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# EXPLORING MYCORRHIZAL SYMBIOSIS: A STUDY OF VAM IN EUCALYPTUS FROM VARIOUS REGIONS OF TELANGANA, INDIA

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## ABSTRACT

Mycorrhizal associations, particularly vesicular arbuscular mycorrhizal fungi (VAM), are crucial mutualistic symbiosis between plants and fungi, enhancing plant growth and nutrient uptake. This study investigated the diversity, distribution, and colonization patterns of VAM associated with *Eucalyptus* plantations in various geographical locations. Using a modified wet sieving and decanting technique for quantitative estimation and PVLG mounting for identification, VAM propagules were isolated and characterized based on their morphological features. Root colonization was determined by the percentage of infected cells after clearing and staining. A total of 28 VAM species across six genera (*Acaulospora*, *Endogone*, *Entrophospora*, *Glomus*, *Sclerocystis*, and *Scutellospora*) were identified. *Glomus fasciculatum* was found to be the most dominant and widely distributed species, followed by *Acaulospora mellea*, *Endogone sp.*, and *Glomus macrocarpum*. The study revealed significant variation in VAM diversity and colonization rates across different soil types and locations. For instance, Bhadrachalam, Kothagudem, and Rampur showed heavy root

infection, while Madikonda and Godavarikhani exhibited lower colonization. Notably, no clear correlation was observed between soil type, number of VAM propagules, soil pH, and the extent of VAM colonization. These findings highlight the widespread presence and ecological importance of VAM in *Eucalyptus* ecosystems, with their distribution and colonization influenced by complex environmental factors beyond simple soil parameters. These findings are crucial for developing sustainable forestry practices in *Eucalyptus* plantations.

**KEYWORDS:** VAM, *Eucalyptus* plantations, Root colonization, Fungal diversity, Soil characteristics.

## I. INTRODUCTION

Frank (1885) first recorded mutualistic symbiosis between roots of higher plants and fungi, calling them mycorrhizae. Mycorrhizae are distinguished into two types: those that grow predominantly intercellular and develop extensively outside the root are called ectomycorrhizae, and those that predominantly develop within the root cortex cells are known as endomycorrhizae. Endomycorrhizae represent the most widespread mutualistic association between plants and fungi. More than 80% of land plants are thought to be susceptible to colonization by a small group of soil-borne fungi belonging to the phylum Glomeromycota (Janowski & Leski, 2022). These are endophytic, intracellular fungi forming characteristic arbuscules and vesicles in the cortical tissues of host plants. Hence, they are also called vesicular arbuscular fungi (VAM) or arbuscular fungi (AM). They typically lack host specificity (Bagyaraj, 2018). Propagation, distribution, and survival occur mainly by asexual spores (chlamydospores or azygospores). VAM produce their hyphae and reproductive structures outside the root system. These fungi colonize the roots of plants, invading only the primary cortex; vascular tissue and the secondary cortex are not infected. VAM are distributed more abundantly in soils deficient in moisture and phosphorus (Cooper, 2018) and facilitate plant nutrient uptake essential for vegetation survival/growth (Hindumathi & Reddy, 2011; 2015; 2016). VAM associated with forest soils have been reported by many workers (Sarkar et al., 2014).

VAM are beneficial to plants in many ways. These fungi have been shown to enhance water and nutrient uptake with concomitant enhancement in plant growth (Abiala et al., 2013). They also make phosphorus, nitrogen, sodium, calcium, zinc, copper, iron, and magnesium available to plants (Ramakrishnaiah & Vijaya, 2013). Lalitha (2017) explained the

mechanism of increased phosphorus uptake by mycorrhizal plants. VAM are also reported to enhance tolerance to heavy metals, saline soils, and drought, and decrease transplantation shock (Chaturvedi & Malik, 2019). Mycorrhizal plants produced in nurseries are less susceptible to transplantation stock than non-mycorrhizal plants (Nerva et al., 2023). VAM are also capable of reducing the effects of various fungal pathogens and parasitic nematodes. Abiala et al. (2013) reported that VAM antagonized the severity of foliar disease. Investigations on the occurrence and seasonal variation of VAM have been carried out Dwivedi (2008). Khanam et al. (2006) observed a lack of correlation between root colonization and VAM spore number.

## I. METHODOLOGY

### Quantitative Estimation of VAM Fungi

To isolate and estimate VAM propagules, a modified wet sieving and decanting technique (Guillén, 2020) was employed. One hundred grams of 2.0 mm sieved soil was divided into four parts, each placed in a 500 mL beaker. A pinch of sodium hexametaphosphate was added to prevent soil particle aggregation. Sieves of mesh sizes 420 µm, 250 µm, 105 µm, and 45 µm were arranged in descending order. The soil suspension was mechanically shaken for 10 minutes and poured through the sieves. Debris from each sieve was washed into separate beakers. Debris from the 420 µm sieve was filtered through synthetic fiber cloth, transferred to petri dishes with water, and observed under a dissecting microscope. VAM propagules were then isolated.

### Mounting and Identification

Permanent slides of isolated propagules were prepared using polyvinyl-lacto-glycerol (PVLG). For PVLG preparation, 1.6 g of polyvinyl alcohol was dissolved in water at 110°C in a water bath. Subsequently, 10 mL of lactic acid and 1 mL of glycerin were added and stirred until fully dissolved. The final volume was made up with distilled water. Spores and sporocarps were isolated using microneedles and mounted in PVLG. Features used for identification included color, size, shape, wall characteristics, hyphal attachments, pore structures, contents, and surface ornamentation (Manoharachary et al., 2002; Reddy, 2005; Souza, 2015).

### VAM Fungal Colonization

The magnitude of root infection by VAM was determined as the percentage of colonized cells (Sylvia, 1994). The fixed root bits were cut into 1 mm pieces. These were autoclaved at 15

psi for 10 minutes in KOH solution and rinsed in tap water until no brown color appeared in the rinsed water. Subsequently, they were acidified in dilute HCl for 3 minutes and stained by autoclaving in lacto-phenol cotton blue at 15 psi for 10 minutes. To ascertain the extent of VAM structures (including arbuscules and other invaded cells) relative to non-VAM structural parts, percentage colonization was calculated as:

$$\text{Percentage of Colonization} = \frac{\text{No. of VAM structures with vesicles}}{\text{Total no. VAM structures observed}} \times 100$$

## II. RESULTS AND DISCUSSION

The VAM identified and surveyed in association with *Eucalyptus* plantations are depicted in Table 1. A correlation was observed between the incidence of this fungal association and plant growth. In total, 28 species representing six genera of VAM were recorded in soils supporting *Eucalyptus* growth. *Acaulospora* (6 species), *Endogone* (2 species), *Entrophospora* (1 species), *Glomus* (15 species), *Sclerocystis* (1 species), and *Scutellospora* (2 species) were associated with *Eucalyptus* roots in different geographical locations in this region. The sandy loam soil at Bhadrachalam supported 7 species belonging to two genera, while soils from Godavarikhani contained 10 species representing four genera of VAM. Similarly, soils from Kothagudem contained 9 species representing 5 genera. Soils from Warangal (Madikonda) and Medak had the least number of VAM fungi, with 4 species each. Soils of Ramagundam and Rampur (Warangal) contained 7 and 8 VAM species, respectively. Overall, *Glomus fasciculatum* was dominant and widely distributed, followed by *Acaulospora mellea, Endogone* sp., and *Glomus macrocarpum* in descending order (Table 2). *Glomus aggregatum* and *G. citricolum* were specific to soils of Manchirial, while *Entrophospora* and *G. multisubstansum* were found in soils of Godavarikhani. Despite being coal-based, the soils of Godavarikhani and Ramagundam were rich in mycorrhizal fungi. Similarly, *Scutellospora* sp. was recorded only in the coal-based soils of Godavarikhani, Ramagundam, and Kothagudem. *Glomus*, followed by *Acaulospora*, were widely distributed in this region. *Entrophospora* and *Sclerocystis* showed restricted distribution, whereas *Endogone* and *Scutellospora* exhibited intermediate distribution in this region. Examination of *Eucalyptus* root bits revealed heavy infection with VAM (Table 3). However, the degree of infection varied with the location and age of the plant. Plants growing at Bhadrachalam, Kothagudem, and Rampur showed heavy infection, while those at Madikonda and Medak had the least. Interestingly, the degree of infection was almost the same in plants growing at Godavarikhani-I and Kothagudem-II, despite different environmental conditions.

**Table 1: Occurrence of VAM species associated with *Eucalyptus*: Locations 1. Badrachalam 2. Godavarikhani –I, 3.Godavarikhani-II, 4. Kothagedem-I, 5. Kothagudem-II, 6. Madikonda 7. Medak 8. Manchirial 9. Karim Nagar 10. Ramagundam 11. Rampur; '+' indicates presence, '-' indicates absence.**

| VAM fungal species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Aculospora bireticola*(Pl-3, Fig.4) | + | - | + | - | - | + | - | - | + | - | + | |
| *A. delicata* (P1-2, Fig.1) | - | + | - | - | + | - | + | - | - | - | - | |
| *A.foveata* (Pl-2, Fig.5) | - | - | + | - | - | + | - | - | - | + | - | |
| *A.locunosa* (Pl-2, Fig.6) | + | - | - | + | - | - | - | - | - | - | + | |
| *A. laevis* (Pl-4, Fig.2) | - | - | + | - | - | - | - | - | - | - | + | |
| *A.* mellea (Plate-2, Fig.4) | - | + | - | + | - | - | + | + | - | + | - | |
| *Endogone pisiformis* | - | - | - | - | + | - | - | + | - | - | - | |
| *Endogone sp.* (Plate-4, Fig.7) | + | + | - | - | + | - | - | - | - | - | - | |
| *Entrophospora sp* (Plate-4, Fig.4) | - | - | - | + | - | - | - | - | - | - | - | |
| *Glomus aggregatum* (Plate-2, Fig.2) | - | - | - | - | - | - | - | + | + | - | - | |
| *G. citricolum* | - | - | - | - | - | - | - | + | - | - | - | |
| *G. constrictum* | - | + | - | - | - | - | - | + | - | + | - | |
| *G. fasciculatum* (Plate-1, Fig.4) | + | + | + | + | - | - | + | + | + | + | + | |
| *G. fuegianum* (Plate-4, Fig.8) | - | - | + | - | + | - | - | - | - | - | - | |
| *G.geosporium* (Plate-3, Fig.4) | + | + | - | - | + | - | - | - | - | - | + | |
| *G.hoi* ( Plate-1, Fig.3) | - | + | - | - | - | + | - | + | - | - | - | |
| *G. intraradices* (Plate-3, Fig.6) | - | - | - | + | - | - | - | - | - | + | - | |
| *G. microcarpum* (Plate-4, Fig.3) | + | - | + | - | - | + | + | - | - | - | - | |
| *G.monosporum* (Plate-2, Fig.3) | - | - | - | - | + | - | - | - | + | - | + | |
| *G.mosseae (Plate-3, Fig.2)* | + | - | - | - | + | - | - | - | - | + | + | |
| *G. multisubstansum* (Plate-4,Fig.5) | - | - | - | + | - | - | - | - | - | - | - | |
| *G.pallidum* (Plate-1, Fig.2) | - | + | - | - | - | - | - | - | + | - | - | |
| *G. tubiforme* (Plate-4, Fig.1) | + | - | + | - | - | - | - | + | - | - | - | |
| *Glomus sp.* | - | - | + | - | - | - | - | - | - | - | - | |
| *Sclerocystis microcarpus* (Plate-3, Fig.3) | - | - | - | - | + | - | - | - | - | - | + | |
| *Scutellospora sp1* (Plate-1, Fig.1) | - | - | - | - | + | - | - | - | - | + | - | |
| *Scutellospora sp2* | - | + | + | - | - | - | - | - | - | - | - | |
| *Un idetified sporocarp of Glomus* (Plate-3, Fig.5) | - | - | + | - | - | - | - | - | - | - | - | |
| | 7 | 9 | 10 | 6 | 9 | 4 | 4 | 8 | 6 | 7 | 8 | |

**Table 2: Number of VAM species isolated in different soils.**

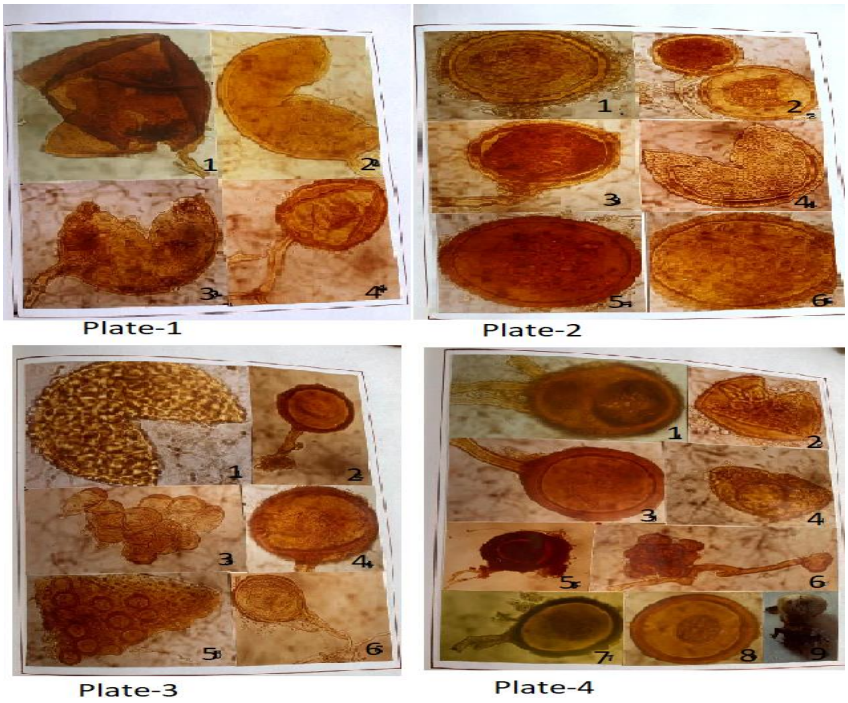
| S. No. | Genus | No. of species isolated in different locations of Telangana | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1 | *Aculospora* | 1 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 3 |
| 2 | *Endogone* | 1 | 1 | - | - | 1 | - | - | - | 1 | - | - |
| 3 | *Entrophospora* | - | - | - | 1 | - | - | - | - | - | - | - |
| 4 | *Glomus* | 5 | 5 | 6 | 4 | 4 | 2 | 2 | 6 | 4 | 4 | 4 |
| 5 | *Sclerocystis* | - | - | - | - | 1 | - | - | - | - | - | 1 |
| 6 | *Scutellospora* | - | 1 | 1 | - | 1 | - | - | - | - | 1 | - |

**Table 3: Colonization of VAM fungi with Eucalyptus in different soils.**

| Place of collection | Percentage of Colonization | No. of propagules in 100g of soil | Soil Type | Soil pH |
|---|---|---|---|---|
| Badrachalam (Khammam | 50.13 | 89 | Sandy loam | 7.3 |
| Godavarikhani-I (Karim Nagar) | 18.51 | 136 | Sandy loam | 7 |
| Godavarikhani-II (Karim Nagar) | 26.53 | 119 | Sandy loam | 6.8 |
| Kothagudem-I (Khammam) | 42.86 | 127 | Coal mine soil | 7.8 |
| Madikonda (Warangal) | 22.45 | 38 | Sandy loam | 6.7 |
| Medak | 29.5 | 55 | Sandy loam | 7 |
| Manchirial | 36.84 | 98 | Black cotton | 7.2 |
| Ramagundam (Karimnagar) | 36.85 | 124 | Black soil | 7.5 |
| Rampur (Warangal) | 56.25 | 103 | Sandy | 7 |

The VAM structures also varied by location. Plants from Godavarikhani, Madikonda, Rampur, and Medak exhibited little or no vesicles or arbuscules, indicating predominantly mycelial infection. In contrast, plants from Kothagudem and Ramagundam showed the formation of both arbuscules and vesicles. Plants at Bhadrachalam, Rajahmundry, and Ramagundam primarily supported vesicle formation only.

No direct correlation was observed between soil type and the number of VAM propagules. For example, soils from Machirial and Ramagundam were black cotton soils, while those from Godavarikhani were sandy; yet, both contained a good number of VAM propagules. Conversely, the soils of Madikonda and Medak were almost identical, but the propagule counts differed. Hepper (2018) reported that the optimum pH for hyphal growth and VAM infection was 6.0. The pH of all soils under study was almost neutral, and no correlation was found between soil pH and VAM colonization.




This study thoroughly investigated the occurrence, diversity, and colonization of VAM associated with *Eucalyptus* plantations across various regions. Our findings confirm the ubiquitous nature of VAM as significant mutualistic partners for *Eucalyptus*, with 28 species identified belonging to six genera. *Glomus fasciculatum* emerged as the predominant species, indicating its broad adaptability across the studied areas.

## III. CONCLUSION

Despite the widespread presence, the diversity and colonization levels of VAM varied considerably between different geographical locations and soil conditions. While some sites like Bhadrachalam, Kothagudem, and Rampur demonstrated high levels of root infection, others such as Madikonda and Godavarikhani showed lower colonization. Interestingly, the study found no direct correlation between soil type, soil pH, or the number of VAM propagules and the extent of VAM colonization. This suggests that VAM associations and their efficacy in *Eucalyptus* may be influenced by a more complex interplay of environmental factors beyond the basic soil parameters investigated. The presence or absence of specific VAM structures like vesicles and arbuscules also differed among locations, hinting at variations in the established symbiotic relationships. These results underscore the complex interplay of environmental factors that might influence VAM associations, perhaps mentioning avenues for future research (e.g., genetic factors of *Eucalyptus*, microbial community interactions, specific nutrient availability.

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