Isolation of *Rizobium* Strains and Conventional Manure of their Difference Effects on Growth and Yield of *Lycopersicon esculentum* Mill.

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Abstract

In the present study, root nodulating bacteria were isolated from the root of *Indigofera tinctoria* L. (Me yine) on Yeast Extract Mannitol agar (YEM) medium. The present investigation was aimed at determining the effect of *Rhizobium* biofertilizers application on the growth and yield of tomato plants. After one week of transplant cultivation in a pot, *Lycopersicon esculentum* Mill. was treated with C-control, R-*rhizobium*, M-manure(cow dung) and R + M-*rhizobium* + manure(cow dung). The effect of *Rhizobium* was shown to have significantly high performance in plant height of 48.2cm, leaf length of 12.1cm, leaf width of 11.5cm and number of leaves of 10.7cm. Similarly, the effect of *Rhizobium* on the yield of *Lycopersicon esculentum* Mill. was significantly higher of 21.92g than the other treatment and control plants. These results indicated that, the highest growth parameter was found in those treated with *Rhizobium.* This result suggests that, the use of *Rhizobium* biofertilizer had a greater positive effect on the growth and yield of tomato plants.

Keywords: *Rizobium* strains, effect on growth and yield of *Lycopersicon esculentum* Mill.

Introduction

Rhizobium is the most well known species of a group of bacteria that acts as the primary symbiotic fixers 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.

Nitrogen is an essential nutrient for plant growth and development. Plants usually depend upon combined, or fixed, forms of nitrogen, such as ammonia and nitrate because nitrogen is unavailable in its most prevalent form, atmospheric nitrogen. Use of these fertilizers has led to worldwide ecological problems as well as affecting human health Vitousek, 1997. The biofertilization of crops with plant growth promoting microorganisms is currently considered as a healthy alternative to chemical fertilization. Biofertilizers are important components of integrated nutrient management. These potential biological fertilizers would play a key role in the productivity and sustainability of soil and also protect the environment as they are ecofriendly and cost effective inputs for farmers.

Beneficial effects of the introduction of specific microorganisms on plant growth have been reported for numerous crops, including tomato (*Lycopersicon esculentum* Mill.) grown under green house in organic media Guo *et al*., 2004. The aim of the present study is to understand the fundamental facts and principles necessary in Microbiology, to inform the isolation of *Rhizobium* from the roots of legume plants, to know the importance factors of physical and chemical requirements for the growth of *Rhizobium* and their effect on the growth of tomato plant.

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Materials and Methods

Collection of plant

In the present research, the root nodules were collected from *Indigofera tinctoria* L. found in Meiktila Township. The collected samples were identified with the help of available literature as Backer, 1963-1368, Cronquist, 1981 and Hooker, 1897.

Preparation of Yeast Extract Mannitol agar (Dubey and Maheshwari, 2007)

The medium containing K_2HPO_4 - 0.5g, $MgSO_4.7H_2O$ - 0.2g, NaCl - 0.1g, Mannitol - 10g and Yeast extract - 1g at pH 7.0 was used then the media were boiled until the agar well dissolved. The pH of the media was adjusted before autoclaving. Then, the media were sterilized by autoclaving at 121°C (15 psi) for 15minutes. After cooling down, the medium was poured into sterilized petridishes.

Isolation procedure (Dubey and Maheshwari, 2007)

Procure healthy root nodules of a young leguminous plant by cutting with a blade. Wash the nodules thoroughly first with tap water and then with sterile distilled water keeping over the nylon mesh under aseptic conditions so as remove contaminants and adhering soil particles. Thereafter, immerse them in 0.1% acidified $HgCl_2$ for 5minutes. Transfer nodules in a sterile beaker containing 10ml of 95% ethanol and wait of 2 to 3minutes. Wash the nodules thoroughly for 5times with sterile tap water, and blot dry by using sterile blotting paper. Aseptically crush the nodules with a glass rod or dissect the nodules by using a nichrome blade and prepare dilutions. Pour 1ml suspension on YEM agar plates. Incubate the inoculated plates at 30°C for 48hours. Thereafter, observe the bacterial colonies that are gummy, translucent or white opaque. Pick up a discrete colony and streak its on a second YEM agar plate for better separation.

Carrier preparation

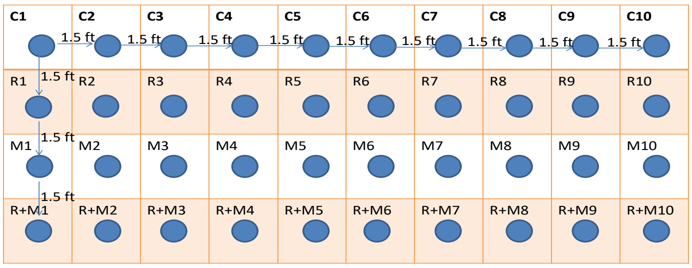
The carrier (charcoal) was powdered and dried in sun to get 5% moisture level. Then it is screened through mesh calcium carbonate powder and sterilized by autocalving.

Preparation of pots for growing of tomatos

The surface loamy soil from a bare ground area of a local field was collected and sieved to discard small pieces of bricks and stones. Four kilograms (4kg) of soil were used for each pot. The distance between the pots and rows was 45cm.

Pot experiment

In this experiment, the seeds of a tomato were loosely wrapped with a piece of cloth and soaked in water over night (about 12hours). Then the seeds were sown in the nursery and covered with a thin layer of soil. After 20days, when the plants become 10cm in height and 4cm foliar leaf area were transplanted to the individual pot containing 4kg of soil as shown in Figure 1.



C=control, R=*Rhizobium*, M=Manure, R+M=*Rhizobium*+Manure

Figure 1. Pot experiment design for cultivation of *Lycopersicon esculentum* Mill.

Measurement of plant growth

Growth of the plants (plant height), leaf length, leaf width and numbers of leaves were monitored for 70days in every 14 days interval.

Data collection and statistical analysis

These data were statistically analyzed using IRRISTAT, 2000 software and LSD (Least significant difference), Friedman test (F - test), and Coefficient for variation percent (CV%) were used to analyze the mean separation of the treatments.

Results

Isolation of a *Rhizobium* strain from the root nodule of *Indigofera tinctoria* L. The morphological characteristics of *Indigofera tinctoria* L. are as follows , as shown in Figure 2 and 3.

Indigofera tinctoria **L.,** Sp. Pl. 751. 1758.
Scientific name - *Indigofera tinctoria* L.
Family - Fabaceae
Myanmar name - Me yine

Figure 2. Habit of *Indigofera tinctoria* L. Figure 3. Root nodule of *Indigofera tinctoria* L.

Culture of an isolated *Rhizobium* strain on YEM solid medium

The isolated *Rhizobium* strains were cultured on YEM agar medium for 5days. After 3days, the cultures of 200ml were used to treat the tomato plants as shown in Figure 4 and 5.

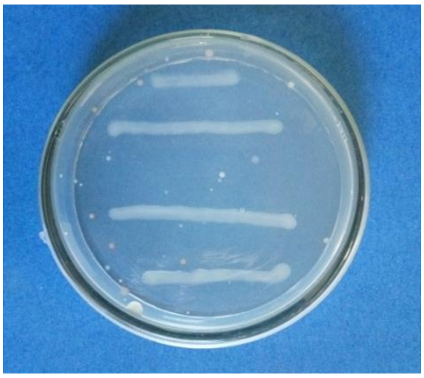


Figure 4 . Morphological character of *Rhizobium* on YEM agar medium

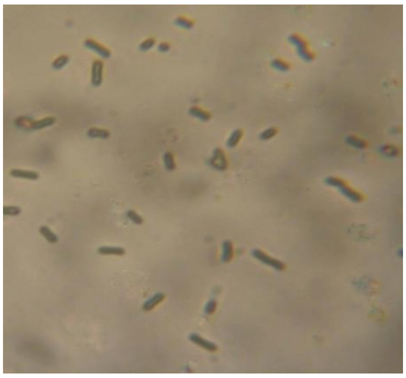


Figure 5. Microscopical character of *Rhizobium* on YEM agar medium

Characters of *Rhizobium* strain

Rhizobium spp. or root nodule bacteria are small-sized, rod-shaped cells, gram-negative. Rhizobia are predominantly aerobic chemoorganotrophs and are relatively easy to culture. They grow well in the presence of oxygen and utilize relatively simple carbohydrates and amino compounds. Optimal growth of rhizobia strains occurs at a temperature range of 25-30 ºC and a pH of 6.0 - 7.0. Generally, rhizobia produce white colonies.

Vegetative growth

The pot experiment of *Lycopersicon esculentum* Mill. was carried out in the open area of Meiktila University campus. In the growing of *Lycopersicon esculentum* Mill., the effect of *Rhizobium* on the growth of the plants was tested. The data of the plants were collected from 14 days after growing of plants to 70days. The flowering was observed at 42days and fruit setting by 55days. A mature fruit was observed at 62 days.

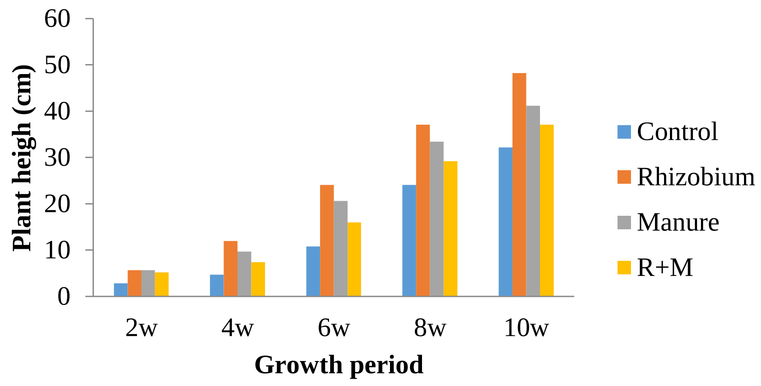
Plant height

The result of the effect of *Rhizobium* and manure on the plant height of *Lycopersicon esculentum* Mill. at 2 weeks to 10 weeks was shown in Table 1 and Figure 6. The highest plant height of 48.2cm was found in *Rhizobium* treatment and the lowest plant height of 37.1cm was found in *Rhizobium* + manure but it was higher than control. All treatment means were significantly different from each other.

Table 1. Effect of biofertilizer on plant height of *Lycopersicon esculentum* Mill.

Plant height (cm) Mean						
Treatment	**2W**	**4W**	**6W**	**8W**	**10W**	**Mean**
Control	2.8	4.7	10.8	24.1	32.2	14.92
Rhizobium	5.7	12.0	24.1	37.1	48.2	25.42
Manure(cow dung)	5.7	9.7	20.6	33.4	41.2	22.12
Rhizobium+Manure(cow dung)	5.2	7.4	16.0	29.2	37.1	18.98
F-test	**	**	**	**	**	
CV%	40.3	40.2	34.9	31.6	22.3	
LSD	0.62	1.07	1.97	3.10	2.80	

Each data was collected from 10 plants samples, W = week, ** = highly significant



R + M = *Rhizobium* + Manure (cow dung)

Figure 6. Effect of biofertilizer on plant height of *Lycopersicon esculentum* Mill.

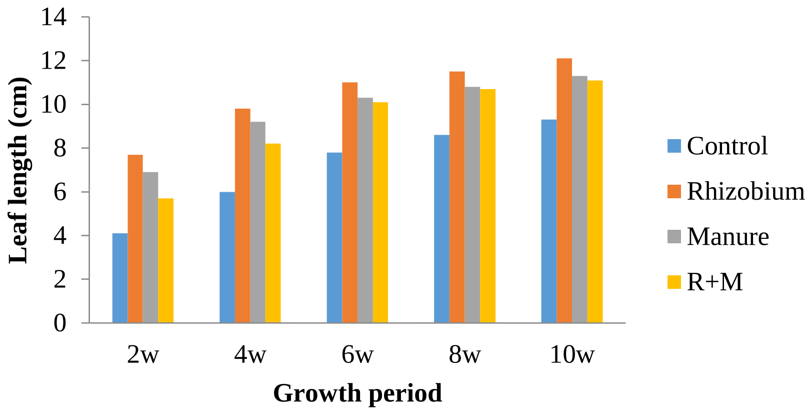
Leaf length

The result of the effect of *Rhizobium* and manure on the leaf length of *Lycopersicon esculentum* Mill. at 2 weeks to 10 weeks was shown in Table 2 and Figure 7. The longest leaf length of 12.1cm was found in the *Rhizobium* treatment and the shortest leaf length of 11.1cm was found in the *Rhizobium* + manure but it was higher than control. All treatment means were significantly different from each other.

Table 2. Effect of biofertilizer on leaf length of *Lycopersicon esculentum* Mill.

Leaf length (cm) Mean						
Treatment	**2W**	**4W**	**6W**	**8W**	**10W**	**Mean**
Control	4.1	6.0	7.8	8.6	9.3	7.16
Rhizobium	7.7	9.8	11.0	11.5	12.1	10.42
Manure(cow dung)	6.9	9.2	10.3	10.8	11.3	9.7
Rhizobium+Manure (cow dung)	5.7	8.2	10.1	10.7	11.1	9.16
F-test	**	**	**	**	**	
CV%	31.1	17.2	10.1	8.8	8.2	
LSD	0.6	0.45	0.31	0.29	0.28	

Each data was collected from 10 plants samples, W = week, ** = highly significant



R + M = *Rhizobium* + Manure (cow dung)

Figure 7. Effect of biofertilizer on leaf length of *Lycopersicon esculentum* Mill.

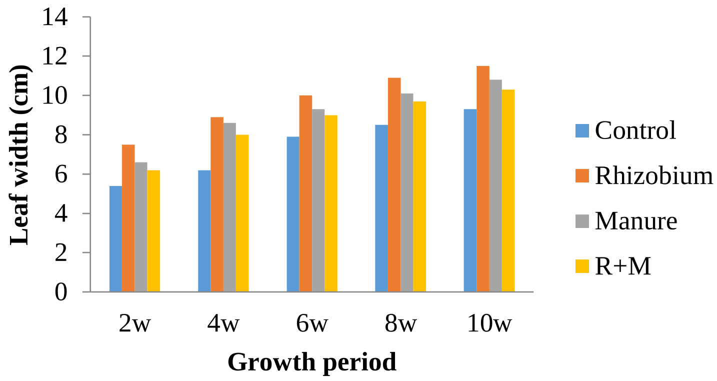
Leaf width

The result of the effect of *Rhizobium* and manure on the leaf width of *Lycopersicon esculentum* Mill. at 2 weeks to 10 weeks was shown in Table 3 and Figure 8. The highest leaf width of 11.5cm was found in the *Rhizobium* treatment and the lowest leaf width of 10.3cm was found in the *Rhizobium* + manure but it was higher than control. All treatment means were significantly different from each other.

Table 3 Effect of biofertilizer on leaf width of *Lycopersicon esculentum* Mill.

Leaf width (cm) Mean						
Treatment	**2W**	**4W**	**6W**	**8W**	**10W**	**Mean**
Control	5.4	6.2	7.9	8.5	9.3	7.46
Rhizobium	7.5	8.9	10.0	10.9	11.5	9.76
Manure (cow dung)	6.6	8.6	9.3	10.1	10.8	9.08
Rhizobium+Manure (cow dung)	6.2	8.0	9.0	9.7	10.3	8.64
F-test	**	**	**	**	**	
CV%	21.4	15.5	12.3	8.4	7.6	
LSD	0.43	0.39	0.35	0.26	0.25	

Each data was collected from 10 plants samples, W = week, ** = highly significant



R + M = *Rhizobium* + Manure (cow dung)

Figure 8. Effect of biofertilizer on leaf width of *Lycopersicon esculentum* Mill.

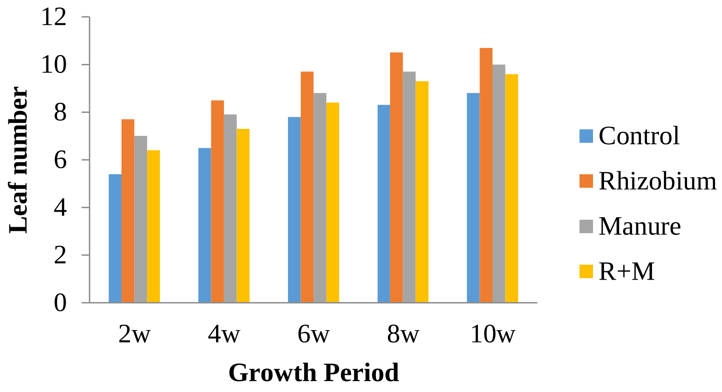
Number of leaf

The result of the effect of *Rhizobium* and manure on the leaf number of *Lycopersicon esculentum* Mill. at 2 weeks to 10 weeks was shown in Table 4 and Figure 9. The highest number of leaves 10.7cm was observed in the *Rhizobium* treatment and the lowest number of leaves 9.6cm was observed in the *Rhizobium* + manure but it was higher than control. All treatment means were significantly different from each other.

Table 4 Effect of biofertilizer on leaf number of *Lycopersicon esculentum* Mill.

Number of leaf (Mean)						
Treatment	**2W**	**4W**	**6W**	**8W**	**10W**	**Mean**
Control	5.4	6.5	7.8	8.3	8.8	7.36
Rhizobium	7.7	8.5	9.7	10.5	10.7	9.42
Manure (cow dung)	7.0	7.9	8.8	9.7	10.0	8.68
Rhizobium+Manure (cow dung)	6.4	7.3	8.4	9.3	9.6	8.2
F-test	**	**	**	**	**	
CV%	18.0	13.3	10.3	7.5	7.5	
LSD	0.6	0.5	0.45	0.35	0.36	

Each data was collected from 10 plants samples, W = week, ** = highly significant



R + M = *Rhizobium* + Manure (cow dung)

Figure 9. Effect of biofertilizer on leaf number of *Lycopersicon esculentum* Mill

Reproductive growth

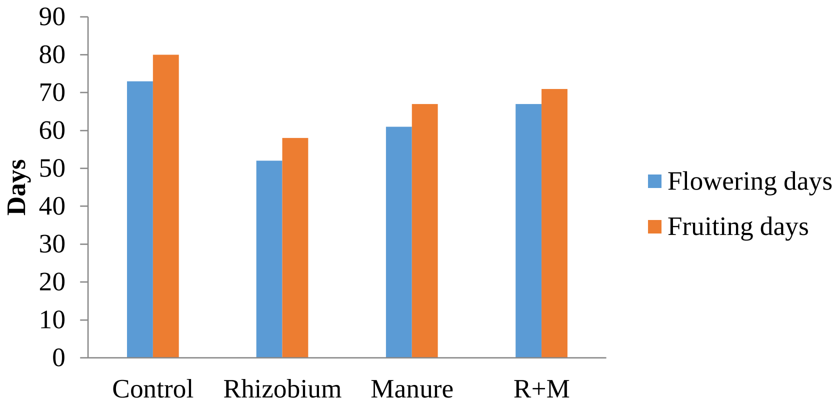
Flowering and fruiting days

The results of the effect of *Rhizobium* on yield components such as flowering and fruiting days in *Lycopersicon esculentum* Mill. were shown in Table 5 and Figure 10. The earliest flowering and fruiting days were found in *Rhizobium* treated plants and the longest was observed in control.

Table 5 Effect of biofertilizer on flowering and fruiting days of *Lycopersicon esculentum* Mill.

Treatment	Flowering Days	Fruiting Days
Control	73	80
Rhizobium	52	58
Manure (cow dung)	61	67
Rhizobium+Manure (cow dung)	67	71
F-test	**	**
CV%	10.4	8.2
LSD	5.99	5.20

Each data was collected from 10 plants samples, ** = highly significant



R + M = *Rhizobium* + Manure (cow dung)

Figure 10. Effect of biofertilizer on flowering and fruiting days of *Lycopersicon esculentum* Mill.

Fruit size diameter and yield weight

The results of the effect of *Rhizobium* and manure on fruit size and weight of *Lycopersicon esculentum* Mill. were shown in Table 6 and Figure 11 . The widest diameter 4.7cm was observed in *Rhizobium* and the smallest diameter 3.35cm was found in *Rhizobium*+ manure (cow dung) but it was higher than control. Similarly, weight 21.92g was observed in *Rhizobium* treatment and the least weight 16.74g was found in *Rhizobium* + manure (cow dung) but it higher than control. All treatment means were significantly different from each other.

Table 6. Effect of biofertilizer on fruit size and yield of *Lycopersicon esculentum* Mill

Fruit size and fruit weight (yield)		
Treatment	**Weight (g)**	**Diameter (cm)**
Control	12.45	3.05
Rhizobium	21.92	4.70
Manure (cow dung)	17.01	3.75
Rhizobium+Manure (cow dung)	16.74	3.35
F-test	**	**
CV%	34.5	19.9
LSD	5.38	0.67

Each data was collected from 10 plants samples, ** = highly significant

Figure 11. Fruit size of *Lycopersicon esculentum* Mill.

Figure12. Pot experiment of *Lycopersicon esculentum* Mill. at 2 weeks

Figure 13. Pot experiment of *Lycopersicon esculentum* Mill. at 4 weeks

Figure 14. Pot experiment of *Lycopersicon esculentum* Mill. at 6 weeks

Figure 15. Pot experiment of *Lycopersicon esculentum* Mill. at 8 weeks

Figure 16. Pot experiment of *Lycopersicon esculentum* Mill. at 10 weeks

Discussion and Conclusion

The present study emphasized the isolation of *Rhizobium* from root nodules of *Indigofera tinctoria* L. and its effects on growth and yield of *Lycopersicon esculentum* Mill. In this experiment, *Lycopersicon esculentum* Mill. plants were treated with *Rhizobium* and manure. Then the growth characters of plant height, leaf length and leaf width, the number of leaves were measured at 2 weeks to 10 weeks interval as shown in Figure 12 to 16. The effect of biofertilizer treatments on the vegetative growth of tomato plants was significantly higher in *Rhizobium* inoculation than in manure, *Rhizobium + manure* and control plants. A statistically significant increase in plant height was recorded at *Rhizobium* treatment at 48.2cm and the lowest plant height 37.1cm was found in *Rhizobium* + manure but it was higher than control in Table 1.

A statistically significant increase in the longest leaf length 12.1cm was found in the *Rhizobium* treatment and the shortest leaf length 11.1cm was found in the *Rhizobium* + manure but it was higher than control as shown in Table 2. Statistically significant increase in the highest leaf width 11.5cm was found in *Rhizobium* treatment and the lowest leaf width 10.3cm was found in *Rhizobium* + manure but it higher than control as shown in Table 3.A statistically significant increase in the highest number of leaves 10.7cm was observed in the *Rhizobium* treatment and the lowest number of leaves 9.6cm was observed in the *Rhizobium* + manure but it was higher than control. Rhizobia used for more than 100 years in legume biofertilization are particularly safe for humans and since they present direct and indirect mechanisms of plant growth promotion they are also excellent candidates to be used for non-legume biofertilization particularly of raw consumed vegetables. However, up to date there was a significant lack of research about the growth promotion of rhizobia on vegetables with edible fruits such as pepper and tomato, the plants analyzed in the present study.

Most rhizobial strains promoting non-legume plant growth described to date belong to *R. biovars, R. phaseoli* and *R. trifolii* from *R.leguminosarum*. Bashan.Y,1998. Biofertilizer help in increasing crop productivity by way of increase BNF, increased availability or uptake of nutrients through solubilization or increased absorption stimulation of plant growth through hormonal action or antibiosis, or by decomposition of organic residues. Furthermore, biofertilizer as to replace part of the use of chemical fertilizers reduces the amount and cost of chemical fertilizers and thus prevents the environment of pollution from extensive application of chemical fertilizers. With the use of biological and organic fertilizers, a low input system can be carried out, and it can help achieve the sustainability of farms.

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