# MYCORRHIZA NEWS

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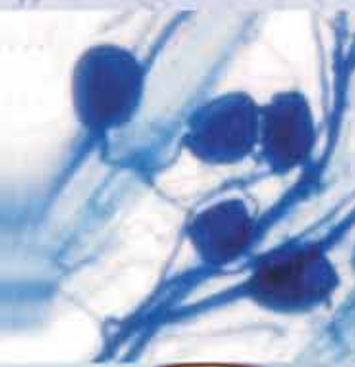

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TERI's Mycorrhiza Network is primarily responsible for establishing the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhiza Culture Collection), and publishing Mycorrhiza News. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

## *Mycorrhiza News*

The Mycorrhiza News provides a forum for dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers complied from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.

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# Research finding papers

## Arbuscular Mycorrhizal Colonization in *Aquilaria malaccensis* Lamk. and in its Surrounding Herbaceous Community


*Atithi Debnath[1], Ajay Krishna Saha[2], and Panna Das***


### Introduction

An increasing demand for herbal products has endangered many traditionally used and pharmaceutically important plant species and their habitats (Fuchs and Haselwandter, 2008). *Aquilaria malaccensis* Lamk. popularly known as agarwood belongs to the family Thymelaeaceae and class Magnoliopsida. It is one of the world's most expensive essential oil yielding tree species. Most of the *Aquilaria* species are found in south-east Asia (Donovan and Puri, 2004). *A. malaccensis* and *A. khasiana* are reported to grow naturally in the plains and in the foothills of north-east India (Hajra, 2000). The oleoresin contained in its wood is widely used in the perfume industry and in medicines (Sumadiwangsa, 1997). Studies have revealed that agarwood has remarkable anticancer activity (Gunasekera *et al.* 1981). A rise in demand for agarwood has resulted in irrational cutting of the tree trunk for extraction of the chemical. Hence, the tree has become endangered now.

Arbuscular Mycorrhizal (AM) fungi are ubiquitous symbionts existing in any ecosystem and colonizing in over two-thirds of the vascular plant species (Koide and Mosse, 2004). AM fungi are naturally occurring soil fungi belonging to a recently new ascribed phylum, the Glomeromycota. This phylum is presumed to have originated at least 460 million years ago (Schüßler *et al.* 2001). AM fungi stimulate uptake of plant nutrients, such as P, Zn, Cu, and Fe in deficient soils. Also, mycorrhizal hyphae can significantly improve N, P, and K uptake (Chen and Zhao, 2009).

The objective of this study was to analyze AM fungal colonization and diversity of *A. malaccensis* and its surrounding herbaceous community growing in Tripura, north-east India.

### Materials and Methods

#### *Sample Collection*

Roots from five plants species, viz., *A. malaccensis*, *Clerodendrum viscosum* Vent., *Phaulopsis dorsiflora* (Retz.) Sant., *Eupatorium odoratum* L., and *Mimosa pudica* L. were collected from Syamalibazar area, Agartala, Tripura. Sample collection was carried out from August 2012 to December 2012. Root samples were collected using a trowel to dig a constant maximum depth of 10cm. Most of the fine roots of the plants were located in the upper 10cm of the soil profile. Roots were traced to their origin to ensure that they were from the desired plants.

The rhizospheric soil, at depths of 10cm, surrounding the roots of the *A. malaccensis* tree was collected from eight different points. The rhizospheric soils from the roots of the five herbaceous plants were also collected. The combined samples of


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approximately 500 g soil of *A. malaccensis* and all the soil samples of herbaceous plants mixed, laced in polythene bags, labelled, and were transported to the laboratory for further analysis.

*Preparation of Roots and Assessment of AMF*

Collected roots were thoroughly washed with tap water several times and cut into uniform pieces approximately 1 cm long . Then the roots were cleaned with 10 per cent NaOH. The cleaned roots were again washed with tap water and bleached in two drops of alkaline $H_2O_2$ before acidification for 2–3 minutes. After acidification, the roots were again washed and stained with black Faber Castell stamp pad ink (Das and Kayang, 2008). These root segments were then mounted on slide and examined for mycorrhizal structures under compound microscope (Olympus 108210).

*Spore Analysis*

The spores were extracted by modified wet sieving and decanting method (Muthukumar, Senthilkumar, and Rajangam *et al.* 2006). The isolated spores were transferred using a wet needle to polyvinyl alcohol-lactoglycerol (PVLG) under a dissecting microscope (Koske and Tessier, 1983) for identification. The complete and broken spores were examined using a compound microscope (Olympus 108210). The taxonomic identification of spores was based on spore size, colour, ornamentation, and wall characteristics by matching original descriptions.

*Soil Analysis*

Soil moisture was determined by drying 10 g of fresh soil at 60 °C for 24 hours in a hot-air oven. For pH and electrical conductivity, 10 g of soil was dissolved in 50 ml of distilled water and stirred for 20 minutes. This solution was kept overnight and then the soil pH and electrical conductivity was measured using a digital meter. The organic carbon, available phosphorus, potassium, and calcium contents of the soil were determined using the Jackson (1978) method. The available nitrogen in the soil was estimated by Black (1982) method.

*Data Analysis*

Standard error of means and Tukey test was conducted to separate the means in Statistica 9.0. Diversity indices were calculated (Hammer, Harper, Ryan *et al.* 2001).

## Results and Discussion

*Soil Properties*

Table 1 depicts the properties of soils collected from the rhizosphere of *Aquilaria malaccensis* and herbaceous plants. Moisture content of soil supporting *A. malaccensis* was higher than the soils harbouring herbaceous plants. The soil pH was found to be acidic while the electrical conductivity was much higher in *A. malaccensis* than herbaceous plants' soil. The other properties of soil, such as organic carbon per cent, available nitrogen, available phosphorus, and available potassium were higher in soils from *A. malaccensis* than the soils of herbaceous plants. The available calcium was higher in soils from herbaceous plants than soils supporting *A. malaccensis*. Nutrient concentration, pH, and soil humidity level can influence fungal distribution, root colonization, and mycorrhizal efficiency (Koide and Li, 1990). In general, soils having pH 6.0–6.3 support greater number of AM propagules than soils having pH 5.3–5.7 (Tabin *et al.* 2009).

*Root Colonization by AM fungi*

All the root samples collected from the tree and the surrounding herbaceous plants exhibited the presence of AM colonization showing arbuscules, vesicles, and hyphae. The mycorrhizal structural colonization in the roots of *Aquilaria malaccensis* and its surrounding herbaceous plants is presented in Table 2. The percentage of arbuscule is highest in *A. malaccensis* and lowest in *Eupatorium odoratum*. The percentage of vesicle was highest in *Mimosa pudica* and lowest in *Clerodendrum viscosum*. The percentage of hyphae was highest in *A. malaccensis* and lowest in *Phaulopsis dorsiflora* (Fig. 1). AM fungal colonization in *A. malaccensis* and its surrounding herbaceous community forms the first report. Similar results were reported in *A. malaccensis* by Tabin *et al.* (2009). Arbuscular, vesicular, and hyphal colonization were found in all the species. The percentage of mycorrhizal colonization showed a variation from 3.86 per cent to 87.88 per cent.

**Table 1:** Properties of soils collected from the rhizosphere of *Aquilaria malaccensis* and herbaceous plants

| Soil Samples | Moisture Content (%) | pH | Electrical Conductivity (mS cm-1) | Organic Carbon (%) | Available Nitrogen (Kg/ha) | Available Phosphorus (Kg/ha) | Available Potassium (Kg/ha) | Available Calcium (Kg/ha) |
|---|---|---|---|---|---|---|---|---|
| *Aquilaria malaccensis* | 11.5 | 4.86 | 119 | 0.75 | 13.84 | 0.35 | 11.37 | 10.73 |
| Herbaceous plants | 7.32 | 5.84 | 49 | 0.59 | 8.44 | 0.23 | 8.55 | 14.93 |

**Table 2:** AM fungal colonization (%) in the roots of *Aquilaria malaccensis* and its surrounding herbaceous plants

| Name of Plants | % RLA[a] | % RLV[a] | % RLH[a] |
|---|---|---|---|
| *Aquilaria malaccensis* | 20.72±3.77[a] | 11.50±2.85[a] | 87.88±2.85[a] |
| *Clerodendrum viscosum* | 11.15±2.92[b] | 8.71±1.79[a] | 69.28±4.54[b] |
| *Phaulopsis dorsiflora* | 4.56±1.45[b] | 8.89±2.98[a] | 50.86±3.30[c] |
| *Eupatorium odoratum* | 3.86±1.21[b] | 17.17±2.82[a] | 61.56±3.70b[c] |
| *Mimosa pudica* | 4.68±1.56[b] | 31.29±3.60[b] | 55.16±3.41b[c] |

Tukey test showing that different alphabetical letters are significantly different ($p$<0.05)

%RLA, %RLV, and % RLH are percent root length with arbuscules, vesicles, and hyphae, respectively.

*AM Fungal Species*

Figure 2 depicts the AM fungal spore density in 25 g/ soil sample. Seven different AM fungal species were found in both the soil samples. These were *Glomus glomerulatum*, *Claroideoglomus etunicatum*, *Glomus* sp 1, *Glomus* sp 2, *Glomus* sp 3, *Glomus* sp 4, and *Acaulospora* sp 1 (Plate 1). These species of AM fungi were collected and identified on the basis of their

**Fig. 1:** Comparison of AM fungal colonization (%) between *Aquilaria malaccensis* and its surrounding herbaceous plants

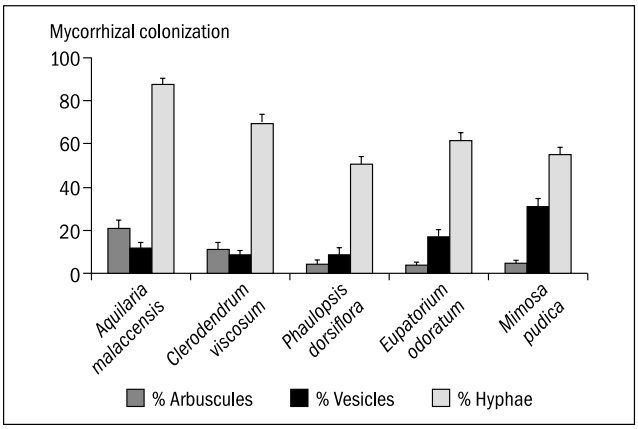



**Fig. 2:** Spore density/25g soil of Aquilaria malaccensis and herbaceous plants

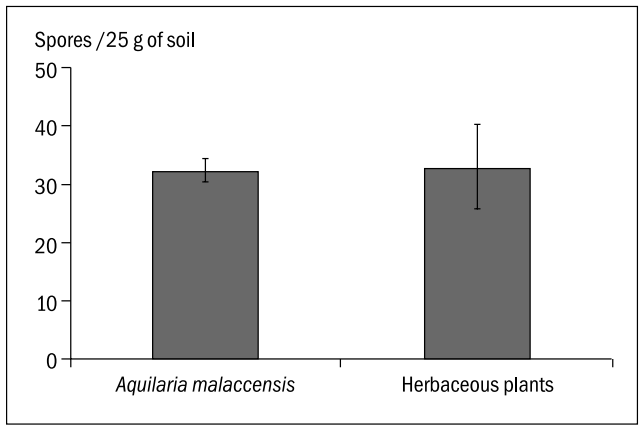
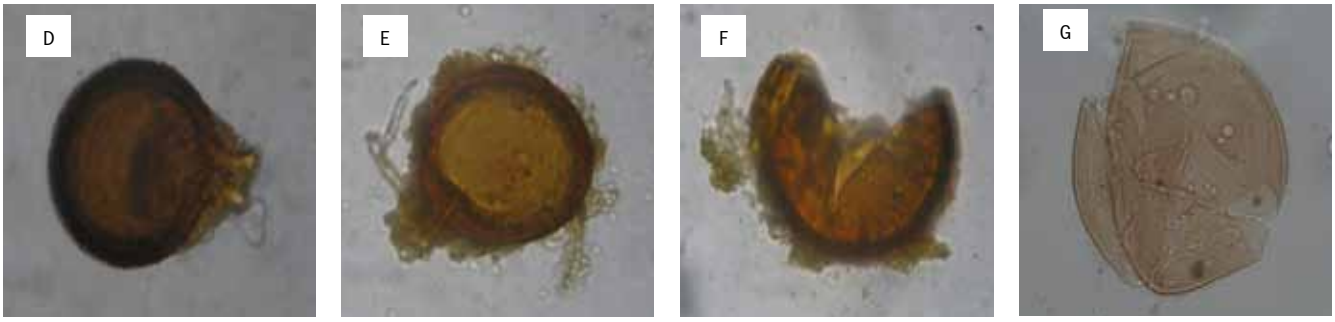



**Plate 1:** AM fungal species (a) *Glomus glomerulatum* (x400) (b) *Claroideoglomus etunicatum* (x400) (c) *Glomus sp* 1 (x400) (d) *Glomus* sp 2 (x400) (e) *Glomus* sp 3 (x400) (f) *Glomus* sp 4 (x400) (g) *Acaulospora* sp 1 (x400)




morphological characteristics. Among these species, *Glomus* sp 2 was common in both the samples. The abundance and relative abundance (in %) of AM fungi was given in Table 3. In herbaceous, *Glomus* sp 1 showed the highest abundance whereas the lowest abundance was by *Acaulospora* sp 1. In *A. malaccensis Glomus* sp 2 showed the highest abundance and the lowest was shown by *Glomus glomerulatum.* In the present study, the AMF spore density for *A. malaccensis* and herbaceous plants was found to be 32 and 33 spores/ 25 g soil, respectively, and the number of spores ranged from 11 to 384 spores per 100g soil reported in forest ecosystems of temperate regions (Read *et al*. 1976). Prasad K (1998) also reported a range of 5–370 spores/100 g of dried soil in India. The spore density was low due to the production of AMF spores in the rhizosphere vicinity of herbaceous plant species (Kruckelmann, 1975). Mohammad *et al*. (2003) observed that the spore production increased with the soil pH and organic carbon. In the present study, the pH was low for both the soil samples which might have influenced the number of spores. Therefore, spore population is affected by a wide range of soil, climatic, fungal, and host factors (Anderson *et al*. 1983).

The diversity indices namely Dominance, Shannon, and Evenness showed no significant difference between the samples. The diversity index is presented in Table 4. Seven species of AMF were obtained from the soil samples of *A. malaccensis* and herbaceous plants, respectively, which were lower than the 8–20 species usually reported in arable lands (Jansa *et al*. 2002). Valsalakumar, Ray, and Potty (2007) also reported very low AMF diversities of one to three taxa for the 21 sampling locations from soil supporting *Phaseolus aureus* in Tamil Nadu and Karnataka, respectively. Out of the seven species, the genus *Glomus* was found to be predominant. Similar result was recorded by Tabin *et al*. 2009 in *Aquilaria*

**Table 3:** Relative abundance (in %) of AM fungal species extracted from the rhizosphere of herbaceous plant and *Aquilaria malaccensis*

| AM fungi | Herbaceous plants | | *Aquilaria malaccensis* | |
|---|---|---|---|---|
| | Abundance | Relative abundance (%) | Abundance | Relative abundance (%) |
| *Acaulospora* sp 1 | 2 | 6.90 | - | - |
| *Claroideoglomus etunicatum* | 4 | 13.79 | - | - |
| *Glomus glomerulatum* sp. nov. Sieverding | - | - | 4 | 14.81 |
| *Glomus* sp 1 | 16 | 55.17 | - | - |
| *Glomus* sp 2 | 7 | 24.14 | 11 | 40.74 |
| *Glomus* sp 3 | - | - | 5 | 18.52 |
| *Glomus* sp 4 | - | - | 7 | 25.93 |

**Table 4:** Diversity index of AM fungal species in herbaceous plants and *Aquilaria malaccensis*

| Diversity Indices | Herbaceous Plants | *Aquilaria malaccensis* |
|---|---|---|
| Dominance_D | 0.3864 | 0.2894 |
| Shannon_H | 1.129 | 1.311 |
| Simpson_1-D | 0.6136 | 0.7106 |
| Evenness_e^H/S | 0.773 | 0.9275 |

plant. Das and Kayang (2009) also reported the dominance of *Glomus* sp. in north-east region of India. Certain species of Glomales are adapted to acidic soils and generally, dominate the AMF community (Sieverding, 1991) or they may be dominant due to their high competitiveness and reproductive capability (Sieverding, 1991). The abundance of AMF species showed the same tendency. Four species of AM fungi were isolated from *A. malaccensis* which is less than nine species observed (Tabin, *et al*. 2009) in *A. malaccensis* from Arunachal Pradesh. Although, spore populations do not exactly reflect the AMF community colonizing the plant roots because there is a possible existence of some non-sporulating AMF species as well (Daniell *et al*, 2001).

The present study showed that the multiplication of indigenous AM fungi associated with *A. malaccensis* and herbaceous plants may possibly help in the sustained growth of *A. malaccensis*.

## Acknowledgement

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# Selection of Efficient VAM Species for Marigold Cultivation and its Effect on Growth, Yield, and Quality Parameters of two Marigold Varieties

*Vijetha Patil, Chaya P Patil*, and B S Kulkarni*

## Introduction

In the present paper, the authors have reported the effect of 12 different species of mycorrhizal inoculation on the germination and growth of marigold raised in pot with soil as the media. This experiment was conducted to know whether all the species of mycorrhizal inoculation would be beneficial for plant growth. Vesicular–arbuscular mycorrhizal (VAM) fungi are obligate parasites that form symbiotic relationships with plant roots. VAM fungi are associated with increased phosphorus uptake, growth, and with increased drought resistance due to a fungal-hyphae-extended nutrient uptake zone around roots of many species (Hayman, 1980). Different species of VAM have different growth promotional effects on particular plant species (Patil and Patil, 2008). Thus, when considering VAM inoculation, it is important to examine several different species of VAM for their effect on plant growth.

Marigold (*Tagetes erecta* L.*)*, which occupies a prominent place in ornamental horticulture, is a commercially exploited flower crop originated from Mexico and belongs to the family Asteraceae. Free flowering habit, short duration to produce marketable flowers, wide spectrum of attractive colours, shape, size, and good keeping quality make marigold an attractive option for flower growers. Apart from its significance in ornamental horticulture, it is valued for its other properties also. The aromatic oil extracted from *Tagetes minuta*, commonly known as 'Tagetes oil', possesses larvicidal properties and is used as a fly repellent. The carotenoids present in *Tagetes* are the major sources of pigments used in poultry ndustry as a feed additive to intensify the yellow colour of egg yolks and broiler skin (Scott *et al.* 1968). The principal pigment present in the flower is xanthophyll. The xanthophyll, lutein accounts for more than 80–90 per cent and is present in the form of esters of palmitic and myristic acids (Alam *et al.* 1968).

## Materials and Method

The present investigation was carried out as a pot experiment in Department of Agriculture Microbiology, Kittur Rani Channamma College of Horticulture, Arabhavi, Gokak taluk, Belgaum, Karnataka during the period from November 2012–February 2013. The details of the materials used and the methods adopted during the investigation are presented below.

### *Planting Material*

The marigold seeds used for the experiment were collected from two sources, a local variety obtained from a farmer in Murgod, Belgaum, and a second variety named Double Orange from Namdhari Seed Company Pvt. Ltd.

VAM species used for the experiment

- Control
- *Glomus bagyarajii*
- *Glomus fasiculatum*
- *Glomus intraradices*
- *Glomus leptotichum*
- *Glomus monosporum*
- *Glomus monihofis*
- *Glomus mosseae*
- *Entrophospora*
- *Aculospora laevis*
- *Sclerocystis dusii*
- *Gigaspora gigantia*
- *Gigaspora margarita*

The marigold seeds of the two varieties were sown in poly bags with 12 VAM species and a control. Mycorrhiza was placed in the poly bag and then soil was added to it. Marigold seeds were placed over the soil and were finally covered with soil. The poly bags were watered regularly. Weeding was done as per the requirement.

### *Inoculation with AM Fungus*

Culture of 12 vesicular arbuscular mycorrhizal fungi (VAM) inoculums was obtained from Department of Agricultural Microbiology, Kittur Rani Channamma College of Horticulture, Arabhavi. The inoculum was multiplied in a sterilized potting mixture using maize (*Zea mays*) as host plant in the shade house of Microbiology Department. The inoculum used consisted of sand and soil in 1:1 ratio, and root segments of maize comprising hyphae, vesicles, arbuscules, and chlamydospores of 12 AM fungus. 5 g of inoculum was applied per poly bag before sowing.

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## Results and Discussion

A mutualistic symbiotic association exists between the endomycorrhizal fungi (AM fungi) and the roots of the horticultural crops (host plants). The AM fungal symbiosis starts with the penetration through the radicle and finally to the roots of the germinating seedlings. The local variety of marigold seeds were inoculated with 12 different AM fungal inoculums in the germinating media. The media constituents help in the early germination of the seeds. The germination percentage and vigour was influenced by different VA mycorrhizal fungi on the Local variety of marigold (Figs. 1, 2, 7, and 8). Least number of days taken for germination was recorded by *Gigaspora margarita* (5 days) followed by *Glomus fasciculatum*, *Glomus monihofis*, *Entrophospora,* and *Gigaspora gigantia* (6 days). The highest germination percentage was recorded in *Gigaspora margarita*, *Glomus bagyarajii, Glomus monihofis, Entrophospora*, and *Gigaspora gigantia* (93.33 per cent).

In Double Orange variety of Marigold, days taken for germination, germination per cent and vigour were also influenced by different VA mycorrhizal fungi existing on it. Least number of days taken for germination was recorded by *Gigaspora margarita* (5 days) followed by *Sclerocystisdusii*, *Entrophospora, Glomus monosporum*, *Glomus leptotichum*, and *Glomus fasciculatum* (6 days). The highest germination percentage was recorded in *Gigaspora gigantia* (100 per cent) followed by *Glomus monihofis* (93.33 per cent), when compared to control (Table 1 and 2). The significant enhancement in germination was also noticed in VAM inoculated seeds when compared to uninoculated control (Figs. 3, 4, 5, and 6). Increased germination due to VAM inoculation is also reported in papaya (Duragannavar *et al.* 2004), and in jamun (Devachandra, 2006).

Enhancement in germination could be due to the fact that soon after sowing of seeds they start imbibing water resulting in triphsic increase in seed fresh weight (Hartmann *et al.* 1997). Another important characteristic of seeds during imbibition is that they become 'leaky'. Several compounds including amino acids, organic acids, inorganic ions, sugars, phenolic compounds, and proteins leak out from the imbibing seeds (Simon, 1984). These solutes/leaked compounds may help VAM fungal propagules to germinate early. The time gap between the first phase and the last phase of germination may help the AM fungi to germinate and establish contacts as soon as

**Fig 1:** Effect of 12 different VAM species on growth of marigold Local variety

**Fig 2**: Effect of different host preferred VAM on growth of marigold Local variety

Con-Control, G.mono- *Glomus monosporum,* Entro-*Entrophospora,* A.l-*Aculospora laeives,* S.d-*Sclerocystis dussii*

**Fig 3**: Effect of 12 different VAM species on growth of marigold Double Orange variety

**Fig 4**: Effect of host preferred VAM on growth of marigold Double Orange variety

Con-Control, Entro-*Entrophospora,* A.l-*Aculospora laeives,* S.d-*Sclerocystis dussii,* Gi.g-*Glomus gigantia*

**Fig 5**: Effect of 12 VAM species on shoot and root growth of marigold Double Orange variety

**Fig 6**: Effect of host preferred VAM on shoot and root growth of marigold Double Orange variety

S.d-*Sclerocystis dussii*, A.l-*Aculospora laeives*, Entro-*Entrophospora*, G.mono *Glomus monosporum*, G.l-*Glomus leptotichum*, Con-Control

**Fig 7:** Effect of 12 VAM species on shoot and root growth of marigold Local variety

**Fig 8**: Effect of host preferred VAM on root and shoot growth of marigold Local variety

Con-Control, G.l-*Glomus leptotichum*, G.moss-*Glomus mossaea*, Entro *Entrophospora*, A.l-*Aculospora laeives*

**Table 1:** Effect of VAM species on days taken for germination and germination percentage in marigold Local variety

| Treatments | Days taken for germination | Germination percentage |
|---|---|---|
| $T_1$- Control | 7.00 | 86.33 |
| $T_2$- *Glomus bagyarajii* | 8.00 | 93.63 |
| $T_3$- *Glomus fasiculatum* | 6.00 | 66.66 |
| $T_4$- *Glomus intraradices* | 8.00 | 86.66 |
| $T_5$- *Glomus leptotichum* | 8.00 | 73.33 |
| $T_6$- *Glomus monosporum* | 8.00 | 66.66 |
| $T_7$- *Glomus monihofis* | 6.00 | 93.33 |
| $T_8$- *Glomus mosseae* | 7.00 | 86.66 |
| $T_9$- *Entrophospora* | 6.00 | 93.33 |
| $T_{10}$- *Aculospora laevis* | 8.00 | 80.00 |
| $T_{11}$- *Sclerocystis dusii* | 7.00 | 80.00 |
| $T_{12}$- *Gigaspora gigantia* | 6.00 | 93.33 |
| $T_{13}$- *Gigaspora margarita* | 5.00 | 93.33 |
| Standard Error Mean (S Em) ± | 0.06 | 0.21 |
| Critical Difference (C. D) @1 % | 0.23 | 0.82 |

**Table 2:** Effect of VAM species on days taken for germination and germination per cent in marigold Double Orange variety

| Treatments | Days taken for germination | Germination percentage |
|---|---|---|
| $T_1$- Control | 9.00 | 80.03 |
| $T_2$- *Glomus bagyarajii* | 9.00 | 73.33 |
| $T_3$- *Glomus fasiculatum* | 6.00 | 80.00 |
| $T_4$- *Glomus intraradices* | 8.00 | 86.66 |
| $T_5$- *Glomus leptotichum* | 6.00 | 80.00 |
| $T_6$- *Glomus monosporum* | 6.00 | 73.33 |
| $T_7$- *Glomus monihofis* | 8.00 | 93.33 |
| $T_8$- *Glomus mosseae* | 5.00 | 73.33 |
| $T_9$- *Entrophospora* | 6.00 | 86.66 |
| $T_{10}$- *Aculospora laevis* | 6.00 | 73.33 |
| $T_{11}$- *Sclerocystis dusii* | 9.00 | 66.66 |
| $T_{12}$- *Gigaspora gigantia* | 7.00 | 100.00 |
| $T_{13}$- *Gigaspora margarita* | 5.00 | 80.00 |
| S Em ± | 0.06 | 0.01 |
| C. D @1 % | 0.23 | 0.04 |

radicles emerge out. Hence, AM fungi help better seed germination by mutualistic symbiosis with roots of the plant and competing with the pathogens for space and nutrition. The differences observed in the efficacy of germination by the different AM fungal species could be attributed to the leached solutes leaked from the appropriate host. Thus, leachet released might play a prominent role in early propagation of AM fungal mycelium, thereby contributing to improved efficiency and early seed germination (Cruz *et al*. 2003).

These plant exudates enhance the hyphal growth as the fungus approach the vicinity of the roots. However, these promoting effects appear to be host specific.

In the Local variety (Table 3), highest shoot height was recorded in *Glomus mosseae* (41.90 cm) as compared to control (21.63 cm). Highest root length was recorded in *Glomus monosporum* (36.00 cm) as compared to control (15.13 cm). Highest number of roots was recorded in *Glomus mosseae* (66.33) followed by *Acaulospora laevis* (51.33*)*, and least in *Glomus bagyarajii* (18.33). Highest root volume was recorded in *Glomus leptotichum* (3.97 ml) followed by *Glomus monihofis* (3.50 ml), *Glomus mosseae* (2.83 ml), and *Aculospora laevis* (3.77 ml) as compared to control (0.43 ml). Highest fresh root weight was recorded in *Glomus leptotichum* (5.40 mg) and least in control (0.15 mg). Highest fresh shoot weight was recorded in *Glomus mosseae* (9.44 mg) and least in the control (0.83 mg). Highest root to shoot ratio was recorded in *Glomus monihofis* (0.81), which was on par with *Glomus leptotichum* (0.63). The least root to shoot weight was recorded in both *Gigaspora margarita* and *Sclerocystis dusii* (0.15).

In the Double Orange variety (Table 4), the highest shoot height was recorded in *Entrophospora* (36.00 cm) on par with all the other treatments. The least shoot height was recorded in control (22.83 cm). Highest root length was recorded in *Glomus fasciculatum* (36.33cm) and the least in control (15.00cm). Highest number of roots was recorded in *Entrophospora* (58.33) which was on par with all the other treatment other than control (22.33). Highest root volume was recorded in *Entrophospora* (5.33 ml) which was on par with *Glomus leptotichum* (4.83 ml), *Aculospora laevis* (4.33 ml), *Glomus intraradices* (4.17 ml), *Glomus fasciculatum* (3.17 ml), and *Glomus mosseae* (3.50ml). The least root volume was recorded in control (0.67 ml). Highest fresh root weight was recorded in *Entrophospora* (5.78 mg) and least in control (0.51 mg). Highest fresh shoot weight was recorded in *Glomus mosseae* (8.33 mg) and the least in control (3.33 mg). Highest root to shoot ratio was recorded in *Entrophospora* (0.72) and the least in *Gigaspora gigantia* (0.20).

In the present investigation on marigold, the morphological and anatomical changes were evident with respect to shoot parameters, such as height of shoot and number of leaves, as well as to root parameters, such as number of roots, length of longest

**Table 3:** Effect of 12 species of VAM on growth of Marigold Local variety

| Treatments | Shoot length (cm) | Root length (cm) | No. of leaves | No. of roots | Root volume (ml) | Fresh root weight (mg) | Fresh shoot weight (mg) | Root: shoot ratio |
|---|---|---|---|---|---|---|---|---|
| $T_1$- Control | 21.63 | 15.13 | 10.67 | 26.33 | 0.43 | 0.15 | 0.83 | 0.40 |
| $T_2$- *Glomus bagyarajii* | 22.00 | 24.93 | 8.33 | 18.33 | 1.10 | 0.62 | 2.68 | 0.22 |
| $T_3$- *Glomus fasiculatum* | 24.63 | 15.37 | 10.00 | 28.33 | 1.13 | 0.71 | 2.93 | 0.24 |
| $T_4$- *Glomus intraradices* | 30.50 | 22.43 | 11.33 | 28.33 | 2.00 | 0.97 | 5.27 | 0.46 |
| $T_5$- *Glomus leptotichum* | 35.67 | 28.33 | 11.67 | 44.33 | 3.97 | 5.40 | 8.12 | 0.65 |
| $T_6$- *Glomus monosporum* | 27.50 | 36.00 | 10.00 | 41.33 | 2.17 | 1.82 | 3.69 | 0.45 |
| $T_7$- *Glomus monihofis* | 31.50 | 28.33 | 10.50 | 33.33 | 3.50 | 4.45 | 5.47 | 0.81 |
| $T_8$- *Glomus mosseae* | 41.90 | 33.17 | 12.67 | 66.33 | 2.83 | 4.39 | 9.44 | 0.46 |
| $T_9$- *Entrophospora* | 36.67 | 22.00 | 12.00 | 30.67 | 2.50 | 1.98 | 7.36 | 0.27 |
| $T_{10}$- *Aculospora laevis* | 34.50 | 30.83 | 11.67 | 51.33 | 3.77 | 3.60 | 8.13 | 0.41 |
| $T_{11}$- *Sclerocystis dusii* | 30.83 | 18.30 | 9.67 | 25.67 | 1.83 | 0.78 | 5.29 | 0.15 |
| $T_{12}$- *Gigaspora gigantia* | 31.67 | 15.67 | 10.33 | 33.33 | 1.23 | 0.97 | 6.27 | 0.16 |
| $T_{13}$- *Gigaspora margarita* | 27.00 | 20.00 | 11.00 | 24.00 | 0.83 | 0.64 | 4.25 | 0.15 |
| S Em ± | 2.99 | 2.91 | 1.55 | 5.34 | 0.31 | 0.05 | 0.08 | 0.06 |
| C. D @1 % | 11.76 | 11.43 | NS | 20.98 | 1.21 | 0.19 | 0.32 | 0.23 |

Table 4: Effect of 12 species of VAM on growth of marigold Double Orange variety

| Treatments | Shoot length (cm) | Root length (cm) | No. of roots | No. of leaves | Root volume (ml) | Fresh root weight (mg) | Fresh shoot weight (mg) | Root : shoot ratio |
|---|---|---|---|---|---|---|---|---|
| $T_1$- Control | 22.83 | 15.00 | 22.33 | 12.00 | 0.67 | 0.51 | 3.33 | 0.28 |
| $T_2$- *Glomus bagyarajii* | 25.17 | 20.83 | 42.67 | 11.33 | 1.17 | 0.89 | 3.66 | 0.24 |
| $T_3$- *Glomus fasiculatum* | 29.33 | 36.33 | 44.67 | 12.00 | 3.17 | 2.76 | 8.33 | 0.33 |
| $T_4$- *Glomus intraradices* | 30.33 | 24.83 | 53.67 | 12.67 | 4.17 | 3.20 | 7.66 | 0.42 |
| $T_5$- *Glomus leptotichum* | 34.00 | 33.00 | 55.33 | 11.33 | 4.83 | 3.65 | 6.66 | 0.55 |
| $T_6$- *Glomus monosporum* | 29.00 | 27.17 | 41.67 | 10.67 | 1.83 | 1.88 | 5.33 | 0.35 |
| $T_7$- *Glomus monihofis* | 29.17 | 28.17 | 50.00 | 13.67 | 1.83 | 1.47 | 5.97 | 0.24 |
| $T_8$- *Glomus mosseae* | 31.83 | 32.67 | 52.67 | 12.00 | 3.50 | 2.88 | 8.33 | 0.35 |
| $T_9$- *Entrophospora* | 36.00 | 32.33 | 58.33 | 12.00 | 5.33 | 5.78 | 7.97 | 0.72 |
| $T_{10}$- *Aculospora laevis* | 33.33 | 35.00 | 48.67 | 12.67 | 4.33 | 2.91 | 6.33 | 0.46 |
| $T_{11}$- *Sclerocystis dusii* | 35.33 | 29.00 | 39.33 | 11.33 | 1.83 | 2.00 | 6.33 | 0.32 |
| $T_{12}$- *Gigaspora gigantia* | 31.00 | 21.50 | 45.33 | 11.33 | 1.33 | 1.14 | 5.66 | 0.20 |
| $T_{13}$- *Gigaspora margarita* | 31.33 | 26.33 | 39.00 | 10.67 | 2.33 | 1.59 | 5.33 | 0.30 |
| S Em ± | 2.76 | 2.90 | 5.69 | 1.03 | 0.59 | 0.19 | 0.01 | 0.03 |
| C. D @1 % | 10.84 | 11.40 | 22.37 | NS | 2.33 | 0.73 | 0.06 | 0.14 |

lateral roots, root volume, fresh root weight, fresh shoot weight, and root to shoot ratio (Table 3 and 4).

Modification in the root geometry and morphology might have a morphogenetic effect mediated by Indole Acetic Acid (IAA) and gibberellins (Allen *et al*. 1980). Hooker and Artkinson (1992) concluded that root morphogenesis can be modified by AM fungal metabolism or by hormones independent of external nutrients supplied. Hence, the root morphogenetic growth (i.e., root geometry) and the promising effect of AM fungi observed in the present investigation and from the literature (Slankis, 1957) could also be attributed to the phytohormones, such as gibberellins and auxins, and vitamins produced by the AM fungi. Further, it is well documented that infection of plant roots by AM fungi has beneficial effects on vegetative parameters and biomass production (fresh weight) of host plants (Duragannavar, 2005).

The increase in these parameters may be attributed to the synthesis of hormones and growth factors by AM fungi, leading to increased cell multiplication and cell division resulting in overall increase in plant height and number of leaves.

Percent root colonization was higher in the entire VAM inoculated seedling as compared to control in both the varieties (Table 5). In Local variety, maximum percentage of root colonization was recorded in *Gigaspora margarita, Sclerocystis dusii,*

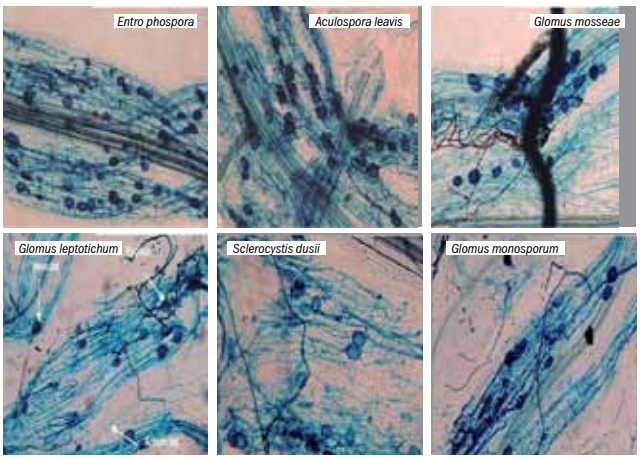
Table 5: Effect of VAM inoculation on root colonization

| Treatments | Percent root colonization | |
|---|---|---|
| | Local | Double Orange |
| $T_1$- Control | 55.00 | 46.00 |
| $T_2$- *Glomus bagyarajii* | 81.00 | 69.00 |
| $T_3$- *Glomus fasiculatum* | 86.00 | 74.00 |
| $T_4$- *Glomus intraradices* | 93.00 | 78.00 |
| $T_5$- *Glomus leptotichum* | 98.00 | 81.00 |
| $T_6$- *Glomus monosporum* | 95.66 | 85.00 |
| $T_7$- *Glomus monihofis* | 99.00 | 90.00 |
| $T_8$- *Glomus mosseae* | 99.00 | 92.00 |
| $T_9$- *Entrophospora* | 97.00 | 87.00 |
| $T_{10}$- *Aculospora laevis* | 98.00 | 89.00 |
| $T_{11}$- *Sclerocystis dusii* | 99.00 | 90.00 |
| $T_{12}$- *Gigaspora gigantia* | 98.00 | 91.00 |
| $T_{13}$- *Gigaspora margarita* | 99.00 | 79.00 |
| S Em ± | 0.58 | 0.57 |
| C D @ 1% | 2.29 | 2.26 |

*Glomus mosseae and Glomus monihofis* (99.00 per cent) which was at par with *Gigaspora gigantia, Aculospora la*evis, *Glomus lepto*tichum (98.00 per cent), and *Entrophospora* (97.00 per cent), as compared to the control (55.00 per cent). Whereas in Double Orange variety, the maximum percentage of root colonization was recorded in *Glomus mosseae* (92.00 per cent) which was at par with Glomus mosseae (92.00 per cent), *Gigaspora gigantia* (91.00 per cent), and *Glomus monihofis* (90.00 per cent). The least root colonization was recorded in the control (46.00 per cent).

The highest root infection/colonization (Fig. 9) and increased root geometry in *Entrophospora, Glomus mosseae, Aculospora la*evis, *Glomus leptotichum*, *Aculospora la*evis, and *Sclerocystis dussii* inoculated marigold seedlings have direct effect on germination and in the development of sound and healthy rhizosphere that would contribute to improved growth, resulting in improved nutrient uptake. Thus, inoculated seedlings are most suited to grow marigold. Similar results were also reported by Hooker *et al.* (1992); Venkat, (2004); and Santosh, (2004).

**Fig 9**: Root staining of marigold inoculated with host preferred VAM Fungi

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# Morphotaxonomy of Arbuscular Mycorrhizal Fungi *Acaulospora foveata*

*Chaitali Bhattacharya*[*] and *Alok Adholeya*[#]

From this edition we shall commence a new series wherein we would be describing each of our precious Arbuscular Mycorrhizal Fungi cultures available in the Centre for Mycorrhizal Culture Collection Bank on a period basis. According to the present classification studies using ribosomal-RNA (rRNA) gene as marker, phylum Glomeromycota has four orders, viz., Archaeosporales, Diversisporales, Glomerales, and Paraglomerales, comprising 10 families and 13 genera. In this periodical, we have given detailed information about *Acaulospora foveata*, a species with undermentioned classification status (http://www.zor.zut.edu.pl/Glomeromycota/Classification.html).

**Glomeromycota** C. Walker & Schuessler

**Glomeromycetes** Cavalier-Smith

**Diversisporales** C. Walker & Schuessler

**Acaulosporaceae** J.B. Morton & Benny

***Acaulospora*** Gerd. & Trappe emend. S.M. Berch

***foveata*** Janos and Trappe

Establishing monosporal (single species) culture for *Acaulospora* spp. was a very challenging process. It took almost two years to get the desired sporulation count (around 50 spores per gram soil). These spores were then isolated for the morphotaxonomic studies (Fig. 1). Undermentioned are the morphotaxonomic features of the organism visualized through voucher specimens study, presented in a similar pattern as adopted by the listed references and sites provided at the end of the article.

**Fig 1.:** Spores isolated from monosporal culture of *Acaulospora foveata*

## Spore Morphology

The spores of *Acaulospora foveata* occur singly in the soil (Fig. 2). As reported by many authors, the ontogenesis of the spore begins with the development of a sporiferous saccule at the top of a sporogenous hypha, which is in continuation with the extraradical hyphae. After the full growth of saccule, the spore begins to develop from the side of the subtending hypha. Spore growth can be observed only when the sample is captured at the right stage under suitable *in-situ* conditions (Fig. 3). Once the spore matures, the saccule loses its contents and eventually slashes off from the fully matured spores.

**Fig. 2:** Singly occurring spores

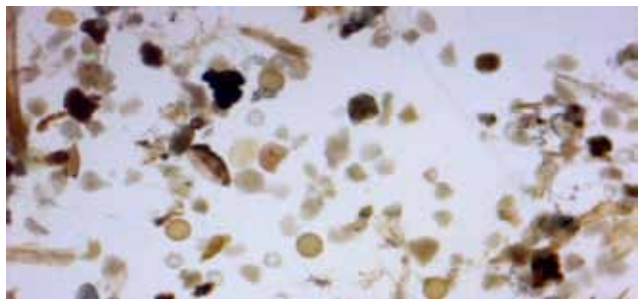

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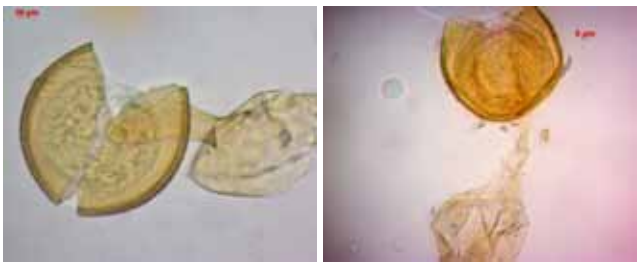
Fig. 3: Sporiferous saccule



## Spore Colour

The newly formed immature spores are initially cream coloured. On reaching to maturity, the spores acquire a dark red and finally a dark brown colour (Fig. 4).

Fig. 4: Single spores representing size

## Spore Shape

Spores are acaulosporiod. Spore shape is mostly globose to subglobose. The spore size is more than 150 µm. Size distribution ranges between 210–378 µm.

*Description of Subcellular Structure of Spore*

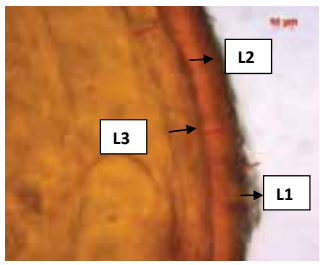
The matured spore wall is composed of three layers (L1, L2, and L3) (Fig. 5). Błaszkowski (2012) has reported the undermentioned details of the spore layer in his book chapter.

**L1:** This layer forms the surface of the spore. It is evanescent and hyaline and remains attached to the layer 2. It is completely sloughed in mature spores. The thickness of the wall ranges between 2–3.63 µm.

**L2:** The second layer helps in providing structural integrity to the spore due to the presence of laminae.

A mature second layer has a thickness of 9–19 µm. It is ornamented with evenly distributed circular pits when observed in plain view (Fig. 6).

Fig. 5: Three different wall layers of spore




**L3:** The third layer is semi-flexible. It is 3–5 µm thick and is usually tightly adhered to the lower surface of layer 2.

## Germinal Walls

There are two hyaline flexible inner walls (gw1 and gw2).

*Reaction with Melzer's Reagent*

It stains pinkish red to a reddish-purple or pastel red with Melzer's reagent (Fig. 7).

Fig. 6: Pitted surface on the spore wall

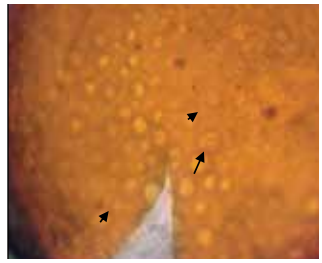
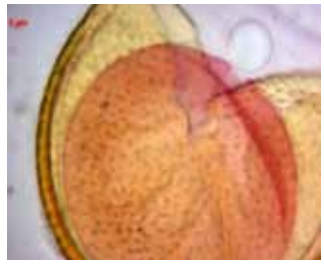


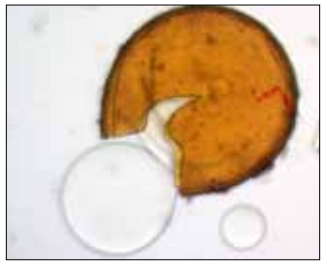
Fig. 7: Pinkish red stain with Melzer's reagent



## Sporiferous Saccule

It consists of a saccule and a neck. It is normally hyaline, light yellow to apricot yellow in colour, globose to subglobose shaped, has a diameter of 200–250 µm, with neck tapering from the saccule towards its end. It usually collapses at maturity and becomes detached from the mature spores.

Fig. 8: Spore showing cicatrix



## Cicatrix

It is circular to ovoid scar, indicating region of contact between spore and saccule neck. It has a slightly raised tapering collar consisting of closely packed tubercles surrounding an unornamented depression. It is about 12–16 µm in diameter (mean = 13.8 µm) (Fig. 8).

Since morphotaxonomy is not considered to be an authentic tool for identification, we are in the process of undertaking molecular as well as biochemical characterization of the spores isolated from the monosporal cultures for establishing its authenticity.

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http://invam.wvu.edu/the-fungi/classification/acaulosporaceae/acaulospora/foveata

http://www.zor.zut.edu.pl/Glomeromycota/Classification.html

# Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals:

- *Applied Soil Ecology*
- *Biological Conservation*
- *Crop Protection*
- *Environmental and Experimental Botany*
- *Forest Ecology and Management*
- *Journal of Plant Physiology*
- *Journal of Theoretical Biology*
- *Microbiological Research*
- *Mycorrhiza*
- *Mycoscience*
- *Phytochemistry*
- *Plant Physiology and Biochemistry*
- *Scientia Horticulturae*
- *Soil and Tillage Research*
- *Soil Biology and Biochemistry*
- *South African Journal of Botany*

| Name of the author(s) and year of publication | Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk) |
|---|---|
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17–20 January, 2014

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*E-mail* info@napatrufflefestival.com

Taipei, **Taiwan**
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APMBC 2014 Congress Secretariat, c/o K&A International Co., Ltd, 3F., No. 183, Kangchien Rd, Taipei, Taiwan 11494

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*Fax* +886-2-8751-2799
*E-mail* apmbc2014@knaintl.com.tw
*Web site* http://www.apmbc2014.com/

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