ISSN No:-2456-2165

Isolation of Mycorrhiza and its Effect on Yield of *Capsicum annuum* L.(Pepper)

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Abstract:- In this experiments were carried out to isolation of mycorrhiza from roots and spore population from rihzosphere in five species of angiosperms and to investigate the effect of mycorrhiza fertilizer on the yield of *Capsicum annuum* L.(pepper) in Magway University during January to December 2017. These experiments included laboratory, pot were conducted at Magway University. In laboratory experiment mycorrhiza colonization of roots were calculated by grid-line intersection method and spore population were collected by spore decanting method. The highest colonization was observed in roots of *Allium cepa* L. (63.4%) and the Lowest colonization was occurred in *Andrographis paniculata* Nees. (22.10 %). Highest number of spore was observed in rhizosphere of *Zea mays* L.. (42). and lowest number of spore was observed in root of *Cleome gynandra* L. (28). In pot experiment, the effect of mycorrhiza infected roots on yield of pepper were also investigated. These results also indicated that yield parameter of the earliest day to flowering and fruiting was found in treated with VAM , and the latest was observed in the control. The yield component of pepper weight were obviously increased the effect of treated with VAM, than control at harvested time. According to result suggested that, the use of mycorrhiza had a higher positive effect of yield on *Capsicum annuum* L.(pepper)

Keywords:- *Mycorrhiza Colonization, Spore Population, Yield of Peppers.*

I. INTRODUCTION

A mycorrhiza ("fungus-root") is a type of endophitic, biotrophic, mutualistic symbiosis prevalent in many cultivated and natural ecosystems. There are three major groups of mycorrhiza: Ectomycorrhiza, Ectendomycorrhiza and endomycorrhiza. Ectomycorrhiza and endomycorrhiza are important in agriculture and forestry.

Davies *et al.* (2000) observed that mycorrhizae can improve P absorption in pepper and increase shoot and root dry weight .General nature of VAM to plant are; (1) Increase plant nutrient supply by extending the volume of soil accessible to plants. (2) Increase plant nutrient supply by acquiring nutrient forms that would not normally be available to plants. (3) Root colonization by VAM fungi can provide protection from parasitic fungi and nematodes. (4) Non nutritional benefits to plants due to change in wate relation, phytohormone levels, carbon assimilation, etc. (5) Mycorrhizal benefit can include greater yield, nutrient accumulation, and/or reproductive success (Brundrett *et al.* 1990).

Mycorrhizal fungi are species of fungi that intimately associate with plant roots forming a symbiotic relationship, with the plant providing sugars for the fungi and the fungi providing nutrients such as phosphorus, to the plants. Mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and release to plant cells in root tissue.

Pepper is one of many horticultural crops grown primarily in mid latitudes, and it is sensitive to high temperature. It is also an important vegetable in Turkey and the rest of the world (Sunsana *et. al.*, 2007).Present agricultural system in Myanmar follow the traditional methods which utilize the available natural resources combined with improved cultural practices. Microbiological fertilizer, more commonly known as biofertilizer, include phosphate solubilizing and plant growth promoting microorganism (Goel 1999). Horticulture is the most promising area for practical use of AMF for nursery. There are two main benefits from introducing mycorrhizal fungi to horticultural crops: stronger growth in the nursery and improved performance after planting in the field. Pepper (*Capsicum annuum* L.) is one of the most common crops produced in nurseries, and one of the most important in central regions of Myanmar.

The aim of this study was to investigate the mycorrhiza infection and spores collection from five species of plants and to determine the effect of the VAM on plant growth and yield of pepper plant in Magway University campus.

II. MATERIAL AND METHODS

❖ *Study of mycorrhiza infection and spore population of five species from Magway University Campus*

➢ *Collection of sample site*

In this research, roots and rhizospheres soil sample were collected from the growing five species of angiosperms in Magway University. These are *Allium cepa* L., *Andrographis paniculata* Nees., *Cleome gynandra* L. Miller, *Helianthus annus* L. and *Zea mays* L.were collected.

In this collection procedure, the soil sample collected from the plant base weighing 200 gm was dug about 5 cm away from the stem of each plant at the depth of 10 cm. The collected species of plants were mounted in the herbarium sheets. The collected soil samples were packed in plastic bags. The root samples were used freshly for isolation of mycorrhiza.

➢ *Plant Identification*

According to the morphology and floral characteristics, the collected plants were identified by using Backer (1963-1968), Cronquist (1981), Dassanayake (1980-2001), Heywood (1978), Hooker (1897), Kress (2003), Lawrence (1951).

➢ *Mycorrhiza colonization by staining method*

The root preliminary surveying and observation of mycorrhizal colonization by staining INVAM (International culture collection of vascular Arbuscular Mycorrhizal Fungi) method. The collected root samples were washed in water to remove adhering soil and sands. After through washing the fresh roots samples were removed all particulates and roots were cut into (1 cm) of root. About 20 segments (1 cm) of root from each plant was cut. They were cleared and stained to determine the intensity of VAM infection using the staining method (Phillips and Hayman 1970). Then the roots were boiled into 10% KOH for 10-15 minutes and after that washed the roots fragments with water.

Acidified into 2% HCL for about 10 minutes. Afterward, the stain was poured off into another container and the roots were immersed in the water in small beaker. Wash again in water and stained with hot 0.05% acid-fuchsine for about 20 minute, the stain was prepared by mixing with water, glycerin and lactic acid (1:1:1). These roots were placed on the slides and the slides were studied under microscope.

➢ *Mycorrhizal colonization analysis*

Mycorrhizal colonization was calculated by grid–line intersection method Newman (1996) 20 fragments of stained roots were randomly placed in petridish (2 cm diameter) which has gird lines of 0.5 cm square block line Counted the intersection of mycorrhizal mycelia on the grid - line under microscope. The percent of VAM infection was determined by the following formula:

$$\text{Percent of infection} = \frac{\text{Intersection of infected roots}}{\text{Total number of intersection roots}} \times 100$$

➢ *Extraction of spores from rhizosphere*

Mycorrhizal spores extraction were carried out from rhizosphere soil of ten species of angiosperms, which was naturally grown in Magway University by wet sieving and spore decanting method (Gerdeman and Nicolson 1963).

Take about 50 g of freshly soil with cut root sample from rhizosphere were taken. These samples were placed in a blender with small amount of water and blended at high speed for just about five second to break up root fragments. The spores were released and liberated from hyphal aggregate attached of roots and from the soil. After that the blended materials immediately poured through three sieves. Most sand remains in the blender. The spore were isolated from the collected soil by passing a suspension of soil through a series of sieves (500µm, 200µm,100µm, 45µm) respectively. The spore size of top sieve generally has 500µm. The capture root debris and large spores. The middle sieve was 100µm and the bottom sieve has about 45µm. The bottom sieve captures the majority of spores. All the spore were isolated from the extract with a fine forceps into a watch glass with small quantity of water. The extract with AM spores were observed under microscope and number of spores were counted.

Fig 1:- Fragment of roots

Fig 2:- staining roots

ISSN No:-2456-2165

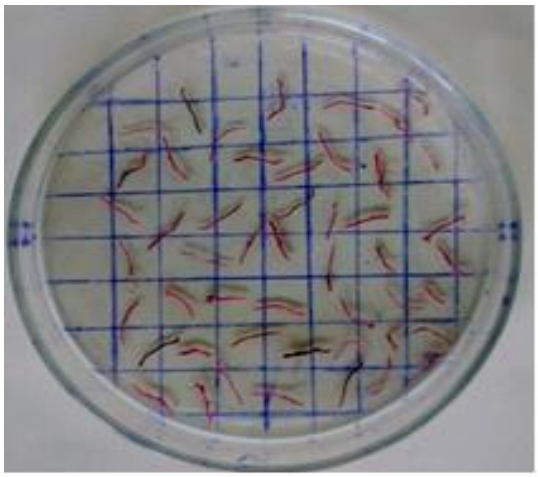
Fig 3:- Grid line intersection method


Fig 4:- Soil suspension blending


Fig 5:- Extraction of spore population

➢ *Preparation of materials*

Firstly, soil were exactly weighed to get 5kg for each pot (37.5cm × 50cm). Two kg of mycorrhiza were taken for each pot and then mixed thoroughly with the soils. In this experiment, totally 20 pots were used (10 for control and 10 for treatment). The seeds of peppers were loosely wrapped and soaked in water over night (at least 12 hours). Only three seeds were directly sown into the individual bags and regularly watered daily. In each pot, only one healthy seedling was left and the rest plants were thinned from the bags. During the planting, the management and cultural practices such as spraying of water and weeds control were done when necessary.

➢ *Data collection and analysis*

The effect of *Capsicum annuum* L. (pepper) such as fruit length and width, day of flowering and fruiting days, fruit weight of yield parameters were measured.

III. RESULTS

In this research, roots and rhizospheres soil sample were collected from the growing five species of angiosperms in Magway University during January to December 2017. The mycorrhiza infection in root of and the collected spore number were counted from rhizosphere were shown in Table 1.

No.	Family	Scientific name	Spore Number (50g Soil)	Mycorrhiza Infection (%)
1	Alliaceae	*Allium cepa* L.	35	63.4
2	Acanthaceae	*Andrographis paniculata* Nees.	32	22.10
3	Capparaceae	*Cleome gynandra* L.	28	41.2
4	Poaceae	*Zea mays* L.	42	30.2
5	Asteraceae	*Helianthus annus* L.	33	54.3

Table 1:- Mycorrhiza infection in roots and spore population in five species

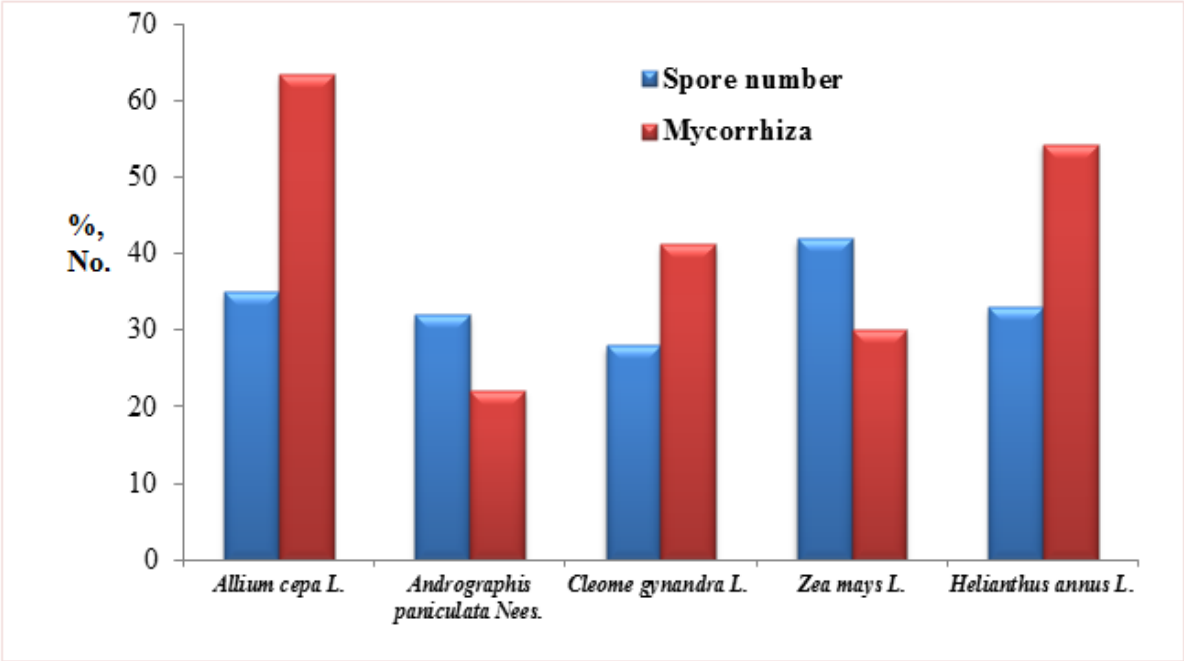

Fig 6:- Mycorrhiza infection in roots and spore population in five species

❖ *Pot experiment*

➢ *Effect of mycorrhiza fertilizer on the yield parameters of peppers*

The earliest flowering days (60) were found in VAM treated plants and the latest (75) was observed in the control. The earliest fruiting days (68) were found in VAM treated plants and the latest (84) was observed in the control.

Treatment	Flowering days (DAS)	Fruiting days (DAS)
Control	75	84
Mycorrhiza	60	74

Table 2:- Effect of mycorrhiza on flowering and fruiting days of *Capsicum annuum* L.
DAS=Day After Sowing

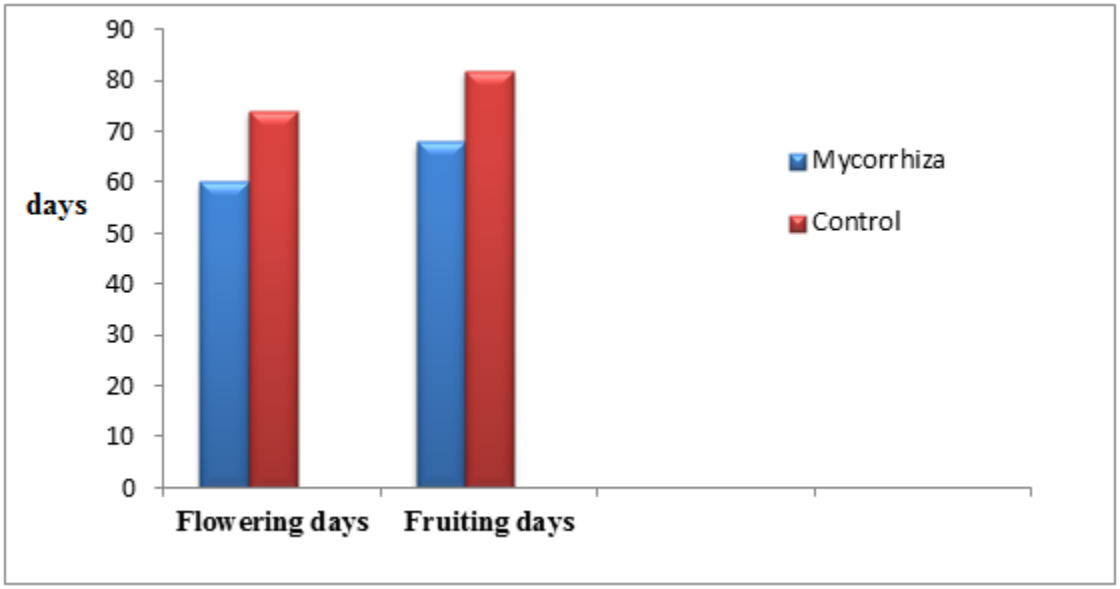

Fig. 7:- Effect of mycorrhiza on flowering and fruiting days of *Capsicum annuum* L.

ISSN No:-2456-2165

Treatment	Size		Weight(g)
	Width(cm)	Length(cm)	
Control	0.64	5.27	1.96
Mycorrhiza	0.80	5.86	2.35

Table 3:- Effect of mycorrhiza on fruiting size and weight of *Capsicum annuum* L.

IV. DISCUSSION AND CONCLUSION

In the present study emphasized on isolation of mycorrhiza from roots and spore number from rhizosphere of five species were collected from Magway University campus. Mycorrhiza colonization was calculated by using grid-line intersection method (Newman 1996). Nearly, all about the selected plants are observed in mycorrhiza colonization. In this investigation, the range of mycorrhiza colonization in roots was 22.10 % to 63.40 %. as shown in Table 1. The results showed that, the highest colonization of mycorrhiza in roots of *Allium cepa* L. is (63.40 %) and the lowest mycorrhiza colonization was *Andrographis paniculata* Nees. 22.10 % . Powell (1997) indicated that, the different species might be considered as good hosts for arbuscular mycorrhiza fungi. In this study, the root colonization ten species of angiosperms were different from one another. The present results agree with the statement of Powell (1997).

The spores extraction from rhizosphere soil were collected by wet sieving and spore decanting method. the highest number of spore was observed in *Zea mays* L. (42) rhizosphere and and the lowest number of spore was found in the *Cleome gynandra* L. (28).Zaw Myo Tun (2009) reported that Variations in present of root colonization were not related to spore population numbers.In this research, the spore number of ten species of angiospermae is different from one another, even at the same temperature and rainfall. The present results agree with the statement of Zaw Myo Tun(2009). In the present study, regarded soils and plants are mainly affected the mycorrhizal fungi and their development in conservation of soil health and soil fertility.

ACKNOWLEDGEMENTS

- We wish to acknowledge my profound gratitude to Dr Khin Mg Oo University of Magway, for providing me all the necessary facilities.
- We are greatly indebted to Dr Aye Aye Kyi, Professor and Head, Department of Botany, University of Magway, for her advices and providing all the necessary facilities from the department.
- Sincerely thanks are all our friends for their helps in the collection of specimens during in laboratory experiment.

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