ISSN No:-2456-2165

Selection of Best Mycorrhizal Inoculum from Five Weed Plants and its Dependency on *Lactuca sativa* L.

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Abstract:- The present study is concerned with investigation of the mycorrhizal dependency on the lettuce plants not only to determine their growth but also for the multiplication of mycorrhiza inoculum. Mycorrhizal spores were collected from five weed plants in Mawlamyine University Campus by using floating adhesion technique (Sutton and Barron, 1972) and wet sieving method (Gerdmann and Nicolson, 1963). 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 (77% in November) were found from source plant of *Eclipta alba* (L.) Hassk. (Kyeik hman). Mycorrhiza isolated from *Eclipta alba* (L.) Hassk. (Kyeik hman) were used as natural mycorrhizal inoculum (nmi) and subjected into seedling of *Lactuca sativa* L.(lettuce) by using polyethylene bag experiments with different treatment. Mycorrhizal dependency of lettuce plants were evaluated with mycorrhizal inoculum potential assay (MIP) method (Jarstfer, A.G. 2002). According to the result, the highest mycorrhiza inoculum potential (MIP) index were obtained in combination of 1 kg (nmi) + 3.5 kg (Soil) T_2 (10.50) and 1 kg (nmi) + 0.5 kg (Cow Dung) + 3.5 kg (Soil) T_4 (10.50) respectively.

Keywords:- *Weed, Mycorrhizal Dependency, Mycorrhizal Inoculum, Spores, Eclipta Alba (L.) Hassk., Lactuca Sativa L.*

I. 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 vitamin 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). In the present study, natural mycorrhiza, commercial mycorrhiza and biofertilizer were treated on seedling of lettuce plants and observed their dependency on it.

II. MATERIAL AND METHODS

- *Source of natural 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, 2017 by floating adhesion technique (Sutton and Barron, 1972) and by wet sieving method (Gerdemann and Nicolson, 1963). The collected spores were identified into genus level by their, size, shape, colour and hyphal attachment according to Smith, 1997 and Miyasaka, 2003. Commercial mycorrhizal were purchased from local market.

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

The collected spores were identified by their, size, shape, colour and hyphal attachment.

- *Experimental Site*

A polyethylene bag experiment was conducted in Taunggyi University, Shan State from January to February, 2018. Each treatment was conducted by twenty replication with randomized design.

- *Preparation of Soil*

Soils are sterilized and added furan (fungicide 0.1% w/v) and maintained it at least 48 hours. Sterilized soil and selected natural mycorrhiza were mixed with 3:2 ratio and use as natural mycorrhiza inoculum (nmi). These natural mycorrhiza inoculum treated on seedling of *Lactuca sativa* L.(lettuce) with following treatment.

T1- 3.5kg (Soil) as control
T2- 1kg (nmi) + 3.5 kg (Soil)
T3- 1kg (nmi)+0.5kg (Biocomposer) +3.5kg(Soil)

ISSN No:-2456-2165

T4- 1kg (nmi) + 0.5 kg (Cow Dung) + 3.5 kg (Soil)
T5- 1kg (Commercial Mycorrhiza Fertilizer) +3.5kg (Soil)

➢ *Mycorrhizal inoculum potential (MIP) Assay (Jarstfer, A.G. 2002).*

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.

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

Where primary ingress refer to the results of differences between middle stage and initial stage infection percent and differences between middle stage and final stage infection percent was assumed as secondary spread.

➢ *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 calculated by using grid-line method (Newman, 1966). The total percentage of root colonization were determined by using the following formula:

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

III. 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. Most of spores were determined as Glomus. Morphological characters of collected VAM spores were 100-310 µm in size, reddish brown to black colour and globose shape, surface ornamentation was smooth, vesicle and bulbous suspensor cell are present (Fig.1).

The collected mycorrhiza spores and root colonization of five weed plants were shown in Tables 1 and 2. Number of mycorrhiza spores and colonization percent of different mycorrhiza inoculated lettuce plants were shown in Tables 3 and 4 Multiplication of VAM with different treatments on *lactuca sativa* L. were shown in Tables 5 and 6.

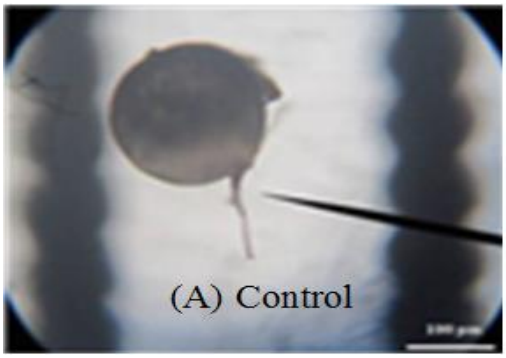

Fig 1:- Morphological characters of collected VAM spores

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

Table 1:- Colonization percent of VAM on roots of different plant sources

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

Table 2:- Number of VAM spores in 50g of different plant's rhizosphere soil

ISSN No:-2456-2165

	Name of source plants				
	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 3:- Infection percent of VAM (from different plant sources) on *Lactuca sativa* L

	Name of source plants				
	Eclipta alba (L.) Hassk.	*Mimosa pudica*	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

Table 4:- Number of VAM spores (from different plant sources) in 50g of *Lactuca sativa*'s rhizosphere soil

	Treatments				
	T_1	T_2	T_3	T_4	T_5
2 weeks	44± 1	62± 2	56± 1	53± 1	59± 1
4 weeks	50± 1	72± 1	59± 1	63± 1	69± 2
6 weeks	52± 1	83± 2	69± 1	74± 1	79± 1

Table 5:- Infection percent of VAM in *Lactuca sativa* L. with different treatments

	Treatments				
	T_1	T_2	T_3	T_4	T_5
2 weeks	35± 1	**73**± 2	41± 1	65± 1	52± 1
4 weeks	39± 1	**80**± 2	58± 1	**69**± 2	62± 1
6 weeks	44± 1	**88**± 1	69± 1	**82**± 1	79± 2

Table 6:- Number of VAM spores (with different treatments) in 50g of *Lactuca sativa*'s rhizosphere soil

Treatment	MIP index
T_1	4
T_2	**10.5**
T_3	6.5
T_4	**10.5**
T_5	10

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

IV. 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 (Jarstfer, A.G. 2002).

In these experiment, highest index of MIP were found in the application of natural mycorrhiza 1 Kg + soil 3.5 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 is suggest that index of MIP was correlated with the number of spores in rhizosphere. Maximum spore number were also found in T_2 and T_4. It was very possible, because other variable of measuring soil infectivity shown in similar concept (Table 6 and 7). Plenchette *et al.* (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 1:3.5 ratio with or without cowdung.

In summarized, among the selected 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 3.5 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 organic fertilizer.

In conclusion, the utilization of mycorrhiza inoculum from wild *Eclipta alba* as organic fertilizers instead of commercial fertilizers show the similar effect to produce more organic products of foods.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to Dr. Aye Pe, Professor and Head, Department of Botany, University of Yangon, for his permission, and for allow me for his permission to carry out this research.

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