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Effects of Root Nodules Rhizobia on Growth of *Vigna unguiculata* subsp. *sesquipedalis* (L.)Verdc.

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Abstract

The effect of rhizobial strains on *Vigna ungiuculata* subsp. *sesequipedalis* (L.) Verdc. have been performed. Four rhizobia strains, MYM-1 from *V. trilobata* (L.) Verdc., MYM-2 from *V. unguiculata* subsp. *sesquipedalis* (L.) Verdc., MYM-3 from *Crotalaria pallida* Ait. and MYM-4 from *Clitoria ternatea* L., were isolated respectively. The host specificity test on *V. unguiculata* subsp. *sesquipedalis* (L.) Verdc. was carried out in the growth chamber for one month. Among the four strains, MYM-2 was found to be most effective in the nodule formation. In pot culture experiment, the germinated seeds of *V. unguiculata* subsp. *sesquipedalis* (L.) Verdc. inoculated with four isolated rhizobia strains were grown from the months of August to October in 2012. In this experiment also, *Bradyrhizobium* strain, MYM-2 showed the most highly significant effects on the fresh and dry weight of plant, nodules and pods and the pod length.

Key words: *Vigna unguiqulata* subsp. *sesquipedalis*, Rhizobia, growth

Introduction

Biological nitrogen fixation by the legume-*Rhizobium* system is the main natural source of nitrogen in agricultural systems. Biological nitrogen fixation (BNF) is an effective alternative natural source of nitrogen made available to the soil (Predeepa & Ravindran 2012).

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Rhizobium is the most well known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of lumps of or nodules where the nitrogen fixation takes place. The bacterium's enzyme system supplies a constant source of reduced nitrogen to the host plant and the plant furnishes nutrients and energy for the activities of the bacterium. About 90% of legumes can become nodulated (Anonymous 2002).

Most *Rhizobium* species nodulate only one or a few genera of legumes. On the other hand, *Bradyrhizobium* species tend to nodulate diverse legumes including a number of the agriculturally most important genera *Arachis, Glycine*, *Vigna* and so forth (Lewin *et al*. 1990).

Legume nitrogen fixation starts with the formation of nodule. A common soil bacterium, *Rhizobium*, invades the root and multiplies within in the cortex cells. The plant supplies all the necessary nutrients and energy for the bacteria. In the field, small nodules are visible 2-3 weeks after planting, depending on legume species and germination conditions (Lindemann & Glover 2003).

Black (1968) suggested that higher grain yield in food legumes inoculated with *Rhizobium* was due to an increase in nodulation.

Biological Nitrogen Fixation (BNF) has been used in farming systems to cut down on fertilizer expenses (Mwangi *et al*. 1994 as cited in Otieno *et al*. 2009). Inoculation with an effective and persistent rhizobium strain has numerous advantages, which include non-repeated application of nitrogen fertilizers and higher pod yield due to increased nodulation (Sanginga *et al*. 1994 as cited in Otieno *et al*. 2009). It has been reported that rates of N_2 fixation of 1 to 2 kg N ha^{-1} growing season day^{-1} is possible in most legumes in tropical cropping systems (Giller 2001 as cited in Otieno *et al*. 2009).

Vigna unguiculata subsp. *sesquipedalis* (L.) Verdc. is a cultivated legume which can be eaten as green pods. It is known as the yardlong bean, bora, long-podded cowpea, asparagus bean, snake bean, or chinese long bean. The crisp, tender pods are eaten both fresh and cooked. They are at their best when young and slender. They are sometimes cut into short sections for cooking uses. As a West Indian dish it is often stir-fried with potatoes and shrimp. Another popular and healthful option is to chop them into very short sections and fry them in an omelette (Anomynous 2012).

The yardlong bean pods are widely cultivated in most of the region of Myanmar, especially in the rainy season. The farmers get some income by cultivation of this crop and the young pods are very common vegetables in Myanmar traditional foods. The nutritionally and healthy young pods can be produced, that is commercially demanded and free of undesirable chemicals by using biofertilizer of rhizobia, it will be very practically applicable research for the country.

Therefore the aim and objectives of the present research is forecasting to study the nature of root nodules and its rhizobia strains that infected in some leguminous species, to investigate the cultural characteristics of infected rhizobia strains on the culture media, to specify the host specificity of studied strains on the *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc., to know the effect of selected strains on cultivation of commercially important crop, yardlong bean and to share the knowledge of the root nodule bacteria in legume reduces the costly plant chemical fertilizers by their nitrogen fixation process and that plant can use for growth.

Materials and Methods

The nodule samples were collected from *Vigna trilobata* (L.)Verdc., *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc., *Crotalaria pallida* Ait. and *Clitoria ternatea* L.

In the isolation of rhizobia strains, Yeast Mannitol Broth (YMB) and Yeast Mannitol Agar (YMA) were used as the basal culture media according to Vincent (1970). N- free nutrient solution for plant experiment of Leonard Jar methods were used sterilized N-free nutrient solution (Broughton and Dilworth 1970 as cited in Somasegaram and Hoben 1994). Isolation of Rhizobia strains and Bacterial Culture followed to Vincent (1970). Isolated bacteria strains were labeled as MYM 1, MYM 2, MYM 3 and MYM 4 depending on sourced plants of *Vigna trilobata* (L.)Verdc., *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc., *Crotalaria pallida* Ait. and *Clitoria ternatea* L. respectively (Figure 1, 2, 3 & 4).

The germinated seeds were inoculated with rhizobia strains and then inoculated germinated seeds were used for testing host specificity and

growing in pots. Germinating seeds were used to record the rhizobia effect on crop for host specificity and pot culture experiment. Leonard Jar Method of Somasegaram and Hoben (1994) was used for test of host specificity.

Soil samples were collected from the cultivated field of leguminous crop from Shangalay Kyun, Mandalay Region. Seeds of *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc. for all experiments were received from Myanmar Agriculture Service, Mandalay Region. The nodulation assays and plant growth promoting were prepared in pots, which is 30 cm in diameter and 35 cm high filled with 9 Kg of soil. After sowing, the pots were placed into the green house in order to receive sufficient light and water. No fertilizers were added to the plants. At 70 days (the completion of growth phase), the plants were harvested and yields characteristics like number and length of pods, fresh weight and dry weight of plant, and root nodules were recorded.

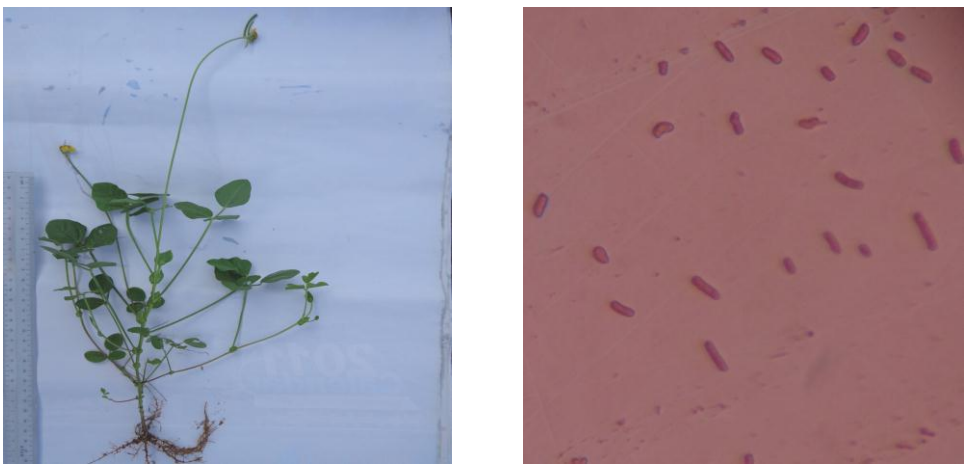


Figure 1 Host plant of *Vigna trilobata* and its infected rhizobial strain (MYM 1)

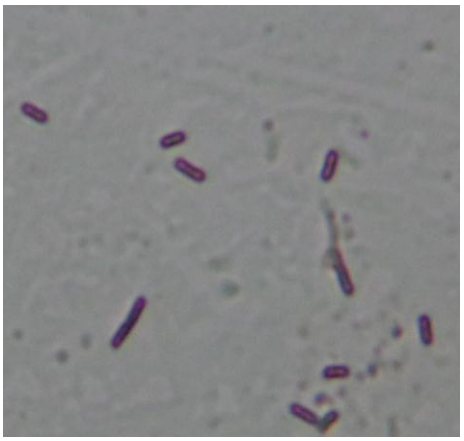
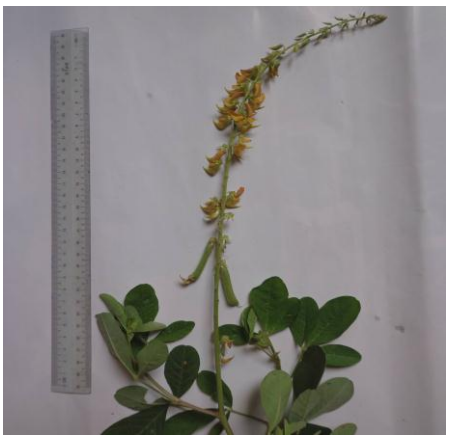


Figure 2 Host plant of *Vigna unguiculata* and its infected rhizobial strain (MYM 2)



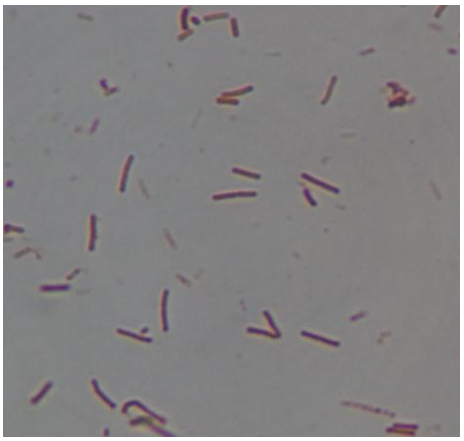


Figure 3 Host plant of *Crotalaria pallida* and its infected rhizobial strain (MYM 3)

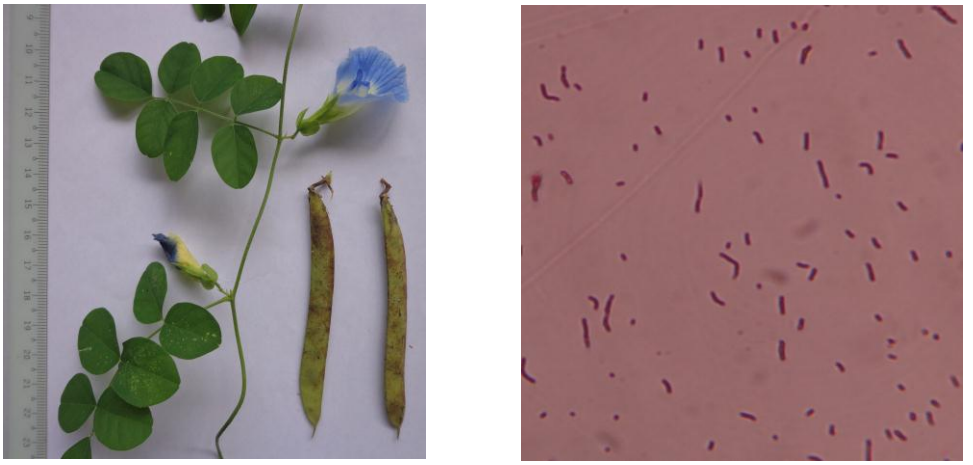


Figure 4 Host plant of *Clitoria ternatea* and its infected rhizobial strain (MYM 4)

Results

Host Specificity

To specify the host specificity of the rhizobia strains the nodule number, fresh weight and dry weight of the seedlings were studied (Figure 5)

In the experiment the nodule number in MYM-2 was higher than the other strains. The average number was found as 4.17 in MYM-1, as 2.9 in MYM-3, as 2.8 in MYM-4. In control there was no formation of nodule.

The fresh weight of the plant was heavier by using the MYM-2 strain with 3.36 g than that grown by other strains. The control was found as 1.84 g, MYM-1 was found as 3.28 g, MYM-3 was found as 3.25 g, and MYM-4 was found as 3.20 g respectively.

The dry weight of the plant also showed by using the MYM-2 with 0.35 g. They were found as 0.23 g in control, 0.34 g in MYM-1, 0.32 g in MYM-3, and 0.29 g in MYM-4.

All the resulting data for host specificity was shown in Table 1.

Figure 5. Experiment of host specificity in Light Control Growth Chamber

A. Seedling at 7DAS B. Seedling at 14DAS

C. Seedling at 21DAS D. Seedling at 28DAS

Table 1 The effect of Rhizobia strains on *V. unguiculata* subsp. *sesquipedalis* (L.) Verdc.

Strain No	Nodule number	Fresh weight	Dry weight
Control	0	1.84 ± 0.30	0.23 ± 0.15
MYM-1	4.17 ± 1.50	3.28 ± 0.25	0.34 ± 0.15
MYM-2	5.17 ± 1.91	3.36 ± 0.29	0.35 ± 0.10
MYM-3	2.9 ± 0.96	3.25 ± 0.42	0.32 ± 0.10
MYM-4	2.8 ± 1.89	3.20 ± 0.17	0.29 ± 0.17

Effect of Rhizobia on Crop Growth

The rhizobia strains MYM-1, MYM-2, MYM-3 and MYM-4 were used as biofertilizer in yardlong bean cultivation (Figure 7)

The results reveal that MYM-2 showed the highest average fresh weight with 15.66 g of plant while the other strain MYM-1 with 15.4 g, MYM-3 with 15.39 g, MYM-4 with 15.22 g, and 15.15 g in control without strains.

In the average dry weight of cultivated plant, MYM-1 and MYM-2 strains showed the significant effect with 2.62 g. MYM-3 strains showed 2.12 g and MYM-4 strain showed 2.09 g, and control showed 1.99 g.

Although the fresh weight of the pods were heavier than the others by using MYM-2 with 8.76 g, 8.18 g was found in control, 8.73 g was found in MYM-1, 8.72 g was found in MYM-3, and 8.39 g was found in MYM-4 .

The dry weight of the pod was found to be 1.50 g by using the rhizobia strain MYM-1 and MYM-2 while 1.11 g in control, 1.32 g in MYM-3 and 1.29 g in MYM-4 .

The fresh weight of the nodule showed the significant effect with the strain of MYM-2 with 4.22 g while 4.19 g in the strain of MYM-1, 4.12 g in MYM-3, 4.12 g in MYM-4 and 4.09 g in control.

The average dry weight of the nodule was 1.89 g in control, 2.01 g in MYM-1, 2.05 g in MYM-2, 1.99 g in MYM-3 and MYM-4 (Table 4.8).

The pod length showed the significant effect with the strain of MYM-2 with 14.55 cm while 14.16 cm in MYM-1, 13.88 cm in MYM-3, 13.72 cm in MYM-4 and control showed 12.13 cm in pod length.

The resulting data on plants, pods, nodules were shown in Table 2.

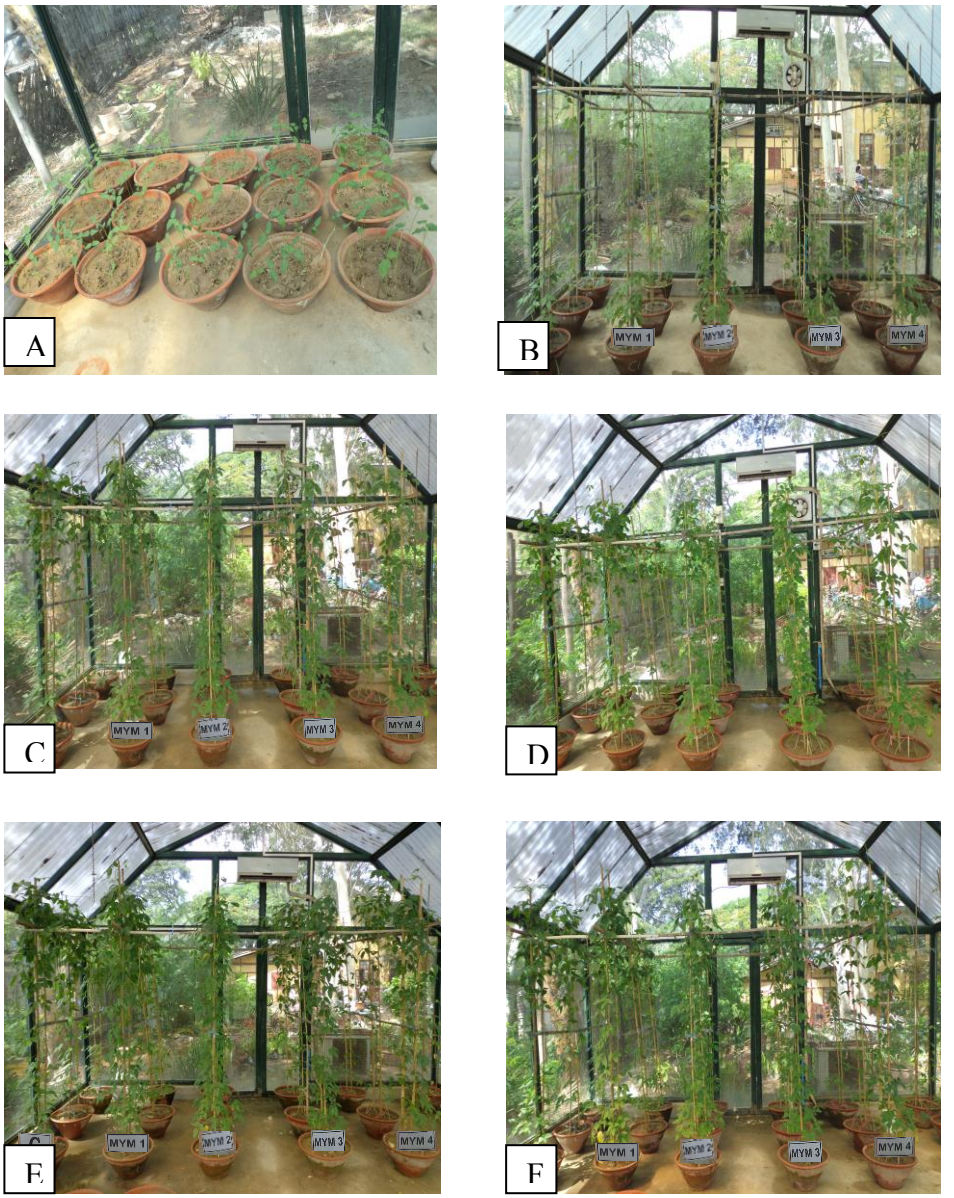


Figure 7 Cultivation of *Vigna unguiculata* subsp *sequipedalis* (L.)

A. Young plants at 7 DAS B. Plants at 21 DAS C. Plants at 35 DAS

D. Plants at 49 DAS E. Plants at 63 DAS F. Plants at 70 DAS

Table 2 Effect of Rhizobia strains on *V. unguiculata* subsp. *sesquipedalis* (L.) Verdc.

Item	Control	MYM-1	MYM-2	MYM-3	MYM-4
Plant fresh weight	15.15±0.17	15.40±0.18	15.66±0.19	15.39±0.16	15.22±0.16
Plant dry weight	1.99±0.13	2.62±0.12	2.62±0.13	2.12±0.12	2.09±0.10
Pod fresh weight	8.18±0.14	8.73±0.18	8.76±0.13	8.72±0.15	8.39±0.13
Pod dry weight	1.11±0.10	1.50±0.10	1.50±0.13	1.32±0.10	1.29±0.13
Nodule fresh weight	4.09±0.08	4.19±0.08	4.22±0.12	4.12±0.12	4.12±0.09
Nodule dry weight	1.89±0.14	2.01±0.15	2.05±0.14	1.99±0.14	1.99±0.11
Pod length	12.13 ±2.18	14.16±2.29	14.55±3.05	13.88±0.64	13.72±2.77

Discussion and Conclusion

The present study deals with the effect of rhizobial biofertilizer on cultivated crop *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc. (yardlong bean). The various rhizobial species, MYM-1, MYM-2, MYM-3 and MYM-4, were isolated from *Vigna trilobata* (L.) Verdc., *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc., *Crotalaria pallida* Ait and *Clitoria ternatea* L. respectively.

Colonies of rhizobia were obtained on YMA agar medium after incubation at 30°C for 3-5 days. The colonies were seemed to be sticky appearance showing that the production of mucous. The morphology of colony indicated that rounded colonies, white colored until 2-4 days of growth and turning yellow in color after 4 days.

Somasegaran and Hoben (1994) stated that rhizobia have specified time for growth, 3 to 5 days for fast growers *Rhizobium* and 7 to 10 days for slow growers *Bradyrhizobium*. In the test of *Bradyrhizobium* strains, the resulting strains of MYM-1, MYM-2 and MYM-3. growed in yeast mannitol agar after 5 days. Lewin *et al*. (1990) also reported that the strains

isolated from the nodule of genus *Vigna* are under *Bradyrhizobium*. Therefore the three strains are the strains under the genus *Bradyrhizobium*. MYM-4 strains are fast-growing root nodule bacteria, medium-sized, rod shaped cells, gram-negative. Therefore this strain of bacteria is under the genus *Rhizobium*.

In the experiment of host specificity, sterilized soil and nitrogen free nutrient solution were used to test the nodule formation of the leguminous plants. It was found that the number of nodules, the fresh weight and dry weight of the plants are the highest in plants inoculated with MYM-2 strains. The other strains (MYM-1, MYM-3 and MYM-4) also showed the better effect compared to control. It was found that there was no nodule formation in the control plants. Therefore, it can be concluded that the inoculation of *Rhizobium* strain to the plants induce the nodulation of the leguminous plant in the nitrogen free nutrient solution. It was observed that the MYM-2 strain (*Bradyrhizobium* strain) from *Vigna unguiculata* sbusp. *sesquipedalis* (L.) Verdc. possess higher host specificity than the other strains.

Although Weaver (1974) postulated that the presence of nodules on roots does not mean on fixation of nitrogen for good growth of host plant, in the present study, it was found that the rhizobia strains can promote nodules formation and also in promoting the plant growth, these characters have in agreement with Ravikumar (2012) on ground nut, Zahran (2001) in several legumes, Warge (1989) in *Acacia*, peanut and cowpea, Mahmood and Athar (2007) in *Leucena leucocephala*.

Khachani (1981) and Million (1989) as cited in Otieno *et al*. (2007) reported that an increase pod yield was due to inoculation of French bean. Mahmood (1992) also noticed that the isolated rhizobia strains of *Albizia lebbeck*, *Pithecellobium dulce* and *Vigna unguiculata* were most effective in nitrogen fixation and the to significant increase of dry weight and nitrogen content of the host plant were found. In the present study, also the inoculation of rhizobial strains were found to be better effect than the control that had not been used by any strain.

In the study on pot culture, all the isolated strains were inoculated to *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc. The *Bradyrhizobium* strain MYM-2 showed the more amount of fresh and dry weight of whole plants and pods. MYM-2 also found to be most significant in fresh weight,

dry weight of total nodule per plant. Otieno *et al*. (2007) suggested that higher grain yield in food legumes inoculated with *Rhizobium* was due to increase in nodulation. Therefore the present research work is in agreement with those findings by Black (1968).

Nitrogen is one of the major components in seed development or seed yield. Nitrogen fertilizer is quite expensive due to the high prize of petroleum. Under these circumstances, the ability of legumes in symbiosis with rhizobia to obtain the atmospheric nitrogen is important in crop production. Biological nitrogen fixation reduces the cost of production and helps to reduce pollution. Therefore, the using of *Bradyrhizobium* strain (MYM-2) in the cultivation of yardlong bean can not only be very applicable for the production of crops but also it may be beneficial to the agroecosystems.

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