Isolation of Agricultural Effective Microorganisms and It's Effect on Growth of Some Crops

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Abstract

In the present study, root nodulating bacteria were isolated from the root of *Phaseolus trilobus* Ait. (Taw-mat-pe) and *Sesbania cannabina* (Retz). (Nyan) on yeast extract mannitol agar (YEM) medium. In the present investigation were aimed to determining the effect of *Rhizobium* biofertilizers application on growth of *Solanum melongena* L. and *Panicum miliaceum* L. (Lu) plants. After 14 days, transplant cultivation in pot, which plant were treated with C-control, R-*Rhizobium* and M-manure. The effect of *Rhizobium* (from root nodule of *Phaseolus trilobus* Ait. Taw-mat-pe) showed that significantly high performance in plant height (33.1 cm), leaf length (18.7 cm), leaf width (14.1 cm) and number of leaf (12.0) on growth of *Solanum melongena* L. The effect of *Rhizobium* (from root nodule of *Sesbania cannabina* (Retz). Nyan) showed that significantly high performance in plant height (78.8 cm), leaf length (77.0 cm), leaf width (1.31 cm), number of leaf (58.7), number of branches (9.6) on growth of *Panicum miliaceum* L. (Lu) plants. These results indicated that the highest growth parameter was found in treated with *Rhizobium*. According to this result suggested that the use of *Rhizobium* biofertilizer had a higher positive effect of growth of eggplant and millet.

Keywords- *Rhizobium*, biofertilizer

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 a unique group called *Rhizobia* has a beneficial effect on the growth of plants (Oblisami, 1995).

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 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 (Kiers *et al*., 2003).

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Biofertilizer is a natural product carrying living microorganisms derived from the root or cultivated soil. So they don't have any ill effect on soil health and environment. Besides their role in atmospheric nitrogen fixation and phosphorous solubilisation, these also help in stimulating the plant growth hormones providing better nutrient uptake and increased tolerance towards drought and moisture stress. A small dose of biofertilizer is sufficient to produce desirable results because each gram of carrier of biofertilizers contains at least 10 million viable cells of a specific strain (Anandaraj and Delapierre 2010).

Many human diseases are due to unbalanced diets or malnutrition. A balanced diet is known to be very important for human health. There is therefore increasing interest in profile ling fruit and vegetables for potential nutrients in order to improve diets and fight malnutrition. Eggplant supplements starchy foods in addition to being good source of protein, minerals, fiber, folate, ascorbic acid, vitamin K, niacin, vitamin B6 and pantothenic acid (Zenia *et al*., 2008).

Common millet has the lowest water requirement among all grain crops; it is also a relatively short-season crop, and could grow well in poor soils. Recently, common millet is frequently cultivated in warm temperate and sub-tropical zones as a late-seeded, short-season summer catch crop with several cultivars (Astarai and Koocheki 1996).

In Myanmar, *Solanum melongena* L. is one of the most important vegetable crops grown in many regions. Although many research have been investigated the effect of *Rhizobium* on growth and yield of various plants, there was no report the effect of *Rhizobium* on growth and yield of *Solanum melongena* L. in Meiktila area. Therefore, in the present study, *Rhizobium* are generally cultured in Yeast Mannitol Agar medium (YEM medium) and which are used as bio-fertilizer in cultivation of *Solanum melongena* L. at Meiktila University.

Materials and Methods

Plant collection

The roots nodules were collected from *Phaseolus trilobus* Ait. (Taw-mat-pe) and *Sesbania cannabina* (Retz) (Nyan) found in Meiktila University Campus during 2017 and 2018.

Isolationof *Rhizobium* from root nodules

Required materials

Root nodules
YEM agar medium
Petri dishes
0.1% acidified $HgCl_2$ (1g $HgCl_2$, 5mL conc. HCl, 1 litre distilled water)
Sterile tap water
Nichrome blade
Plates containing YEM (Yeast Extract Mannitol agar) medium

Preparation of YEM medium

K_2HPO_4	0.5 g
$MgSO_4$. $7H_2O$	0.2 g
NaCl	0.1 g
Mannitol	10.0 g
Yeast extract	1.0 g
Distilled water	1 litre
Agar	15 g
pH	6.8

Isolation procedure

The procedure was followed by (Dubey and Maheshwari Method, 2007)

1. The healthy root nodules of a young plant were cut with a blade.
2. Wash the nodules thoroughly with sterile distilled water, after washing the nodules under aseptic conditions so as to remove contaminants and adhering soil particles.
3. Thereafter, immerse them in 0.1% acidified $HgCl_2$ for 5 minutes.
4. Transfer nodules in a sterile beaker containing 10 ml of 95% ethanol for 2-3 minutes.
5. Wash the nodules thoroughly 5 times with sterile tap water, and dry by sterile blotting paper.
6. Aseptically crush the nodules with glass rod or dissect the nodules by using nichrome blade and prepare dilutions.
7. Take 1 ml suspension for serial dilution and then 1 ml suspension from final dilution tube into YEM agar plates for spread culture.
8. Incubate the inoculated plates at 28°C for 48 hours. Thereafter, observe the bacterial colonies using microscope or with naked eyes which are gummy, translucent or white opaque.
9. Pick up a discrete colony and streak on a second YEM agar plates for better separation.

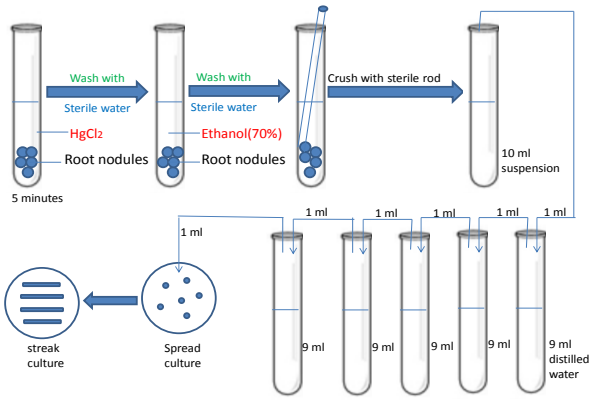

Fig. 1. Isolation procedure of *Rhizobium* from root nodules

Biofertilizer making

***Rhizobium* inoculation**

1. The isolated organisms are grown in Petri plate with YEM agar medium.
2. After two days culture they were transferred into conical flask containing 200 ml broth medium.

Preparation of carrier material

1. The carrier material charcoal is ground to pass through a mesh sieves.
2. The fine charcoal powder was sterilized by autoclave.
3. Then broth medium were mixed with sterilize charcoal powder 200 g. Carrier material charcoal is the medium in which organisms are allowed to multiply.
4. The survival of *Rhizobia* is also poor in coarser carrier materials. After two to three days they were used for pot cultivation experiment.

Pot experiment

In this experiment, the seeds of eggplant and millet were loosely wrapped with a piece of cloth and soaked in water over night (about 12 hours). Then the seeds were sown in the nursery and covered with the thin layer of soil. After 20 days, plants were transplanted to the individual pot containing 4 kg of soil. After one week cultivation, eggplants were treated with C-control, M-manure and R-*Rhizobium* from *Phaseolus trilobus* Ait. (Taw-mat-pe), millet plant were treatment C-control, M-manure and R-*Rhizobium* from *Sesbania cannabina* (Retz) (Nyan) and control (non treatment) plants were prepared. During the planting, the crop management and cultural practices such as spraying of water and weeds control were done when it was necessary.

Measurement of plant growth

The vegetative growth such as plant height, leaf length, leaf width, number of leaves and numbers of branches were collected biweekly interval for 70 days. These data were statistically analyzed using IRRISTAT (2000) software.

RESULTS

Isolation of *Rhizobium* strain from the root nodule

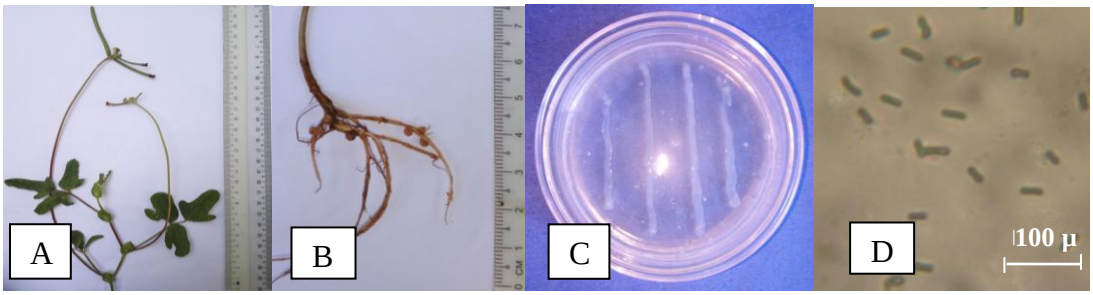

Figure. 2. A. Habit of *Phaseolus trilobus* Ait. B. Root nodule of *Phaseolus trilobus* Ait.
C. Morphological character of *Rhizobium* on YEM agar medium.
D. Microscopical character of *Rhizobium* on YEM agar medium.

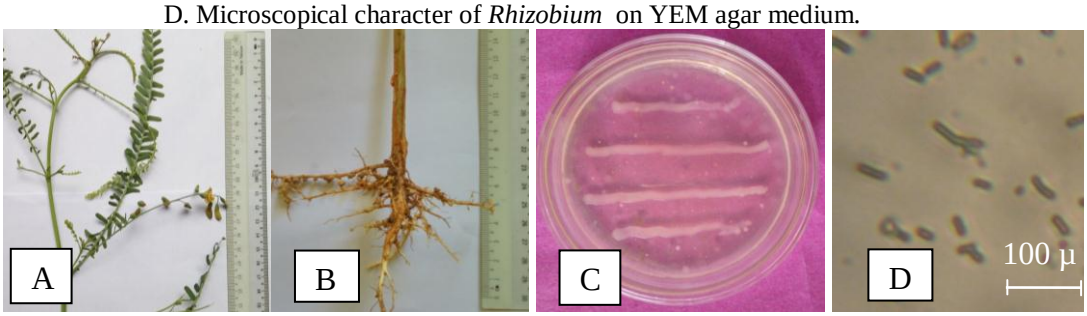

Figure. 3. A. Habit of *Sesbania cannabina* (Retz). B. Root nodule of *Sesbania cannabina* (Retz).
C. Morphological character of *Rhizobium* on YEM agar medium.
D. Microscopical character of *Rhizobium* on YEM agar medium

Characters of *Rhizobium* strain on YEM solid medium

In the present study, *Rhizobium* was isolated from the root nodules of *Phaseolus trilobus* Ait. (Taw-mat-pe) and *Sesbania cannabina* (Retz) (Nyan). After 3 to 5 days of growth on YEMA at 30°C, diameter of all colonies ranged from 2.0 to 2.5 mm, circular colonies with regular borders, convex in elevation, creamy in color, showing high production of mucus and gram negative.

Pot experiment for the growth of *Solanum melongena* L.

Table.1. Effect of biofertilizer on plant height of *Solanum melongena* L.

		Plant height (cm)			
Treatment	No. of Plants	14 days	28 days	42 days	56days
Control	10	9.7	17.9	25.2	25.3
Rhizobium	10	15.4	25.4	32.6	33.1
Manure	10	13.3	21.9	31.2	31.5
F-test		**	**	**	**
CV%		22.7	21.0	16.3	15.2
LSD		0.93	1.42	1.56	1.47

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Table. 2. Effect of biofertilizer on leaf length of *Solanum melongena* L.

Leaf length (cm)					
Treatment	No. of Plants	14 days	28 days	42 days	56days
Control	10	13.5	14.9	15.9	16.2
Rhizobium	10	17.1	17.8	18.3	18.7
Manure	10	15.0	16.4	16.9	17.4
F-test		**	**	**	**
CV%		14.1	11.4	9.4	8.6
LSD		0.69	0.59	0.51	0.48

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Table. 3. Effect of biofertilizer on leaf width of *Solanum melongena* L.

Leaf width (cm)					
Treatment	No. of Plants	14 days	28 days	42 days	56days
Control	10	10.0	11.1	11.7	12.1
Rhizobium	10	12.8	13.7	13.7	14.1
Manure	10	11.8	12.6	12.8	13.1
F-test		**	**	**	**
CV%		16.0	12.0	10.6	9.9
LSD		0.59	0.48	0.43	0.41

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Table. 4. Effect of biofertilizer on leaf number of *Solanum melongena* L.

Number of leaf					
Treatment	No. of Plants	14 days	28 days	42 days	56days
Control	10	7.4	8.5	9.5	10.1
Rhizobium	10	9.9	10.9	11.3	12.0
Manure	10	9.2	10.2	10.6	11.5
F-test		**	**	**	**
CV%		15.6	12.3	10.4	8.6
LSD		0.44	0.38	0.35	0.31

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Table. 5. Effect of biofertilizer on number of branches of *Solanum melongena* L.

Number of branches					
Treatment	No. of Plants	14 days	28 days	42 days	56days
Control	10	1.0	1.2	2.9	3.3
Rhizobium	10	1.0	1.5	4.3	5.2
Manure	10	1.0	1.3	4.1	4.8
F-test		**	**	**	**
CV%		0.0	33.4	21.3	20.7
LSD		0.0	0.14	0.26	0.30

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Pot experiment for the growth of *Panicum miliaceum* L.

Table 6. Effect of biofertilizer on plant height of *Panicum miliaceum* L.

Plant height (cm)						
Treatment	No. of plants	14 days	28 days	42 days	56 days	70 days
Control	10	27.3	38.3	54.7	65.3	66.9
Rhizobium	10	30.1	47.1	66.1	76.9	78.8
Manure	10	27.8	46.6	62.9	73.1	75.2
F-test		**	**	**	**	**
CV%		3.6	7.8	3.6	2.6	2.7
LSD		0.32	1.10	0.70	0.60	0.62

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Table 7. Effect of biofertilizer on leaf length of *Panicum miliaceum* L.

Leaf length (cm)						
Treatment	No. of plants	14 days	28 days	42 days	56 days	70 days
Control	10	12.0	30.0	52.9	66.7	67.5
Rhizobium	10	12.7	33.1	67.9	75.9	77.0
Manure	10	12.0	32.6	63.6	71.4	72.5
F-test		**	**	**	**	**
CV%		6.2	5.3	5.9	4.6	4.5
LSD		0.24	0.53	1.17	1.05	1.03

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Table 8. Effect of biofertilizer on leaf width of *Panicum miliaceum* L.

Leaf width (cm)						
Treatment	No. of plants	14 days	28 days	42 days	56 days	70 days
Control	10	0.68	0.85	0.96	1.06	1.09
Rhizobium	10	0.89	1.09	1.17	1.28	1.31
Manure	10	0.77	0.88	0.98	1.14	1.17
F-test		**	**	**	**	**
CV%		11.2	10.3	8.0	5.7	4.5
LSD		0.27	0.30	0.26	0.21	0.17

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Table 9. Effect of biofertilizer on number of leaf of *Panicum miliaceum* L.

Number of leaf						
Treatment	No. of plants	14 days	28 days	42 days	56 days	70 days
Control	10	7.7	21.3	33.2	43.4	47.3
Rhizobium	10	9.7	26.7	44.2	55.9	58.7
Manure	10	8.0	24.1	39.2	47.7	50.7
F-test		**	**	**	**	**
CV%		13.3	22.2	19.2	19.3	17.5
LSD		0.36	1.72	2.42	3.04	2.92

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

Table 10. Effect of biofertilizer on number of branches in *Panicum miliaceum* L.

		Number of branches				
Treatment	No. of plants	14 days	28 days	42 days	56 days	70 days
Control	10	1.8	3.5	5.3	6.2	6.4
Rhizobium	10	2.2	5.6	7.9	9.3	9.6
Manure	10	1.9	4.5	6.9	8.1	8.5
F-test		**	**	**	**	**
CV%		16.5	26.3	17.2	22.1	18.1
LSD		0.10	0.39	0.37	0.55	0.47

** = highly significant, CV = Coefficient of variation, LSD = Least Significant Difference

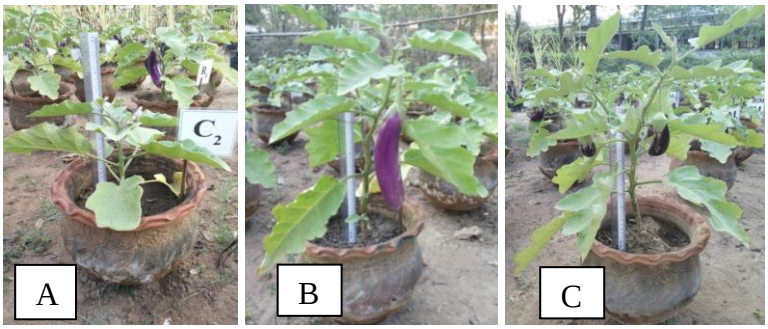

Figure 4. Pot experiment of *Solanum melongena* L.

A.Control plant **B**. *Rhizobium* treatment plant **C.** Manure treatment plant

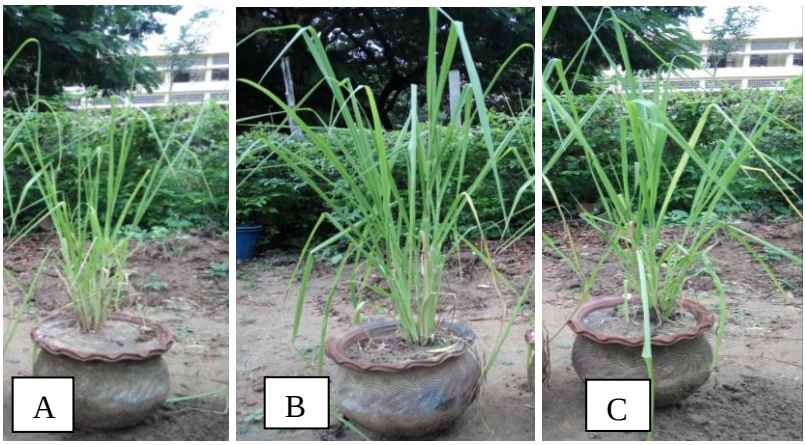

Figure 4. Pot experiment of *Panicum miliaceum* L.

A.Control plant **B**. *Rhizobium* treatment plant **C**. Manure treatment plant

DISCUSSION AND CONCLUSION

In this experiment, crops plants were treated with *Rhizobium* and manure. Then the growth characters of plant height, leaf length, leaf width, the number of leaf and number of branches were measured at two weeks interval. Statistically significant increase in the tallest plant height was recorded at *Rhizobium* treatment (33.1 cm) (Table 1), in leaf length was recorded at *Rhizobium* treatment (18.7 cm) (Table 2), in leaf width was recorded at *Rhizobium* treatment (14.1 cm) (Table 3), in number of leaf was recorded at *Rhizobium* treatment (12.0) (Table 4), in number of branches was recorded at *Rhizobium* treatment (5.2) (Table 5), on the growth of *Solanum melongena* L.

Statistically significant increase in the tallest plant height was recorded at *Rhizobium* treatment (78.8 cm) (Table 6), in leaf length was recorded at *Rhizobium* treatment (77.0 cm) (Table 7), in leaf width was recorded at *Rhizobium* treatment (13.1 cm) (Table 8), in number of leaf was recorded at *Rhizobium* treatment (58.7) (Table 9), in number of branches was recorded at *Rhizobium* treatment (9.6) (Table 10), on the growth of *Penicum miliaceum* L. This study showed the positive effects of *Rhizobium* treatment on *Solanum melongena* L. and *Penicum miliaceum* L. during growing seasons.

According to Burd *et al*., 2000, reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens.

In conclusion, the results from the present research are very applicable in the agriculture especially in the better production of quality crops and soil health. The biofertilizer technology is one of the advantages for the agriculture sector.

Morevoer, bio treatments are important components of integrated nutrients management. These potential biological fertilizers would play key role in productivity and sustainability of soil and also protect the environment as ecofriendly and cost effective inputs for the farmers.

Acknowledgements

I wish to express my heartfelt thanks to Dr Thanda, Professor and Head, Department of Botany, Meiktila University for permitting to undertake this research work and for providing me the necessary facilities. Finally, I would like to express my heartfelt gratitude to my beloved parents for their financial support and continual encouragement throughout my life.

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