

Deciphering Root-Fungus Symbiosis: Assessment of Arbuscular Mycorrhizal Colonization in Medicinal Flora of Melghat

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) establish beneficial symbiotic associations with plant roots and contribute to nutrient acquisition, plant growth and ecological adaptation. The present investigation aims to assess the extent of arbuscular mycorrhizal fungal root colonization in two medicinally important plant species *Plumbago zeylanica* L. and *Ricinus communis* L., naturally occurring in the Melghat region of Maharashtra, India. Root samples collected from three different sites were processed using standard root-clearing and staining techniques and the percentage of root colonization was determined using the grid-line intersect method. The assessment focused on the occurrence of characteristic AMF structures including intraradical hyphae, vesicles, arbuscules and intraradical spores, wherever observed. The study is designed to document the extent of root colonization and compare the mycorrhizal associations of the selected plant species across the sampling sites. The findings may contribute to a better understanding of AMF associations in the medicinal flora of the Melghat region and provide a basis for further investigations into their potential role in sustainable medicinal plant cultivation and soil health management.

Keywords: Arbuscular Mycorrhizal Fungi, Medicinal Plants, *Plumbago zeylanica* L., *Ricinus communis* L., Root Colonization and Mycorrhizal Symbiosis

INTRODUCTION

Melghat, situated in the Satpura hill ranges of Maharashtra, India, is an ecologically significant region rich in biodiversity and medicinal plant resources. Indigenous communities of this region have traditionally relied on medicinal plants for primary healthcare. The conservation and sustainable utilization of these plants are important for preserving biodiversity and traditional medicinal knowledge.

Plumbago zeylanica L. (Plumbaginaceae) and *Ricinus communis* L. (Euphorbiaceae) are medicinally important plant species known for their traditional applications and diverse phytochemical constituents. The growth and physiological performance of these plants are influenced by soil conditions and interactions with beneficial microorganisms. Therefore, understanding their association with soil fungi is relevant to their ecology and sustainable management.

Arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota, establish symbiotic associations with the roots of numerous terrestrial plants. These fungi facilitate nutrient acquisition, particularly phosphorus and may improve plant growth, soil structure and tolerance to environmental stresses. AMF associations may also influence secondary metabolite production in medicinal plants,

although these effects vary with the host plant, fungal species and environmental conditions (Smith *et al.*, 2011; Zhao *et al.*, 2022).

Root colonization is an important indicator of AMF association and can be assessed through microscopic examination of cleared and stained roots. The presence of characteristic structures, including hyphae, arbuscules, vesicles and intraradical spores, provides evidence of fungal colonization. The grid-line intersect method is commonly used to estimate the percentage of colonized root segments and compare colonization among plant species and sampling sites.

Considering the ecological and medicinal importance of these plants, the present study aimed to assess arbuscular mycorrhizal fungal root colonization in *Plumbago zeylanica* and *Ricinus communis* growing naturally in selected sites of the Melghat region, Maharashtra, India. The study also examined the occurrence of characteristic AMF structures and compared root colonization patterns using standard clearing, staining and grid-line intersect methods. The findings provide baseline information on AMF associations in these medicinal plants and may support further research on sustainable medicinal plant management.

MATERIALS AND METHODS

Study Area: The study was conducted in the Melghat region located in the southwestern Satpura mountain ranges of Maharashtra, India, between latitudes 21°11'–21°46' N and longitudes 77°34'–78°38' E.

Selected Medicinal Plants

- *Plumbago zeylanica* L.
- *Ricinus communis* L.



Collection of Root Samples

Healthy root samples were collected from three different sites (S1, S2, and S3) within the Melghat region. Roots were gently washed to remove soil particles and fixed in FAA solution (Formalin: Acetic acid: Alcohol, 5:5:90 v/v). Samples were stored at 4°C until further processing.

Estimation of AMF Root Colonization

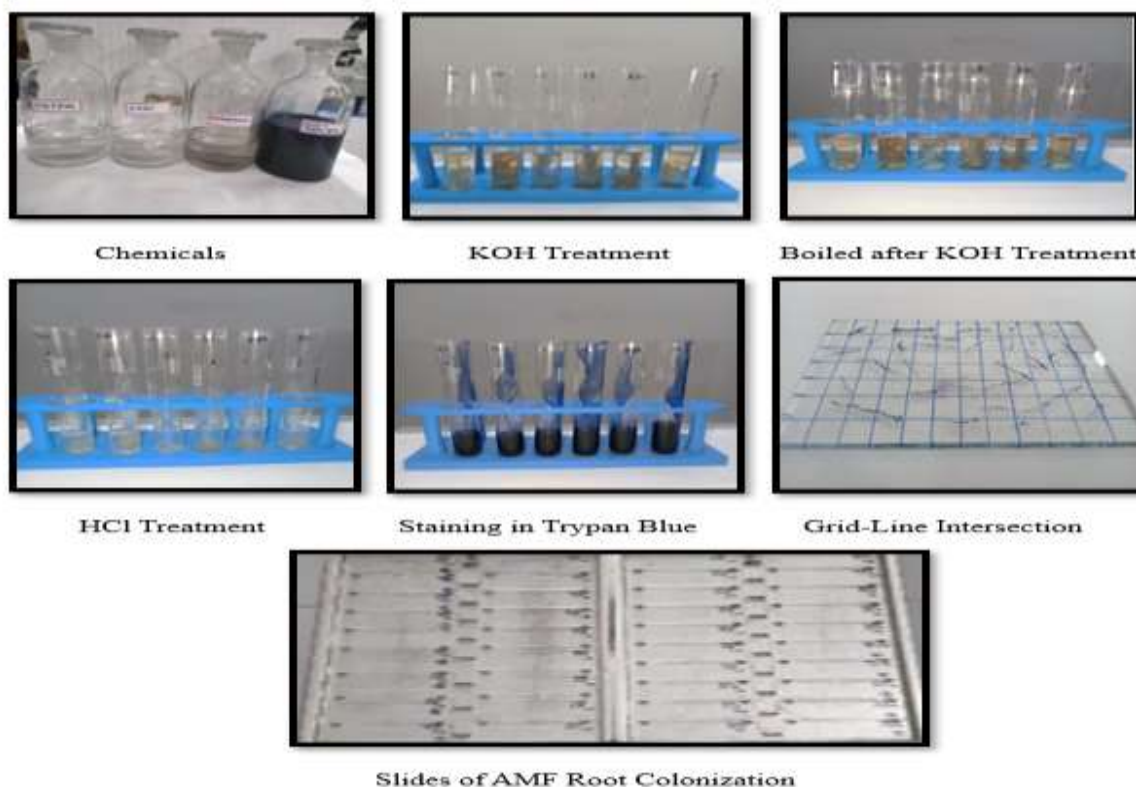
Clearing and Staining of Roots

Root samples were processed following the method of Phillips and Hayman (1970)

1. Clearing (Sathiyadash *et al.*, 2010): Fixed roots were washed 4–5 times with tap water to remove FAA solution and cut into 1 cm segments. The segments were cleared in 2.5% KOH at 100°C for 4–5 min

in test tubes using a water bath. The pigment-containing KOH was poured off, and the root segments were rinsed 4–5 times with tap water to remove residual KOH.

2. Bleaching: If highly pigmented roots remained uncleared after KOH treatment, they were bleached in freshly prepared alkaline H_2O_2 at room temperature for 10–20 min and rinsed 2–3 times with tap water. Alkaline H_2O_2 was prepared using 30% H_2O_2 (1 mL), NH_4OH (1 mL), and distilled water (8 mL) (Kaminskyj, 2008).
3. Acidification: Cleared and bleached root segments were treated with 2% HCl for 15 min. The HCl was then poured off without washing the roots with water.
4. Staining (Phillips and Hayman, 1970): The acidified root segments were covered with 0.05% trypan blue staining solution and left overnight for adequate staining of AMF structures.
5. Destaining: After 24 hours, the stain was poured off, and the root segments were covered with lactoglycerol for 1–3 hours or longer, depending on staining intensity. This removed excess stain from root cortical regions without AMF structures, improving the visibility of fungal structures. Lactoglycerol was prepared using lactic acid (40 mL), glycerol (40 mL), and distilled water (20 mL) (v/v).
6. Mounting: Permanent slides were prepared by placing 9–10 properly stained root segments lengthwise in a thin layer of PVLG mountant on a glass slide. A coverslip was placed carefully to avoid air bubbles and pressed gently. The slides were stored in slide boxes for further AMF analysis and photography.



Estimation of root colonization by AMF using Gridline intersection Method

Assessing the AM infection and % colonization, roots from preserved samples were processed. The Grid line intersect method (Giovannetti and Mosse, 1980) was used for quantifying the AM colonization.

The stained root samples were randomly spread on a plane square glass. A grid of lines was marked at the bottom of glass to form 0.5-inch squares. Horizontal and vertical grid lines were scanned and the total

number of roots intersecting grid- lines and the total numbers of intersections involving infected roots were recorded.

The % root colonization was calculated by the formula:

$$\text{(\% Root Colonization)} = \frac{\text{\% Infection} \times \text{Total no. of roots intersecting grid line}}{\text{Total no. of infected roots intersecting grid line} \times 100}$$

The fine roots were screened for presence of septate hyphae, vesicle, arbuscules and the intra-radical spore.

Photography

Photography of both AMF structures in roots isolated from rhizospheric soil was done by using Carl Zeiss inverted compound microscope with Tucsen Camera (0.5 MP).

RESULTS AND DISCUSSION

Percentage of Root Colonization

Plant Species	Site	Infected Intersections	Total Intersections	Root Colonization (%)
<i>Plumbago zeylanica</i> L.	S1	13	14	92.86
	S2	15	15	100.00
	S3	17	17	100.00
<i>Ricinus communis</i> L.	S1	14	14	100.00
	S2	14	15	93.33
	S3	16	16	100.00

Mean percentage of root colonization

Plant species	Mean root colonization (%)	Range (%)
<i>Plumbago zeylanica</i> L.	97.62	92.86–100.00
<i>Ricinus communis</i> L.	97.78	93.33–100.00

Comparative Analysis of AMF Root Colonization

Site-wise percentage root colonization in *Plumbago zeylanica* L. and *Ricinus communis* L.

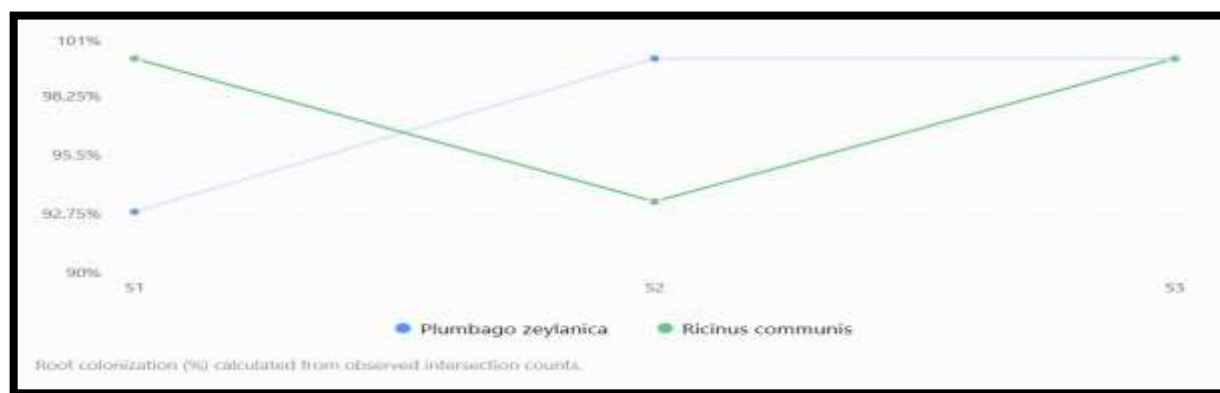


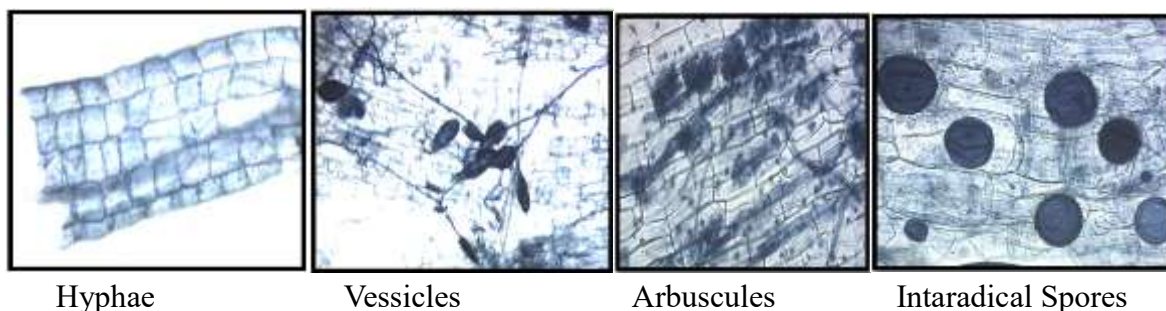
Figure 1. Comparative line graph showing the percentage of AMF root colonization. *Plumbago zeylanica* showed an increase in colonization from S1 to S2, which remained constant at S3. In contrast, *Ricinus communis* showed a decrease at S2 followed by an increase at S3. Both species recorded

100% colonization at S3, while their mean colonization percentages were 97.62% and 97.78%, respectively.

Qualitative Root Analysis

Plant species	Site	Hyphae	Vesicles	Arbuscules	Intraradical spores
<i>Plumbago zeylanica</i> L.	S1	+++	+++	+++	++
	S2	+++	+++	+++	++
	S3	+++	+++	+++	+++
<i>Ricinus communis</i> L.	S1	+++	++	+	++
	S2	+++	+++	+++	++
	S3	+++	++	+++	+++

– = absent; + = low occurrence; ++ = moderate occurrence; +++ = high occurrence



In *Plumbago zeylanica*, hyphae, vesicles, and arbuscules were consistently recorded at high levels across all three sites. Intraradical spores showed moderate occurrence at S1 and S2 and high occurrence at S3. These observations indicate the presence of several characteristic structures associated with AMF colonization.

In *Ricinus communis*, hyphae showed consistently high occurrence at all sites. Vesicles were recorded at moderate levels at S1 and S3 and at high levels at S2. Arbuscules showed low occurrence at S1 but high occurrence at S2 and S3. Intraradical spores showed moderate occurrence at S1 and S2 and high occurrence at S3.

Overall, the observations demonstrate variation in the occurrence of AMF structures between plant species and sampling sites. The comparatively consistent occurrence of arbuscules in *Plumbago zeylanica* and their site-dependent occurrence in *Ricinus communis* provide useful descriptive evidence of differences in the observed root associations.

CONCLUSION

The present investigation demonstrated the occurrence of arbuscular mycorrhizal fungal (AMF) associations in the roots of *Plumbago zeylanica* L. and *Ricinus communis* L. growing in the Melghat region of Maharashtra, India. Root colonization analysis revealed high colonization percentages in both species with mean values of 97.62% in *Plumbago zeylanica* and 97.78% in *Ricinus communis*. Microscopic observations further confirmed the occurrence of characteristic AMF structures including hyphae, vesicles, arbuscules and intraradical spores, although their observed occurrence varied among sampling

sites. These findings provide baseline information on AMF root colonization in the selected medicinal plants and highlight the importance of investigating plant-fungus associations in natural ecosystems. Further studies involving AMF identification, soil physicochemical properties and experimental assessment of plant growth and secondary metabolites are recommended to clarify the ecological and potential practical significance of these associations in sustainable medicinal plant management.

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