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Vesicular Arbuscular Mycorrhizal Fungal status on some medicinal plants of Gopegarh, Midnapore and VA-mycorrhizal experiment on yield with *Catharanthus roseus* (L.) G. Don.

Pampi Ghosh[1] and N K Verma[2]
1-Department of Botany, Seva Bharati Mahavidyalaya, Kapgari, Paschim Medinipur, W.B.
2-Ex-Professor of Botany & Forestry, Vidyasagar University, Midnapore-721102

ABSTRACT : *Medicinal plants are important for our existence that supplies us many components for drug formulation. In nature the plant of particular kind invades with so many microorganisms. Among them one beneficial one is Va-mycorrhizal fungi. It helps in various ways to promote growth and yield of biomass better in natural habitats. So, to promote growth in garden or manmade environment application of VA-fungi as bio-fertilizer is beneficial. In this study 41 medicinal plants have been studied and application of VAM fungi inocula on Catharanthus roseus (L.) G. Don. have been done. Monsoon showed highest colonization percentage followed by winter and summer where as spore density showed highest during winter followed by summer and monsoon.*

Key words: *Medicinal Plants, VA-mycorrhizae,Colonization, spore density, yield.*

I. INTRODUCTION

Medicinal plants are important for sustenance of life in a present day scenario. The plant parts are used in the treatment of various ailments and the constituents are protein, alkaloid, steroid, saponin, phenol, tannin etc. which are biologically active and are capable to which may regulate or stimulate physiology of human system (Altschul and Von, 1973; Ross, 1999; Melchior *et al*. 2000; Merzouki *et al*., 2000).

As our natural environment is complex one which consists of a large and diverse number of flora, fauna and abiotic components, so a wide variation is always exist. All components are influenced by each other. Alteration of abiotic components of our natural environment, obviously affect the health of flora and fauna of the said ecosystem. The negative effects caused by alteration of environmental abiotic factors make deleterious effect and caused pollution. Using various chemical fertilizers we unknowingly or knowingly decreasing the soil fertility and at the same time loosing the rhizosphere organisms which help to make an eco-friendly soil. Soil microorganisms such as bacteria, fungi, algae, symbiotically abode at the roots of the plants and helps in many ways. Some bacteria and algae help to increase soil nitrogen and at the same time supply the nutrients to the plant.

Almost all land plants form symbiotic associations with mycorrhizal fungi. These below-ground fungi play a key role in terrestrial ecosystems as they regulate nutrient and carbon cycles, and influence soil structure and ecosystem multi-functionality (Marcel *et al*., 2015).

Some fungi like vesicular arbuscular mycorrhizae (VAM) symbiotically penetrate into the host plants feeder root cortex and make 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. 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 inoculoated *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. AREA UNDER STUDY

The selected study site was near the downhill side on Medinipur-Dherua metallic road under Southwest Bengal. 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 (Das and Ghosh,

2006). It consists of dry deciduous vegetation covered with plantation species like *Anacardium, Acacia, Eucalyptus, Ailanthus, Cassia* etc. Other shrubby species found there are *Helicteres isora, Premna latifolia, Smilax macrophylla, Ichnocarpus frutescens, Ipomoea* spp., and *Butea superba*. Herbs found luxuriously during monsoon on lateritic bed are species like *Ilysanthes* spp., *Leucas* spp., *Paspalum* spp., *Phyllanthus* sp., *Glenus* sp., *Andrographis paniculata* etc. Net house of Botany & Forestry Department, Vidyasagar University, Midnapore, West Bengal was used to conduct the experiment.

III. MATERIALS AND METHODS

Regular and periodical survey for consecutive two years (2013 to 2015) of study area of forests was conducted in Gopegarh of Southwest Bengal to collect medicinal plants with roots and rhizospheric soils. Medicinal plants along with the soil samples were collected from the selected marked small plots (20m x 20m) at regular intervals following Verma (2000).

Available and easy to uprooting medicinal plants with their intact roots and rhizospheric soil up to 10 cm depth was collected randomly from selected study areas. For seasonal study monsoon (July, August, September and October); winter (November, December, January, and February) and summer (March, April, May and June) were considered round the year.

Species wise fine feeder roots of the medicinal 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 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 H_2O_2 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 use.

Percent of root colonization = Number of root segments colonized/Number of root segments observed x 100

Quantification and separation of VA-mycorrhizal spores from each medicinal plant rhizosphere soil sample 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 Liter 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 soil. Spores were separated by wooden dowel and mounted in lacto phenol for temporary work. For permanent slide preparation for further work spores were mounted in Polyvinyl-alcohol-lacto-glycerol (Koske and Tessier, 1983). Sometimes glue was used for the same purpose in absence of PVLG (Anonymous, 1994).

The starter culture of *Glomus mosseae*, *Acaulospora laevis* and *Gigaspora margarita* were obtained from Centre for 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 30cm 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 20gm 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.

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 *Catharanthus 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.

Polypots 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 (*Glomus mosseae, Gigaspora margarita* and *Acaulospora laevis*), 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). Qualitative tests for alkaloid were done by Dragendroff's reagent using standard method.

IV. RESULTS AND DISCUSSION

During the period 2013-2014, monsoon (rainy) season showed highest colonization percentage in case of *Cissus adnata* Roxb. (96.6±1.2) followed by *Cassia occidentalis* L. (96±1.6) and lowest colonization percentage was observed in case of *Sporolobus indicus* (L.) R. Br. (29.3±0.9). In winter highest colonization percentage was observed in case of *Evolvulus nummularius* L. (80.3±1.2) and lowest colonization percentage was observed in case of *Ocimum sanctum* L. (19.3±0.9). Plants like *Curculigo orchioides* Gaertn.; *Lindernia ciliata* (Colsm.) Pennell and *Lindernia crustacean* (L.) F. Muell. were not visible in winter and summer but visible during monsoon. During monsoon colonization percentage of these three plants were 70±1.6, 87±4.2 and 59.6±2.05 respectively (Table 1). In the summer highest colonization percentage was observed in case of *Holarrhena pubescens* Wall. ex G. Don. (41.6±1.2) and lowest colonization percentage was observed in case of *Sebastiania chamaelea* (L.) Muell.-Arg. (11.6±1.2). In the next year (2014-2015), the result showed similar type of observation i.e. monsoon showed highest colonization percentage followed by winter and summer. Here, highest colonization percentage was observed in case of *Cassia occidentalis* (93.6±1.2) plus *Evolvulus nummularius* (93.6±1.2), *Evolvulus nummularius* (87±0.8) and *Holarrhena pubescens* (41.6±1.2) in monsoon, winter and summer respectively. The lowest colonization percentage was observed in case of *Sporolobus indicus* (24.6±2.62), *Ocimum sanctum* (16.6±1.24) and *Hemidesmus indicus* (10.6±0.94) in monsoon, winter and summer respectively. The average annual rainfall was more in the year 2013-2014 compared to 2014-2015 and it was observed that except few cases almost all plants showed more percentage of colonization in the year 2013-2014. Plants like *Aegle marmelos, Atylosia scarabaeoides, Bridelia retusa, Evolvulus nummularius, Rungia pectinata, Sida cordata, Spermacoce hispida, Tridax procumbens* and *Zizyphus oenoplia* showed more percentage of colonization in the year 2014-2015.

Highest Spore density of rhizospheric soil during monsoon was observed in case of *Andrographis paniculata* Nees. (609.3±8.9) followed by *Phyllanthus simplex* Retz. (579.6± 7.7) and lowest spore density was observed in case of *Dicliptera bupleuroides* Nees. (138.3± 6.9) in the year 2013-2014. The range between highest to lowest spore densities during the first year observation in monsoon was 609.3±8.9 to 138.3±6.9 (Table 2). In the next year observation spore number ranged from 485.3± 4.1 to 115.3± 11.11. Highest number of spore in the rhizosphere soil was observed in case of *Phyllanthus simplex* Retz. and lowest number was observed in case of *Dicliptera bupleuroides* Nees.

In the first year observation (2013-2014) it was found that highest spore density of rhizospheric soil during winter was recorded in case of *Cassia occidentalis* L (800±14.1) followed by *Sida cordifolia* L. (793.3±4.7) and lowest spore density was observed in case of *Hemidesmus indicus* R. Br. (419.3±6.5). In the second year observation (2014-2015) the range between highest to lowest spore densities during winter was 746.6±12.4 to 380.3±12.6 per 100 gm rhiozospheric soil (Table 11). It was observed that spore density in rhizosphere soil of the study area was little beat lower during second year than the first year observation.

Highest Spore density of rhizospheric soil during summer was observed in case of *Rungia pectinata* (L.) Nees. (667.3±9.9) followed by *Aegle marmelos* Corr. (630±8.6) and lowest spore density was observed in case of *Hemidesmus indicus* R. Br. (328±7.4) in the year 2013-2014. The range between highest to lowest spore densities in

rhizospheric soil during summer in the next year observation was 585.3±9.2 to 301.6 ± 6.2 per 100 gm rhizosphere soil respectively in case of *Ziziphus oenoplia* (L.) Mill. and *Hemidesmus indicus* R. Br. In the study site all studied rhizosphere soils contained a good number of VAMF spore.

In total, 41 medicinal plant species was selected and studied under 23 families and 35 genera (Table 1) from Gopegarh. Family Euphorbiaceae showed highest species number (five) and colonization percentage ranged between 96.3±1.47 (*Phyllanthus simplex* Retz.) to 11 ± 0.8 (*Sebastiania chamaelea* (L.) Muell.-Arg.) in the successive two years study.

The effect of VAM inocula on the wet matter and dry matter on *Catharanthus roseus* are presented in table 3. The wet weight of whole plant of VAM inoculated plants were increased over control and were highly significant in all cases. Highest total green weight increase was observed in case of *Gigaspora margarita* inoculated plant (1.88 gm/plant) followed by *Acaulospora laevis* inoculated plant (1.82 gram/ plant) at 180 days harvest (Fig 1) and which was significant at $p<0.01$ level .The highest percentage of total wet weight increment was observed in *Gigaspora margarita* inoculated plant (1243 %) followed by *Acaulospora laevis* inoculated plat (1200 %) at 180 days harvest. Lowest percentage of wet weight increase over control counterpart was observed in case of *Glomus mosseae* inoculated plant in all harvesting periods and total wet weight increase was significant at $p<0.01$ and $p<0.001$ level.

The dry weight of whole plant of VAM inoculated plants was increased gradually over control in all harvesting periods and significant in all cases except one. Highest total dry weight increase was observed in case of *Gigaspora margarita* inoculated plant (865 %) at 120 days harvest and (781 %) at 180 days harvest. *Acaulospora laevis* inoculated plant showed high percentage of increase of dry weight over control plants and the result was significant (Fig 2.). Lowest percentage of total dry weight increase over control counterpart was observed in case of *Glomus mosseae* inoculated plant in all harvesting periods and was highly significant ($P<0.001$). Chlorophyll pigment is main pigment for photosynthesis in plant and mainly distributed in leaf tissue. The chlorophyll content was influenced by the effect of three different VAM fungi treatment in context of control at four different harvesting periods. The data are listed in table 4. The total chlorophyll content in the leaf tissue of VAM inoculated *C. roseus* showed increase of chlorophyll content over control plant and the result was highly significant in all cases except one. Highest percentage of increase of chlorophyll content over control plants leaf tissue was observed in *Gigaspora margarita* inoculated plant at 90 days harvest (189.85 %) followed by 120 days harvest (167.94 %). Highest amount of chlorophyll (2.12mg/gm leaf tissue) was observed in *Gigaspora margarita* inoculated plant at 150 days harvest followed by (2.09 mg/gm leaf tissue) 120 days harvest of same VAM inoculated plant (Fig.3). Least amount of chlorophyll increase was observed in *Glomus mossea* inoculated plant in all harvesting periods (Table 4). The total carbohydrate content in the leaf tissue of VAM inoculated *C. roseus* showed increase of carbohydrate content over control plant and the result was highly significant in all cases. Highest percentage of increase of carbohydrate content over control plants leaf tissue was observed in *Gigaspora margarita* inoculated plant at 150 days harvest (90 %) followed by 180 days harvest (85 %). Highest amount of carbohydrate content (159 mg/gm leaf tissue) was observed in *Gigaspora margarita* inoculated plant at 180 days harvest followed by 150 days harvest (156 mg/gm leaf tissue) of same VAM inoculated plant (Fig. 4). Least amount of carbohydrate increase was observed in *Glomus mosseae* inoculated plant in all harvesting periods but the result is highly significant at $p<0.01$ level (Table 5).

The total protein content in the leaf tissue of VAM inoculated *C. roseus* showed increase of protein content over control plant though the result was not highly significant in all cases. Highest percentage of increase of protein content over control plants leaf tissue was observed in *Acaulospora laevis* inoculated plant (586 %) at 180 days harvest followed by *Gigaspora margarita* inoculated plant (488 %) at 180 days harvest. Highest amount of protein content (7.0 mg/gm leaf tissue) was observed in *Acaulospora laevis* inoculated plant followed by *Gigaspora margarita* inoculated plant (6.0 mg/gm leaf tissue) at 180 days harvest period (Fig. 5). Least amount of protein content increase was observed in *Glomus mosseae* inoculated plant in all harvesting periods and showed that the result is not significant at all harvesting periods (Table 6).

The qualitative test for alkaloids on experimental plants leaf tissue showed different range of precipitation on same amount of tissue extract. The increase of precipitation was observed in VAM inoculated plants over un-inoculated one. Leaf tissue extract of *Catharanthus roseus* showed highest degree of precipitation for alkaloid test in *Acaulospora laevis* inoculated plants followed by *Gigaspora margarita* and *Glomus mosseae* at 180 days harvesting periods (Table 7).

TABLES

Table-1 Seasonal aspects of Vesicular Arbuscular Mycorrhizal colonization status on selected medicinal plants of Gopegarh forest area of Paschim Medinipur District

Sl No.	Scientific Name of the selected plants	Family	Season wise root Colonization (R- rainy, W-Winter, S-Summer) 2013-2014			Season wise root Colonization (R- rainy, W-Winter, S-Summer) 2014-2015		
			Rainy/Monsoon (Mean of 3)	W inter (Mean of 3)	Summer (Mean of 3)	Rainy/Monsoon (Mean of 3)	W inter (Mean of 3)	Summer (Mean of 3)
1	*Aegle marmelos* Corr.	Rutaceae	70.3±1.24^{c-f}	42.6±2.05^{f-k}	34±3.2^{a-e}	73.3±2.6^{d-h}	43±1.6^{g-m}	33.3±1.2^{a-d}
2	*Andrographis paniculata* (Burm.f.)Wall.ex Nees.	Acanthaceae	90.6±0.8^{ab}	56±3.2^{c-f}	32±3.2^{a-e}	88±1.6^{a-c}	49±0.8^{fj}	31.3±0.4^{a-e}
3	*Anisomeles indica* O. Kuntze	Lamiaceae	45.6±1.6^{k-n}	28±1.6^{k-n}	PNV	45.6±1.6^{m-o}	25.3±1.2^{n-o}	PNV
4	*Atylosia Scarabaeoides* (L.) Benth.	Fabaceae	69±3.7^{c-g}	64.3±3.8^{b-c}	25.6±3.09^{b-g}	83.3±3.3^{a-e}	81.6±3.8^{ab}	27.6±2.05^{a-e}
5	*Azadirachta indica* Adr. Juss.	Meliaceae	60±1.4^{f-k}	32.6±2.4^{i-m}	25.6±1.2^{b-g}	58±0.8^{i-m}	30±0.8l-p	26.3±0.4a-f
6	*Bonnaya brachiata* Link. & Otto.	Scrophulariaceae	90.3±1.2^{ab}	PNV	PNV	87.6±1.6^{a-d}	PNV	PNV
7	*Bridelia retusa* (L.) Sperng.	Euphorbiaceae	56 ±3.2^{f-l}	47.3±1.4^{d-i}	22±1.6^{b-g}	57±0.8^{i-m}	55±0.8^{fg}	22±0.8^{d-h}
8	*Cassia occidentalis* L.	Caesalpiniaceae	96±1.6^{a}	50.6±4.1^{c-h}	37±3.2^{a-d}	93.6±1.2^{a}	44.6±1.2^{g-l}	33±1.41^{a-e}
9	*Cassia tora* L.	Caesalpiniaceae	60±1.4^{f-k}	35.3±3.3^{h-l}	25.3±4.1^{b-g}	51±1.41^{k-o}	32±0.8^{k-o}	24.3±0.47^{c-h}
10	*Catharanthus roseus* (L.) G. Don.	Apocynaceae	56±1.6^{f-l}	42.3±2.05^{f-k}	29.6±1.2^{a-e}	52.3±2.05^{j-n}	38.6±1.2^{h-n}	29±2.44^{a-e}
11	*Cissus adnata* Roxb.	Vitaceae	96.6±1.2^{a}	51.3±4.1^{c-g}	PNV	91.3±1.2^{ab}	48±1.4^{fj}	PNV
12	*Clerodendrum viscosum* Vent.	Verbenaceae	89.6±2.04^{ab}	75.3±4.1^{ab}	40±1.6^{a-c}	87.3±2.49^{a-d}	70.3±1.6^{b-e}	39.6±2.05^{a-c}
13	*Combretum decandrum* Jacq.	Combretaceae	68.6±1.6^{c-g}	40.6±1.6^{g-k}	27.6±1.6^{a-f}	65.6±1.2^{f-k}	35.6±1.2^{j-n}	25.3±2.05^{b-h}
14	*Curculigo orchioides* Gaertn.	Hypoxidaceae	70±1.6^{c-f}	PNV	PNV	66.3±0.47^{f-k}	PNV	PNV
15	*Dicliptera bupleuroides* Nees.	Acanthaceae	70.3±2.6^{c-f}	50±2.1^{c-h}	PNV	66.6±1.2^{f-j}	45.6±1.69^{f-k}	PNV
16	*Eupatorium odoratum* L.	Asteraceae	93±3.5^{ab}	75.3±1.2^{a-b}	21.3±0.4^{e-g}	92±1.63^{ab}	73±2.86^{bc}	21.6±1.24^{d-h}
17	*Evolvulus nummularius* L.	Convolvulaceae	92±1.6^{ab}	80.3±1.2^{a}	26±0.8^{b-d}	93.6±1.24^{a}	87±0.8^{a}	26.3±0.47^{a-f}
18	*Flacourtia vulgare* Mill./	Flacourtiaceae	68±1.6^{c-g}	49.6±1.2^{c-h}	40.6±0.9^{ab}	65.6±1.24^{f-k}	49.3±0.9^{fj}	40.3±1.69^{ab}
19	*Hemidesmus indicus* R. Br.	Asclepiadaceae	38.6±2.6^{no}	19.6±1.2^{m}	12.3±2.6^{f-h}	36.6±1.24^{op}	17±0.8^{p}	10.6±0.94^{hi}
20	*Holarrhena pubescens* Wall. ex G. Don.	Apocynaceae	61.3±3.8^{e-j}	60.3±0.4^{c-e}	41.6±1.2^{a}	61.6±1.69^{g-l}	60.6±0.47^{c-f}	41.6±1.24^{a}
21	*Jatropha gossypifolia* L.	Euphorbiaceae	55.3±4.4^{f-l}	48.3±1.2^{d-h}	30±1.6^{a-e}	53.6±1.69^{j-n}	48±1.6^{fj}	30.3±1.24^{a-e}
22	*Lantana camara* L.	Verbenaceae	55±4.08^{f-l}	45.3±2.4^{e-j}	34±1.6^{a-e}	52.6±1.69^{j-n}	43.6±1.24^{g-l}	32.6±0.9^{a-e}
23	*Lindernia crustacean* (L.) F. Muell.	Scrophulariaceae	59.6±2.05^{f-k}	PNV	PNV	57.6±2.05^{i-m}	PNV	PNV
24	*Mimosa pudica* L.	Mimosaceae	55.3±1.2^{fl}	48±1.6^{d-h}	22.3±1.6^{d-g}	51.6±1.24^{j-n}	47.6±1.69^{fj}	22.3±1.24^{d-h}

25	*Ocimum americanum* L.	Lamiaceae	61±1.4 $^{e-j}$	40.3±1.2^{g-k}	29.3±1.6^{a-e}	59±0.8^{h-m}	39±1.63^{h-n}	29±2.1^{a-e}
26	*Ocimum sanctum* L.	Lamiaceae	79±1.4 $^{b-d}$	19.3±0.9^{m}	PNV	75±2.44^{c-g}	16.6±1.24^{p}	PNV
27	*Phyllanthus amarus* Schum & Thonn.	Euphorbiaceae	65.6±3.2 $^{d-h}$	19.6±1.2^{m}	PNV	60.3±1.24^{g-m}	18±1.63^{op}	PNV
28	*Phyllanthus simplex* Retz.	Euphorbiaceae	96.3±1.47 ^{a}	60.6±1.6^{c-e}	PNV	88.3±1.24^{a-d}	58±1.63^{d-g}	PNV
29	*Polygala chinensis* L.	Polygalaceae	57±5.09 $^{f-l}$	41.6±1.2^{f-k}	PNV	55.6±2.62^{j-m}	38.3±1.24^{h-n}	PNV
30	*Rothia indica* (L.) Druce	Fabaceae	65.3±4.1^{d-i}	53±0.8^{c-g}	PNV	63.3±1.69^{f-k}	49.3±1.69^{fj}	PNV
31	*Rungia pectinata* (L.) Nees	Acanthaceae	80±1.6^{b-d}	62±1.6^{b-d}	12.3±2.05^{f-h}	83±0.8^{a-e}	76.3±1.24^{ab}	11.6±1.24^{f-i}
32	*Sebastiania chamaelea* (L.) Muell.-Arg.	Euphorbiaceae	82±1.6^{a-c}	74.6±2.4^{a-b}	11.6±1.2^{g-h}	77.6±1.69^{b-f}	71±2.1^{b-d}	11±0.8^{f-i}
33	*Sida acuta* Burm.f.	Malvaceae	39.6±1.2^{m-o}	31.3±0.8^{j-m}	19.6±1.24^{e-g}	37±0.8^{op}	28.3±1.24^{m-p}	19±0.8^{d-h}
34	*Sida cordata* (Burm. f.) Borss.	Malvaceae	57±2.1^{f-l}	49.6±0.4^{e-h}	23.3±2.4^{d-g}	59±0.8 $^{h-m}$	52±1.6^{fi}	23±0.8^{d-h}
35	*Sida cordifolia* L.	Malvaceae	50±2.1^{i-n}	39.3±0.9^{g-k}	29.6±1.2^{a-e}	47.6±2.05^{l-o}	38±1.63^{i-n}	23.6±1.69^{d-h}
36	*Spermacoce hispida* Linn.	Rubiaceae	52±1.6^{h-n}	29.6±1.08^{km}	27.6±1.08^{a-f}	52±1.63^{j-n}	32±1.63^{k-o}	27±0.8^{a-e}
37	*Sporolobus indicus* (L.) R. Br.	Poaceae	29.3±0.9^{O}	22±1.6^{lm}	PNV	24.6±2.62^{p}	20.3±1.69^{op}	PNV
38	*Stephania japonica* (Thunb.) Miers.	Menispermaceae	75.3±4.1^{c-e}	59±1.4^{c-e}	19.6±1.2^{e-g}	72±1.63^{e-i}	56.6±1.24^{e-g}	17.6±1.24^{e-h}
39	*Tridax procumbens* L.	Asteraceae	54±4.3^{g-m}	45.3±2.4^{e-j}	31±2.1^{a-e}	57±2.1^{i-m}	53.6±2.05^{fh}	30.3±0.47^{a-e}
40	*Ziziphus oenoplia* (L.) Mill.	Rhamnaceae	47.3±2.0^{j-n}	38.6±2.6^{g-k}	25±0.81^{c-g}	57.6±2.05^{i-m}	55.3±2.49^{fg}	25±0.8^{b-h}
41	*Zornia diphylla* (L.) Pers.	Fabaceae	43±2.5^{l-o}	32±1.6^{j-m}	PNV	40.3±1.69^{no}	30±0.8^{l-p}	PNV

Note: PNV-Plant not visible, NA-Not Applicable, DP-Dead Plant, , *Holarrhena antidysenterica* (L.) Wall. *ex* Dc. = Syn. *H. Pubescens* Wall. ex G.

Don. , Each value represents mean of three soil samples. Mean values followed by the same superscript in each column do not differ significantly

at P=0.05 level by Duncan's Multiple Range Test (DMRT).

Table-2 Seasonal aspects of Vesicular Arbuscular Mycorrhizal Fungal spore density in rhizospheric soil of selected medicinal plants at Gopegarh, Paschim Medinipur District.

Sl. No.	**Scientific Name of the selected plants**	**Season wise Spore density in 100g rhizospheric soil for one 2013-2014 (R- rainy, W-winter, S-summer)**			**Season wise Spore density in 100g rhizospheric soil for a year 2014-2015 (R- rainy, W-winter, S-summer)**		
		R	W	S	R	W	S
1	*Aegle marmelos* Corr.	436.6±12.4^{j-m}	660±4.24^{g-i}	630±8.6^{b}	325±4.08^{op}	566.6±12.4^{h}	486.6±12.4^{fg}
2	*Andrographis paniculata* Nees.	609.3±8.9^{a}	697.6±7.4^{e}	623±5.09^{b}	417±12.56^{e-g}	685±4.08^{b}	510±8.16^{cd}
3	*Anisomeles indica* O. Kuntze	402±5.8^{no}	535±8.3^{o}	PNV	313.3±12.47^{pq}	506.6±16.9^{jk}	PNV
4	*Atylosia Scarabaeoides* (L.)	442±8.6^{h-k}	596.6±10.	466.3±12.	313.3±12.4^{p}	483.3±12.4	400±8.16^{j}

	Benth.		8[jk]	4[j]	q	mn	
5	*Azadirachta indica* Adr. Juss.	433.3±12.4[k-m]	542±6.04[no]	486.6±9.4[i]	383.3±4.7[kl]	502±10.7[kl]	428.3±8.4[i]
6	*Bonnaya brachiata* Link & Otto	471.3±15.6[g]	PNV	PNV	402.±10.7[hj]	PNV	PNV
7	*Bridelia retusa* (L.) Sperng.	424.3±4.7[m]	550.3±6.7n	485±10.8[i]	356.6±4.7[1m]	506.6±9.4[jk]	440±16.32[i]
8	*Cassia occidentalis* L.	475.3±4.1[g]	800±14.1[a]	525±10.8[fg]	405.3±15.17f-j	746.6±12.4a	503.3±12.4de
9	*Cassia tora* L.	454.3±4.1[h]	648±11.2[i]	530±8.1[f]	400±8.1[ij]	518.6±6.59[j]	480±8.1[gh]
10	*Catharanthus roseus* (L.) G. Don.	316.6±23.6[s]	576±17.2[m]	424±17.2[l]	234±5.88[t]	500.6±8.99kl	390±8.16[j]
11	*Cissus adnata* Roxb.	525.3±3.6[c]	757.6±13[c]	PNV	413.6±10.65e-i	655.3±12.2c	PNV
12	*Clerodendrum viscosum* Vent.	453±4.9[hi]	658±7.4[hi]	548.6±6.5[e]	335±14.71[no]	543.3±4.71[i]	470±8.16[h]
13	*Combretum decandrum* Jacq.	340.6±7.3[r]	545.6±6.7no	448.6±6.5[k]	233.3±12.47t	486±4.32[m]	396±4.32[j]
14	*Curculigo orchioides* Gaertn.	524.3±13.8[c]	PNV	PNV	434.6±4.98[c]d	PNV	PNV
15	*Dicliptera bupleuroides* Nees.	138.3±6.9[u]	547.6±3.6no	PNV	115.3±11.11u	446±4.89[o]	PNV
16	*Eupatorium odoratum* L.	478±10.7[fg]	792.6±8.9ab	546.3±6.9[c]	346.6±12.47mn	676.6±12.4b	510.6±8.21cd
17	*Evolvulus nummularius* L.	426.6±6.7[lm]	548.3±5.3no	460±7.3[jk]	315.3±4.10[p]q	480.6±7.36mn	386.6±4.71[j]k
18	*Flacourtia vulgare* Mill./	371.6±6.5[q]	603±3.7[jk]	390±6.5[n]	278.6±9.84[s]	580.6±8.2[fg]	372.33±6.12[l]
19	*Hemidesmus indicus* R. Br.	286±11.7[t]	419.3±6.5q	328±7.4[o]	280.6±4.71[rs]	380.3±12.6p	301.6±6.23m
20	*Holarrhena pubescens* Wall. ex G. Don.	321.6±6.9[s]	544.6±8.0no	409±6.9[m]	281±2.94[rs]	486.6±10.6m	373.3±6.18kl
21	*Jatropha gossypifolia* L.	390±8.16[op]	572.3±7.1m	507.3±5.2[h]	373±8.83[l]	471.3±6.59n	438.6±6.59[i]
22	*Lantana camara* L.	433.3±9.4[k-m]	609.3±8.9[j]	523.6±4.4[f]g	392.6±8.9[jk]	580.6±8.2[fg]	488±7.48[fg]
23	*Lindernia crustacea* (L.) F. Muell.	493.6±9.6[e]	PNV	PNV	419.3±7.36[ef]	PNV	PNV
24	*Mimosa pudica* L.	384.3±9.1[pq]	579±9.4[m]	451±6.9[k]	308.6±13.29q	481.3±6.59mn	386±4.89[jk]
25	*Ocimum americanum* L.	439.6±8.1[i-l]	680.6±8.9[f]	520.6±7.6[f-h]	323.3±6.79[o]p	568.6±3.3[gh]	498±9.79[d-f]
26	*Ocimum sanctum* L.	537.6±5.6[c]	669.3±8.9[f-h]	PNV	440.6±22.23c	598±10.7[e]	PNV
27	*Phyllanthus amarus* Schum & Thonn.	449.3±7.3[h-j]	681.3±6.5[f]	PNV	330±4.8[o]	513.3±10.6[j]k	PNV
28	*Phyllanthus simplex* Retz.	579.6±17.7[b]	768±10.7[c]	PNV	485.3±4.1[a]	691.3±6.5[b]	PNV
29	*Polygala chinensis* L.	533.3±11.6[c]	675±4.5[f]	PNV	422.6±8.9[de]	596.6±4.7[e]	PNV
30	*Rothia indica* (L.) Druce	535.3±4.1[c]	673.3±8.4[f]g	PNV	410±8.1[e-i]	586±4.3[ef]	PNV
31	*Rungia pectinata* (L.) Nees	568±16.0[b]	783.6±5.7b	667.3±9.9[a]	421.3±6.5[de]	690.6±8.2[b]	522±5.8[c]
32	*Sebastiania chamaelea* (L.) Muell.-Arg.	533.6±12.9[c]	674.3±4.4[f]	571.6±4.6[d]	434.6±10.8[c]d	588.6±9.8[ef]	490±8.1[e-g]
33	*Sida acuta* Burm.f.	498.3±6.9[e]	769±8.2[c]	552.3±11.1[e]	415.3±11.1[e-h]	678.6±8.3[b]	506.6±4.7[d]
34	*Sida cordata* (Burm. f.) Borss.	410.3±8.6[n]	593.3±4.7kl	515.6±11.4[gh]	293.3±12.47r	506.6±23.5[j]k	470±8.16[h]

35	*Sida cordifolia* L.	511.3±6.5^{d}	793.3±4.7^{ab}	573±5.3^{d}	456.6±4.7^{b}	690.6±7.3^{b}	506±9.93^{d}
36	*Spermacoce hispida* Linn. /Rubiaceae	474.6±5.5^{g}	706±9.9^{de}	582±5.8^{d}	402.6±5.24^{g-h}	662±8.64^{c}	562±5.8^{b}
37	*Sporolobus indicus* (L.) R. Br.	351.3±8.3^{r}	432.3±7.09^{p}	PNV	314±10.19^{pq}	384±4.3^{p}	PNV
38	*Stephania japonica* (Thunb.) Miers.	405±8.5^{n}	716.6±11.9^{d}	514.3±8.2^{g-h}	321.3±8.37^{o-q}	682±5.8^{b}	489.3±7.3^{fg}
39	*Tridax procumbens* L.	495.6±4.1e	677.3±7.5^{f}	550.3±1.7^{e}	399±8.2^{ij}	635.3±4.1^{d}	508.6±6.59^{cd}
40	*Ziziphus oenoplia* (L.) Mill.	570.3±5.3b	769.6±8.1^{c}	602.3±7.1^{c}	440.6±8.21^{c}	680.6±8.2^{b}	585.3±9.2^{a}
41	*Zornia diphylla* (L.) Pers.	490.3±8.1ef	582.2±5.8^{l-m}	PNV	399.3±9.2^{ij}	492±8.6^{lm}	PNV

Note: PNV-Plant not visible, NA-Not Applicable, DP-Dead Plant, , *Holarrhena antidysenterica* (L.) Wall. *ex* Dc. = Syn. *H. Pubescens* Wall. ex G.

Don. , Each value represents mean of three soil samples. Mean values followed by the same superscript in each column do not differ

significantly at P=0.05 level by Duncan's Multiple Range Test (DMRT).

Table- 3 Green matter versus dry matter production of *Catharanthus roseus* (L.) G. Don. at different harvesting periods

DAT	Green weight of whole plant in gram (g plant^{-1})				% increase of green wt. over control			Dry weight of whole plant in gram (g plant^{-1})				% increase of dry wt. over control		
	Con.	**+M (Gom)**	**+ M (Gim)**	**+ M (Acl)**	**Gom**	**Gim**	**Acl**	**-M (Con)**	**+M (Gom)**	**+ M (Gim)**	**+ M (Acl)**	**Gom**	**Gim**	**Acl**
90 Days	0.11	0.782 **	0.81 NS	0.90 *	611	636	718	0.022	0.099 ***	0.121 NS	0.126 *	350	450	473
120 days	0.13	0.872 ***	1.675 *	1.54**	571	1188	1085	0.026	0.110 ***	0.251 *	0.215 **	323	865	727
150 days	0.14	1.03 ***	1.72 *	1.76**	636	1129	1157	0.032	0.130 ***	0.258 *	0.246 **	306	706	669
180 days	0.14	1.07 ***	1.88 **	1.82**	664	1243	1200	0.032	0.135 ***	0.282 *	0.254 **	322	781	694

Note: Con.=Control *i.e.* without any inoculums, +M=with mycorrhizal inoculation, -M= without any inoculum *i.e.* for control plant, Gom-*Glomus mosseae*, Gim-*Gigaspora margarita*, Acl-*Acaulospora laevis*., Values are mean of three samples. Statistical significance as calculated between the VAM (+M) and non-VAM (-M) treatment. * = p<0.05, **=p<0.01, ***=p<0.001 and NS=Not significant.

Table-4 Total Chlorophyll of *Catharanthus roseus* (L.) G. Don. at different harvesting periods

DAT	Total Chlorophyll of plant (mg./g tissue)				% increase of chlorophyll content over control plant		
	Con.	**+M (Gom)**	**+ M (Gim)**	**+ M (Acl)**	**Gom**	**Gim**	**Acl**
90 days	0.69	1.11 ***	2.0 ***	1.26 *	60.86	189.85	82.60
105 days	0.78	1.21 ***	2.09 ***	1.32 *	55.12	167.94	69.23
150 days	0.83	1.31 ***	2.12 ***	1.53 *	57.83	155.42	84.33
180 das	0.82	1.30 ***	1.88***	1.09 NS	58.53	129.26	32.92

Note: Con.=Control *i.e.* without any inoculums, +M=with mycorrhizal inoculation, -M= without any inoculum *i.e.* for control plant, Gom-*Glomus mosseae*, Gim-*Gigaspora margarita*, Acl-*Acaulospora laevis*., Values are mean of three samples, Statistical significance as calculated between the VAM (+M) and non-VAM (-M) treatment. * = p<0.05, **=p<0.01, ***=p<0.001 and NS=Not significant.

Table 5-Total Carbohydrate content of *Catharanthus roseus* (L.) G. Don. leaves at different harvesting periods

DAT	Total Carbohydrate of plant leaves (mg./g) tissue)				% increase of carbohydrate content over control plant		
	Con.	**+M (Gom)**	**+ M (Gim)**	**+ M (Acl)**	**Gom**	**Gim**	**Acl**
90 days	70	98 **	120 **	111 **	40	71.42	58.57
120 days	78	100 **	140 **	122 **	28	79	56.41
150 days	82	110 **	156 **	138 **	34	90	68
180 das	86	120 **	159 **	150 **	39	85	74

Note: Con.=Control *i.e.* without any inoculums, +M=with mycorrhizal inoculation, -M= without any inoculum *i.e.* for control plant, Gom-*Glomus mosseae*, Gim-*Gigaspora margarita*, Acl-*Acaulospora laevis*., Values are mean of three samples. Statistical significance as calculated between the VAM (+M) and non-VAM (-M) treatment. * = p<0.05, **=p<0.01, ***=p<0.001 and NS=Not significant.

Table- 6 Total Protein content of *Catharanthus roseus* (L.) G. Don. Leaves at different harvesting periods

DAT	Total protein Content of plant leaves (mg./g tissue)				% increase of protein content over control plant		
	Con.	**+M (Gom)**	**+ M (Gim)**	**+ M (Acl)**	**Gom**	**Gim**	**Acl**
90 days	0.68	2.21 NS	2.6 NS	3.2 *	225	282.35	420
120 days	0.75	2.25 NS	3.0 *	4.0 *	200	300	433
150 days	1.00	3.75 **	5.0 **	5.5 **	275	400	450
180 das	1.02	4.6 **	6.0 **	7.0 **	350	488	586

Note: Con.=Control *i.e.* without any inoculums, +M=with mycorrhizal inoculation, -M= without any inoculum *i.e.* for control plant, Gom-*Glomus mosseae*, Gim-*Gigaspora margarita*, Acl-*Acaulospora laevis*., Values are mean of three samples. Statistical significance as calculated between the VAM (+M) and non-VAM (-M) treatment. * = p<0.05, **=p<0.01, ***=p<0.001 and NS=Not significant.

Table 7- Comparative aspects of Alkaloid content (qualitative) in leaves of of *Catharanthus roseus* at 180 days harvesting period

Plant leaves	**Control**	**Treatment**		
		Glomus mosseae	***Gigaspora margarita***	***Acaulospora laevis***
Catharanthus roseus	+	++++++++	++++++++++++	++++++++++++++++

Note: (+) sign indicates present, Treatment shows multiplication of alkaloid production against control under different VAM inocula.

FIGURES

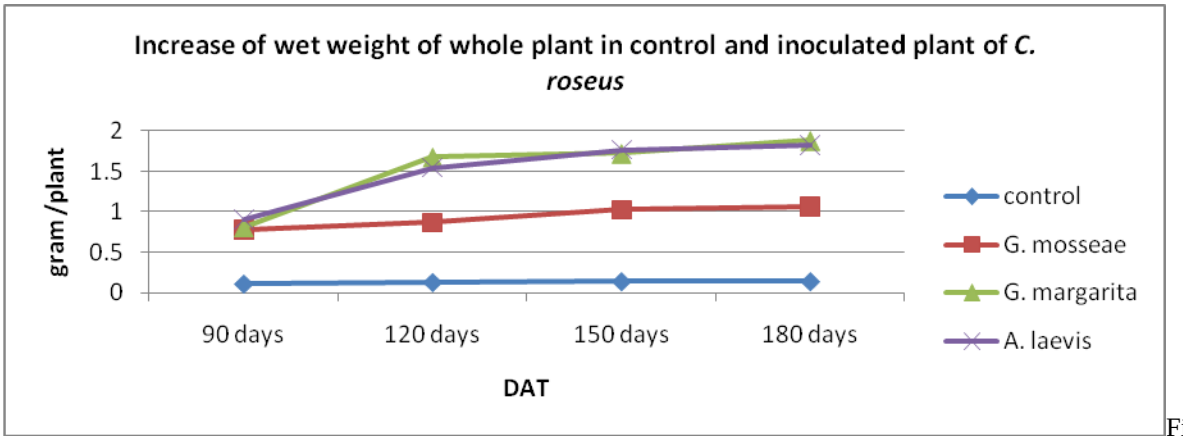

Fig. 1 Increase of wet matter of control and VAM inoculated whole plant of *Catharanthus roseus*

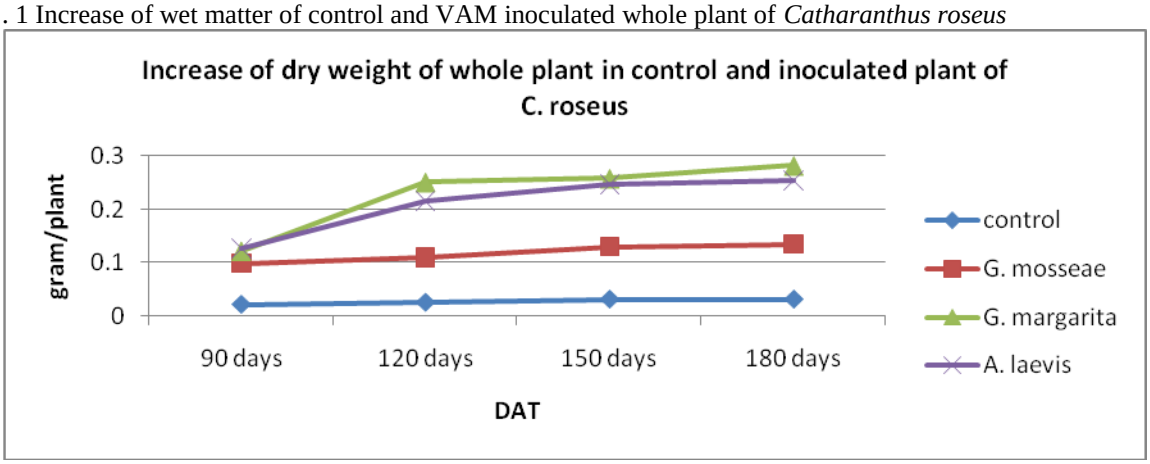

Fig. 2. Increase of dry matter of control and VAM inoculated whole plant of *Catharanthus roseus*

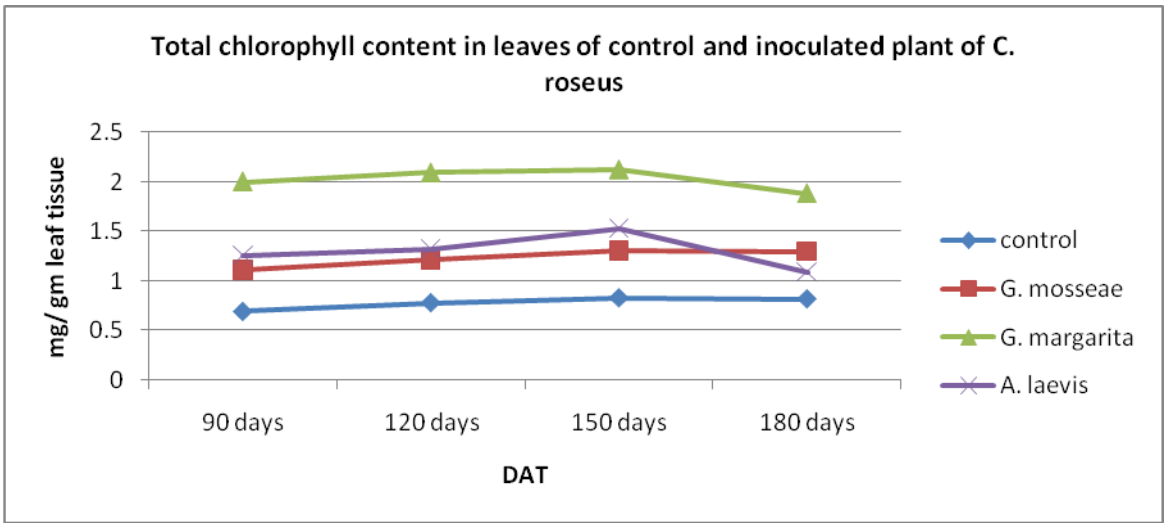

Fig. 3. Increase of total chlorophyll content in leaves of control and VAM inoculated plant of *Catharanthus roseus*

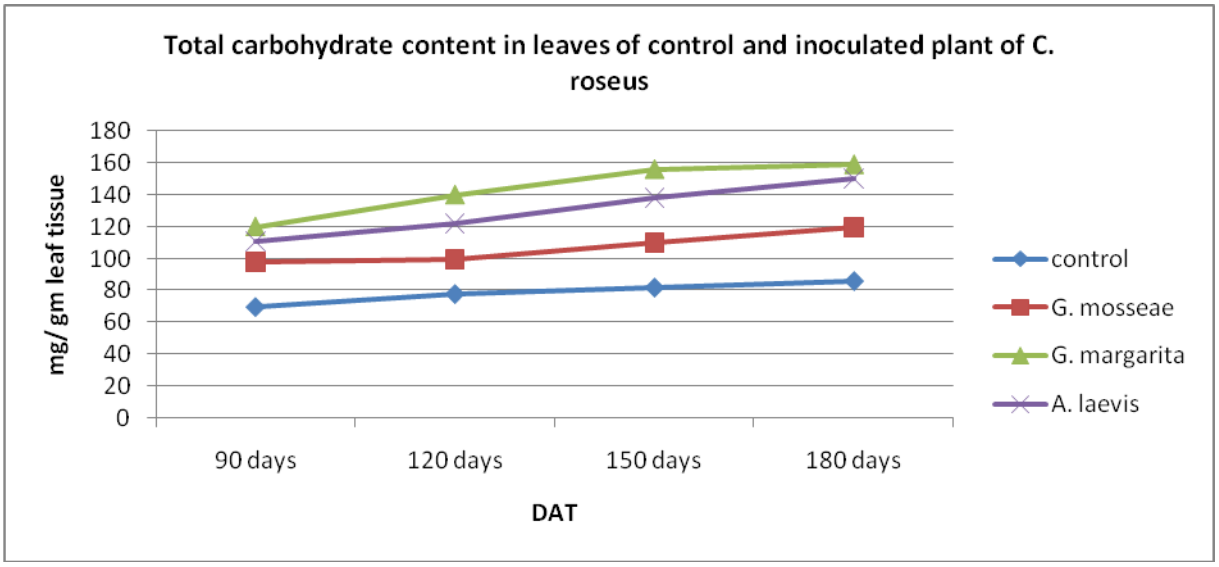

Fig. 4. Increase of total carbohydrate content in leaves of control and VAM inoculated plant of *Catharanthus roseus*

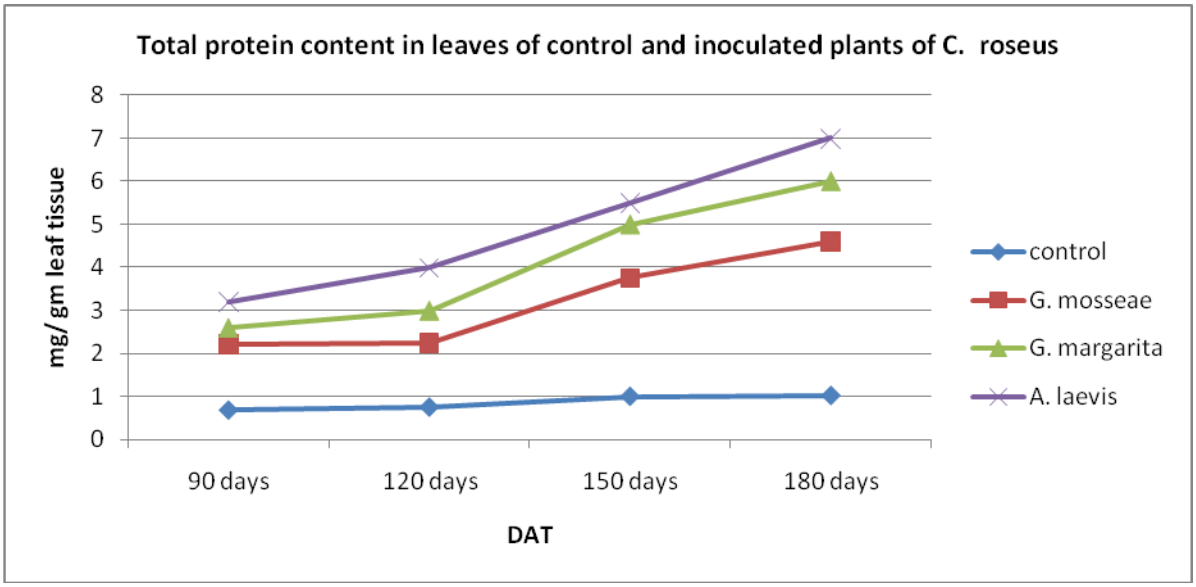

Fig. 5. Increase of total protein content in leaves of control and VAM inoculated plant of *Catharanthus roseus*

VESICLES AND SPORES

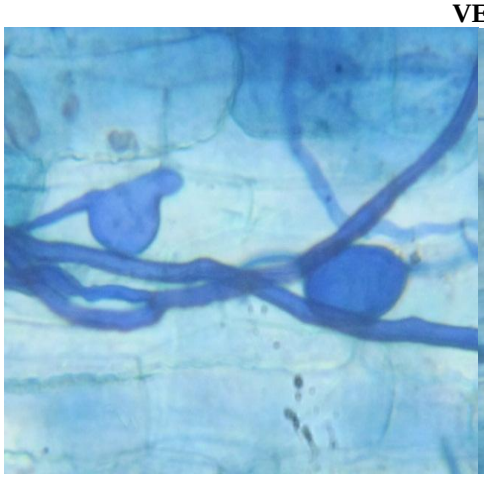

Fig. 6. Vesicles with hyphae

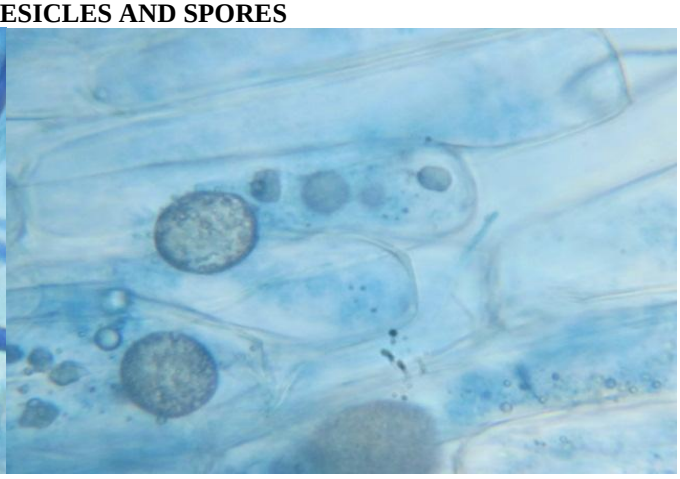

Fig. 7. Internal spores

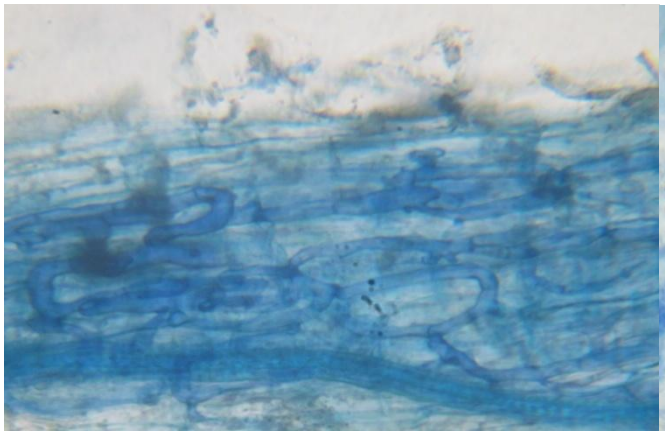

Fig 9. Coiled hyphae and hyphae

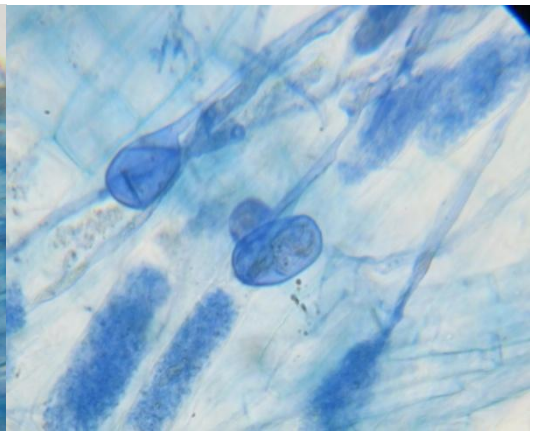

Fig. 10. Arbuscules and Vesicles

Fig 11. *Acaulospora laevis*

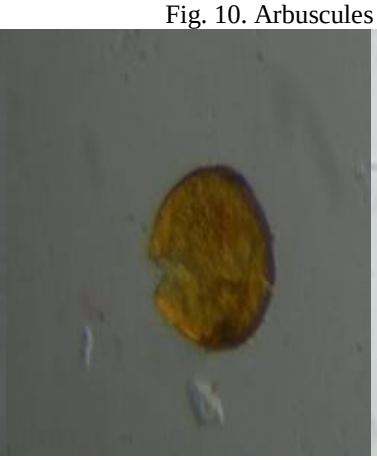

Fig. 12. *Gigaspora margarita*

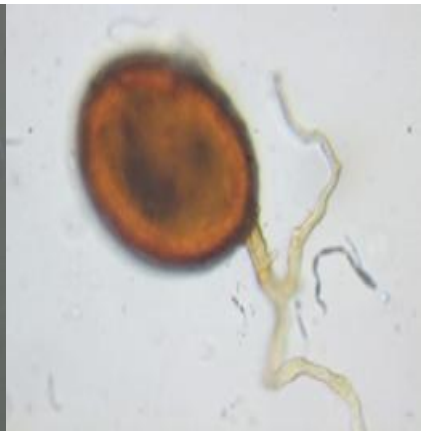

Fig. 13. *Glomus mosseae*

V. CONCLUSION

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

VI. ACKNOWLEDGEMENTS

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