MULTIPLICATION OF MYCORRHIZA INOCULUM FROM FIVE WEED PLANTS AND ITS EFFECT ON *Lactuca sativa* L.

Han Su Kyi[1], Ko Tin[2]

Abstract

The present study is concerned with investigation of the mycorrhiza on the lettuce plants not only to determine their growth but also for the multiplication of mycorrhiza inoculum. Mycorrhiza spores were collected from five weed plants in Mawlamyine University Campus during June to December, 2014 by using floating adhesion technique and wet sieving method. Mycorrhiza, collected from five selected weeds plants were mixed with sterile soil and inoculate on lettuce plants. Spores density and mycorrhizal colonization from rhizosphere of lettuce plants were recorded in every two weeks. Among five selected weed plants, the maximum rate of spore number and the highest colonization percent of mycorrhiza on lettuce plants were isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman). Mycorrhiza isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman) were used as inoculum and subjected into multiplication procedure. The polyethylene bag experiments were conducted on *Lactuca sativa* L. (lettuce). There are five treatment namely soil 5 kg soil (T_1), natural mycorrhiza 1 kg + soil 4 kg (T_2) , natural mycorrhiza 1 kg + biocomposer 0.5 kg + soil 4 kg (T_3), natural mycorrhiza 1 kg + cow dung 0.5 kg + soil 4 kg (T_4) and commercial mycorrhiza fertilizer (MF) 15 g + soil 5 kg (T_5) in polyetylene bag culture were used in the study. According to the result, the highest mycorrhiza inoculum potential (MIP) index were also found in T_2 (10.50) and T_4 (10.50).

Keywords: Weed, mycorrhizal colonization, mycorrhizal fertilizer, spores**,** *Eclipta alba* (L.) Hassk.

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Introduction

Mycorrhiza refers to an association between plant roots and soil borne fungi that colonize the cortical tissue of plant roots during period of active growth (Smith and Read 1997). Soil microorganism plays a major role in nutrients cycling and plant growth in contrast the chemical fertilizers, organic manures and are less expensive and increase productivity without harming the environment. It is therefore important to use vesicular-arbuscular mycorrhizal (VAM) fungi as biofertilizer. Mycorrhizal fungi are associated in the roots of most species and effectively increase the volume of soil that can be explored by the plant (Morton and Benny, 1990). Biocomposer is one of the organic fertilizers which are widely used in Myanmar Agriculture Service (MAS). It contains many organic nutrients. Biocomposer is the product of many basic sugar cane bubble waste (MAE, 2006).

Cowdung has high percentage of nitrogen and potassium, which plays an important role in a accelerating the translocation of photosynthesis from the leaves and shoots to the tuberous roots for bulking (Forbe and Watson, 1994)

Lettuce plants are planted just only for consuming as a vegetable. The leaves rich in vitamins and minerals are popularly used as salad. Lettuce has been cultivated for more than 2,500 years. The Romans grew many varieties, and it became widely appreciated in Asia and Europe (Grigson, 1978).

Present study, natural mycorrhiza, cormmerical mycorrhiza and biofertilizer were treated on cultivation of lettuce plants.

Material and Methods

Source of mycorrhiza

Natural mycorrhiza were collected from five weed plants such as *Eclipta alba* (L.) Hassk., *Mimosa pudica* L., *Phyllanthus urinaria* L., *Tridax procumbens* L., *Urena lobata* L. in Mawlamyine University Campus during June to December, 2014 by floating adhesion technique (Sutton and Barron, 1972) and by wet sieving method (Gerdemann and Nicolson, 1963). The

collected spores were identified by their, size, shape, colour and hyphal attachment according to Smith, 1997 and Miyasaka *et al.*, 2003.

Experimental Site

A polyethylene bag experiment was conducted in Taunggyi University, Shan State from September to October, 2015.

Preparation of Soil

Soils are sterilized by furan (fungicide) and maintained it at least 48 hours. Sterilized soil and selected natural mycorrhiza were mixed with 3:2 ratio.

Multiplication of mycorrhiza inoculum in different fertilizers used

Treatment 1- 5 kg (Soil)

Treatment 2- 1 kg (NM) + 4 kg (Soil)

Treatment 3- 1 kg (NM) + 0.5 kg (Biocomposer) + 3.5 kg (S0il)

Treatment 4- 1 kg (NM) + 0.5 kg (Cow Dung) + 3.5 kg (Soil)

Treatment 5- 15 g (Commercial Mycorrhiza Fertilizer) + 5 kg (Soil)

Quantification of Mycorrhiza fungi propagules (Mycorrhizal inoculum potential (MIP) Assay Methods, Bracker, 1999)

The MIP assay measures the percentage mycorrhizal colonization in a host plant over time, after the host plant has been grown in a series of inoculum and root colonization is estimated after 2 to 6 weeks. MIP technique is less complex and time consuming.

$$MIP = \frac{\text{Primary ingress + Secondary spread}}{2}$$

MIP = Mycorrhizal inoculum potential index

Primary ingress = % of infection (4 weeks) - % of infection (2 weeks)

Secondary spread = % of infection (6 weeks) - % of infection (4 weeks)

Figure 1. Soil mixture of natural VAM (4:1 Kg)

Figure 2. Soil mixture of biocomposer with VAM (3.5: 0.5: 1 Kg)

Figure 3. Soil mixture of cowdang with VAM (3.5: 0.5: 1 Kg)

Figure 4. Soil mixture of commercial with VAM (5 Kg per 15 g)

Figure 5. Preparation of polyethylene bags for planting

Estimation of VAM root colonization and collection of spores

Spores were collected from the rhizosphere soil by floating adhesion technique (Sutton and Barron, 1972) and by wet sieving method (Gerdemann and Nicolson, 1963). AM root colonization in the hosts were studied and caculated by using grid-line method (Newman, 1966). The total percentage of root colonization were determined in the following formula:

$$\text{Root colonization (\%)} = \frac{\text{Intersection of infected roots}}{\text{Total number of intersection roots}} \times 100$$

Results

In the present study, mycorrhiza were collected from five weed plants, such as *Eclipta alba* (L.) Hassk., *Mimosa pudica* L., *Phyllanthus urinaria* L., *Tridax procumbens* L., and *Urena lobata* L. The collected mycorrhiza spores and root colonization of five weed plants were shown in Tables 1and 2. Number of mycorrhiza spores and colonization percent of different mycorrhiza inoculated lettuce plants were shown in Figures 7-11 and Tables 3 and 4 Multiplication of VAM with different treatments on *lactuca sativa* L. were shown in Figures 12-16 and Tables 5 and 6.

Table 1. Determination of colonization percent on roots during June to December,2014

No	Scientific Name	June	July	Aug	Sep	Oct	Nov	Dec
1	*Eclipta alba* (L.) Hassk.	67%	79%	57%	55%	61%	77%	70%
2	*Mimosa pudica* L.	42%	46%	39%	32%	46%	50%	53%
3	*Phyllanthus urinaria* L.	54%	50%	44%	45%	54%	53%	56%
4	*Tridax procumbens* L.	51%	54%	30%	50%	49%	55%	53%
5	*Urena lobata* L.	59%	63%	45%	47%	57%	61%	57%

Table 2. Comparison of spores from rhizophere soil during July to December, 2014

No	Scientific Name	June	July	Aug	Sep	Oct	Nov	Dec
1	*Eclipta alba* (L.) Hassk.	50	67	75	100	160	81	89
2	*Mimosa pudica* L.	30	50	25	40	64	52	34
3	*Phyllanthus urinaria* L.	35	48	58	45	65	59	50
4	*Tridax procumbens* L.	41	54	60	58	75	65	75
5	*Urena lobata* L.	39	63	65	67	75	69	65

Identification of VAM Fungal Spores (Smith and Read, 1997; Miyasaka, 2003)

The collected spores were identified by their, size, shape, colour and hyphal attachment. Collected spores from *Eclipta alba* (L.) Hassk. (Kyeik-hman) were determined as Glomus.

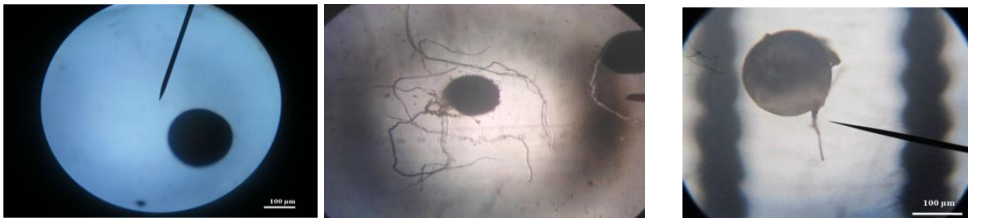
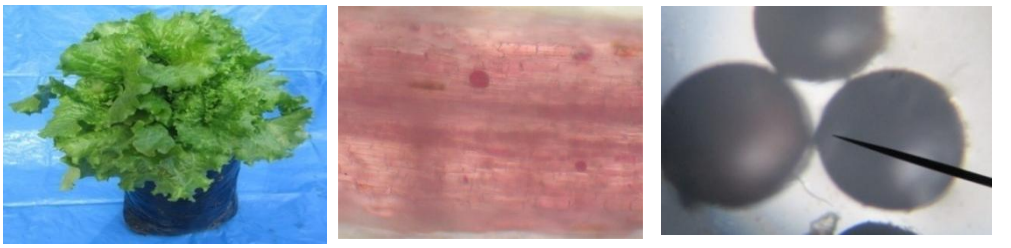


Figure 6. Morphological characters of collected VAM spores

Size	- 100 –310 μm
Colour	- reddish brown to black
Shape	- globose
Surface ornamentation	- smooth
Suspensor cell	- terminally on a bulbous cell present
Vesicle	- present



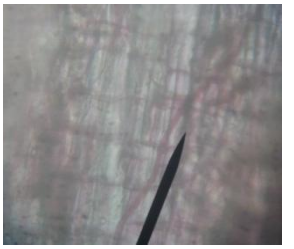
(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Eclipta alba* (L.) Hassk.

(B) Infected vesicle in root X 100

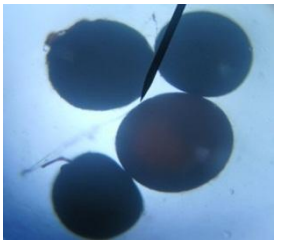
(C) Collected spores

Figure 7. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Mimosa pudica* L.



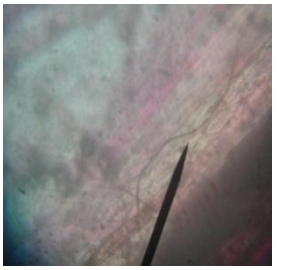
(B) Infected root (X 100)



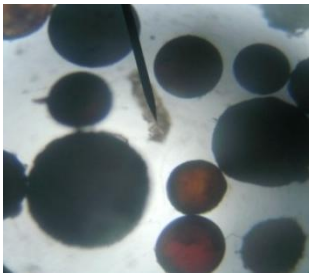
(C) Collected spores

Figure 8. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Phyllanthus urinaria* L.



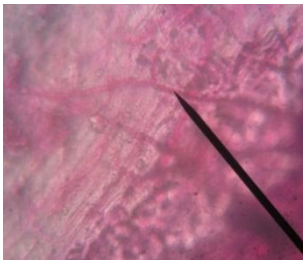
(B) Infected root (X 100)



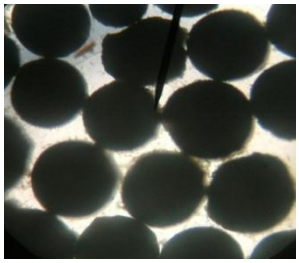
(C) Collected spores

Figure 9. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Tridax procumbens* L.



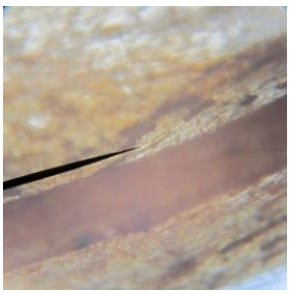
(B) Infected root (X 100)



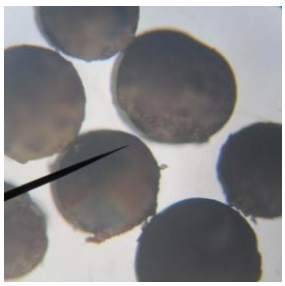
(C) Collected spores

Figure 10. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

(A) Innoculated lettuce plant growing by Mycorrhiza isolated from *Urena lobata* L.



(B) Infected root (X 100)



(C) Collected spores

Figure 11. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere

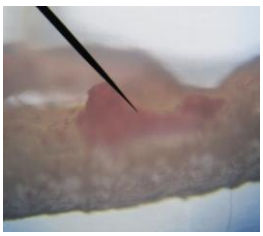
Table 3. Summarized infection percent of different mycorrhiza on letttuce plants

	% of infection				
	Eclipta alba (L.) Hassk.	*Mimosa pudica* L. .	*Phyllanthus urinaria* L. .	*Tridax procumbens* L.	*Urena lobata* L.
2 weeks	**61**	37	46	48	55
4 weeks	**69**	41	50	51	58
6 weeks	**71**	51	61	59	63

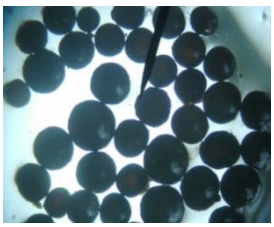
Table 4. Summarized different mycorrhiza spores number on lettuce plant

	Number of spores				
	Eclipta alba (L.) Hassk.	*Mimosa pudica* L. .	*Phyllanthus urinaria* L. .	*Tridax procumbens* L.	*Urena lobata* L.
2 weeks	**41**	29	31	37	29
4 weeks	**61**	38	49	42	38
6 weeks	**75**	45	52	48	45

Treatment - 1



(B) Infected root (X 100)



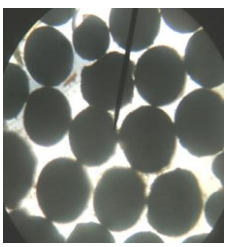
(C) Collected spores

Figure 12. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere. Treatment-1 (control)

Control

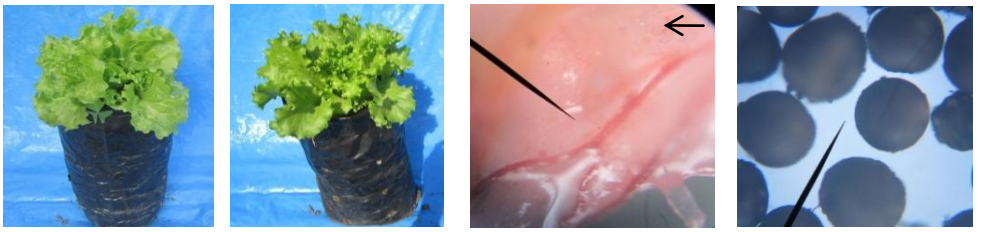
Treatment - 2

(B) Infected vesicle in root (X 100)



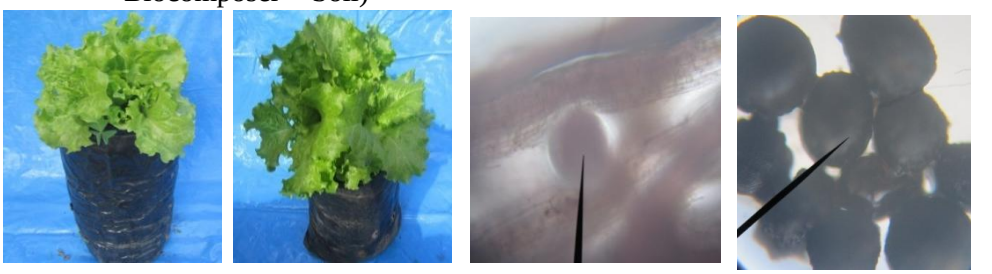
(C) Collected spores

Figure 13. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere. Treatment-2 (Natural Mycorrhiza + Soil)



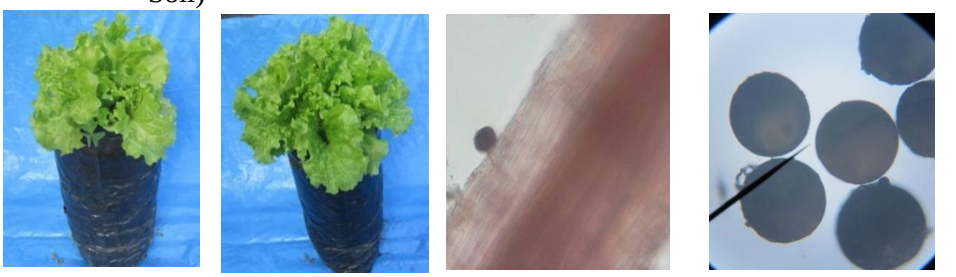
Control Treatment - 3 (B) Infected root (X 100) (C) Collected spores

Figure 14. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere. Treatment-3 (Natural Mycorrhiza + Biocomposer + Soil)



(A) Control Treatment - 4 (B) Spore forming inroot (X 100) (C) Collected spores

Figure 15. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere . Treatment-4 (Natural Mycorrhiza + Cow dung + Soil)



(A) Control Treatment - 5 Infected root and spore attachment X 100 (C) Collected spores

Figure 16. Growing of *Lactuca sativa* L. and mycorrhizal infection in its roots and spores in its rhizosphere. Treatment-5 (Mycorrhiza Fertilizer + Soil)

Table 5. Summarized on the infection percent of different treatments

% of infection					
	T1 (Control)	T2 (Natural mycorrhiza + Soil)	T3 (Natural mycorrhiza + Biocomposer + Soil)	T4 (Natural mycorrhiza + Cowdung + Soil)	T5 (Commercial Mycorrhiza Fertilizer+ Soil)
2 weeks	44	62	56	53	59
4 weeks	50	72	59	63	69
6 weeks	52	83	69	74	79

Table 6. Summarized on the number of spores of different treatments

Number of spores					
	T1 (Control)	T2 (Natural mycorrhiza + Soil)	T3 (Natural mycorrhiza + Biocomposer + Soil)	T4 (Natural mycorrhiza + Cowdung + Soil)	T5 (Commercial Mycorrhiza Fertilizer+ Soil)
2 weeks	35	**73**	41	65	52
4 weeks	39	**80**	58	**69**	62
6 weeks	44	**88**	69	**82**	79

Table 7. Index of Mycorrhizal inoculum potential (MIP) on *Lactuca sativa* L.

Treatment	**MIP index**
T_1	4.00
T_2	**10.50**
T_3	6.50
T_4	**10.50**
T_5	10.00

Discussion and Conclusion

In this research, mycorrhiza were collected from five weed plants: *Eclipta alba* (L.) Hassk., *Mimosa pudica* L., *Phyllanthus urinaria* L., *Tridax procumbens* L., and *Urena lobata* L. These mycorrhiza were introduced into *Lactuca sativa* L. The maximum rate of spore number and the highest colonization percent of VAM on lettuce plants were isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman). Root colonization percent were calculated into MIP assay method, because this techniques is simple and it can estimate the long term survival of VAM in the host (Bracker, 1999).

In these experiment, highest index of MIP were found in the application of natural mycorrhiza 1 Kg + soil 4 Kg (T_2) and natural mycorrhiza 1 kg + cow dung 0.5 Kg + soil 3.5 Kg (T_4) on lettuce cultivation. These findings were agreed with Hawkins and George (1999).

It was correlated with the number of spores in rhizosphere. Maximum rate of spore number were also found in T_2 and T_4. It was very possible, because other variable of measuring soil infectivity have been described (Table 6). Plenchette (1982) described an experiment whereby soil infectivity can be related from host infectivity relationship.

In fact VAM are obligate symbionts and therefore cannot be multiplied on laboratory media apart from a living host (Habte, 2000). According to present research, *Lactuca sativa* L. (lettuce) plant was suitable habitat for VAM, isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman). Best multiplication of VAM, isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman) can be obtained by mix with sterilized soil in 1:4 ratio.

In summarized, among the weed plants, *Eclipta alba* (L.) Hassk. (Kyeik hman) should be chosen as tracking plants for the multiplication of VAM in cultivation of lettuce plants. Furthermore, natural mycorrhiza 1 Kg + soil 4 Kg (T_2) and natural mycorrhiza 1 Kg + cow dung 0.5 Kg + soil 3.5 Kg (T_4) should be applied on the lettuce cultivation to be produced high MIP (Mycorrhizal Inoculum Potential) for the utilization of biofertilizer.

In conclusion, the utilization of organic fertilizers instead of inorganic fertilizers should be used in the growing plants to promote the organic farming system and to produce the high yield and more organic products of foods.

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