International Journal of Scientific & Engineering Research Volume 9, Issue 12, December-2018
ISSN 2229-5518

Characterization of Faba bean (*Vicia faba*) nodulating rhizobia from producing areas around Ambo and their effect on growth and nitrogen nutrition of the plant

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ABSTRACT

Biological nitrogen fixation with symbiotic relationship between Rhizobia and leguminous plants is considered to be very efficient in supplementing or replacing the chemical nitrogen input in the agricultural system. Minimizing the chemical nitrogen input can also reduce the negative effect of chemical nitrogen fertilizer in the environment which in turn contributes in the sustainability of the environment. The main objective of this study was to isolate and characterize Rhizobial strains from Faba bean growing areas of Ambo Woreda and check their nitrogen fixing efficiency with faba bean. Standard microbiological procedures were used for the isolation and characterization Rhizobium isolates from the field collected faba bean nodules, and the green house experiment was done in triplicate in the randomized block design, and percent of nitrogen and total proteins in dry biomass of the faba bean was estimated by Kjedahl method. A total of 21 Rhizobium strains were isolated from the faba bean (*Vicia faba*) root nodules collected from the five Kebeles of Ambo Wareda. All the Rhizobium isolated from different study sites showed circular colony shape, and 6 of them white colony color while the rest were colorless in their colony color. All Rhizobium isolates were gram negative, rod shaped and arranged in straight chain. 19 of the 21 isolates of the Rhizobium isolates showed negative test for methyl red, while two of them were positive. Four of the 21 isolates were positive for citrate test, while the rest of the isolate was negative. All the isolates of the Rhizobium were positive for the motility test. Only one of the 21 isolates of the Rhizobium isolate showed urease positive. Inoculation alone or together with nitrogen fertilization highly initiated nodule formation and the highest nodule number per plant were observed on T2, T3, T4, T5 and T7 (71.11, 51.11, 45.66, 64.44 and 56.44) respectively, while the nodules fresh/wet weight per plant were collected from T2, T3, T4, T5 and T7 (0.61gm, 0.49gm, 0.44gm, 0.56gm and 0.58gm) respectively. Inoculation with the different Rhizobium isolates alone greatly improved the percent of nitrogen in the dry biomass of the faba bean T2, T3, T4 and T5 (3.54%, 3.52%, 3.75% and 3.49%) respectively. Nitrogen fertilization alone minimized the percent of nitrogen content in T6 that is 2.22%. Similar trends were observed in relation to total protein content in the dry biomass of faba bean received different treatments. Our agricultural system has been facing supplying sufficient food for rapidly growing human population, while minimizing the negative effect of chemical fertilizer used for boosting crop yields. Symbiotic nitrogen fixation between Rhizobia and leguminous plants can reduce these hurdles since it is the efficient way of supplying fixed nitrogen for the growth of plants. This small scale investigation had clearly indicated the possibility of supplementing or replacing chemical nitrogen fertilizer by locally isolated Rhizobium strains.

Key phrases: Biological Nitrogen fixation, Nitrogen nutrition, Rhizobium and Symbiotic Nitrogen fixation

INTRODUCTION

Nitrogen is one of the most important macronutrients for the survival of living things. Plants are supplied this important element from different source which include chemical fertilizer and biological nitrogen fixation. Nitrogen fixation is very important for all living organisms on the world and sources of nitrogen without pollution. All organisms cannot use atmospheric nitrogen. They need conversion of atmospheric nitrogen into usable forms of nitrogen compound by biological

nitrogen fixation. Biological nitrogen fixation is very important sources of nitrogen for all organisms. Soil has many different types of microorganisms like bacteria, actinomycetes, fungi, and algae that are extremely important because they determine the fertility of the soil. From these large number of microorganisms soil bacteria a unique group called Rhizobia has a beneficial effect on the growth of and productivity of leguminous plants *(Neeraj et al., 2008)*. Rhizobia are selectively infecting the roots of legume plants and they have characteristics such as gram negative, motile, rod-shaped and heterotropic *(Bouhmouch et al., 2005)*. Root nodule bacteria generally grow under of conditions 25-30°C, (optimum) in the pH range of 6-7 *(Bouhmouch et al., 2005)*. Rhizobia generally grow in aerobic condition, however, fixation of nitrogen by this bacteria needs low level of oxygen, that is why formation of nodules is required *(Singh and Prasad, 2011)*. At this time almost all farmers use chemical fertilizer as agricultural input, which has number of problem including pollution, poor quality in nutirition and it is too expensive. However, it improves the productivities of crops, the quality of crops that produced by chemical fertilization is very poor in nutrition. The use of Rhizobium strains as agricultural input can solve all these problem, which include quality of nutrition, environmental pollution and expensiveness of the cost of the fertilizer.

Rhizobia are a genetically diverse and physiologically heterogenousgroup of bacteria and they are able to elicit nodule formation on legumes plants *(Zahran, 2001)*. They are a ubiquitous of the soil micro-flora in a free-living state in the rhizosphere of legumes until the point where nodulation reaches *(Hungrıa et al., 2006)*. The number of symbiotic relationships that can form between rhizobia and hosts is restricted and Rhizobia elicit on their host and the formation of nodules in which they fix. Rhizobium can live either in the soil or within the root nodules of host legumes and these bacteria colonize the root nodules, where it transforms atmospheric nitrogen to ammonia and provides organic nitrogenous compounds to the plants that the usable forms of nitrogen for plants as well as indirectly for all living organisms that live on the world *(Neeraj et al., 2008)*. Plant growth stimulating rhizobacteria are a very few in number (about 2–5%) of the total rhizobacterial population *(People et al., 2011)*. Plant growth stimulating rhizobacteria use direct or indirect mechanisms to improve the growth and health of plants. These mechanisms can be active simultaneously or independently at different stages of plant growth *(People et al., 2011)*. Legumes have been used in agriculture during early time and legume seeds or pulses were from the primary source of human food and for other living things. The bacteria that are able to form nodules on the leguminous plants are belonging to the genera Rhizobium, Bradyrhizobium, Allorhizobium, Azorhizobium and Mesorhizobiumwhich are collectively known as *Rhizobium (Singh et al., 2011)*. International emphasis on environmentally sustainable development with the use of renewable resources is likely to focus on the potential role of biological nitrogenfixationin supplying nitrogen for agriculture *(Zahran, 2001)*. The cooperative interaction between rhizobia and other plant root colonizing bacteria is of relevance in improvement of nodulation and N_2 fixation in legume plants *(Mensah et al., 2006)*. Currently, the subject of BNF is of great practical importance because the use of chemical nitrogenous fertilizers has resulted in unacceptable levels of water pollution (increasing concentrations of toxic nitrates in drinking water supplies) and the eutrophication of lakes and rivers *(Zahran, 2001)*. Biofertilizer is the key for agriculture as well as breeding different animals, which used as source of food. The importance of biofertilizer is extremely required for the farmer that their live based on

agriculture directly or indirectly. Because of access useof chemical fertilizer which is capable of destroying soil microorganism and polluting different natural resources. Pollution of different natural resource is the most dangerous aspects of chemical fertilizer. To overcome this problem farmer must use biofertilizer, which involves the use of nitrogen fixing bacteria (rhizobium species) is extremely important *(Zahran, 2001)*. In this case, Ethiopian farmershaveinformation aboutbiofertilizer in their agriculture. However, they do not use biofertilizer effectively in their agriculture. So to inform our farmer agriculture development workers must work strongly. Biofertilizerinitiates plant growth and increasesproductivity has internationally been accepted as an alternative source of chemical fertilizer. The soluble form of nitrite and nitrate can be absorbed by plant roots and utilized in the formation of proteins and nucleic acids. This form of nitrogen can be transformed to ammonia by plants, animals and microorganisms. Animals return nitrogenous wastes to the environment as uric acids.

In low soil pH does not allow the rhizobial cells to survive in adequate numbers in free living state. As a result it becomes inevitable to inoculate the crop in adequate rhizobium *(Hungrıa et al., 2006)*. It provides the major biological source of fixed nitrogen in agricultural soils. A well-established practice for keeping soil fertility has been the cultivation of leguminous plants which change atmospheric nitrogen through symbiosis with rhizobia in rotation with non-leguminous plants. *Singh et al (2008)* suggest that a world decrease in agricultural based on biological nitrogen fixation was incompatible with the need to increase world protein production from a notably deteriorating area of global suitable land. Additionally, bean plants which cultivated by biofetilizerare better in the growth and in their nutrition for the health of society *(Zahran, 2001)*.

Despite the widespread distribution of leguminous crops, many soils remain void of rhizobialstrains *(Hungrıa et al., 2006)*. Nitrogen fixation effectiveness of a legume-Rhizobium symbiotic association is dependent on biological factors such as host-strain specificity and environmental factors which affect the multiplication and growth of rhizobia in the environment. Faba bean also improves soil fertility by capturing nitrogen from the atmosphere. An amount of 300 kg of nitrogen per hectare has been reported *(Hungrıa et al., 2006)* and range of 31-110kg per hectare reported by a study, *Mensah et al., (2006)*. The crop has high protein content (40%) and high gross output of protein (20%) among the cultivated crops in the world *(Zahran, 2001)*. The importance of faba bean is source of protein and this is due to ability of fix nitrogen from the atmosphere through root nodules formed symbiotic association with Rhizobium bacteria *(Bouhmouch et al., 2005)*. A study conducted by *Mensah, et al., (2006*) has shown that promiscuously nodulating varieties of fababeannodulate abundantly and effectively in most soils in Southern Africa. *Mishra et al., (2008)* observed that strains of Rhizobium varied in ability to survive in soil. Ability to survive depends on tolerance by the strain to prevailing unfavorable conditions. For example, *Hung et al (2005)* observed that an indigenous strain of Rhizobium was more adapted to prevailing soil temperatures than three introduced strains.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Ambo Woreda, West shoaZone, Oromia regional state Western part of Ethiopia. The capital city of Ambo Woreda is Ambo town. The study was conducted in five Kebeles around the Ambo town which were known to grow faba bean. These Kebeles are Awaro, Gosu, Feris, Senkele and Wadesa). Gosu is located to words the south, Awaro to the east, Wadesa to north and Senkele and Feris to the west of Ambo town. These areas have an elevation of 2101 meters above sea level and located between latitudes 8054` and 8059` North and longitudes 37048` and 38002` East. Microbiological experiments were done in microbiology laboratory, department of biology, College of natural and computational sciences, Ambo Universityand the nitrogen content of the fababean biomass was done in chemistry laboratory, chemistry department, college of natural and computational sciences, Ambo University.

Isolation of rhizobia

The *Vicia faba* were collected from five study sites of Ambo Woreda. Three health and young plant of *Vicia faba* were uprooted carefully from each fields of faba bean. The nodules were carefully removed by sterilized forceps. The root nodules were surface sterilized with 95% ethyl alcohol and 0.2% acidified mercuric chloride for three minutes according to *Vincent (1970)*. After washing with distilled water for several times to remove the remains of acidified mercury chloride,

the nodules were taken to sterilized petr-dish and crushed carefully with sterilized glass rod. Then, 0.1ml of the suspensions was taken and spreaded on the petri-dish containing Yeast extract mannitol agar. This was incubated for 24-48hrs at 28^0C. Then, single colony was picked and streaked on petri-dish containing Yeast Extract Mannitol Agar again and incubated for 48hrs at 280C in order to check the purity of the isolate. The purified colonies were kept at 40C on YEMA slants. The composition of Yeast Extract MannitolAgar gram/liter: Mannitol 10.00, Dipotassium phosphate 0.50, Magnesium sulphate 0.20,Yeast extract 1.00, Sodium chloride 0.10, Agar 20.00 and Congo red 0.10 *(Vincet, 1970).*

Characterization of Rhizobium isolates

Morphological characterization

The different Rhizobium isolates where morphologically characterized by growing on YEMA plates prepared as described previously. The isolated colony of each isolates were streaked on YEMA plate and incubated at 28 0C for 2-3 days. After the incubation period the different isolates were examined for the growth period, gram reaction, colony color and shape *(Kumari et al., 2010)*.

Gram staining

The gram stain of the different Rhizobium isolate was prepared using the 24-48hrs young culture grown on YEMA plate. The smear was prepared on the clean microscopic slide and air dried and passed through the flame in order to heat fix. Then the heat fixed smear was flooded with crystal violet solution for one minute and the excess stain was washed with the tap water. Then the smear was flooded with crystal iodine solution for one minute. The excess iodine solution was washed with the tap water. And the alcohol is flooded on the smear for 30 seconds and washed with water. Finally the smear was flooded with safaranin and the stained smear was observed under microscope with 100 magnifications with oils and the observation was recorded.

Biochemical characterization

Methyl Red Test

To perform methyl red test, Glucose phosphate broth (GPB) and methyl red indicator were required. The composition of Glucose phosphate

broth Buffered peptone 7.000gm, Dextrose 5.00gm and Dipotassium phosphate 5.00gm. Final pH (at 25°C) 6.9±0.2).Then, these components were dissolved well in 1000 ml of distilled water. The medium were heated to dissolve completely. The medium were distributed in test tubes in 10 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Then, the test culture were inoculated into GPB and incubated at 28^0C-30^0C for 48-72hr. After incubation 5 drops of methyl red indicator were added to the medium.The Isolate that forms red color at the top of medium is positive, while yellow color indicates negative tests.

Citrate Utilization Test

The simmon's citrate agar slant was prepared and the culture was streaked heavily on the surface of the agar slant and incubated for 24-48hrs at 28^0C-30^0C.After incubation 24-48hrs color change of the slants for 21 isolates were observed and recorded.The blue color indicates the positive, while green color indicates negative tests.

Motility Test

Motility Test Medium is recommended for detection of bacterial motility. Composition of culture media that was used to detect the motility of rhizobium, gms/L Tryptose10.000gms, Sodium chloride 5.000gms and Agar 5.000gms. Final pH (at 25°C) 7.2±0.2 was adjusted and the ingredient were dissolved in 1000 ml distilled water. The medium was heated to dissolve completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The tube medium was allowed to cool in an upright position; the medium was inoculated with culture and incubated for 24-48hrs at 28^0C-30^0C.

Urease Test

To perform this test Christensen's Urea Agar was prepared. The composition of urea agar slant in gram per liter of de ionized water: Urea 20.00, Sodium Chloride 5.00, Mono potassium Phosphate 2.00, Peptone 1.00, Dextrose 1.00, Phenol Red 0.12 and agar 15.00. The medium was heated to dissolve completely. Test culture was streaked on the surface of a urea agar slant. The cap was left loosely and the tube was incubated at 28-30^0Cin ambient air for 48hrs to 7 days.

Pot Experiments

The effects of inoculation of rhizobium on growth and nitrogen fixation of Vicia faba were evaluated on soil samples in the pot experiments.

Soil

The soil sample was ground to pass through 2mm sized mesh. Farmyard manure at a rate of 2% was incorporated to maintain the organic matter content of 2% level (Mishra, 2008). Then it was sterilized at 1210C at 15 psi for 1hour and two kilogram of sterilized soil was filled in plastic pots

of three-liter capacity (*Mishra and Mishra, 2008).*

Inoculum preparation

Four isolate were selected to inoculate the plants and three of them were selected for their fast growth (Gosu, Feris and Wadesa isolate) and one isolate (Senkele) was selected for its unique characteristic (positive to urease test).The four-rhizobium isolates were grown on Yeast Extract Mannitol Broth (YEMB) until the population reached 109 cells/ml of culture for five days. Volume of each flask in which isolates grown was half liter.

Treatments

From the 21 isolates of the Rhizobium strains, from of them namely (G, F, S and W) from Gosu, faris, senkele and wodesa were selected respectively. The rhizobium isolates were selected based up on the growth on the Yeast Extract Mannitol Agar. Forty eight sterilized pots were used for four selected

isolates. All pots were sterilizer carefully with 97% alcohol. Each pot was about three liter volume capacity. The treatments were for the four different rhizobium isolates dome in the same manner.

Table 1. Treatment and contents of treatments

Treatment 1	T1	Soil + Seed (Control)
Treatment 2	T2	Soil + Faris I. + seed
Treatment 3	T3	Soil+ Gosu I. +seed
Treatment 4	T4	Soil+ Senkele I.+seed
Treatment 5	T5	Soil + Wadesa I. +seed
Treatment 6	T6	Soil + CF + seed
Treatment 7	T7	Soil + CF + Bacterial I. + seed

The experiment was set in three replications and arranged in a randomized block design in the green houses around the microbiology laboratory.

Seed sterilization, inoculation and sowing

Faba bean seeds which were obtained from local market, were surface sterilized with 1% HgCl2 for 3 minutes and subsequently dipped into 70% alcohol for three minutes and repeatedly washed with sterilized water. Just before sowing, seeds dipped with rhizobium isolate each about 109 cells per Seed. Five seeds per pot were planted and later thinned to three. Plants were watered at fields' capacity with distilled water every other day.

Data collection

Data collection method that used in this research varies based on types of data that required for this study, which include direct counting, measuring, laboratory analysis and weighing.

Data analysis

The data gathered through the instruments repeatedly stated earlier was mathematically organized, interpreted and analyzed qualitatively and quantitatively. In both quantitative and qualitative analysis of the data that was collected through experiment were analyzed by SPSS version 20 in terms mean and SD deviation of descriptive statistics.

Finally, all data obtained from different sources were analyzed to arrive at sound conclusions and recommendations regarding to the practices to the isolation of rhizobium species bacteria from the roots of *Vicia faba* and their effectiveness on growth and total nitrogen content of the plants.

RESULTS AND DISCUSSION

A total of 21 Rhizobium strains were isolated from the faba bean *(Vicia faba)* root nodules collected from the five Kebeles of Ambo Wareda. Of these 3 Rhizobium isolates were obtained from Awaro site,3 Rhozobium isolates were from Farisi site, 9 Rhizobium isolates were from Gosu site,3 Rhizobium were from Senkele site and 3 Rhizobium isolates were from Wodessa site. The number of isolates and the codes of the isolates from the different Kebeles of Ambo Woreda are shown in the Table 2. The results of Rhizobium strain isolation has been reported by different investigators from different localities of the country *(Destaand Angaw, 1987; Aynababa et al., 2001).*

Morphological characteristics the Rhizobium isolates

All the different Rhizobium strains isolated from different study sites were characterized morphologically, except the Farisi and Awaro isolates which showed colony color white, all the other study sites isolates of Rhizobium showed white colony. The growth periods (time) of the different Rhizobium strains isolated from different sites show similar which ranges from 2-5 days. All the Rhizobium stains isolated from different sites were observed to have circular colony shape. The results of gram staining of the different rhizobium isolated from different study sites were the 26 same.

All isolates were gram negative. All the Rhizobium isolated from the different sites has rod shaped cells which arranged in chains. The morphological characteristics of Rhizobium strains recorded in this study are similar with the results reported by *Abere et al., (2009).*

Table 2. Biochemical Characteristics of Rhizobium isolation

S.No.	Isolates	Methyl red test	Motility test	Urease test	Citrate Utilization Test
1	A1.1	_	+	_	_
2	A1.2	_	+	_	_
3	A1.3	_	+	_	_
4	F1.1	_	+	_	_
5	F1.2	_	+	_	_
6	F1.3	_	+	_	_
7	G1.1	_	+	_	_
8	G1.2	_	+	_	_
9	G1.3	+	+	_	+
10	G2.1	_	+	_	+
11	G2.2	_	+	_	_
12	G2.3	_	+	_	_
13	G3.1	_	+	_	_
14	G3.2	_	+	_	_
15	G3.3	_	+	_	_
16	S1.1	+	+	+	_
17	S1.2	_	+	_	_
18	S1.3	_	+	_	+
19	W1.1	_	+	_	_
20	W1.2	_	+	_	_
21	W1.3	_	+	_	+

Effects of Rhizobium isolate inoculation on growth and N-nutrition of faba bean

In order to check the ability of the different Rhizobium isolated from different localities to form nodule and nitrogen fixation ability four different Rhizobium strains from the 21 isolates were selected for pot experiment trials. These isolates were designed as F1.1, G3.1, S1.1 and W1.2.

On order to determine the effect of the Rhozobium isolated from Farisi, Gosu, Senkele and Wadessa site on the height of faba bean seven treatments were carried out: Treatment 1 (T1) sterilized soil only, Treatment 2 (T2) sterilized soil inoculated with Farisi Rhizobium isolate, Treatment 3 (T3) sterilized soil inoculated with Gosu Rhizobium isolate, Treatment 4 (T4) sterilized soil inoculated with Senkele Rhizobium isolate, Treatment 5 (T5) sterilized soil inoculated with Wadessa Rhizobium isolate, Treatment 6 (T6) sterilized soil treated with nitrogen fertilizer and Treatment 7 (T7) sterilized soil inoculated with four Rhizobium isolate (Faris, Gosu,Senkele and Wadessa) and Nitrogen fertilizer. The effects of the different treatment on faba bean height are shown in table 3. Inoculation of the Rhizobium isolates significantly affected the faba bean hight as compared to un incoculated soil. There is no significant variation between T2, T3 T4, T5 and T6, which means plants that were inoculated with rhizobium isolate and treated with nitrogen fertilizer were almost the same (P~0.05). Rhizobium isolates (Faris, Gosu, Senkele and Wadessa) inculation together with that of the chemical nitrogen fertilizer greatly improved the faba bean height. And from four isolates slightly Senkele isolate was effective than other three isolates Faris,Gosu and Wadessa).

Effect of inoculation of Rhizobium isolates on faba bean shoot dry weight

In order to determine the effect of the Rhizobium isolated from four sites (i.e faris, Gosu, Senkele and Wadesa) on shoot dry weight of faba bean seven (7) treatments were carried out: Treatment 1 (T1) uninoculated sterilized soil, Treatment 2 (T2) sterilized soil inoculated with Faris Rhizobium

isolate, Treatment 3 (T3) sterilized soil inoculated with Gosu Rhizobium isolate, Treatment 4 (T4) sterilized soil inoculated with Senkele Rhizobium isolate, Treatment 5 (T) sterilized soil inoculated with Wadesa Rhizobium isolate, Treatment 6 (T6) sterilized soil treated with nitrogen fertilizer and Treatment 7 (T7) sterilized soil inoculated with four isolates (Faris, Gosu, Senkele and Wadessa) and nitrogen fertilizer. Inoculation of the Four isolates (Faris, Gosu, Senkele and Wadesa) significantly affected the shot dry weight of faba bean. There is only slight difference was observed between T2 (2.39gm), T3 (2.4gm), T4 (2.49gm), T5 (2.4gm) and T6 (2.44gm) on the faba bean shoot dry. Rhizobium isolates (Faris, Gosu, Senkele and Wadesa) inoculation together with nitrogen fertilization significantly improved the faba bean shoot dry mass as compared to the rest of the treatment (3.91gm).

Effect of inoculation of Rhizobium isolates on the root dry weight of faba bean

In order to determine the effect of the Rhizobium isolated from four sites (i.e faris, Gosu, Senkele and Wadesa) on root dry weight of faba bean seven (7) treatments were carried out: Treatment 1 (T1) uninoculated sterilized soil, Treatment 2 (T2) sterilized soil inoculated with Faris Rhizobium isolate, Treatment 3 (T3) sterilized soil inoculated with Gosu Rhizobium isolate, Treatment 4 (T4) sterilized soil inoculated with Senkele Rhizobium isolate, Treatment 5 (T) sterilized soil inoculated with Wadesa Rhizobium isolate, Treatment 6 (T6) sterilized soil treated with nitrogen fertilizer and Treatment 7 (T7) sterilized soil inoculated with four isolates (Faris, Gosu, Senkele and Wadessa) and nitrogen fertilizer. Treatment with nitrogen fertilizer significantly affected the root dry weight of faba bean. There is great difference was observed between T6 and all other treatments (T1,T2, T3, T4, T5 and T7). T6 was greater in root dry weight than all treatments. T2 (1.79gm), T3(1.81gm), T4 (1.79gm) and T5 (1.81gm) almost have the same root dry weights which were all inoculated with different Rhizobium isolates. T1(1.69gm) was the least in root dry weight. And T7 (2.00) was average in root dry weight.

Percent of nitrogen and total protein

The effects of the different treatments on the percent of nitrogen and total protein of faba bean are shown in table 3. Inoculation of the Rhizobium isolates (faris, Gosu, Senkele and Wadesa) significantly affected the percent of nitrogen and total protein content of the faba bean. The sterilized soil which is not inoculated with Rhizobium isolate but fertilized with nitrogen (T6) gave the least percent of nitrogen and total protein content as compared to the rest of the treatments (T6) 2.22%. The Rhizobium inoculated (T2,T3, T4 and T5) gave the highest percent of nitrogen and total

Protein content as Compared the other treatments (3.54%, 3.52%, 3.75% and 3.49%) respectively. The rest of the treatments gave values between the lowest and the highest for percent of nitrogen and total protein.

Table 3.Height, Nodule number, Nodule weight, Dry weight of shoot and root, Percentage of Nitrogen and Protein contents of plants.

S. No.	Treatments	Height (in cm)	Nodule number and weight (in gm)		Dry weight of Shoot and Root (in gm)		Percentage of Nitrogen and Proteins (in %)	
			Number	Weight	Shoot	Root	Nitrogen	Protein
1	T1	45.30 ± 0.15	-	-	1.78	1.69	2.65	16.56
2	T2	48.56 ± 0.23	71.11 ± 4	0.61± 0.08	2.39	1.79	3.54	22.13
3	T3	48.10 ± 0.40	51.11± 10	0.49± 0.01	2.4	1.81	3.52	22.00
4	T4	48.70 ± 0.34	45.66± 6	0.44± 0.05	2.49	1.79	3.75	23.44
5	T5	48.50 ± 0.32	64.44±12	0.56± 0.10	2.4	1.81	3.49	21.81
6	T6	49.67 ± 0.13	-	-	2.44	2.91	2.22	13.87
7	T7	52.80 ±0.41	56.00± 8	0.58± 0.02	3.91	2.00	2.90	18.13

Discussion

Biological nitrogen is used traditional with chemical nitrogen fertilizer by Ethiopian farmers. In Ethiopia number of researchers conducts study on isolation, characterization, and effects of Rhiozbium isolates on growth and nitrogen nutrition of faba bean (*Vicia faba) (Destaand Angaw, 1987; Aynababa et al., 2001)*. In the case of Isolation and characterize the isolates of Rhizobia, result of this study and other Ethiopian researchers were almost the same *(Aynababa et al., 2001).* The effects of the rhozobia isolates on the growth were very amazing in this study which show that inoculated plants with rhizobium isolates and treated with nitrogen fertilizer almost the same in their dry weight of shoot and height. Uninoculated one is less in their dry weight of shoot and height from the rest. A plant which was inoculated with rhizobium isolates and treated with nitrogen fertilizer was higher in their dry weight of shoot and height. This is confirms the study of other Ethiopian researchers, but there is in this study Plants which is inoculated with rhizobium isolates and plants which were treated with nitrogen fertilizer was higher than that of other Ethiopian researchers in their dry weight of shoot and height *Wondwosen et al., (2016).* Again the height of plants of inoculated with rhizobium isolates and plants which were treated with nitrogen fertilizer were almost the same in this study. This result almost the same with other Ethiopian researchers. In this study the percentage of nitrogen and protein of plants that inoculated with the rhizobium isolates were higher than all of the treatments which include Plants that do not inoculatesd and not treated with nitrogen fertilizer (T1), plants that are inoculated with rhizobium isolates and treated with nitrogen fertilizer (T7), which confirms the study of *Assefa et al.,(2016).*

CONCLUSION

Rhizobium species of bacteria can isolated easily from the root nodules of the *vicia faba* and characterized morphologically and biochemically. From most of their characteristics rather than motility and gram-reaction, there were unique species of rhizobium isolates that shows different from the most of rhizobium species of bacteria. Rhizobium isolate of bacteria supports the growth of plants, increase height, dry weight of shoot, nitrogen contents, improve total nitrogen contents, increase protein contents and increase fertility of soil. Rhizobium species of bacteria are slightly different in nitrogen fixing ability.

Some of strains of rhizobium were effective than the others. Treatments that inoculated with bacteria and treatments that treated with chemical fertilizer were almost the same in above treats. But in total nitrogen and protein contents of plants, inoculated plants with rhizobium isolate contains high level of nitrogen and protein than any plants in treatments. Having high total nitrogen contents of inoculated plants have number of advantages in society. From number of advantages some were free from pollution, not expensive and advisable for health of society for food.

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