Arbuscular Mycorrhizal Fungal (AMF) Inocula As Potent Bio-Fertilizers

Pampi Ghosh[1], N K Verma[2]
[1]Dept of Botany
[2] Retired Professor, Dept of Botany & Forestry
[1]Seva Bharati Mahavidyalaya, Kapgari, Jhargram, W.B., India
[2]Vidyasagar University, Midnapore-721 102, India

Abstract- *Bio-fertilizers are living organisms used as fertilizer which can change the rhizospheric environment and promote growth and development of the host plant without causing any harm. Arbuscular Mycorrhizal Fungal (AMF) bio-fertilizer is important one because it affects symbiotically in the roots of almost all plants and its rhizosphere surroundings. An AM fungal inoculation process is simple as they need no artificial media. It symbiotically penetrates in to the host plants feeder root cortex and makes a bridge between soil and roots. Various nutrients like N, P, K, Ca, Fe, Mn, Cu, Zn etc. absorbed by VAMF from the soil and transfer these to their hosts. VAM fungi improve soil structure, suppress plant diseases and improve plant tolerance to water stress, salinity, soil acidity and heavy metal toxicity. Large scale applications of AM fungi on selected plants show better yield as bio-fertilizer that increases chlorophyll content of leaves, carbohydrate and protein contents. Hope that isolates from locally available VAM fungi would be a boon to restore the ecosystem pristine.*

Keywords- AM-Fungi, Catharanthus roseus, AM-inoculation and Yield

I. INTRODUCTION

The symbiotic relationship between fungus and plant roots is called as mycorrhizae which was first time noticed by Vittadini (1842) and later the term mycorrhizae was coined by Frank (1885). There were several types of mycorrhizae and Vesicular Arbuscular Mycorrhizae are the most common symbiotic component of the roots of higher plants. Vesicular Arbuscular Mycorrhizal Fungi (VAMF) symbiotically penetrates into the host plants feeder root cortex and makes a bridge with soil. The VAMF absorb various nutrients like N, P, K, Ca, Fe, Mn, Cu, Zn etc. from the soil and transfer these to its hosts. Not only that, these fungi improve soil structure, suppress plant diseases and improve plant tolerance to water stress, salinity, soil acidity and heavy metal toxicity. In agriculture VAM colonized plants increases uptake of soil minerals so, it is possible to consider substantially reducing applications of fertilizers and pesticides and at the same time obtain equivalent or higher crop yields (Abbott and Robson, 1991). Mycorrhizal fungi contribute to carbon storage in soil by altering the quality and quantity of soil organic matter (Ryglewicz and Aderson, 1994). It is clear that mycorrhizal fungi play a vital role in nutrient cycling and productivity of crops (Smith and Read, 1997).Large scale applications of VAM fungi on selected plants show better yield as biofertilizer that increases chlorophyll content of leaves, carbohydrate and protein contents. Seema and Garampalli (2015) showed the root, shoot length and whole weight of fresh plant increased in mycorrhizae inoculated *Piper longum* plant against non-inoculated plant. Three AM fungi *viz. Glomus fasciculatum, Acaulospora fovata* and *Gigaspora margarita* were used as inocula in the study. Among these three, *Gigaspora margarita* showed better result in biomass yield. Kasliwal and Srinivasamurthy (2016) in their current study established influence of Arbuscular Mycorrhiza (*Glomus mosseae*) inoculation on *Hibicus rosa sinensis* and better result on application of VAM fungi. Hope that, the isolates from locally available VAM fungi from Southwest Bengal would be a boon to restore the ecosystem pristine. Remembering this, the present study was undertaken to isolate the VAM fungi and study in experimental condition.

II. STUDY AREA

The selected study site was conducted at net house of Vidyasagar University, Midnapore, West Bengal, India. Spores of VAM fungi were isolated from soil of Gopegarh near the downhill side of Medinipur-Dherua metallic road, Paschim Medinipur District. Geographically it is located in western part of the town nearly 4 kms away from the centre of the town and nearer the bank of river Kansai. It is situated nearly 3.5 kms away from Vidyasagar University nearer to Gopoegarh Eco-tourism and nature interpretation centre (Das and Ghosh, 2006, Ghosh, 2016).

III. MATETIALS AND METHODS

The starter culture of Glomus mosseae, Acaulospora laevis and Gigaspora margarita were obtained from Centre for Natural Biological Resources and Community Development (CNBRCD), Anand Nagar, Bangalore through email communication to Prof. D. J. Bagyaraj's Laboratory. For mass culture, soil was collected from the field and mixed with sand in 1:1 proportion. The sand soil mixture was sterilized by autoclaving at 15lb pressure for 1 hour followed by three

consecutive days. Earthen pots with 30 cm diameter and 15cm depth were surface sterilized with raw formalin solution and sun dried for three days. After that each pot was filed with 2 kg sterilized sand-soil mixture. Seeds of Sudan grass (Sorghum sp.) were surface sterilized with 0.5% NaOCl solution for 15 minutes. Starter culture of 20 gm each was spread on sand soil mixture and sterilized sorghum seeds were placed just over the substratum. It was covered by a thin film of sterilized sand soil mixture. The pots were watered with sterile distilled water as and when required. The pot culture was maintained up to 90 days and intermediate samplings were done to study the root infection. Similarly spore density was recorded from the culture soil to know the spore population. After 90 days the shoot portion was discarded and entire root system was chopped off and mixed thoroughly with sand soil mixture followed by air drying. The soil inoculums containing spores, mycelia, and infected root pieces were preserved in airtight polythene bags for future use at room temperature. The mass culture of the specimens contained ± 5.4 spores in 1 gm soil. Soil and sand mixture (1:1) was sterilized by autoclaving at 15lb pressure for 1 hour followed by three consecutive days. The prepared and sterilized sand soil mixture was used to mass production of VAM-fungal inocula as a whole.

Fine feeder roots of *Catharanthus roseus* plants were collected and cut into approximately 1cm pieces. Fragments were washed under tap water properly. Root samples were taken into labelled glass test tubes and 20% KOH solution was added to them so that samples were immersed into the solution properly. The test tubes were kept in the laboratory for three days. The cold treatment is though time consuming at the same time labour saving and easy (Utobo, *et al*., 2011, Zubek *et al.*, 2013, Ghosh, 2014). After three days roots were taken in nylon tea-sieves and washed under tap water. Then these pieces were soaked in dilute HCl solutions (1%) for 3- 4 minutes and again washed in tap water. Cleared root segments were stained by writing ink (Camel, Royal Blue) as a dye/stain. The samples with stain were kept in the same condition for at least 30 minutes prior observation after rinsing with acidified water. Pigmented root segments after cold treatment were immediately placed in freshly prepared alkaline H2O2 Solution at room temperature for 10 to 20 minutes or until roots are bleached (Utobo, et al., 2011). For accessing the root colonization following formula was used.

$$\text{Percent of root colonization} = \frac{\text{Number of root segments colonized}}{\text{Number of root segments observed}} \times 100$$

Quantification and separation of VA-mycorrhizal spores from rhizosphere soil of *Catharanthus roseus* plant was done by using wet sieving and decanting method (Gerdemann and Nicolson, 1963). From each stock soil sample 100 gm soil was taken and mixed with 1 L normal tap water in large beaker and stirred by glass rod until all the aggregates dispersed to leave a uniform suspension. Heavier particles were allowed to settle down. The suspension was passed through stack of sieves, 780 µm, 150 µm, 75 µm, 53 µm and 32 µm consecutively for several time repeats. The residues of respective sieves were collected in separate beaker. Then the aliquots were passed through filter paper placed in a glass funnel. To accumulate spores in a single circle clear water drops should be tickled through dropper. Now the filter papers were placed in wet Petri dish and spores were counted and observed through stereomicroscope (×40). Total spores were counted by adding the spore numbers of each respective filter paper spores. Spore density was calculated by counting the spores in the 100 gm of rhizosphere soil. Spores were separated by wooden dowel and mounted in lacto phenol for temporary work.

Soil was collected from the premise of Vidyasagar University garden at the depth of 20 cm. Soil was sterilized by formalin (38% formaldehyde) diluted with water (1:4) applied at the rate of 1 liter per 50 Kg. soil. The soil was sealed airtight with plastic bags for 15 days and then opened and spread to aerate for 20 days. Soil was examined for formalin free condition through direct testing by smell. Surface sterilization of selected seeds of experimental plant like *C. roseus* was done using 0.5% NaOCl solution for 15 minutes. All the plantlets were raised in square plastic tray using sterilized sand as medium and after a certain height similar seedlings were transferred in proper sterile polythene bags filled with 2 kg prepared sterile soil. Poly-pots were filled with 2 kg sterilized soil. A small hole of 5 cm in depth and 2 cm in diameter was dug out. Twenty five gm of selected three VAM fungal inoculums i.e. *Glomus mosseae, Gigaspora margarita* and *Acaulospora laevis*(Fig. 3, 4 and 5)), consisting of root fragments, mycelia and about 130 spores were placed into the hole of respective treatments. Then 2-3 previously raised aseptic plantlets were placed in the hole of respective pots so that inoculums and roots of plantlet could come close to each other. The treatments were: (i) control (sterilized soil without inoculums) - 24 (6 x 4) replicates (ii) sterilized soil + *Glomus mosseae* - 24 (6 x 4) replicates (iii) sterilized soil + *Gigaspora margarita* - 24 (6 x 4) replicates and (iv) sterilized soil + *Acaulospora laevis*- 24 (6 x 4) replicates for each experimental plant. The pots were placed in trays and were kept on raised iron tables specially made for it. Pots were watered on every alternate day initially but later watering was done on requirement basis. After two weeks thinning was done to maintain single seedlings per pot. The experiment was continued for six months in net house of Vidyasagar University. The experiment was laid out in eight (4 x 2) randomized blocks with 24 replicates of each treatment. For study of Chlorophyll, Arnon (1949); for Protein, Lowry (1951) and for Carbohydrate, Dubois (1956) method was followed which was appended in the reference book (Misra, 1968).

IV. RESULTS AND DISCUSSION

Periwinkle (*Catharanthus roseus*) is a potent medicinal plant as per the standard literature and known as

common garden and found in degraded stand of forest as well as in shrubberies of roadside. The effects of VAM inocula on the leaf number, leaf area, shoot length, root length, wet matter and dry matter production on *Catharanthus roseus* after 120 days harvest are presented in table 1. The effect of different VAM fungi inoculated C. *roseus* plant on different parameters as leaf number, leaf area, shoot length and root length showed increase over their control counterpart (Figure. 1). The wet weight of whole plant of VAM inoculated plants were increased over control. Highest total green weight increase was observed in case of *Gigaspora margarita* inoculated plant (1.675 g/plant) followed by *Acaulospora laevis* inoculated plant (1.54 g/ plant) and *Glomus mosseae* inoculated plant (0.872 g/plant) at 120 days harvest (Fig 2). The dry weight of whole plant of VAM inoculated plants was increased gradually over control. Highest dry weight increase was observed in case of *Gigaspora margarita* inoculated plant (0.251 g/plant) and followed by *Acaulospora laevis* inoculated plant (0.215 g/ plant) and *Glomus mosseae* inoculated plant (0.11 g/plant) at 120 days harvest (Fig 2).

Table1.Yield of Catharanthus roseus VAMF inoculated and un-inoculated control plant at 120 days after treatment (DAT)

Sl.No.	Inocula	Leaf area Sq.cm	Mean leaf number	Shoot length (cm/plant)	Root length (cm/plant)	Dry biomass of whole plant (g/plant)	Wet biomass of whole plant(g/plant)
1.	Un-inoculed control	1.36	4.6	8.166	5.5	0.026	0.13
2.	Glomus mosseae	3.20	7.5	11.66	12.00	0.11	0.872
3.	Gigaspora margarita	6.79	8.0	13.00	13.00	0.251	1.675
4.	Acaulospora laevis	4.11	7.0	12.25	21.75	0.215	1.54

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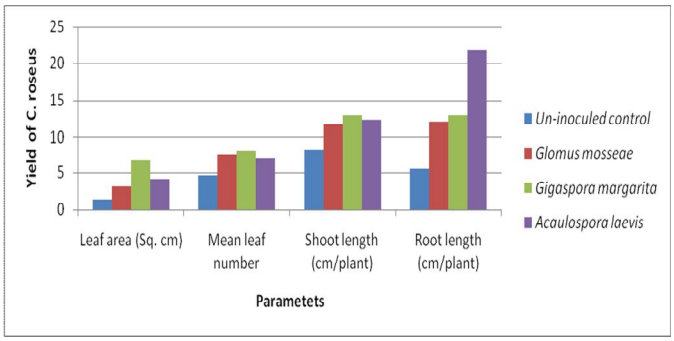

Fig. 1 Un-inoculated and VAM inoculated *C. roseus plant* yield at 120 DAT

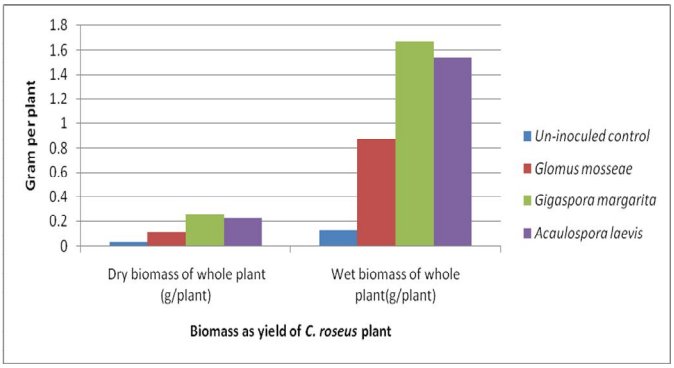

Fig. 2 Biomass production of VAM inoculated and un-inoculated control *C. roseus* plant at 120 day after treatment.

V. PHOTOPLATES

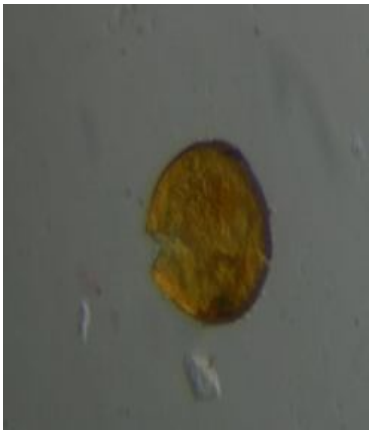

Fig. 3 Gigaspora margarita

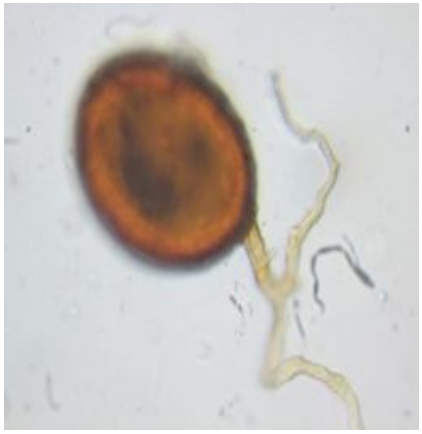

Fig. 4 Glomus mosseae

Fig. 5 Acaulospora laevis

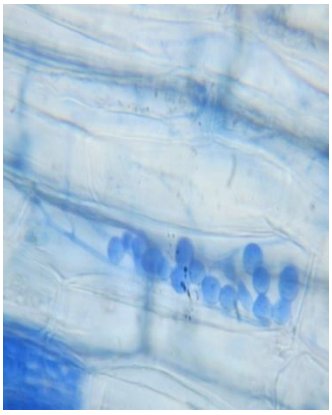

Fig. 6 Vesicles

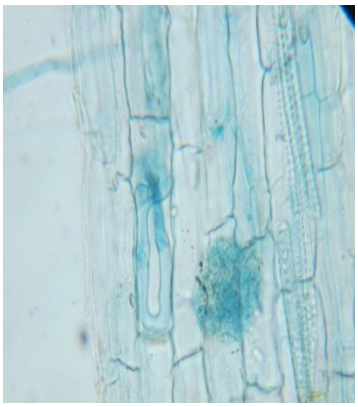

Fig. 7 Coiled hyphae

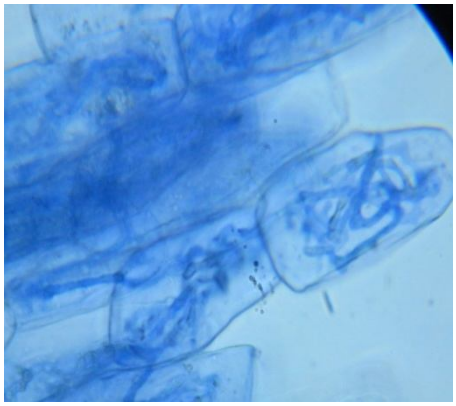

Fig. 8 Coiled hyphae

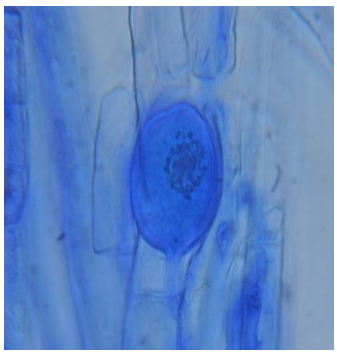

Fig. 9 Vesicle

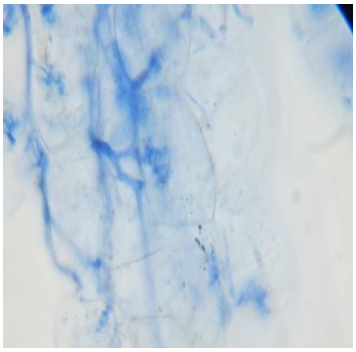

Fig. 10 Arbuscles

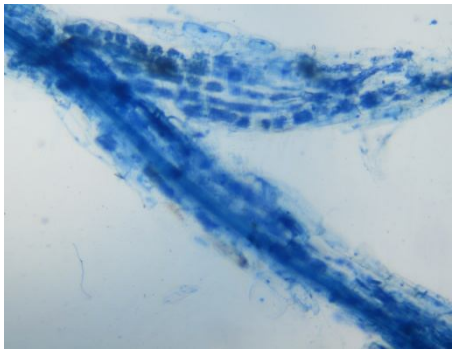

Fig. 11 Arbuscles

VI. CONCLUSION

It is concluded that more detailed study of spores and fungal taxonomy is required. From the large number of local variants VAMF spores may be isolated which may be a boon for further inoculums production and application to field in near future for betterment of the society.

VII. ACKNOWLEDGEMENTS

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