

Comparative physico-chemical study on *Catharanthus roseus* (L.) G. Don and *Kalanchoe pinnata* (Lam.) Pers plant after inoculation with *Glomus mosseae* as AM biofertilizer

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Abstract

Soil contains various microorganisms *i.e.* fungi, bacteria, algae, protozoa, millimoles and centipedes. Among them fungi is an important group of organisms. Among the fungi, one important type is arbuscular mycorrhizal fungi (AMF) which is ubiquitous in nature and found in rhizosphere soil which penetrates in root cortical cells of almost all plants as root associates and show mutual association. AM fungi are important tool to develop soil aggregation and soil structure development due to presence of glomalin. This means high AM fungi in an indicator of high root development and better resistance of host plants against soil borne pathogens. AMF help plants to grow better and resist against different stress prone condition. Therefore, rapid and wide application of AM fungi in any field procure good response for its host plants for better growth even to develop healthy seedlings in nursery condition. In this paper application of monoculture of AM fungi have been placed after study on Cape periwinkle and life plant. Result showed a comparative account on their growth behaviour which has been recorded as better due to inoculation by *Glomus mosseae* under experimental condition in compare to controlled condition. This AM fungi may be used as fungal biofertilizer in any trial or even in field condition for better growth and yield of plants for future generations.

A common symbiotic association of plant and fungi is regarded as AM when they produce arbuscles and make a relation of beneficial kind. The fungus which produces vesicle in plant's cortical cell is regarded as vesicular arbuscular mycorrhizal fungi (VAM).

These fungi are the most common and abundant coexistent fungi in soil and can coexist with more than 90 % of plant species to establish a symbiotic relationship¹⁰. These symbiotic relationships are found in almost all habitats and provide important ecological

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services including growth and health of the host plants in addition to the resistance of abiotic and biotic kind in harsh environment. AM fungi are beneficial mycorrhizae available in all types of rhizosphere soil and can infect almost all types of plants. These beneficial association in a particular kind helps to grow a better environment for plants to grow in a specific area. It is evident that AM-related host mycorrhizal pathways can also stimulate plant growth and physiology in nutrient-independent way. Arbuscular mycorrhizae help the host plant to accumulate nutrients such as nitrogen, phosphorus, and other macro and micro nutrients from rhizosphere soil to get its own carbohydrate from host plant in return⁷. Study revealed that AM inoculated plants show increase of root volume, root length and root biomass in comparison with un-inoculated plants in experimental conditions⁴. Host plants get benefit from AM fungal colonization that depends largely on the environmental and microclimatic conditions of the host plant¹. AM fungi are not host specific and remain either in active or dormant phase within the host plant rootlets and in rhizosphere soil. Distributions of AM fungi are ubiquitous. Soil with diverse AM fungal flora can support more diverse plant communities than if only one or few AM fungi are present in the rhizosphere or surrounding soil¹². Arbuscular mycorrhizal fungi exhibit 50 to 100% fungal root colonization both inter or intra cellular in way during infection. Study showed that mycorrhizal root is more efficient in phosphorus uptake than a non mycorrhizal root¹⁰.

In our lateritic West Bengal, *Glomus mosseae* is an important biological component of ecosystem and has significant role in increasing ecosystem efficiency and popular

as a useful organism in laboratory study. So, studies on some medicinal plants and *Glomus mosseae* now *Funneliformis mosseae* has been taken into account to know the efficacy in particular cases. *G. mosseae* was taken as laboratory AM organism to study the interactions between this AM fungi and chick pea to study the salinity stress in relation to nitrogen fixation, plant growth and nitrogen accumulation. Therefore the present AM fungi was applied on the growth of *C. roseus* and *K. pinnata* in experimental condition in red lateritic zone of southwest Bengal.

Pure culture of *Glomus mosseae* was purchased from CNBRCD for mass culture which was used during experimental inoculation. Sterilized sand-soil mixture (1:1) was prepared to use as base substratum for mycorrhizal development and sterilized sudan grass seeds were used as host plant for initial inoculation. After 60 days culture above part of sudan grass was copped out and rest of the soil and root parts were kept for dry out. It was used as pure culture inoculum. Two medicinal plants namely periwinkle and life plants were taken as host plant for inoculation with *Glomus mosseae* as symbiotic AMF organism. Seed of *C. roseus* and leaves of *K. pinnata* in sterilized condition was placed for germination and propagation respectively. After 20 days a single selected plant was transferred in sterilized soil containing pot (2 kg each) previously added with 50g *G. mosseae* inoculum against control (without *G. mosseae*). For each type of plant 6 replicas with 6 control sets were prepared. Experimentaion was completed at 120 dyas after transplantation (DAT). Samples of selected plant roots were taken separately into marked glass test tubes and 20% KOH solution was added to the container so that

samples were immersed properly in KOH solution for 3 days following^{3,11,14}. Treated roots were taken in nylon tea-sieves and washed under tap water to remove the KOH solution. After that roots were soaked in 1% dilute HCl solutions for 3 to 4 minutes and again washed in tap water to remove acid. Staining was done using Royal Blue writing ink. Stained root samples were kept in the same condition for at least 30 minutes prior to observation after rinsing with acidified water. Vesicular arbuscular mycorrhizal colonization in roots was assessed following slide method as per Giovannetti *et al.*⁶ and Mc Gonigle *et al.*⁹. Approximately 1cm stained root pieces were randomly placed on glass slide in groups of 5 to observe fungal hyphae, vesicles, arbuscules, coiled hyphae and other related structures under light microscope. The root colonization % was calculated as per the following formula

$$= \frac{\text{Number of root segments colonized/Number of root segments observed}}{\frac{\text{Number of root segments colonized/Number of root segments observed}}{X}} \times 100$$

Root colonization observation is very essential to know the host-AM interaction. The more colonization percentage may be due to more dependency on the AM fungi of the host plant.

Natural forest soil and agricultural lands contain several AMF species and

Glomus was a dominant genus over other genera of AMF⁵. Experimental study on two medicinal plants has been done after inoculation with *Glomus mosseae*. Two experimental plants used in this study were *Catharanthus roseus* and *Kalanchoe pinnata*. Experimental trials on these plants were made and 90, 120 days after transplantation (DAT) result was taken for control as well as for AM inoculated plants under controlled conditions. The effect of AM inoculums on the production of mean number of leaves on *Catharanthus roseus* is also increased (Table-1). Lowest percentage of green total chlorophyll was observed in case of *G. mosseae* inoculated plant compared to *K. pinnata* plant against control. The mean leaf number of AM inoculated *Kalanchoe pinnata* whole plant was increased over control plant (Table-2). Result showed that the effects of AM culture inocula on the total chlorophyll production on *Kalanchoe pinnata* was increased over control plant at 90 and 120 days after transplantation (Table-3). Similar result observed by Yaghoubian *et al.*¹³ on wheat treated with *G. mossaeae* and *Pyriformospora indica* in green house condition. Similar kind of experiment done by Chen *et al.*² on liquorice (*Glycyrrhiza uralensis*) and showed *G. mosseae* inoculated plant under stressed condition exhibit improvement of root system architecture and photosynthesis efficiency.

Table-1. Increase in leaf number and leaf size of *C. roseus* plant inoculated with *Glomus mosseae* at 120 days after transplantation (DAT)

DAT	Mean No of Leaves		% increase over control	Mean Leaf area in sq. cm.		% increase over control
	Control	+M	M	Control	+M	M
90 days	4.0	7.0**	75.00	1.20	3.20***	166.0
120 days	4.6	7.5**	63.04	1.36	3.20***	135.2

Table 2. Increase in leaf number and leaf size of *K. pinnata* plant inoculated with *Glomus mosseae* at 120 days after transplantation

DAT	Mean No of Leaves		% increase over control	Mean Leaf area in sq. cm.		% increase over control
	Control	+M	M	Control	+M	M
90 days	2.36	3.20 NS	35.5	2.2	4.3*	95.45
120 days	7.33	7.60 NS	3.68	3.55	7.4**	108.45

N.B. NS –Non significant

Table-3. Total chlorophyll content on *K. pinnata* and *C. roseus* plant inoculated with *G. mosseae* at 120 DAT

DAT	Total chlorophyll of <i>K. pinnata</i> Leaves (mg/g of tissue)		% increase of total chlorophyll over control for <i>K. pinnata</i>	Total chlorophyll of <i>C. roseus</i> Leaves (mg/g of tissue)		% increase of total chlorophyll over control for <i>C. roseus</i>
	-M(Con)	+M(Gom)	Gom	-M(Con)	+M(Gom)	Gom
90 days	0.06	0.101 NS	68.33	0.69	1.11 ***	60.86
120 days	0.07	0.136 NS	94.28	0.78	1.21 ***	55.12

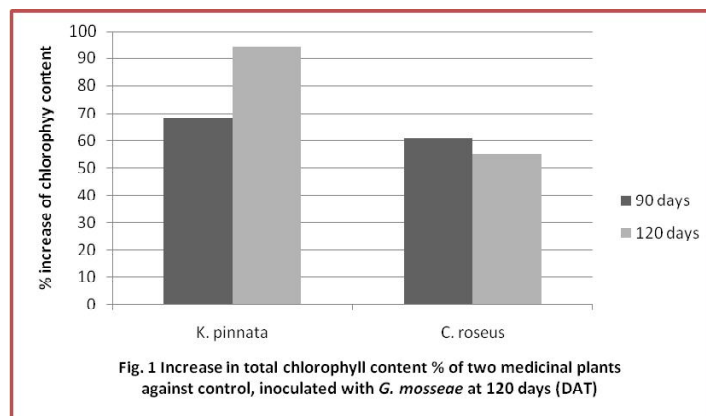


Fig. 1 Total chlorophyll content (%) of *C. roseus* and *K. pinnata* plant inoculated with *G. mosseae* at 90 and 120 DAT

It is concluded that *Catharanthus roseus* plant shows highest increase % of total chlorophyll content at 90 days after that increase in % over control plant decrease this means that there is a tendency of rapid growth of chlorophyll content induced by *Glomus mosseae* (Fig. 1). Opposite trend found in case

of *Kalanchoe pinnata*, here increase in chlorophyll content observed at 120 days after transplantation compared to 90 days result. In case of *K. pinnata*, higher chlorophyll content was observed at 90 days after transplantation compared to *C. roseus*. Not only total chlorophyll, separate study on different types of chlorophyll

content on yield of both the medicinal plant species after inoculation with same AM fungi may be done in near future to know the trends of yield in lateritic areas of West Bengal. This is because AM can help to grow better yield under high temperature stress condition and present study area fall under such category. Related research on such stress prone situation and AM fungi on yield of plant done by Mathur *et al.*,⁸. Therefore *G. mosseae* may be used as biofertilizer to grow better crop in a managed system under eco-friendly sustainable basis in near future.

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Conflicts of interest

None

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