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Samson Zemikael Haftu

zemikael23@gmail.com

Aksum University

Hiwot Kasa Abera

Aksum University

Melaku Mekonen Kasegn

Mekelle University

Etsay Mesele

Mekelle University

Brhane Gerensea Gebrekidan

Aksum University

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Isolation and Biochemical Characterization of Indigenous *Rhizbium* from Root nodules of Field Pea (*Pisum Sativum L.*) and Assessing their Bioinculant Potential for *Bursa* variety in Tigray, Ethiopia

Samson Zemikael Haftu[1], Hiwot Kasa Abera[2], Melaku Mekonen Kasegn[3], Etsay Mesele[3], Brhane Gerensea Gebrekidan[1]

[1]Aksum University, Department of Biotechnology (MSc. in Plant Biotechnology), Aksum, P.O.Box.1010, Ethiopia

[2]Aksum University, Department of Chemistry (MSc. in Analytical Chemistry), Aksum, P.O.Box. 1010, Ethiopia

[3]Mekelle University, Department of Biotechnology (MSc. in Biotechnology), Mekelle, P.O.Box. 231, Ethiopia

Corresponding author: zemikael23@gmail.com, Phone no: +251943664694

Abstract

The world's population is growing exponentially, and farmers have been using chemical fertilizers to boost crop yields to meet the demand for food. In Tigray, food security is still challenging. The region has been using inappropriate amount of Urea and DAP as a primary sources of fertilizer since 1960s to maintain this difficulty. Long-term overuse of chemical fertilizers caused soil infertility, and raising serious concerns about agriculture's future. This study was focused on creation of local biofertilizer as an alternative for chemical fertilizers for sustainable farming in the area. About 50 root nodules of pea samples were collected from two zones of Tigray. The samples were subjected to biochemical and standard confirmatory testing. As a result, 21 Rhizobia isolates were identified. Out of 21, seven potent isolates were screened based on in-vitro and colony forming unit's tests, and grown in a broth culture. Finally, Bursa seed varieties were inoculated with biofertilizers and grown in greenhouse to see how they affected the crop's growth parameters. According to the study's findings, *Bursa* seeds inoculated with biofertilizer significantly impacted the *bursa* growth parameters when compared to the control. Highest mean on shoot length (33.55 ± 1.37) cm, root length (18.08 ± 0.38) cm, chlorophyll content (3.19 ± 0.3) mg, and dry shoot weight (1.32 ±0.02) mg was recorded on T_5 which treated with *Rhizobium*-RS4 biofertilizers. All of the data generated from this study were statistically examined using SPSS version 20 by evaluating the mean and standard deviation at $p < 0.05$.

Keywords: Rhizobia, Isolates, Local-Biofertilizer, *In-vitro* test, *Bursa* Variety

1. Introduction

1.1. Background and Justification

Agriculture in the twenty-first century faces numerous challenges, including the need to produce more food to feed a growing population. To increase production, the global agricultural systems use a lot of chemicals, such as chemical fertilizers, pesticides, and herbicides (1). However, using higher doses or excess than the ideal of these chemicals and fertilizers for ensuring the world food security causes several issues, including environmental pollution, decreased input efficiency, decreased quality of food, the emergence of pest and disease resistance, soil degradation, and a lack of micronutrients (2). As plants absorb fertilizers through the soil, their application in excessive amounts allows them to enter the food chain (3). Certain chemical fertilizers used in agriculture cause a large number of environmental problems as they contain heavy metals (e.g., Cadmium and Chromium) and high concentrations of radionuclides (2). Later these fertilizers constitute the main source of heavy metals and radionuclides in plants and some result in the accumulation of inorganic pollutants. Moreover, greenhouses and aquaculture use huge amounts of chemical fertilizers, especially during the peak season, which leads to dangerously polluted underground water, especially water resources and crop production deteriorates both in terms of quality and quantity (4).

Ethiopia is one of the few nations in Sub-Saharan Africa where agriculture is the backbone of the economy (5), (6). Within the agriculture sector, cereals are the principal staple crops in terms of planted area (81.46%) and output volume (88.52%) (7). To increase the production of the cereals, Ethiopia including Tigray region have been used urea and DAP fertilizer since the 1960s, and there has been no change in the composition of the use of fertilizers in Ethiopian agriculture. This long time and overuse of these synthetic fertilizers pose to diminish the fertility and mineral compositions of the soil in the nation (1). The national soil data base reported that, the Ethiopia Soil Information Systems (EthioSIS) soil fertility mapping project in Ethiopia identified deficiencies of K, S, Zn, B and Cu in addition to N and P in major Ethiopian soils and hence recommended customized and balanced fertilizer (EthioSIS, 2013) applications for sustainable crop production (8), (9). In addition synthetic fertilizers are manufactured industrially using non-renewable petroleum products under high temperature and high pressure (10). The petroleum cost, day by day affects the cost of the chemical fertilizers. As a result, these chemical fertilizers are not cost efficient to the low level income farmers.

Thus, biofertilizers are an excellent alternative to chemical fertilizers in agriculture due to their positive effects in the environment (11). Research on the applications and benefits of biofertilizers indicates that though they may not replace chemical fertilizers totally, they do provide an excellent alternative source of fertilization (12). Biofertilizers are microbial inoculants which are capable of mobilizing important

nutritional elements in the soil from non-usable to usable form through biological processes natural (13). These are cost effective, self-perpetuating and self-propagating, eco-friendly and not contributing to nitrate contamination in soil and water or causing eutrophication. They bring about an improvement in soil health by replenishing the nutrient supply over a period of time, which is a sustainable agricultural practice (14).

The nitrogen fixing biofertilizers make a net addition to nitrogen supplies by fixing atmospheric nitrogen for the soil-plant system (15). *Rhizobium* is one of the promising nitrogen fixing bacteria that enhances soil nutrient by using microorganisms that establish symbiotic relationships with legumes plants (16).This bacteria assures attainment in crop productivity and reduces the need for synthetic fertilizers that are expensive and cause environ mental matters (17). Inoculation of *Rhizobium* appears to be very important for legume cultivation and sustainable agriculture (18).

Bursa' is the newly released field pea variety characterized by a prostrate growth habit. Materials 'EH 04053' and 'EH 04051', which was selected from the last stage of variety trial, was crossed together and resulted breeder ID designation of 'EH05027-2' (Bursa). The crossing was done at Holeta Agricultural Research Center during 2002cropping season. Its flower color is purple. The seed coat and cotyledon colors are Light Brown and Light Yellow, and with seed character of Wrinkled. It's Pod Character and Pod Color are Full (not constricted) and green) (19).

The objectives of this experiment were to produce potent *Rhizobium* biofertilizer as an alternative of chemical fertilizers, and evaluate its effect on growth parameters of bursa variety crop.

2. MATERIALS AND METHODS

2.1. Description of the study area

The study was conducted at molecular biotechnology and microbial laboratory in Mekelle University College of veterinary medicine Kalamino campus Tigray, Ethiopia. It is located 13°29'33.6" N and 39°28'44.99" E; and altitude of 2080 meters above sea level. The mean minimum and mean maximum an average monthly temperatures of area are 8.7, 26.8 and 17.6°c respectively and laboratory setup at 25°c temperature and 60-80 humidity (https://www.distancesto.net› ethiopia › mekele).

2.2. Research design

The study was conducted in complete randomized design (CRD) includes eight treatments with four replications. Treatments were arranged as T_0 (uninoculated), T_1 (*Rhizobium*-TS1), T_2 (*Rhizobium*-TS3), T_3 (*Rhizobium*-TS11), T_4 (*Rhizobium*-TS6), T_5 (*Rhizobium*-RS4), T_6 (*Rhizobium*-RS5) and T_7 (*Rhizobium*-RS6).

2.3. Sample collection

A total number of 50 healthy, dark pinkish or red and medium sized root nodules of pea were collected from two distinct ecological districts of Tigray region, Ethiopia: southern Tigray (Adishu) and central Tigray (Kola Tembien). To obtain intact nodules, carefully uprooted pea plants were used and delivered right away to the lab. Cut the roots at about 0.5 cm on either side of the healthy nodules that were seen at the lateral and main roots. Only medium-sized nodules that were dark pinkish or red were sampled (Table1).

Table 1. Location of root nodule sample

Sr.No.	Location of root nodule samples	Samples
1	Southern Tigray Adishu (from farmers field)	RS1, RS2, RS3, RS4, RS5, RS6, RS7, RS8, RS9 and RS10..... RS25
2	Central Tigray Kola tembien (from farmers field)	TS1, TS2, TS3, TS4, TS5, TS6, TS7, TS8, TS9, TS10 and TS11.....TS25

Note: RS= Root nodule sample from Adishu, TS= Root nodule sample from kola tembien

2.4. Isolation of *Rhizobium* isolates

Each pea sample's root nodules underwent five thorough washes with regular water to clean them of all soil and other debris. The nodules were then submerged in 95% ethanol for 10 seconds to dissolve surface tension and flush out air bubbles. Nodules were then submerged in 3% hydrogen peroxide for 5 minutes, followed by five dips in sterile water, to surface sterilize them. The nodules were transferred with sterile forceps to prevent contamination, and the forceps were sterilized in between transfers by dipping them in alcohol and torching them. Pure and surface-sterilized root nodules were separated by a blade, held in place by a scalpel to prevent damage, and then released into 0.1 milliliters of saline solution. On Yeast Mannitol Agar Congo red (YEMA-Cr), a loopful of liquid streaked. On Congo red YEMA medium, Rhizobium was isolated by separating colonies that did not take on the color of Congo red after 3- 4 days of incubation. YEMA medium was streaked with a loop of isolated colonies for further purification, and the loop was kept alive by sub-culturing on Yeast Extract Mannitol Agar medium (20).

2.5. Morphological characterization of *Rhizobium* isolates

By observing the colonies on YMA plates after growth at 30 ^{o}C, it was possible to determine the colony characteristics (i.e. shape, size, color, opacity of the bacterial colony, and their growth rate) (21). The isolates were examined under the microscope using the Gram staining method, which (22) described. Additionally, growth on glucose peptone agar (GPA) (23), the keto-lactase test (24), and Yeast Mannitol Agar (YMA) media mixed with 5ml/l bromothymol blue (YEMA-Brtb) (25) were used to determine the purity of the Rhizobium isolates.

2.6. Biochemical characteristics of the isolates

Isolates were incubated in various media for each physiochemical test to determine whether the isolates were Rhizobium other contaminants, and then incubated at 30 ºC depending on their growth rate (26). (25) conducted a catalase test to determine catalase enzyme production, and (23) assessed the ability to hydrolyze starch. The production of the gelatinase enzyme, the citrate utilization test, the production of urease (27), and the oxidase tests were carried out (28).

2.7. *In-vitro* assessment of plant growth promoting substance

The production of Indole-3- Acetic Acid (IAA), phosphate solubilization, and ammonia production were investigated under the in-vitro conditions for the different isolates of Rhizobium (29, 30).

2.7.1. Liquid bioinoculant production

Under sterile conditions, mother culture of different Rhizobium strains was created in 250 ml of growth medium using Yeast Extract Mannitol (YEM) medium (31). The inoculated broth was incubated for one week at 28 ºC in a shaking incubator. The viable cell count should have risen to 1x108cfu/ml and the pH level should have reached 6.8–7.0 after one week. The liquid bioinoculants were used immediately, packed, and stored once the necessary cell count was reached (32, 33).

2.8. Evaluation of symbiotic effectiveness of *Rhizobium* isolates on the growth of pea variety *Bursa* in pot experiment

The effectiveness of isolates in promoting plant growth and atmospheric nitrogen fixation were examined in a pot experiment. To accomplish this, standard protocols were followed for the inoculum preparation(34, 35), seed preparation (36), inoculation process (29), and determination of chlorophyll content (37) experiments.

2.9. Data collection and analysis

The information was gathered from the outcome of *in-vitro*, *in-vivo,* and biochemical tests. The results of their colorimetry tests were used to describe the data collected from biochemical tests and in-vivo tests, which were then subjected to analysis of variance (ANOVA) using statistical package software—SPSS Version 20—and the means obtained were compared using the Least Significant Difference (LSD) test at ($p<0.05$).

3. RESULTS

3.1. Cultural and microscopic characteristics of *Rhizobium* isolates

The cultural selection media of YEMA containing 0.0025% were applied to a total of fifty samples of root nodules**.** Only 21 samples out of these produced white colonies after 24 hours at 30 ºC and exhibited similar colony morphology (Fig. 1). In addition, all 21 isolates tested positive for the confirmatory keto-lactose test, and all isolates tested positive for the GPA test, with the exception of RS7 and RS8. Furthermore, microscopic analysis of the 21 isolates showed that they were all rod-shaped, gram-negative bacteria (Fig. 2).

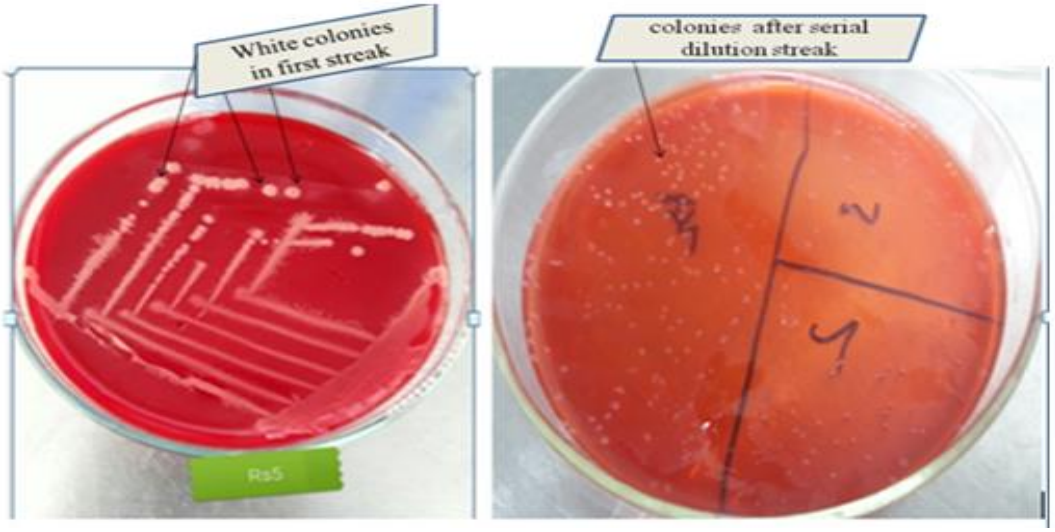


Fig. 1 Growth of *Rhizobium* in YEMA-Cr medium

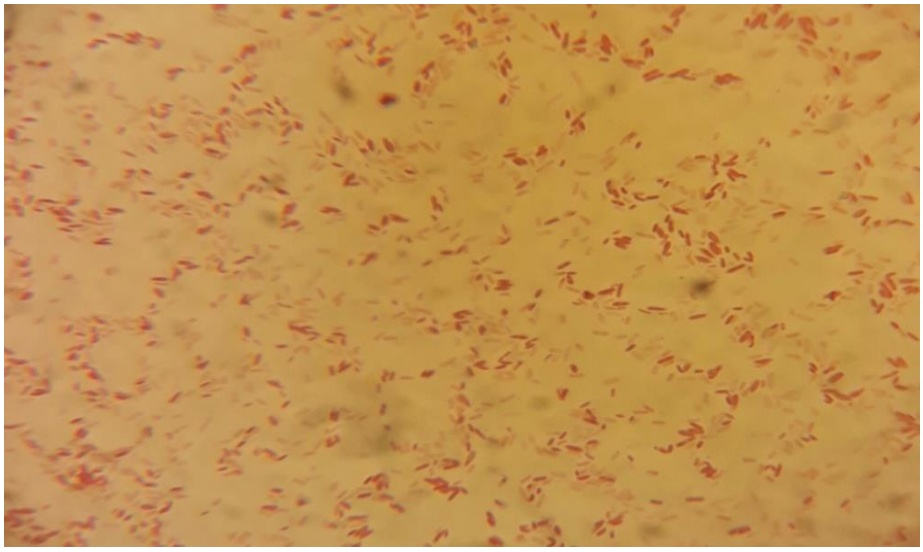


Fig. 2 Microscopic observation of *Rhizobium* isolates

3.2. Biochemical characterization

In the catalase test, all the isolates except RS9 were positive as confirmed by the liberation of the buble of oxygen around the *Rehizobium* colonies when a drop of 3% hydrogen was added to the taste isolates and fourteen isolates except RS3, RS7, RS10, TS8, TS9 and TS10 were oxidase positive as confirmed by change in color of oxidase strip to blackish purple (Table 2 and Fig 3). Only fourteen isolates produced clear zones around their colonies in the starch hydrolysis test after dousing the inoculated plates in iodine solution. In the urase hydrolysis test, only 12 *Rhizobium* isolates had successfully converted the urea medium from yellow to pink color after incubation. This indicating that the test isolates were urease positive and contributing to the medium's alkaline nature as a result of ammonia production (Table 2 and Fig 3). In the citrate test, the majority of the isolates saw a color change from green to blue in Simmon's citrate agar medium after 24 hours of incubation, indicating that the test isolates were citrate positive. The exceptions were RS1, RS9, TS7, TS8, TS9, and TS10. Only 13 isolates, however, liquefied the medium in the tubes during the gelatin hydrolysis test and created a clear zone around the line growth (Table 2 and Fig 3).

Table 2. Biochemical characteristics of *Rhizobium* isolates from pea plants.

Isolates	Catalase test	Oxidase test	Urease test	Starch hydrolysis test	Citrate test	Gelatin test
RS1	+	+	+	-	-	+
RS2	+	+	+	+	+	+
RS3	+	-	-	+	+	+
RS4	+	+	+	+	+	+
RS5	+	+	+	+	+	+
RS6	+	+	+	+	+	+
RS7	+	-	-	-	+	-
RS8	+	+	-	-	+	-
RS9	-	+	-	+	-	-
RS10	+	-	-	+	+	+
TS1	+	+	-	-	+	+
TS2	+	+	+	+	+	+
TS3	+	+	+	+	+	+
TS4	+	+	+	+	+	-
TS5	+	+	+	+	+	+
TS6	+	+	+	+	+	+
TS7	+	-	+	-	-	-
TS8	+	-	-	+	-	-
TS9	+	-	-	-	-	-
TS10	+	-	-	-	-	-
TS11	+	+	+	+	+	+

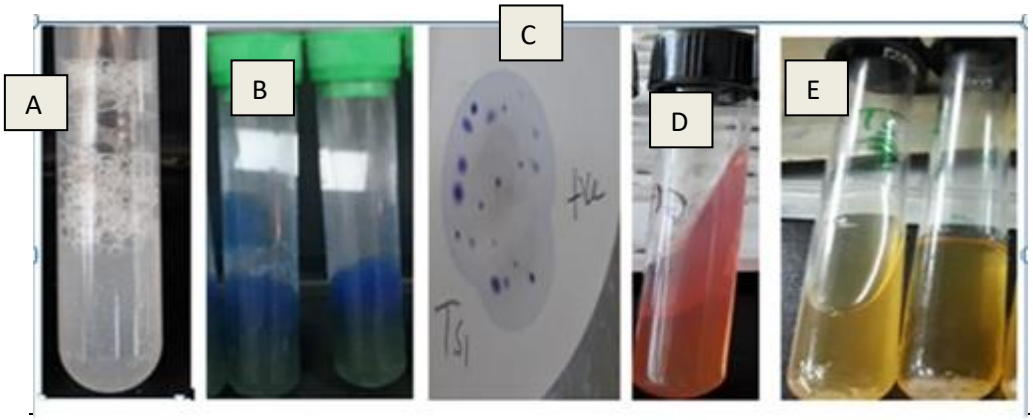


Figure 3. Biochemical test; (A). Catalase test, (B). Citrate test, (C). Oxidase test, (D). Urease test, and (E) .Gelatin hydrolysis test

3.3. *In-vitro* assessment of plant growth-promoting substance by isolates

The production of indole acetic acid, solubilization of phosphate, and production of ammonia were all tested *in-vitro* on all 21 isolates. The study's findings of these tests were showed that all of the isolates responded favorably to the production of ammonia and all but three of the isolates RS10, TS2, and TS4 responded favorably to the production of IAA. The phosphate solubilization test revealed positive results for 18 isolates (Table 3 and Fig. 4).

Table 3. *In-vitro* tests of plant growth promoting activities of *Rhizobium* isolates

Isolates	IAA production test	Phosphate Solubilization test	Ammonia Production test
RS1	+	+	+
RS2	+	+	+
RS3	+	+	+
RS4	+	+	+
RS5	+	+	+
RS6	+	+	+
RS7	+	+	+
RS8	+	+	+
RS9	+	+	+
RS10	-	-	+
TS1	+	+	+
TS2	-	-	+
TS3	+	+	+
TS4	-	-	+
TS5	+	+	+
TS6	+	+	+
TS7	-	-	+
TS8	+	+	+
TS9	+	+	+
TS10	+	+	+
TS11	+	+	+

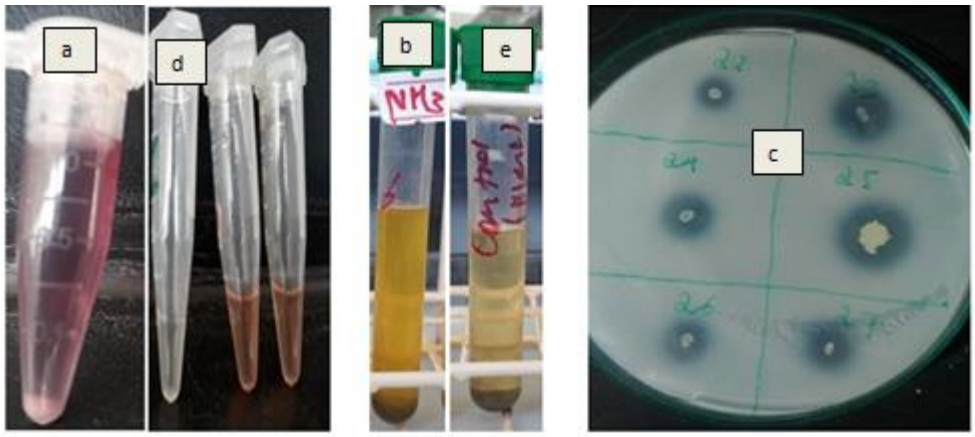


Fig 3. *In-vitro* tests; (a). IAA test, (b). Ammonia test, (c). Phosphate solubilization test,

(d). Control and (e). Control

3.4. Estimation of viability of cell

Only 7 isolates (TS1, TS3, TS11, TS6, RS4, RS 5, and RS6) scored 10^8 cfu/ml when all the isolates were estimated for colony forming unit per milliliter (Table 4).

Table 4. Colony counts for different *Rhizobium* isolates from pea plants

S. No.	Isolates	Dilution factor	Count(cfu/ml)
1	RS1	10^{-6}	1.8×10^7
2	RS2	10^{-6}	2.6×10^7
3	RS3	10^{-6}	1.6×10^7
4	RS4	10^{-6}	1.23×10^8
5	RS5	10^{-6}	1.12×10^8
6	RS6	10^{-6}	1.36×10^8
7	RS7	10^{-6}	3.21×10^8
8	RS8	10^{-6}	3.19×10^8
9	RS9	10^{-6}	2.3×10^7
10	RS10	10^{-6}	1.7×10^7
11	TS1	10^{-6}	1.16×10^8
12	TS2	10^{-6}	3.13×10^8
13	TS3	10^{-6}	1.11×10^8
14	TS4	10^{-6}	2.3×10^7
15	TS5	10^{-6}	2.0×10^7
16	TS6	10^{-6}	1.32×10^8
17	TS7	10^{-6}	3.26×10^8
18	TS8	10^{-6}	3.31×10^8
19	TS9	10^{-6}	3.27×10^8
20	TS10	10^{-6}	2.5×10^7
21	TS11	10^{-6}	1.22×10^8

3.5. Effect of *Rhizobium* isolates on growth promotion activity of pea variety of *bursa* in pot experiment or *In-vivo* test

The study was used a pot experiment to test the effectiveness of *Rhizobium* isolates in promoting the growth and development of field pea variety of *bursa.* Each isolate was discovered to have a favorable impact on the diversity of growth promotion activity. This indicates that after four weeks of planting, the growth parameters *bursa*, including shoot length (cm), root length (cm), fresh shoot weight (g), dry shoot weight (mg), fresh weight (g), and dry weight (mg), chlorophyll content, and number of root nodules per plant, were recorded. According to the analysis of variance; the effects of the *Rhizobium* inoculation treatments and their interaction on the shoot length, root length, root nodule, fresh shoot weight, dry shoot weight, fresh shoot weight, dry root weight, and chlorophyll content were significant at ($p< 0.05$) compared to the control (Table 5, Table 6, Fig 5,6 and 7).

Table 5. ANOVA of shoot length, root length, chlorophyll content, and number of nodules pea variety of *bursa* as influenced by *Rhizobium* inoculants

Treatments	**Shoot length(cm)**	**Root length(cm)**	**Total chlorophyll (mg)**	**No. of nodules per root**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
T_0 (control)	$15.98^{c} \pm 1.49$	$6.02^{e} \pm 1.76$	$0.21^{e} \pm 0.02$	$0.00^{d} \pm 0.000$
T_1 (*Rhizobium*-TS1)	$27.38^{b} \pm 1.65$	$12.85^{cd} \pm 1.79$	$2.77^{ab} \pm 0.25$	$22.00^{c} \pm 2.94$
T_2 (*Rhizobium*-TS3)	$27.63^{b} \pm 1.65$	$15.00^{bc} \pm 1.83$	$1.68^{d} \pm 0.24$	$24.00^{abc} \pm 1.29$
T_3 (*Rhizobium*-TS11)	$31.20^{a} \pm 2.97$	$15.50^{b} \pm 1.29$	$1.87^{cd} \pm 0.19$	$25.00^{ab} \pm 1.41$
T_4 (*Rhizobium*-TS6)	$27.30^{b} \pm 1.86$	$11.98^{d} \pm 1.34$	$2.16^{cd} \pm 0.46$	$23.00^{bc} \pm 1.718$
T_5 (*Rhizobium*-RS4)	$33.55^{a} \pm 1.37$	$18.08^{a} \pm 0.38$	$3.19^{a} \pm 0.3$	$26.00^{a} \pm 0.96$
T_6 (*Rhizobium*-RS5)	$32.48^{a} \pm 1.15$	$15.00^{bc} \pm 0.82$	$2.74^{ab} \pm 0.21$	$26.00^{a} \pm 1.89$
T_7 (*Rhizobium*-RS6)	$31.63^{a} \pm 1.75$	$12.93^{cd} \pm 1.09$	$2.38^{bc} \pm 0.75$	$21.00^{c} \pm 2.16$

SD= Standard deviation**.** Means in the same column followed by the same letter are not significantly different at LSD test ($p<0.05$).



Fig 5. *Rhizobium* inoculated plants and control plants growing in greenhouse conditions.

T0= Control and T1, T2, T3, T4, T5, T6 and T7=Treatments inoculated with different rhizobial isolates

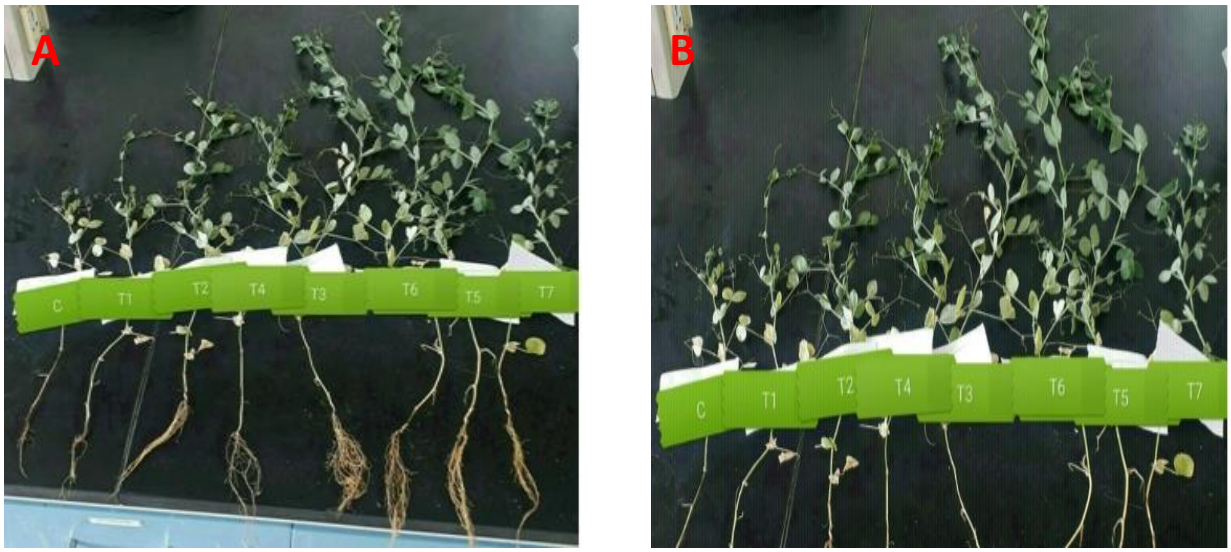


Fig 6. (A). Shoot and root length of various treatments (B). Shoot length of various treatments

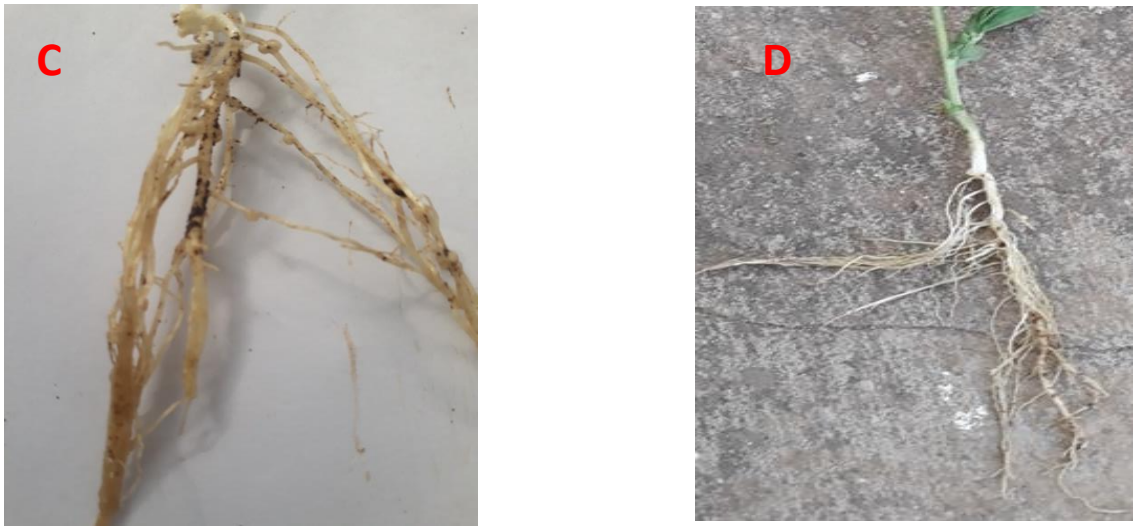


Fig 7. Nodule formations in *Rhizobium* inoculated plants (C), and absence of nodule formations in control plant (without *Rhizobium* inoculations) (D)

Table 6. ANOVA of fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight of pea variety of *bursa* as influenced by *Rhizobium* inoculants

Treatments	**Fresh shoot weight (g)**	**Fresh root weight (g)**	**Dry shoot weight (mg)**	**Dry root weight (mg)**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
T_0 (control)	1.20^{f} ±.11	0.61^{e} ±0.10	0.33^{e} ±0.1	0.13^{g} ±0.01
T_1 (*Rhizobium*-TS1)	2.05^{e} ±.03	1.35^{bc} ±0.14	0.97^{cd} ±0.04	0.70^{d}±0.05
T_2 (*Rhizobium*-TS3)	2.42^{d} ±.05	1.28^{c} ±0.03	0.95^{cd} ±0.05	0.76^{bc} ±0.04
T_3 (*Rhizobium*-TS11)	2.93^{c} ±.09	1.43^{b} ±0.04	1.13^{b} ±0.07	0.80^{ab} ±0.04
T_4 (*Rhizobium*-TS6)	2.49^{d} ±.07	1.03^{d} ±0.04	0.88^{d} ±0.04	0.57^{f} ±0.02
T_5 (*Rhizobium*-RS4)	3.47^{a} ±.19	1.73^{a} ±0.12	1.32^{a} ±0.02	0.84^{a} ±0.03
T_6 (*Rhizobium*-RS5)	3.13^{b} ±.02	1.25^{c} ±0.02	1.17^{b} ±0.07	0.75^{cd} ±0.01
T_7 (*Rhizobium*-RS6)	2.93^{c} ±.15	1.35^{bc} ±0.10	0.99^{c} ±0.02	0.65^{e} ±0.03

SD= Standard deviation**.** Means in the same column followed by the same letter are not significantly different at LSD test ($p<0.05$).

4. DISCUSSION

Among 21*Rhizobium* isolates isolated from the root nodules of field pea plant collected from field. Seven isolates were selected from the total 21 *Rhizobium* isolates based on their characteristics. The rhizobia isolates utilized in this investigation were assigned to the genus *Rhizobium* as a result of *in-vitro* characterization, biochemistry, and colony morphology. The microscopic analysis of the colony and morphological characteristics the isolates in this study were white, mikly, rod-shaped, and gram-negative (Fig 1 and Fig 2). This result was agreed with studies (38-40). Rhizobial isolate colonies grown in Congo red in this study were white; they are unable to absorb Congo red, but other contaminants, such as agrobacterium, can. Similar to this, after adding 1.0 ml of Benedict's reagent to each isolate's plate in the keto-lactose test; none of the isolates produced a yellow color on the keto-lactose media. This means that every isolate tested positive for both the GPA test and the keto-lactose test, with the exception of RS7 and RS8, which did not grow on GPA media. This shows that rhizobia isolates grew poorly or not at all on glucose peptone agar medium and barely affected pH. These findings thus proved that the isolates were Rhizobium (41, 42).

In the present study, all isolates except RS9 were positive for catalase test as confirmed by liberation of bubble of oxygen around the Rhizobium colobies when drop of 3% hydrogen peroxide was added to the test isolates and oxidase test; fourteen isolates were positive except the isolates of RS3, RS7, RS10, TS7, TS8, TS9 and TS10 as confirmed by change in color of oxidase strip to blackish purple. This outcome was in great agreement with (43) (44, 45).

Only fourteen isolates in the current study produced clear zones around their colonies in the starch hydrolysis test after dousing the inoculated plates in iodine solution. In the urease hydrolysis test, only 12 *Rhizobium* isolates had changed the color of the urea medium from yellow to pink after incubation, indicating that the test isolates were urease positive and that the ammonia production was the cause of the medium's alkaline nature. The findings of) (46, 47) were relatively similar to this result.

In the citrate test, the majority of the isolates saw a color change from green to blue in the simmon's citrate agar medium after 24 hours of inoculation, indicating that the test isolates wer citrate positive. The exceptions were RS1, RS9, TS7, TS8, TS9, and TS10. Only 13 isolates, however, liquefied the medium in the tubes and created a clear zone around the line growth in the gelatin hydrolysis test. The findings from the studies published by (45) (47) (48) were in agreement with this outcome.

All of the isolates in this study responded favorably to ammonia production. This result showed that despite the nodule's low oxygen concentration, the isolates were still able to reduce N_2 to ammonia using nitrogenase. Additionally, these isolates were reluctant to give their host plant the ammonia they produced, so by choking their metabolism, they are forced to do so. This result was align with studies (49, 50).

Except for RS10, TS2, TS4, and TS7, all isolates tested positive for IAA production. This suggested that the isolates could produce IAA from organic substances like tryptophan excreted from plant roots to promote and facilitate plant growth. On Pikovskaya agar, 18 isolates created a clear zone during the phosphate solubilization test. This demonstrated the isolates' ability to dissolve tricalcium phosphate. Studies by Othman and Tamimi (2016), (51) (52) (53) were comparable to the current study.

Only 7 isolates (TS1, TS3, TS11, TS6, RS4, RS5, and RS6) were estimated for colony forming unit per milliliter in the country. According to this estimation, the seven isolates (TS1, TS3, TS11, TS6, RS4, RS5, and RS6) satisfy the requirement of 10^8 colony forming units per milliliter (10^8cfu/ml) to be used as bioinoculants. The findings of studies by (54) (55) are all consistent with this conclusion.

After 4 weeks of planting, the following plant characteristics were measured: shoot length (cm), root length (cm), fresh shoot weight (g), dry shoot weight (mg), fresh root weight (g), dry root weight (mg), and the number of root nodules per plant. According to an analysis of variance, there was a significance differences between the *Rhizobium* inoculation treatments and the control terms of shoot length, root length, root length, root nodule, fresh shoot weight, dry shoot weight, fresh root weight, and chlorophyll content. The length of the shoots varies from 15.98 to 33.55 cm. T5 (33.55 cm), inoculated with Rhizobium-RS4, had the longest shoots, followed by T6 (32.48 cm), T7 (31.63 cm), and T3 (31.20 cm).

A minimum shoot length of 15.98 cm was noted in the control group or T0 (uninoculated). In comparison to T1 (*Rhizobium*-TS1), T2 (*Rhizobium*-TS3), and T4 (*Rhizobium*-TS6), T5 (*Rhizobium*-RS4), T6 (*Rhizobium*-RS5), T7 (*Rhizobium*-RS6), and T3 (*Rhizobium*-TS11) had the longest shoots. Table 5 summarizes the results of the treatments T1 (*Rhizobium*-TS1), T2 (*Rhizobium*-TS3), and T4 (*Rhizobium*-TS6). 6.02 to 12.93 cm is the size range of the roots. Maximum root length was noticed in T5 that had received Rhizobium-RS4 inoculation. A minimum root length of 6.02 cm was noted in the T0 (uninoculated) or control group. The differences between the inoculated treatments and the control were all statistically significant. The differences in root length between treatments T5 (*Rhizobium*-RS4), T1 (*Rhizobium*-TS1), T2, T3, T4, T6, and T7 (*Rhizobium*-RS6) was statistically significant. *Rhizobium*-TS1, *Rhizobium*-TS6, and *Rhizobium*-RS6 were identical in T1 but different from the control in T4 and T7 (Table 5). This study was comparable with the studies (56-58).

The range of chlorophyll content is 0.21- 3.19 mg. The most chlorophyll was found in T5 (3.19 mg), which had been exposed to Rhizobium-RS4. The control group or T0 (0.21mg) had the least amount of chlorophyll. The *Rhizobium* inoculated plants' treatments all outperform the control group statistically. In terms of their chlorophyll contents, the treatments T5 (*Rhizobium*-RS4) and T7 (*Rhizobium*-RS6) differ significantly from T2 (*Rhizobium*-TS3), T3 (*Rhizobium*-TS11), T4 (*Rhizobium*-TS6), and T6 (*Rhizobium*-RS5). When compared to the treatments T1 (*Rhizobium*-TS1), T5 (*Rhizobium*-RS4), and T6 (*Rhizobium*-RS5), the T2 (*Rhizobium*-TS3), T3 (*Rhizobium*-TS11), and T4 (T4 (*Rhizobium*-TS6) results were identical but significantly different. This study result was concurrent with the studies (58-60)

In comparison to un-inoculated or control plants, the number of root nodules per plant infected by *Rhizobium* isolates was significantly ($p<0.05$) different (Table 5). All of the treatments in a greenhouse increased the number of root nodules on each plant in comparison to the control. Root nodules ranged in number from 0.00 to 26.00. Most root nodules were seen in the T5 (*Rhizobium*-RS4) and T6 (*Rhizobium*-RS5) treatments. In the group T0 (control), there were no root nodules observed. The *Rehizobium* inoculated plants had significantly more nodules than the control. T5 (*Rhizobium*-RS4) and T6 (*Rhizobium*-RS5) were significant among the treatments, outperforming all others. Similar results were found for T1 (*Rhizobium*-TS1) and T7 (*Rhizobium*-RS6) (Table 5). *Brusa* variety in this experiment had longer shoots, longer roots, more chlorophyll, and more nodules due to the inculation of *Rhizobium* isolates. This shows that Rhizobium isolates effectively interact symbiotically with *Bursa* variety roots. The investigation conducted by (30, 39, 61, 62) coincided with the above-mentioned results.

The experimental findings in the current study showed that the *Rhizobium* inoculated *bursa* variety performed better than the control group in terms of fresh shoot weight, fresh root weight, dry shoot

weight, and dry root weight. The range of fresh shoot weight per plant was found to be between 1.20 and 3.47 g. T5 (3.47 g/plant) had the highest shoot fresh weight, followed by T6 (3.13 g/plant), T7 (2.93 g/plant), T3 (2.93 g/plant), T4 (2.49 g/plant), T2 (2.42 g/plant), T1 (2.05 g/plant), and T0/control (1.20 g/plant). The plants that received *Rhizobium* inoculation outperformed the control group. Overall treatments, the highest mean fresh shoot weight T5 was significant. Similar results were found for the treatments T3 (Rhizobium-TS11) and T7 (*Rhizobium*-RS6) (Table 5). *Rhizobium* inoculated treatments' root fresh weights demonstrated significant differences from the control and ranged from 0.61 to 1.73 g per plant. In comparison to the control group, root fresh weights of *Rhizobium* inoculated treatments were statistically significant. T5 (*Rhizobium*-RS4) recorded the highest root fresh weight, followed by T3 (*Rhizobium*-TS11), and T0 (Control) recorded the lowest root fresh weight (0.61g/plant). The treatment T5 (*Rhizobium*-RS4) outperformed all others in terms of the root fresh weight. The results of the treatment T1 were comparable to those of T2, T3, T6, and T7, but they differed significantly from those of T4 and T5 (Table 5). Shoot dry weights between 0.33 and 1.32 mg were noted. The plants that received *Rhizobium* inoculation outperformed the control group. *Rhizobium*-RS4 showed the highest shoot dry weight at T5, followed by *Rhizobium*-TS11 at T3 and *Rhizobium*-RS5 at T6, and control showed the lowest shoot dry weight at T0. T5 (*Rhizobium*-RS4) was the most significant treatment overall. T1 and T2 among the treatments produced results that were comparable but noticeably different from T3 and T6. The root's dry weight was measured in the 0.13-0.84 mg range. T5 (*Rhizobium*-RS4) 0.84 mg, which is statistically significant compared to all other treatments, recorded the highest root dry weight. The *Rhizobium* inoculated plants were significantly superior to the control in all growth performance parameters of the plants at p-value ($p<0.05$). This showed that the isolated strains that had been inoculated performed better at fixing nitrogen and enhancing plant growth. This might be because the organism has the genetic capacity to fix nitrogen. Thus, the current findings of the bursa pot experiment were in line with those of the studies published by (30, 63-66).

5. CONCLUSIONS

Based on biochemical and *in-vitro* tests, twenty-one *Rhizobium* isolates were obtained for this study from the root nodule of field pea plants. Seven isolates were chosen from this group, and their efficacy was evaluated based on their performance in *in-vitro* tests and their ability to produce 10^8 colony-forming units (10^8cfu) when used as bioinoculants to inoculate bursa seeds and assess the bursa's ability to grow. The outcome demonstrated that *Rhizobium* isolate inoculated *bursa* variety outperform uninoculated *bursa* variety in terms of performance. Even though all of the isolates improved the *bursa*'s ability to grow, isolate *Rhizobium*-RS4 was the most effective in terms of nodulation efficiency, fresh weight shoot/root, dry weight shoot/root, and chlorophyll content. The efficiency of variation among the *Rhizobium* isolates in nodulation and other growth parameters on the crop may be due to variations in their symbiotic traits and genetic makeup. The unique aspect of this study was the screening of isolates from field pea root nodules and their inoculation to the other newly released field pea *bursa* variety seeds to enhance plant growth. As a result, the isolated inoculants that this study produced were not variety-specific. This discovery may provide us with fundamental knowledge about bioinoculants, which are not variety-specific in their ability to promote the growth of legumes.

6. REFFERENCES

1. Pahalvi HN, Rafiya L, Rashid S, Nisar B, Kamili AN. Chemical fertilizers and their impact on soil health. Microbiota and Biofertilizers, Vol 2: Ecofriendly tools for reclamation of degraded soil environs. 2021:1-20.
2. Jote CA. The impacts of using inorganic chemical fertilizers on the environment and human health. Org Med Chem Int J. 2023;13:555864.
3. Angon PB, Islam MS, Das A, Anjum N, Poudel A, Suchi SA. Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. Heliyon. 2024;10(7).
4. Das H, Devi N, Venu N, Borah A. Chemical fertilizer and its effects on the soil environment. Research and Review in Agriculture Sciences. 2023;7:31-51.
5. Baye TG. Poverty, peasantry and agriculture in Ethiopia. Annals of Agrarian Science. 2017;15(3):420-30.
6. Yigezu Wendimu G. The challenges and prospects of Ethiopian agriculture. Cogent Food & Agriculture. 2021;7(1):1923619.
7. Seifu W, Elias E, Gebresamuel G, Tefera W. Soil type and fertilizer rate affect wheat (Triticum aestivum L.) yield, quality and nutrient use efficiency in Ayiba, northern Ethiopia. PeerJ. 2022;10:e13344.

8. Bekele D, Abera G, Gobena A. Effects of chemical fertilizer types and rates on tuber yield and quality of potato (Solanum tuberosum L.) at Assosa, Western Ethiopia. African Journal of Plant Science. 2020;14(4):155-64.
9. Redda A, Abay F. Agronomic performance of integrated use of organic and inorganic fertilizers on rice (Oryza sativa L.) in Tselemti District of North-Western Tigray, Ethiopia. Journal of Environment and Earth Science. 2015;5(9):2224-3216.
10. Pathak AK, Dubey P, Pandey S. Overview of fertilizer industry in India. Int J Soc Sci Interdiscip Res. 2014;3(7):7-15.
11. Chaudhary P, Singh S, Chaudhary A, Sharma A, Kumar G. Overview of biofertilizers in crop production and stress management for sustainable agriculture. Frontiers in Plant Science. 2022;13:930340.
12. Nosheen S, Ajmal I, Song Y. Microbes as biofertilizers, a potential approach for sustainable crop production. Sustainability. 2021;13(4):1868.
13. Soni SK, Manhas R, Jakhar Y, Sharma A, Soni R. Biofertilizers for sustainable agriculture: current trends and future perspective. Genomic, Proteomics, and Biotechnology. 2022:331-56.
14. Pereira F, Pereira A, Menezes LD, Sawaikar R. Rhizobium as a biofertilizer for non-leguminous plants. Discover Food. 2024;4(1):85.
15. Sharma U, Datta M, Sharma V. Soil microbes and biofertilizers. Soils in the Hindu Kush Himalayas: Management for agricultural land use: Springer; 2023. p. 117-44.
16. Abd-Alla MH, Al-Amri SM, El-Enany A-WE. Enhancing rhizobium–legume symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change. Agriculture. 2023;13(11):2092.
17. Arjumend T, Sarıhan EO, Yıldırım MU. Plant-bacterial symbiosis: an ecologically sustainable agriculture production alternative to chemical fertilizers. Revisiting Plant Biostimulants: IntechOpen; 2022.
18. Goyal RK, Mattoo AK, Schmidt MA. Rhizobial–host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability. Frontiers in Microbiology. 2021;12:669404.
19. Gutu DT, Haile GA, Kebede GY, Gala TS, Tolessa TT, Ertiro TA. Field Pea Variety Development for Yield and Disease Resistance for Potential Areas-Registration of a Field Pea Variety Named ‘Bursa’. World Scientific Research. 2020;7(1):5-11.
20. Rasool S, Sharma B, Rasool S. Isolation and characterization of Rhizobium spp. from a wild legume from BGSBU campus of District Rajouri, J & K. International Journal of Agricultural Sciences. 2015;5(3):407-13.

21. Kneen BE, LaRue TA. Congo red absorption by Rhizobium leguminosarum. Applied and Environmental Microbiology. 1983;45(1):340-2.
22. Pervin S, Jannat B, Al Sanjee S. Characterization of Rhizobia from Root Nodule and Rhizosphere of Lablab purpureus and Vigna sinensis in Bangladesh. Turkish Journal of Agriculture-Food Science and Technology. 2017;5(1):14-7.
23. Rai R, Sen A. Biochemical characterization of French bean associated rhizobia found in North Bengal and Sikkim. Journal of Academia and Industrial Research. 2015;4(1):10-8.
24. Deshwal VK, Chaubey A. Isolation and characterization of Rhizobium leguminosarum from root nodule of Pisum sativum L. Journal of Academia and Industrial Research. 2014;2(8):464-7.
25. Tyagi A, Kumar V, Tomar A. Isolation, identification, biochemical and antibiotic sensitivity characterization of Rhizobium strains from Vigna mungo (L.) Hepper, Cicer arietinum (L.) and Vigna radiata (L.) Wilczek in Muzaffarnagar, Uttar Pradesh India. International Journal of Current Microbiology and Applied Sciences. 2017;6(12):2024-35.
26. Prajapati S, Dadke M, Surekha S, Godika S, Krishna VP. Isolation and characterization of Rhizobium meliloti isolated from rhizosphere soil and roots of fenugreek from different locations. Int J Curr Microbiol Appl Sci. 2018;7(6):1460-7.
27. Mashifane DC. The effect of chemomutagenesis on root nodulation and seed protein in tepary bean (Phaseolus acutifolius). 2018.
28. HALOPHYTES CORF. Morphological, biochemical and molecular characterization of rhizobia from halophytes of Khewra Salt Range and Attock. Pak J Bot. 2009;41(6):3159-68.
29. Tamiru G, Muleta D. The effect of rhizobia isolates against black root rot disease of Faba bean (Vicia faba L) caused by Fusarium solani. The Open Agriculture Journal. 2018;12(1).
30. Rajeshwari P, Aishwaryaalakshmi B, Jeyagowri C. Isolation, identification and screening of Rhizobium for plant growth promotion. International Journal of Applied Research. 2017;3(1):732-3.
31. Majeed A, Abbasi MK, Hameed S, Imran A, Rahim N. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in microbiology. 2015;6:198.
32. Ruíz-Valdiviezo VM, Canseco LMCV, Suárez LAC, Gutiérrez-Miceli FA, Dendooven L, Rincón-Rosales R. Symbiotic potential and survival of native rhizobia kept on different carriers. Brazilian Journal of Microbiology. 2015;46(3):735-42.
33. Hajjam Y, Alami I, Udupa S, Cherkaoui S. Isolation and evaluation of phosphate solubilizing rhizobia from root nodules of faba bean (Vicia faba L.) in Morocco. Journal of Materials and Environmental Science. 2016;7(11):4000-10.

34. Swenson JM, Thornsberry C. Preparing inoculum for susceptibility testing of anaerobes. Journal of clinical microbiology. 1984;19(3):321-5.
35. Yshita KN. Isolation, identification and characterization of Rhizobium from hyacinth bean (Lablab niger) root nodule and study its effect on plant growth and nitrogen fixation ability: BRAC University; 2016.
36. Su S, Zhou Y, Qin JG, Yao W, Ma Z. Optimization of the method for chlorophyll extraction in aquatic plants. Journal of Freshwater Ecology. 2010;25(4):531-8.
37. Sumanta N, Haque CI, Nishika J, Suprakash R. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Res J Chem Sci. 2014;2231:606X.
38. Al-Mujahidy S, Hassan M, Rahman M, Mamun-Or-Rashid A. Isolation and characterization of Rhizobium spp. and determination of their potency for growth factor production. International Research Journal of Biotechnology. 2013;4(7):117-23.
39. Abdul Waheed AW, Aftab Afzal AA, Tariq Sultan TS, Inayat-ur-Rahman I-u-R, Sohail S, Farhana Ijaz FI, et al. Isolation and biochemical characterization of rhizobium from pea crop at Swabi. 2014.
40. Datta A, Singh RK, Tabassum S. Isolation, characterization and growth of Rhizobium strains under optimum conditions for effective biofertilizer production. Int J Pharm Sci Rev Res. 2015;32(1):199-208.
41. Küçük Ç, Kivanç M, Kinaci E. Characterization of Rhizobium sp. isolated from bean. Turkish Journal of Biology. 2006;30(3):127-32.
42. Elakiya N, Ragupathi K. Utilisation of Green Manures as Organic Substrates in Soilless Culture: A Review.
43. Mousa N, Abdul Hassan M. Biodegradation of Glyphosate by Four Plant Growth Promoting Bacteria (4PGPB). Pollution. 2025;11(1):15-22.
44. Hussien E, Sultan R, Edavana V. Tolerance of Rhizobium Isolates to Certain Heavy Metals. Journal of Education and Science. 2024;33(3):101.0-11.0.
45. Vashistha H, Kumar P, Kumar S. PGP attributes of non-rhizobial bacterial endophytes from root nodules of wild leguminous plant Tephrosia purpurea (L) Pers. Environment Conservation Journal. 2024;25(4):942-55.
46. Islam MM, Mandal S. Unveiling growth-promoting attributes of peanut root endophyte Micromonospora sp. Archives of Microbiology. 2024;206(4):182.

47. Massadeh MI, Alkhataibeh EA, Al-Ghzawi ALA, Aukour F. Enhancement of Medicago sativa plant growth and yield after treatment with Rhizobium spp. Journal of Ecological Engineering. 2024;25(2).
48. Adu Oparah I, Deaker R, Hartley JC, Gemell G, Hartley E, Sohail MN, et al. Symbiotic Effectiveness, Rhizosphere Competence and Nodule Occupancy of Chickpea Root Nodule Bacteria from Soils in Kununurra Western Australia and Narrabri New South Wales Australia. Plants. 2025;14(5):809.
49. Beshah A, Muleta D, Legese G, Assefa F. Isolation and Screening of Effective Rhizobium Species Nodulating and Fixing Atmospheric Nitrogen on Groundnut (Arachis hypogaea) Grown in Some Important Regions of Ethiopia. Eurasian Soil Science. 2024;57(Suppl 1):S78-S91.
50. Adeyemi O, Feruke-Bello Y, Odeyemi O. Improvement of yield parameters of cowpea (Vigna unguiculata) by Rhizobia sp. isolated from Mucuna pruriens and Centrosema pubescens at Ile-Ife, Nigeria. Sustainable Microbiology. 2025:qvaf004.
51. Tiwari T, Sharma S, Kumar D, Kumar C. Potential of Biofertilizers in Sustainable Agriculture and Soil Health. Vigyan Varta. 2024;5(1):228-32.
52. Attar B, Kitson JJ, Cuff JP, Howard B, Lages A, Gomez D, et al. Identifying the Fusarium species involved in foot rot disease of faba beans in the UK using a combined molecular and microbiological approach. bioRxiv. 2024:2024.10. 25.620281.
53. N Hegazy W, S Abdel-Lateif K, Ibrahim Fahmi A, Nagaty H, M Zayed O. DEVELOPMENT AND CHARACTERIZATION OF LEAD-TOLERANT RHIZOBIUM MUTANTS FOR ENHANCING PEA GROWTH. Menoufia Journal of Agricultural Biotechnology. 2025;10(1):1-19.
54. Shaheb MR, Islam MT, Sarker A. of Sustainable Agriculture. Soil Bacteria: Biofertilization and Soil Health. 2024:3.
55. Messias M, de Brito Ferreira EP, da Silva OF, Wander AE. Economic Assessment of Rhizobium tropici and Azospirillum brasilense Co-Inoculation in Common Bean. Agricultural Research. 2024;13(2):216-24.
56. Mir MI, Kumar BK, Gopalakrishnan S, Vadlamudi S, Hameeda B. Characterization of rhizobia isolated from leguminous plants and their impact on the growth of ICCV 2 variety of chickpea (Cicer arietinum L.). Heliyon. 2021;7(11).
57. Arafa M, El-Batanony N, Nofal A. Inoculation effect of rhizobial strains on growth, yield and chemical composition of some legume crops in new reclaimed soil. Middle East Journal of Agriculture Research. 2018;7(2):352-63.
58. Qureshi MA, Shahzad H, Saeed MS, Ullah S, Ali MA, Mujeeb F, et al. Relative potential of rhizobium species to enhance the growth and yield attributes of cotton (Gossypium hirsutum L.). Eurasian Journal of Soil Science. 2019;8(2):159-66.

59. Mushtaq Z, Faizan S, Gulzar B, Hakeem KR. Inoculation of Rhizobium alleviates salinity stress through modulation of growth characteristics, physiological and biochemical attributes, stomatal activities and antioxidant defence in Cicer arietinum L. Journal of Plant Growth Regulation. 2021;40:2148-63.
60. Athul PP, Patra RK, Sethi D, Panda N, Mukhi SK, Padhan K, et al. Efficient native strains of rhizobia improved nodulation and productivity of French bean (Phaseolus vulgaris L.) under rainfed condition. Frontiers in Plant Science. 2022;13:1048696.
61. Demissie N, Degefu T, Ergena A, Ojiewo C. Phenotypic characteristics of rhizobial and non-rhizobial isolates recovered from root nodules of chickpea (Cicer arietinum L.) grown in Ethiopia. African Journal of Microbiology Research. 2018;12(4):73-85.
62. Mendoza-Suárez M, Andersen SU, Poole PS, Sánchez-Cañizares C. Competition, nodule occupancy, and persistence of inoculant strains: key factors in the rhizobium-legume symbioses. Frontiers in Plant Science. 2021;12:690567.
63. Soumaya T-H, Sana DF, Faysal BJ, Imran H. Effect of Rhizobium inoculation on growth and nutrient uptake of sulla (Hedysarum coronarium L.) grown in calcareous soil of northern Tunisia. Romanian Biotechnological Letters. 2016;21(4):11632-9.
64. Kandil AE, Özdamar Ünlü H. Effect of rhizobium inoculation on yield and some quality properties of fresh cowpea. Cogent Food & Agriculture. 2023;9(2):2275410.
65. Gedamu SA, Tsegaye EA, Beyene TF. Effect of rhizobial inoculants on yield and yield components of faba bean (Vicia fabae L.) on vertisol of Wereillu District, South Wollo, Ethiopia. CABI Agriculture and Bioscience. 2021;2:1-10.
66. Desta M, Akuma A, Minay M, Yusuf Z, Baye K. Effects of Indigenous and Commercial Rhizobia on Growth and Nodulation of Soybean (Glycine max L) under Greenhouse Condition. The Open Biotechnology Journal. 2023;17(1).

Statements and Declarations

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Author Contribution

Samson Zemikael Haftu was responsible for conceptualization, drafting methodology, conducting experimental activities, and writing the original manuscript. Melaku Mekonen Kasegn contributed supervising the study, writing review, reading, and editing the manuscript. Hiwot Kasa Abera contributed to writing review, reading, and editing the manuscript. Brhane Gerensea Gebrekidan contributed to writing review, reading, and editing the manuscript. Etsay Mesele contributed to reading, and editing the

manuscript. Finally, the authors had worked by sharing their ideas, reading and approving the final manuscript.

Declarations

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].