

The first report on diversity of AM fungi from Leucas stelligera Wall. ex Benth. Malaxis versicolor (Lindl.) Abeyw from Matheran Hills, Maharashtra

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ABSTRACT

The present study assesses the diversity and root colonization of arbuscular mycorrhizal (AM) fungi associated with Leucas stelligera Wall. ex Benth and Malaxis versicolor (Lindl.) from the Matheran Hills of Maharashtra, India. Rhizosphere soil samples were collected during the peak flowering seasons (November and August) over a three-year period (2022–2024). The analysis revealed abundance of spores and higher root colonization by AM fungi in both the plant species. A total of 16 AM fungal species were identified from L. stelligera and 20 species from M. versicolor, predominantly belonging to the genera Glomus and Acaulospora, with Glomus emerging as the dominant genus. Mean spore density was recorded as 241.34 ± 8.62 and 276.68 ± 24.13 per 50 g of rhizosphere soil for L. stelligera and M. versicolor, respectively. Root colonization levels averaged $91 \pm 0.74\%$ in L. stelligera and $70 \pm 1.33\%$ in M. versicolor. Several AM fungal species reported in this study have not been previously documented in other species of Leucas or Malaxis, highlighting the uniqueness of the fungal communities in this region. This is the first report on AM fungal diversity associated with L. stelligera and M. versicolor, as well as the first account from Matheran Hills.

Key words: Arbuscular mycorrhizal fungi, Glomus, Acaulospora, Leucas stelligera, Malaxis versicolor, Matheran hills.

INTRODUCTION

The symbiotic association of AM fungi with the roots of higher plants is well documented. Various geographical factors affect the diversity of Am fungi and the diverse assemblage of Am fungal species can enhance the ecosystem productivity [1]. These fungi increase the root surface area of the plants which helps them in the maximum absorption of the nutrients. Soil conditions, geographical isolation, climate, habitat helps in shaping the diversity of AM [2, 3,4, 5]. Additionally, Anthropogenic activities also affect the diversity of AM [6]. According to Gollotte [7] the local AMF diversity is influenced by the condition of soil as well as by the host plants.

Moreover, the composition of specific AM fungal communities is influenced by interactions among the host species [8]. The nutritional requirements and physiological activities of the naturally growing plants are highly dependent on the AM fungi [9]. AM fungi plays a crucial role in protecting the plants form abiotic stress while increasing the absorption of more nutrients and water uptake [10,11].

The composition of arbuscular mycorrhizal fungi (AMF) communities in the Western Ghats remains inadequately understood, as comprehensive studies have been limited to specific regions such as Goa and Kerala [12,13]. Matheran is a part of Western Ghats, and the rhizosphere soil has not been so far studied for AM fungal colonization. It is important to examine the geographical patterns and root association of AM fungi with hosts in specific geographical regions, since edaphic conditions often have an impact on AM fungal species in various environments [14].

Empirical investigations revealed a broad spectrum of Arbuscular mycorrhizal (AM) fungal species members of the Lamiaceae family, including Salvia [15], Mentha [16], and various Leucas species such as L. lantana [17], L. aspera [18], and L. hirta [19]. Even though the extensive research on AM fungal associations within Lamiaceae family, the rhizospheric diversity related of Leucas stelligera remains unexplored. Similarly, AM fungal associations and quantification of mycorrhizal colonization was studied in Malaxis versicolor (Lindl.) Santapau & Kapadia from Orchidaceae [20]. AM fungal association in Malaxis rheedei has been documented by Ram et al. [21]. Govindaraj



Bagyalakshmi [22], Sathiyadash *et al.* [23], and Jyothsna *et al.* [24] reported the mycorrhizal associations and root colonization percentages in *M. rheedei*. However, no previous records are available on the distribution of the diversity of arbuscular mycorrhizal (AM) fungi associated with *Leucas stelligera* and *Malaxis versicolor* from the Matheran region. Hence, study seeks to explore and record the AM fungal diversity associated with the rhizosphere soil of *Leucas stelligera* and *Malaxis versicolor* from Matheran forest.

Leucas stelligera has been observed to occur predominantly during the months of October and November, corresponding to its flowering season, while *Malaxis versicolor* typically flowers between July and August each year in Matheran. Therefore, rhizosphere soil samples were collected from both species during their respective flowering periods to assess the density and AM fungal diversity.

MATERIAL AND METHODS

Description of the plant

Leucas stelligera Wall ex Benth. (Starry Leucas, Burumbi, Goma) belongs to the family Lamiaceae, it is an erect branched herb, dense many flowered terminal and axillary whorls and found mostly in the Western Peninsula of India [25]. The plant is herbaceous, erect, branched, softly pubescent, 0.6-1 m high; stem and branches quadrangular and hairy. Leaves are 5-10 X 1.5-2.5 cm, obtuse or sub-acute, elliptic or oblong-lanceolate, obtusely serrate green on top, and pale underneath, long, soft hair covers the base, which taper into the petiole, which is 3-10mm long. Sessile flowers in thick terminal and axillary whorls with numerous flowers that can be sometime 4 cm in diameter. Calyx pubescent, teeth about 1.5 mm long, linear, soft. Corolla white, rather less than 10 mm long, lips of limb nearly equal, the upper surface is thickly covered with long white hair, the middle lobe of the lower lip sub-orbicular. Fruit is nutlets 2 mm long, oblong- obovoid, rounded at the apex. Flowering: November [26]. The plants were collected from Matheran hill, Raigad, Maharashtra Situated in the Western Ghats at an altitude of about 800 m (2,625 ft) above sea level



Figure 1: a. Leucas stelligera Wall. ex Benth. b. Malaxis versicolor (Lindl.)

Malaxis versicolor (Lindl.) Abeyw is one of the greatly varying species in the Western Ghats. It is terrestrial, lithophytic, and infrequently epiphytic. The stem is sheathed in a greenish-purple sheath and stands erect, 3-25 cm tall, with a slight swollen base. There are three to five leaflets, which can be broadly ovate, elliptic-lanceolate, or ovate-lanceolate. Length of petioles: 2-6 cm. The inflorescence is 8-35 cm long. It shows pedicellate yellow flowers with a tinge of purple when they are young, maturing into deep reddish-purple flowers measuring 4x4 mm in diameter. Shows the Green, $3.5 \times 1-1.5$ mm bracts. While the petals are purple, entire, reduplicate, reniform, slightly curved, and fanshaped, the sepals are uneven, purple, and

obtuse. While flowering occurs from July - August and fruiting occurs from September -March, [27].

Collection and processing of the soil

To examine the spore diversity in *Leucas stelligera* and *Malaxis versicolor* soil from the root zone area was collected during their respective flowering seasons (November for *L. stelligera* and August for *M. versicolor*) over the period 2022–2024. The soil samples were kept at 4°C in zip locked polythene bag till further analysis. The root samples taken for the analysis of colonization and later fixed with FAA.

Estimation of AM fungal colonization and isolation of spores Spore Isolation

Am fungal spores were isolated by sieving decanting technique of Gerdeman and Nicolson [28]. 50gms of rhizosphere soil were added to 500 ml of water. The soil suspension was allowed to stand undisturbed to facilitate the settling of soil particles at the bottom. The supernatant was then passed through different mesh sizes such as $210\mu m$, $120\mu m$, $103\mu m$, $4.5\mu m$, $2.5\mu m$ to collect the spores. It is then isolated on Whatman's filter paper No.1. The trapped spores or sporocarps were observed under the stereomicroscope after mounting in PVLG.



Examination of root colonization

The collected roots were washed and further examination was performed by the Phillips and Hayman method [29]. Firstly, the roots were washed and then kept in 10% KOH at 121°C for 10 minutes and then treated with 5N HCl for 3-4 minutes then again after washing with water they kept overnight in trypan (0.05%) blue for staining. Mounting of spores was done in PVLG. The percentage of root colonization was recorded after the analysis.

$$Root\ colonization\ percentage = \frac{\textit{No.of\ infected\ root\ fragments}}{\textit{Total\ No.of\ fragments\ seen}} \times 100$$

Identification of the isolated AM spores

Identification of spores and sporocarps was done by using manuals of Schencz and Perez [30] also by using standard sites such as www.invam.caf.wvu.edu and www.zor.zut.edu based on colour, hyphae, shield, wall layers etc. The identification of spores was confirmed by Dr. V. Mohan, Scientist- G and Head (RETD), University of Madras, Chennai.

RESULTS AND DISCUSSION

The dominant species of AM fungi associated with the rhizosphere soil of *Leucas stelligera* and *Malaxis versicolor* are presented in Table 2. A total of 16 AM fungal species in the rhizosphere of *L. stelligera* (fig. 3.) and 20 species associated with *M. versicolor*(Fig.4) were recorded. *Leucas stelligera* and *Malaxis versicolor* exhibited mean spore densities of 241.34 ± 8.62 and 276.68 ± 24.13 per 50 g of rhizosphere soil, respectively. Over the three-year period of study, the mean root colonization rates recorded were $91.00 \pm 0.74\%$ for *L. stelligera* and $70.00 \pm 1.33\%$ for *M. versicolor* (Table. 1) (Fig.2). The increased colonization in hosts may be due to the release of oxidizable compounds, which may attract the Am fungi [31]. Since variety of factors such as moisture, pH, temperature of soil, hosts, seasonal variations of spore germination, hence the current study did not found a co-relation between the colonization rate and spore density [32, 33]. According to Bever [34], the host species can provide either positive or negative feedback on the sporulation of several AM fungi. Low soil nutrient content, good aeration, undisturbed soil, or ideal moisture levels that provided enough time for mycorrhizal spore colonization could also be the cause of the high AM spore density in the present study [35].

Table 1: The percentage of root colonization, mean AM spore density

Sr. No	Host species	AM structure present in root H V A		oot	Average percent of root colonization	No. of AM species recorded	Mean spore density/50gm of rhizosphere soil
1.	Leucas stelligera	+	+	-	91± 0.737	16	241.34±8.621
2.	Malaxis versicolor	+	-	-	70±1.34	20	276.68 ±24.131

Where, #H - Hyphae, V-Vesicles, A- Arbuscules; +: Present, -: Absent, ±: Standard deviation

Table 2: Diversity of spores

Sr. No.	Name of the plant	Am fungal spores
1	Leucas stelligera	a. Glomus geosporum b. Glomus caledonium c. Glomus claroideum d. Glomus multicaulae e. Glomus fulvus f. Glomus albidum g. Glomus macrocarpum h. Glomus occultum i. Glomus fecundisporum j. Glomus pubescens k. Glomus constrictm l.Glomus microcarpum m. Glomus intraradice n. Aculospora scrobiculata o. Acaulospora laevis p. Glomus aggregatum (sporocarp)
2	Malaxis versicolor	a. Glomus pubescens, b. Acualospora denticulate, c. Glomus multisubstensum, d. Glomus occultum, e. Glomus convolutum, f. Glomus albidum, g. Glomus fecundisporum, h. Glomus multicaulae, i. Glomus intraradices, j. Glomus geosporum, k. Glomus macrocarpum,l. Acaulospora scrobiculata, m. Glomus monosporum, n. Glomus fasciculatum, o. Glomus microcarpum, p. Glomus caledonium, q. Glomus deserticola, r. Glomus constrictum, s. Glomus diaphanum, t. Glomus multisubtensum



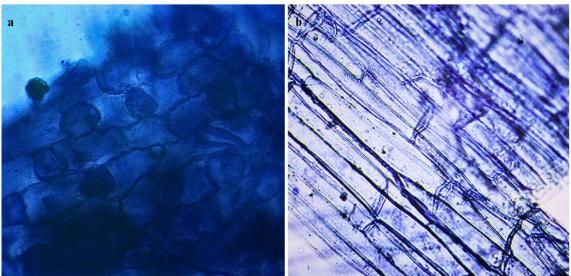


Fig: 2 Mycorrizal infection in roots of a. Leucas stelligera Wall. ex Benth. b. Malaxis versicolor (Lindl.) Abeyw

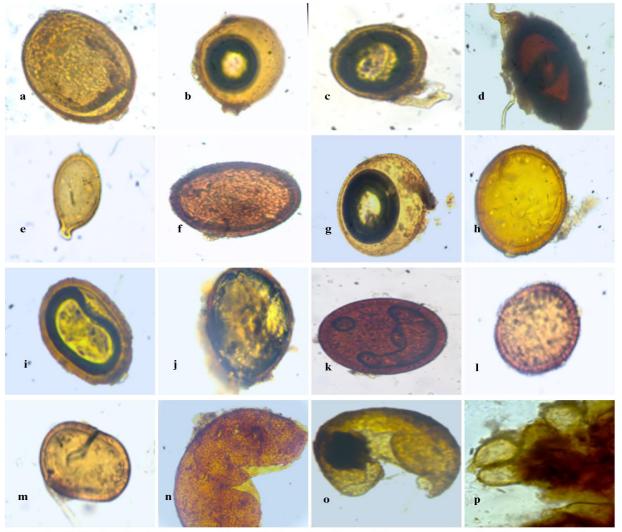


Figure 3: AMF diversity in Leucas stelligera Wall. ex Benth: a. Glomus geosporum b. Glomus caledonium c. Glomus claroideum d. Glomus multicaulae e. Glomus fulvus f. Glomus albidum g. Glomus macrocarpum h. Glomus occultum i. Glomus fecundisporum j. Glomus pubescens k. Glomus constrictm l. Glomus microcarpum m. Glomus intraradice n. Aculospora scrobiculata o. Acaulospora laevis p. Glomus aggregatum (sporocarp)



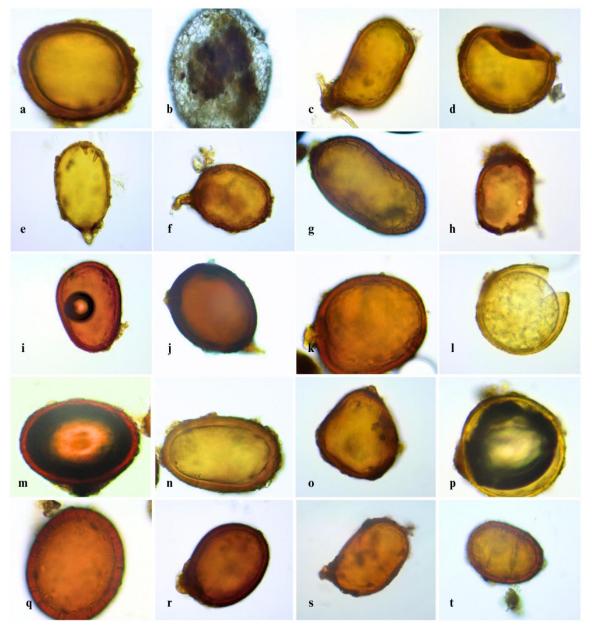


Figure 4: AMF diversity in Malaxis versicolor (Lindl.) Abeyw: a. Glomus pubescens, b. Acualospora denticulate, c. Glomus multisubstensum, d. Glomus occultum, e. Glomus convolutum, f. Glomus albidum, g. Glomus fecundisporum, h. Glomus multicaulae, i. Glomus intraradices, j. Glomus geosporum, k. Glomus macrocarpum, l. Acaulospora scrobiculata, m. Glomus monosporum, n. Glomus fasciculatum, o. Glomus microcarpum, p. Glomus caledonium, q. Glomus deserticola, r. Glomus constrictum, s. Glomus diaphanum, t. Glomus multisubtensum

Previous studies on Leucas lantana have reported the presence of various AM fungal species, including Acaulospora foveata, A. laevis, A. bireticulata, A. denticulata, Entrophospora sp., Glomus ambisporum, G. clavisporum, G. fasciculatum, G. macrocarpum, G. magnicauli, G. mosseae, G. pallidus, G. pansihalos, G. sinuosum, Sclerocystis ceremoides, and Scutellospora sp. [36]. Rajkumar et al. [37] reported G. rubiforme, G. luteum, G. fasciculatum, G. manihotis, A. denticulate, Gigaspora sp. from Leucas hirta and G. fasciculatum, A. myriocarpa, A. scrobiculata from Leucas hirta. A. laevis and G. luteum were reported from Leucas aspera [38]. Also studies on various Leucas species have demonstrated associations with a diverse range of AM fungal species. In present study, except for Acaulospora scrobiculata, A. laevis, and Glomus macrocarpum, which were also reported in earlier Leucas species, all other AM fungal spores identified in L. stelligera were distinct, suggesting a unique assemblage of AM fungi potentially influenced by host-specific traits or environmental conditions.

N. Raman and N. Nagarajan [39] were the first to report *Glomus aggregatum* and *Glomus mosseae* in association with *Malaxis rheedi*. In contrast, the other AM fungal species found in *Malaxis versicolor* appear to be unique and have not been recorded in any other *Malaxis sp*.



The dominance of *Glomus sp.* in the host trees of in the Western Ghats region was documented by Muthukumar and Manian [40] and Vasanthakrishna *et al.* [41]. Consistent with these findings, the present study identified *Glomus* as the most prevalent AM fungal genus associated with both *Leucas stelligera* and *Malaxis versicolor*. This dominance may be attributed to the evolutionary adaptability of *Glomus*, which is believed to have developed efficient and versatile symbiotic mechanisms with a broad variety of host plants as part of a co-evolutionary process [42].

Members of the genera *Acaulospora* and *Glomus* are known to produce spores more rapidly than genera such as *Gigaspora* and *Scutellospora*. Some AM fungi develops mycelia but produce relatively few spores, particularly those from families other than *Acaulosporaceae* and *Glomeraceae*. This may explain the observed dominance of *Acaulospora* and *Glomus* species in the present study [43,44]. Moreover, evidence suggests that plants can actively select specific AM fungal partners, resulting in distinct AM fungal communities even among individuals of the same species [45,46]. The present findings further suggest that the symbiotic association of AM fungi with *L. stelligera* and *M. versicolor* is likely governed more by host preference than strict host specificity, in line with earlier observations by Smith and Read [47] and Yang *et al.* [48].

CONCLUSION

Present study, the genus *Glomus* was consistently prominent, indicating its ecological adaptability and possible coevolution with a variety of plant species. When compared to those found in other species of *Leucas* and *Malaxis*, the unique AM fungal assemblages found in the study suggest that selection of host by fungus is influenced by regional soil and microclimatic conditions. The dominance of fast-sporulating genera such as *Glomus* and *Acaulospora* is due to their adaptive advantage in colonizing diverse hosts. The documentation of AM fungal associations with *L. stelligera* and *M. versicolor* contributes to the understanding of host–fungus interactions in less-studied medicinal and endemic species. Specifically, the identified AM fungal taxa, particularly those from the genus *Glomus*, could be utilized in the development of targeted AM fungal inoculants aimed at enhancing plant growth, improving nutrient uptake, and potentially increasing the biosynthesis of secondary metabolites during cultivation of these species.

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