mixture of AMF morpho-species with indigenous and commercial rhizobia isolates resulted in a significant increase in all growth parameters except the number of haricot bean nodules, according to the current greenhouse study. This demonstrates that combining effective Rhizobia with AM fungi had a greater effect on plant growth, nodulation, and N_2 fixation. The importance of AM fungi as P suppliers to legume root nodules cannot be overstated. *Glomus intraridices* was found to be more effective when co-inoculated with Rhizobia spp. NR 4, whereas Glomus coronatum was effective when co-inoculated with Rhizobia spp. NR9 (Kathiresan and Selvam, 2006). According to research findings, the genetic pathway of AM symbiosis is shared in part by other root-microbe symbioses, such as N_2-fixing rhizobia (Spaink et al., 1998). Such interactions between AM fungi, Rhizobia, and plant growth promoting rhizobacteria (PGPR) have provided insight into the functional compatibility relationships between AMF and PGPR and their management when used as bio-fertilizers or bio-control agents.

Effect of AMF and Rhizobia inoculation on Haricot bean growth parameters in the field

Unlike the greenhouse controlled experimental studies, this study was conducted in the field, exposing the inoculants to natural factors and soil inherent

characteristics (soil nutrients, fungal and bacterial densities, and other physical and chemical properties) to investigate the effectiveness of the bio-inoculant on haricot bean growth and productivity under natural conditions. Despite the influence of natural environmental factors, the results showed that the inoculants promoted all of the pulse's tested growth parameters. In the greenhouse study, sole AMF inoculation improved performance and nearly all growth parameters. However, the opposite result was obtained in the field study for sole rhizobia inoculation, with better growth parameters recorded when compared to sole AMF inoculation and the control.

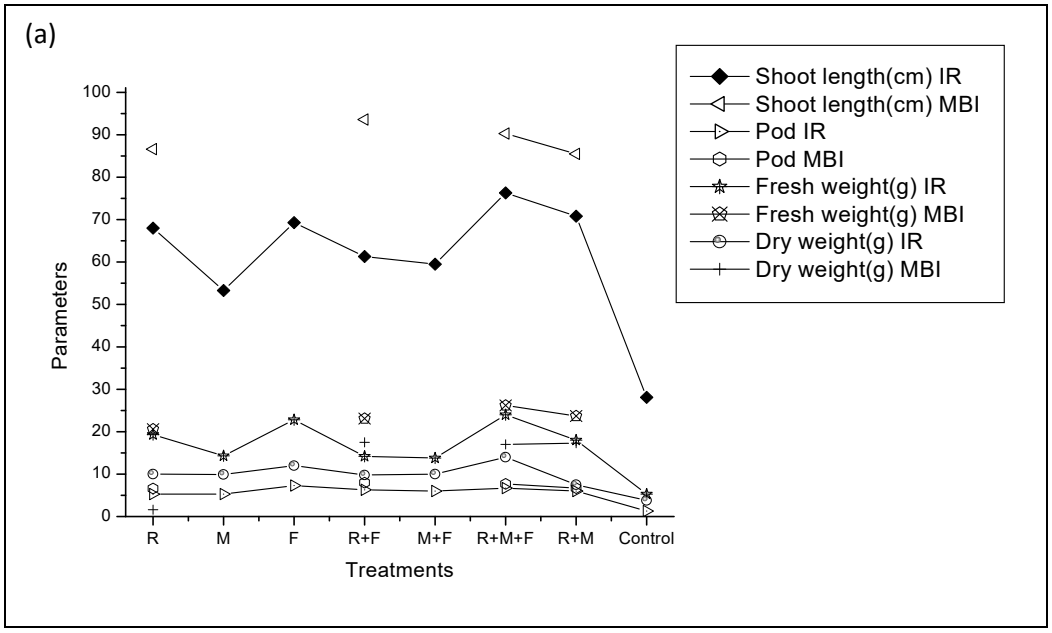
Furthermore, the difference in seed yield for AMF inoculated plants was significantly higher in field conditions with limited P and N supply. Interestingly, in the field, seed yield of AM + rhizobia+ fertilizer inoculated haricot bean was significantly higher than without fertilizer (R+M) application. Taken together, tripartite interactions of legumes with AM fungi and rhizobial bacteria promote plant growth and seed yield in low soil nutrient conditions, implying that tripartite (AMF + Rhizobia + Legume) interactions may have a greater potential to sustain agricultural activities in haricot bean production.

When the effect of sole and co-inoculation treatments on each growth parameters of haricot bean in the field was observed; the highest (76.3±`1.7cm) shoot length, 6.3±0.3 seed number/plant, 27.6±0.3 total yield/Kg, 8.9±0.1 100 seed weight and 7.8±0.3 husk weight/g was recorded for indigenous M+HBIR4+F co-inoculation treatment while the highest10.8±3.6 pod number and 10.8±3.6 dry weight was recorded for sole fertilizer and sole rhizobia treatments respectively (Figs. 2a & b).

However, when effect of the indigenous rhizobial inoculant was compared with the control and commercial (MBI) inoculants, in haricot bean growth and yield in the field, for the MBI inoculants was recorded better performance in shoot length (93.6±1.2), number of pods/plant (8.0±0.6) for R+F treatment and dry weight (19.2±0.4), seed number/plant (7.3±0.3), total yield kg/ha (28.0±0.3), 100 seed weight (10.0±0.2) and husk weight (10.0±0.2) in M+R+F treatment (Figs. 2a & b).

According to Silveira and Cardoso (2004) most of the legumes possess two types of microbial symbionts namely mycorrhizal fungi and nitrogen fixing bacteria thereby establishing triple association, capable of supplying N and P contents to the plants. Dual inoculation with both microorganisms results in a tripartite mutualistic symbiosis and generally increases plant growth to greater extent than inoculation with only one (Chalk *et al*., 2006).

Inoculation alone or in combination of beneficial microorganisms including AMF, rhizobia, PGPR and PSB (Phosphate Solubilizing Bacteria) have been shown to increase haricot bean production, nitrogen fixation, and nutrient uptake (Thenua *et al*., 2010). Murat *et al*. (2011) found that AMF inoculation, alone or in combination with rhizobial inoculation, increased yield, root colonization, and seed and shoot phosphorus content.



(b)

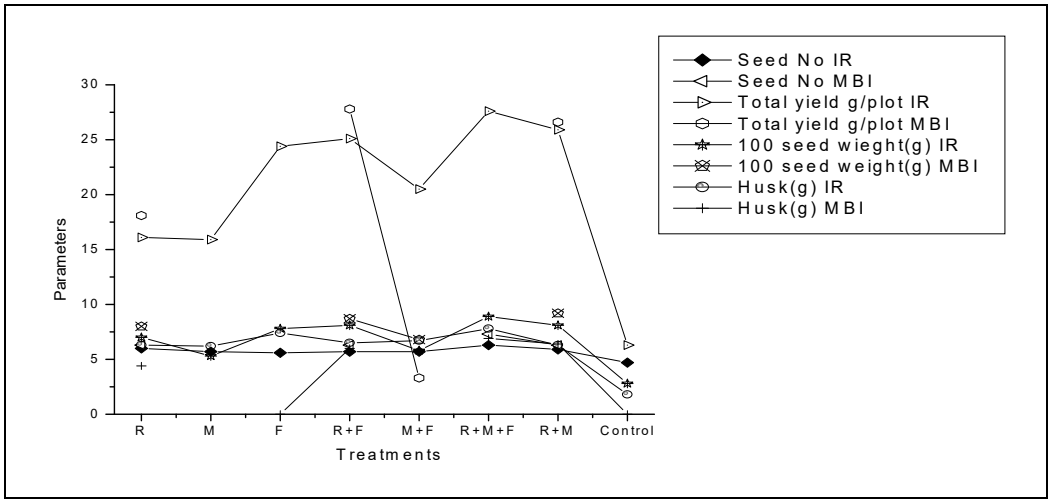


Figure 2 (a, b). Growth parameters*: R-rhizobia; M-mycorrhiza; F-fertilizer; IR-indigenous rhizobia; MBI-Menegasha biotechnology industry.*

Mycorrhizal root colonization and spore density in the greenhouse

All haricot bean treatments inoculated with sole AMF, AMF + fertilizer, AMF + rhizobia and AMF + Rhizobia + fertilizer treatments were found colonized with AMF in the greenhouse. But those treatments inoculated with rhizobia alone (R), R+F, only fertilizer (F) and the control were found not colonized (Tables 7). When compared sole and co-inoculation treatments with commercial and indigenous rhizobia, the highest root length colonization (80.0±2.9) was recorded for R+M+F (R-commercial) treatments in haricot bean. Similarly the highest root length (85.0± 5.8) was recorded for R+M+F (IR-indigenous) treatments in haricot bean (Table 7).

The rate of AM colonization is normally attributed to tree and crop species, as well as environmental factors. According to Smith *et al.* (1997), the extent to which typical AM fungi colonize root systems varies with plant species. This is because some plants are less mycotropic or show low mycorrhizal dependency when compared with plants such as Maize and Sorghum with highest mycorrhizal dependency.

The extent of AM infection in root systems is also known to be influenced by environmental conditions, the most important of which are the age of the plants, the level of phosphate (P) in the soil relative to the plant's requirements, and the population of AMF propagules in the soil's capacity to form AMF. In this study, the percentage colonization ranged from 60% ((M) to 85% in R+M+F for haricot bean, indicating that dual inoculation favored root colonization more. The current study contradicts the findings of (Yinsuo et al., 2004), who found that single AMF inoculation produced better results than dual R + M inoculation in *Vacia faba*. In haricot bean, the spore density ranged from 280(M+F) to 600/50g (M) dry soil.

Table 77 AMF root colonization and spore density in *Phaseolus vulgaris* both commercial rhizobia (MBI) and Indigenous Rhizobia (IR) with mixture of indigenous arbuscular mycorrhizal fungi (AMF) isolates in sole and co-inoculation trials in greenhouse.

Treatments	(Mean±SEM) of Growth parameters (MBI)				(Mean±SEM) of Growth parameters (IR)			
	A	V	RLC%	SD/50g dry soil	A	V	RLC%	SD/50g dry soil
R	-	-	-	-	-	-	-	-
M	-	-	-	-	25.3±1.5^{a}	35.0±0.6^{a}	65.0±2.9^{a}	600.0±57.7^{d}
R+M	25.0±0.6^{a}	25.0±2.9^{a}	65.0±2.9^{a}	520.0±11.5^{b}	25.0±5.8^{a}	35.0±2.9^{a}	70.0±2.9^{b}	440.0±34.6^{c}
F	-	-	-	-	-	-	-	-
R+F	-	-	-	-	-	-	-	-
M+F	-	-	-	-	25.0± 2.9^{a}	35.0± 2.9^{a}	84.0± 2.9^{c}	280.0± 11.5^{a}
R+M+F	30.0±1.2^{b}	42.0±5.8^{b}	80.0±2.9^{b}	400.0±28.9^{a}	25.0± 2.9^{a}	35.0± 2.9^{a}	85.0± 5.8^{d}	400.0± 28.9^{b}
Control	-	-	-	-	-	-	-	-

Key: A-arbuscules; V-vesicles; RLC- Root length colonization; SD-Spore density. Similar letters in columns show not significant difference between treatments at α 0.05.

Better spore density/50g dry soil was recorded for MBI R+AMF mix (520.0±11.5) and for Indigenous Rhizobia inoculum sole AMF mix (600.0±57.7) and HBIR4+AMF mix (440.0±34.6) in haricot bean (Table 7). In these

experiment, for R, F, R+F and the control treatments was not recorded spore formation.

Mycorrhizal dependency of Haricot bean

The highest MD value (70%) was recorded for co-inoculation and fertilizer application treatment in haricot bean followed by co-inoculation without fertilizer application (M+R) (68%). The least value (30.7%) was recorded in sole mycorrhizal treatment (Fig. 3). The current findings are consistent with the findings of (Arumugam et al., 2010), who demonstrated that co-inoculation of rhizobia and AMF increases mycorrhizal dependence more than AMF inoculation alone.

Nutrient content

The soil for the greenhouse experiment collected from Fate Kebele of Wolaita zone, Ethiopia, was analyzed for its physicochemical properties with emphasis on nitrogen, phosphorus, organic matter and soil texture. The soil inherent available nitrogen was 0.12%, phosphorus 6mg/kg (Olson), soil organic matter 0.82% and soil texture was clay. After the greenhouse experiment plant aboveground tissue analysis showed that all sole inoculated, co-inoculated and sole-fertilized-applied treatments showed an increase in haricot bean tissue nutrient uptake as compared with the control (Fig. 4). Plant tissue nitrogen, phosphorus and potassium concentrations were higher in the co-inoculated (M+R+F) treatments with fertilizer application, while the next better nutrient uptake was recorded for co-inoculation treatment (M+R) without fertilizer application (Fig. 4). Besides, for sole mycorrhizal inoculation treatments better nutrient uptake was recorded as compared with sole rhizobia inoculation (Fig. 4). Also, for sole mycorrhizal inoculums with fertilizer application was recorded better performance as compared with sole rhizobia inoculation with fertilizer application.

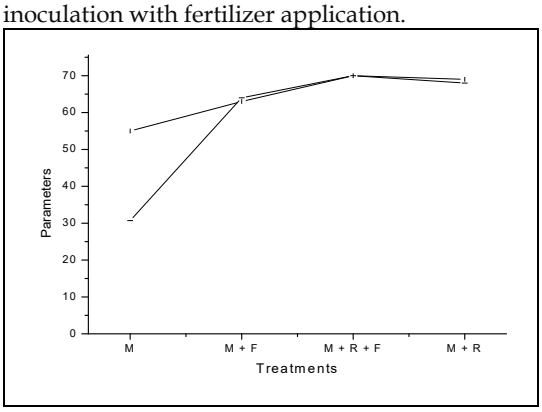


Figure 3. Mycorrhizal dependency of haricot bean in the greenhouse

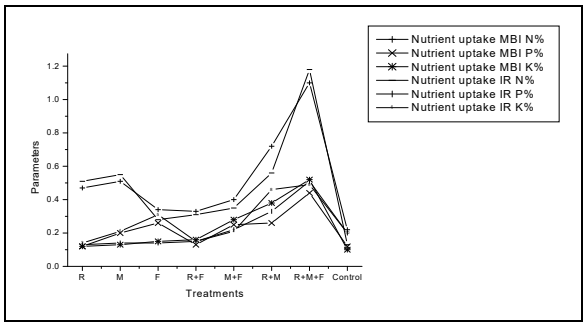


Figure 4. Nutrient uptakes: R-rhizobia; M-mycorrhiza; F-fertilizer; N-nitrogen; P-phosphorus; K-potassium; IR-indigenous rhizobia; MBI- menagesha biotechnology industry.

Sole and tripartite interactions can have a synergistic effect on plant biomass, especially when N and P levels are low. The current study discovered that plants in tripartite (M+R+Hb) interactions had significantly higher root and shoot biomass, as well as N and P contents, than plants colonized only by rhizobia or AM fungi. Khan et al. (2008) discovered that dual inoculation improved the dry weight of shoot and root while also increasing nutrient uptake (NPK), which is consistent with the current finding. Dual inoculation, according to Bhattacharjee and Sharma (2012), has the potential to increase the nutrients and chlorophyll content of pigeon pea. Other authors have also described synergistic responses in tripartite interactions, particularly under low P and N supply conditions (Bournaud et al., 2018).

Dual inoculation of legumes with rhizobia and AM fungi increases photosynthetic rates and improves the harvest index (the proportion of seed yields in relation to total plant biomass) (Kaschuk et al., 2009). The positive impact of tripartite interactions on plant growth was primarily due to increased biological nitrogen fixation BNF activity of the nodules and improved plant N nutrition in this study. Higher BNF rates in tripartite interactions have been attributed primarily to improved P supply caused by AM fungi colonization (Mortimer et al., 2008; Püschel et al., 2017). Root nodules are extremely effective P sinks, and P deficiency can result in lower BNF rates and inhibit nodule growth (Kossmann and Valentine, 2014). According to Mensah et al. (2015), the ability of some AM fungi to deliver N can even lead to a strong growth response in legumes such as Medicago sativa.

CONCLUSION

This study demonstrated that the mycorrhiza-rhizobia-plant interaction significantly improved plant growth by increasing phosphate and nitrogen uptake. The findings suggest that the host's nutrient demands and fungal nutrient access are important factors in controlling nutrient (photosynthetic carbon) allocation to individual root symbionts in mycorrhiza-rhizobia-

plant interactions. Mycorrhiza-rhizobia-plant interactions synergistically effect host plant growth response because AMF deliver phosphate from soil beyond root access and rhizobial bacteria provide nitrogen to the host plant through biological nitrogen fixation. The effects of indigenous AMF, indigenous and commercial rhizobia inoculums on plant growth and seed yield of haricot bean cultivars in greenhouse and field conditions show that application of AMF and rhizobia inoculums increased all studied plant growth parameters including biomass and seed yield except number of nodules in greenhouse and field conditions when compared to the control and sole rhizobia inoculums. The effects of AMF and rhizobia inoculants on plant growth and seed yield were noticeably greater in soils with limited nutrient availability. Taken together, the use of indigenous rhizobia, commercial rhizobia, and AMF inoculant improves plant productivity and seed yield, particularly in areas with limited nutrient availability, and could be an alternative to chemical fertilizers in haricot bean production. The results also show that AM colonization promotes plant growth by increasing nutrient uptake. There was no root colonization or spore density in the control or fertilizer treatments without AM and rhizobia inoculants. Un-inoculated treatments had lower dry biomass, height growth, root number, and length. In conclusion, this study clearly demonstrated that using only AMF and rhizobia, as well as dual-inoculation with and without fertilizer, increased all studied yield and yield contributing characteristics in haricot bean in the greenhouse and in the field when compared to the control.

REFERENCES

Agah, MV, Orj, JO, Nnach, AV, Chukwu, OS, Udu-Ibiam, OE, Nwuchi, AC and Olaozebikan, OO. 2016. Isolation and identification of Rhizobia species from Root Nodules of *Arachis hypogea* L. and *Telfairia occidentalis* in South-East, Nigeria. *International Journal of Science and Research* **5:** 227-230.

Arumugam, R., Rajasekaran, S. and Nagarajan, S.M. 2010. Response of Arbuscular Mycorrhizal fungi and *Rhizobium* inoculation on growth and chlorophyll content of *Vigna unguiculata* (L) Walp Var. Pusa 151. *J. Appl. Sci. Environ. Manage.* **14:** 113 – 115.

Beaty, RD and Kerber, J.D. 1993. Concepts, Instrumentation and Techniques in Atomic Absorption Spectrophotometry.2nd Edition. The Perkin-Elmer Corporation, Norwalk, CT, USA.

Beyene Dobo, Fasil Asefa and Zerihun Asfaw. 2016. Response of Selected Perennial Crops and Trees to AMF Inoculation and NPK Application in Pot Culture. *Acad. J. Biotechnol*. **4:** 325-336.

Bhattacharjee, S. and Sharma, GD. 2012. Effect of dual inoculation of Arbuscular Mycorrhiza and Rhizobium on the Chlorophyll, Nitrogen and Phosphorus Contents of Pigeon Pea. *Advan. Microbiology*. **2:** 561-564.

Black, C, Evans, AD, White, JL, Ensminger, LE and Clark, FE. 1965. Methods of Soil Analysis Part 1. Physical and Mineralogical Properties Including Statistics of Measurement and Sampling, American Society of Agronomy, Madison, Wis, USA.

Bournaud, C, James, EK, Fariam, SM, Lebrun, M, Melkonian, R, Duponnois, R,Tisseyre, P, Moulin, L and Prin, Y. 2018. Interdependency of efficient nodulation and arbuscular mycorrhization in *Piptadenia gonoacantha*, a Brazilian legume tree. *Plant, Cell & Environment*. **41:** 2008 - 2020.

Bremner, JM and Mulvaney, CS. 1982. Nitrogen-Total. In: Methods of soil analysis. Part 2. Chemical and microbiological properties, Page, A.L., Miller, R.H. and Keeney, D.R. Eds., American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin, p. 595-624.

Cavell, A.J. 1995. The colorimetric determination of phosphorus in plant materials. *Journal of the Science of Food and Agriculture*. ***6:*** 479–480.

Chalk, PMR, Souza, S, Urquiaga, BJ, Alves, R and Boddey, RM. 2006. The role of Arbuscular mycorrhizae in legume symbiotic performance. *Soil Biol. Bioch*. **38:** 2944-2951.

Franche, C, Lindstrom, K and Elmerich, C. 2009. Nitrogen fixing bacteria associated with leguminous and non-leguminous plants. *Plan Soil*. **321:** 35-59.

George, E, Marschner, H and Jakobsen, I. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Crit. Rev. Biotechnol.* **15:** 257–270.

Gerdemann, JW, Nicolson, TH. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans Brit Mycol Soc*. **46:**235–244.

Gianinazzi-Pearson, V. 1996. Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. *Plant Cell*. **8:** 1871–1883.

Giller, KM. 2001. Nitrogen Fixation in Tropical Cropping Systems. Second edition. CABI Publishing, Wallingford, UK.

Hartman, GL, West, ED and Herman, TK. 2011. Crops that feed the World 2. Soybean-Worldwide production, use, and constraints caused by pathogens and pests. *Food Sec.* **3**:1–5.

Hassen, AI, Bopape, FL and Trytsman, M. 2014. Nodulation Study and Characterization of Rhizobial Microsymbionts of Forage and Pasture Legumes in South Africa. *World*. **2:** 93-100.

Jones, BJJ. 2001. Laboratory Guide for Conducting Soil Tests and Plant Analysis, CRC press, LLC.

Jordan, DC. 1984. Transfer of *Rhizobium japonicum* Buchanan 1980 to Bradyrhizobium genus of slow-growing, root nodule bacteria from leguminous plants. *Int J Syst Bacteriol*. **32:**136–139.

Kaschuk, G, Kuyper, TW, Leffelaar, PA, Hungria, M and Giller, KE. 2009. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses?. *Soil biology & biochemistry*. **41:** 1233 – 1244.

Kathiresan, K and Selvam, MM. 2006. Evaluation of beneficial bacteria from mangrove soil. *Botanica Marina*. **49:** 86-88.

Khan, IA, Ayub, N, Mirza, SN, Nizam, SM and Azam, M. 2008. Synergistic effect of dual inoculation (Vesicular-Arbuscular Mycorrhizae) on the growth and nutrients uptake of Medicago sativa. *Pak. /;J. Bot.* **40:** 939-945.

Kossmann and valentine. 2014. the reallocation of carbon in P deficient lupins affects biological nitrogen fixation. *Journal of Plant Physiology*. **171:**1619-1624.

Lupwayi, N and Haque, I. 1994. Legume-Rhizobium Technology Manual. Environmental Sciences Division International Livestock Center for Africa, Addis Ababa, Ethiopia, pp: 1-93.

McGonigle, TP, Miller, HM, Evans, GD, Fairchild, LG. and Swan, AJ. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **115:** 495-501.

Mensah, JA, Koch, AM, Antunes, PM, Kiers, ET, Hart, M and Bücking, H. 2015. High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. *Mycorrhiza.* **2:** 1-14.

Mhango, J, Mahamadi, D, Ndusa, B, Woomer, PL, Abaidoo, R, Karanja, NK and Uwizerwa, M. 2013. Rhizobium collection, testing and the identification of candidate elite strains.

Mortimer, PE, Pe´rez-Ferna,´ ndez, MA and Valentine, AJ. 2008. The role of arbuscular mycorrhizal Colonization in the carbon and nutrient economy of the tripartite symbiosis with Nodulated *Phaseolus vulgaris*. *Soil Biol. Biochem*. **40:** 1019-1027.

Murat, E, Demir, S, Tufenkci, EOS, Oguz, F and Akkopru, A. 2011. Effects of Rhizobium, Arbuscular mycorrhizae and whey applications on some properties in chickpea (*Cicer arietinum* L.) under irrigated and rain fed conditions. *Field Crops Res*. **122:** 14-24.

Mylona, P, Pawlowski, K and Bisseling, T. 1995. Symbiotic nitrogen fixation. *Plant Cell*. **7:** 869–885.

Okakira, E and Kawaguchi, M. 2006. Long-distance signaling to control root nodule number. *Curr Opin Plant Biol.* **9:** 496–502.

Olsen, SR and Dean, LA. 1965. "Phosphorous," in Methods of Soil Analysis Part 2: Chemical and Microbiological Properties, C. A. Black, Ed., vol. 9, pp. 1035–1049, American Society of Agronomy, Madison, Wis, USA.

Perret, X, Staehelin, C and Broughton, WJ. 2000. Molecular basis of symbiotic promiscuity. *Microbiol Mol Biol Rev.* **64:**180–201.

Plenchette, CA, Fortin, A and Forlan, N. 1983. Growth response of several plant species to Mycorrhiza in a soil of moderate P-fertility. I. Mycorrhizae under field conditions. *Plant Soil.* **70:**199-203.

Püschel, D, Janoušková, M, Hujslová, M, Slavíková, R, Gryndlerová, H and Jansa, J. 2017. Plant–fungus competition for nitrogen erases mycorrhizal growth benefits of Andropogongerardii under limited nitrogen supply. *Ecology and Evolution.* **6:** 4332-4346.

Schnitzer, M. 1982. "Total carbon, organic matter and carbon," in Methods of Soil Analysis-Part 2, Agronomy Monograph, A. L. Page, R. H. Miller, and D. R. Keeney, Eds., vol. 9, pp. 539- 577, American Society of Agronomy, Madison, Wis, USA, 2nd edition.

Schußler, A, Schwarzott, D and Walker, C. 2001. A new fungal phylum the glomeromycota:

Silveira, P.D. and Cardoso, E..J. 2004. Arbuscular Mycorrhizal and kinetic parameters of Phosphorus absorption by bean plants. *Agri. Sci*. **61:** 203-209.

Simon, Z, Mtei, K, Gessesse, A and Ndakidemi, PA. 2014. Isolation and Characterization of Nitrogen Fixing Rhizobia from Cultivated and Uncultivated Soils of Northern Tanzania. *American Journal of Plant Science.* **5:** 4050 - 4056.

Smith, SE and Read, DJ. 2008. Mycorrhizal symbiosis. Third edition. Academic Press, London, UK.

Smith, SE. and Read, DJ. 1997. Mycorrhizal Symbiosis. Academic, San Diego, CA

Smith, SE, Smith, FA and Jacobsen, I. 2003. Mycorrhizal Fungi Can Dominate Phosphate Supply to Plants

Irrespective Of Growth Responses. *Plant Physiol.* **133:** 16-20.

Solomon Legesse, Fasil Asefa. 2014. Symbiotic And Phenotypic Characteristics Of Rhizobia Nodulating Faba Bean (*Vicia faba*) From TahtayKoraro, Northwestern Zone Of Tigray Regional State, Ethiopia. *International journal of technology enhancements and emerging engineering research*. **2:** 15-23.

Somasegaran, P and Hoben, HJ. 1994. Handbook for Rhizobia: Methods in Legume *Rhizobium* Technology. Springer-Verlag New York, USA, pp. 450.

Somasegaran, P and Hoben. HJ. 2012. Handbook for Rhizobia: Methods in Legume *Rhizobium* Technology. Springer Science and Business Media, Berlin.

Soto, MJ, Sanjuan, J and Olivares, J. 2006. Rhizobia and plant-pathogenic bacteria: common Infection weapons. *Microbiology*. **152**:3167–3174.

Spaink, HP, Kondorosi, A and Hooykaas, PJJ. 1998. The Rhizobiaceae (Kluwer Academic Publishers, Dordrecht, The Netherlands.

Thenua, OVS, Singh, SP and Shivakumar, BG. 2010. Productivity and economics of chickpea (*Cicer arietinum*) fodder sorghum (*Sorghum bicolor*) cropping system as influenced by P sources, bio-fertilizers and irrigation to chickpea. *Ind. J. Crop Sci.* **12:** 253-261.

Utobo, EB, Ogbodo, EN and Nwogbaga, AC. 2011. Techniques for Extraction and Quantification of Arbuscular Mycorrhizal Fungi. *Libyan Agriculture Research Center Journal International*. **2:** 68-78.

Vincent, JM. 1970. A manual for the practical study of root-nodule bacteria, Blackwell Scientific, Oxford. I.B.P handbook.

Weir, BS. 2016. The current taxonomy of rhizobia. NZ Rhizobia website. https://www.rhizobia.co.nz/taxonomy/rhizobia. last updated x Jan, 2016. Accessed, May 11, 2020

Yinsuo, J, Vincent, MG and Colin, JS. 2004. The Influence of Rhizobium and Arbuscular Mycorrhizal Fungi on Nitrogen and Phosphorus Accumulation by *Vicia faba*. *Annals of Botany*.**94:** 251–258.