The Effect of Isolated *Rhizobium* Spp. on Germination and Nodulation Activities of *Vigna Radiata* (L.) R. Wilczek Plant

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Abstract

The present study deals with three *Rhizobium* strains (Rhizo-1, 2 and 3) which were isolated from root nodules of *Clitorea ternatea* L. (Aung-me-nyo), *Tephrosia purpurea* (L.) Pers. (Me-yaing) and *Cullen corylifolium* (L.) Medik. (Ne-hle) of Fabaceae family. The growth responses of *Vigna radiata* (L.) R. Wilczek (green gram) inoculation with indigenous isolated strains were studied. The experiments were carried out with 3 isolated strains to know the germination percentage, seedling length and vigour index. The inoculation of isolated strains increased in germination up to 5.16%, 12.82% in seedling length and 27.74% in vigour index over the control by isolated strain Rhizo-3. In Leonard Jar experiment, among the isolated strains, Rhizo-3 showed the most significant than among the other strains and control in plant height (5.44%), leaf area (14.43%), nodule number (4.22%), fresh weight (24.39%) and dry weight (19.44%) over the control. These results indicated that certain *Rhizobium* spp. could promote germination percentage, seedling length and vigour index, plant height, leaf area, nodule number, fresh and dry weight of *Vigna radiata*. Therefore, this experiment concerning the effects of *Vigna radiata* by isolated strains must be followed by investigation of yield productivity in natural condition.

Key words: *Rhizobium*, inoculation, vigour index

Introduction

Soil contains many types of microorganisms such as bacteria, actinomycetes, fungi, and algae, which are important because they affect the physical, chemical, and biological properties of soil. Amongst the soil bacteria, there is a unique group called Rhizobia that has a beneficial effect on the growth of plants. The bacteria colonize within root nodules, where converts atmospheric nitrogen to ammonia and provides organic nitrogenous compounds to the plants (Oblisami, 2005).

Increasing production in the modern agriculture is extensively dependent upon the optimum use of chemical fertilizers and other inputs, given that using these types of fertilizers triggers even more salinity in addition to high charges, low efficiency, and high risk of environmental pollution (Piranooshe *et al*., 2011).

To overcome these problems, Integrated Nutrient Management (INM) would be the best alternate, which includes judicious use of organic, inorganic and biological (BNF) sources of nutrients so as to sustain optimum yields, to improve or maintain the soil chemical, physical and biological properties and to provide crop nutrition packages which are technologically sound, economically attractive and environmentally safe (Dhar, 2010).

The aims and objective of the present study were to isolate *rhizobium* strains from root nodule of indigenous root nodules, to investigate the growth characters and biochemical characters of isolated strains and to know the effects of germination and host specificity of isolated strains on *Vigna radiata* (green gram).

Materials and Methods

Plants and Root nodules of *Clitorea ternatea* L. (Aung-me-nyo), *Tephrosia purpurea* (L.) Pers. (Me-yaing) and *Cullen corylifolium* (L.) Medik. (Ne-hle) were collected from Monywa University campus, Monywa Township in Sagaing District and identified during 2019.

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Isolation of *Rhizobium* Strains from Root Nodules (Vincent, 1970)

Rhizobium strains were cultured in following Yeast extract Mannnitol Agar (YMA) culture medium (K_2HPO_4 , 0.5g; $MgSO_4$.$7H_2O$, 0.1g; NaCl, 0.2g; Yeast extract, 0.5g; Manitol, 10g; Agar, 17g; pH, 7; DW, 1L). The colony morphology of isolates was examined on YMA agar plates. After an incubation of 2-3 days at room temperature, individual colonies were characterized based on their color, shape, transparency, borders, elevation and biochemical tests.

Seedling germination test

The seeds were surface sterilized by immersion in hydrogen peroxide (H_2O_2) for 30s. The seeds were rinsed for three times with distilled water. Sterilized seeds were placed into the bacterial culture medium for 1 hour and controls are inoculated with only nutrient medium. And then the seeds were placed in sterilized petriplate were containing moist cotton wool and germinated in dark at 30 °C for 120 hours.

$$\text{Germination \%} = \frac{\text{Number of emerged seedling}}{\text{Number of seed sown}} \times 100$$

Vigour Index = Standard germination (%) × Average seedling length (cm)

Leonard Jar Method for Host Specificity

The test crop seeds were surface sterilized in 3% sodium hypochlorite solution for 3 minutes. The seeds were placed in a petridish containing moist cotton wool for pre-germination. Five healthy seedlings were planted in every Leonard jar and then 10 ml of *Rhiozobium* bacterial suspension were added to each of the jar. After 7 days after sowing, the seedlings were thinned to 3 plants per jar. Plants were allowed to grow in natural condition for 45 days of experimental period.

Results

Morphological characters of the isolated *Rhizobium* spp.

The *Rhizobium* colonies were round, convex semi-translucent, raised and having sticky appearance showing the production of mucous.Typical colonies of the isolated strains had a diameter of 2 - 5 mm. The size of the isolates varied within the range of 0.5 - 1.0 in diameter and 1.5 - 2.5 µm long (Figure 1).

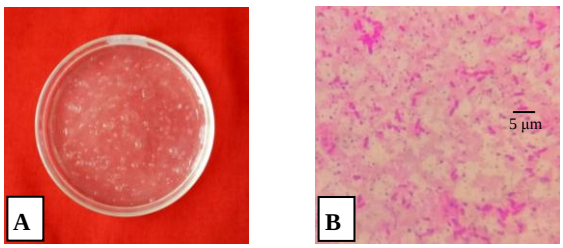


Figure 1. Isolated *Rhizobium* spp.
A. Spread on YMA medium **B**. Photomicrograph of *Rhizobium* spp.

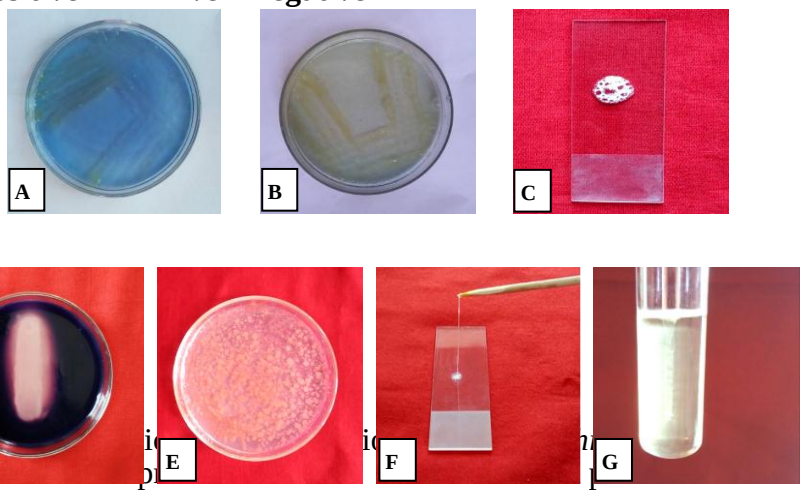
Biochemical Tests for Isolated Bacteria

Biochemical tests of the isolated *Rhizobium* spp. were found positive tests for alkaline or acid producer, starch hydrolysis, catalase, potassium hydroxide solubility, ability to absorb Congo red and motility test as shown in Table 1 and Figure 2.

Table 1. Biochemical characterization of isolated *Rhizobium* spp.

No.	Biochemical tests	Isolated *Rhizobium* spp		
		Rhizo - 1	Rhizo - 2	Rhizo - 3
1.	Acid production	-ve	+ve	-ve
2.	Alkaline production	+ve	-ve	+ve
3.	Gram staining	-ve rod	- ve rod	- ve rod
4.	Starch hydrolysis	+ve	+ve	+ve
5.	Catalase	+ve	+ve	+ve
6.	Potassium hydroxide solubility	+ve	+ve	+ve
7.	Congo red absorption	+ve	+ve	+ve
8.	Motility	Motile	Motile	Motile

+ve = Positive **-ve = negative**



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C. Catalase test D. Starch hydrolysis test
E. Congo red absorption test F. Potassium hydrolysis test
G. Motility test

The results of the tests has indicated that among the isolated strains, Rhizo-3 was found to be the most effective in germination percentage, seedling length and vigour index as shown in Table 2 and Figure 3.

Table 2. Effects of inoculation on germination %, root and shoot length

Treatment	Germination %	Seedling length (cm)	Vigour index
Control	92	9.34	859.28
Rhizo -1	95	11.81	1121.95
Rhizo -2	94	10.15	954.10
Rhizo -3	97	12.26	1189.22

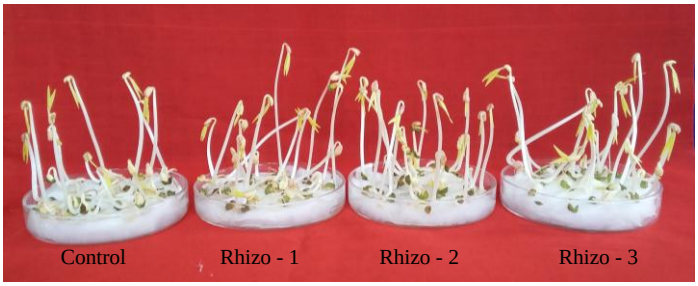

Figure 3. Effect of *Rhizobium* spp. on Germination of *Vigna radiata*

Leonard Jar Experiment

The inoculations of isolated strains were significant in plant height, leaf area, nodule number, fresh weight and dry weight of plant when compared to control as shown in Table 3 and Figure 4.

Table 3. Effects of isolated strains and control on *Vigna radiata* after 45 days

Treatment	Plant Height (cm)	Leaf Area (cm^2)	Nodule Number	Fresh Weight (g)	Dry Weight (g)
	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd
Control	11.29± 0.67	8.30 ± 1.77	0.00 ± 0.00	1.24± 0.39	0.29 ± 0.09
Rhizo- 1	11.44± 0.77	9.10 ± 1.45	2.24 ± 1.88	1.45± 0.57	0.33 ± 0.14
Rhizo- 2	11.39± 0.70	8.70 ± 1.42	1.33 ± 1.23	1.37± 0.36	0.31 ± 0.11
Rhizo - 3	11.94± 0.68	9.70 ± 1.34	4.22 ± 2.95	1.64± 0.46	0.36 ± 0.09

Figure 4. Effect of isolated strains and control on plant height of *Vigna radiata* after 45 days

Effect of isolated *Rhizobium* strains on plant height

In the present study, the mean of plant height ranged from 11.29 to 11.94 cm at 45 days after sowing. The highest length was found in treated plant with Rhizo -3 (11.94 cm) where as the lowest length was observed in control (11.29 cm).

Effect of isolated *Rhizobium* strains on leaf area

In the present study, after 45 days, the result observed that the leaf area arranged from 8.70 – 9.70 cm^2 in the treatment while 8.30 cm^2 in the control.

Effect of isolated *Rhizobium* strains on nodule number

In this study, after 45 days, nodule number arranged from 2.24 – 4.22 while 0.00 in the control (Table 3).

Effect of isolated *Rhizobium* strains on dry weight

In the present study, the result indicated that the dry weight of plant ranged from 0.31 – 0.36 g with inoculation while the control has 0.29 g.

Effect of isolated *Rhizobium* strains on fresh weight

The mean of fresh weight was ranged from 1.37 - 1.64 g in the treatment and 1.24 g in the control. The highest nodule number was found in treated plant with Rhizo -3 (1.64g) but the lowest length was observed in control (1.24 g).

Discussion and Conclusion

In the present study, the *Rhizobia* were isolated from the root nodules of *Clitorea ternatea* L. (Aung-me-nyo), *Tephrosia purpurea* (L.) Pers. (Me-yaing) and *Cullen corylifolium* (L.) Medik. (Ne-hle). These isolates were designated as rhizobia on the basis of their colony characteristics, cell morphology, and inability to absorb Congo red dye. All the isolates were Gram-negative and had rod-shaped cells. The inability of the isolates to absorb Congo red dye is a distinctive character of rhizobia (Somasegaran and Hoben, 1994).

Sanzida *et al*. (2008) stated that inoculation effect of *Azospirillum* spp. on growth of wheat at 30 days was up to 43.24% over the control in germination percentage. Similarly, Hnin Ei Phyu (2013) reported that inoculation of *Azospirillum* strains on germination increased up to 12.12% in wheat over the control. Htar Htar (2017) stated that inoculation of endophytic bacteria strains on germination increased up to 20.0% in rice over the control.

In this study, the inoculations of isolated *rhizobium* strains (Rhizo-1, 2 and 3) were 95%, 94% and 97% while the control had 92% in germination percentage, 11.81 cm, 10.15 cm and 12.26 cm and control has 9.34 cm in seedling length and 1121.95, 954.10 and 1189.22 and 859.28 in control in vigour index respectively.

Ravikumar (2012) showed significant increased in plant height up to 48.89% in *Vigna Mungo* and 40.00% in *Vigna radiata* over the uninoculated control. Similarly, Rajpoot and Panwar (2013) stated that inoculation effect of *Rhizobium* spp. on growth of *Vigna radiata* at 30 days up to 29.67% over the control in shoot length. In this result, the plant height was observed that 5.44 % over the control was given by strain Rhizo-3.

Ahmed *et al*., (2006) stated that inoculation effect of *Rhizobium* spp. on growth of *Vigna radiata* was up to 23.36% over the control in number of nodule at maturity. Rajeswari *et al*., (2017) who had reported 18.00% in number of nodule per plant of *Vigna unguiculata* was due to *rhizobium* inoculation over uninoculated control. In this study, the highest number in plants inoculated with Rhizo -3 is 4.22% over the control.

Ravikumar (2012) who had reported there were 47.27% increase in fresh weight per plant of *Vigna mungo* and 32.50 % in *Vigna radiata* with the Rhizobium inoculation. Soe Myint Aye *et al*., (2014) stated that inoculation effect of *Rhizobium* spp. on fresh and dry weight of *Vigna unguiculata* was up to 45.24% and 34.29% over the control.

In this study, among the isolated strains, Rhizo-3 show the most significant among the other strains and control in germination percentage (5.16%), seedling length (12.82%), vigour index (27.74%), plant height (5.44%) leaf area (14.33%), nodule number (4.22%), fresh weight (24.39%) and dry weight (19.44%) over the control. Therefore, this experiment concerning the effects of green gram by isolated strains must be followed by investigation of yield productivity in natural condition.

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