



Original Article

Assessment of Arbuscular Mycorrhizal Fungal Root Colonization in Selected Medicinal Plants of the Melghat Region

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Abstract:

Arbuscular mycorrhizal fungi (AMF) play a crucial role in enhancing plant nutrition, growth, and secondary metabolite production, particularly in medicinal plants. The present investigation evaluates the extent of AMF root colonization in three important medicinal plant species, *Caesalpinia bonduc*, *Clerodendrum serratum*, and *Physalis minima*, naturally growing in the Melghat region of Maharashtra, India. Root samples collected from three different sites were processed using standard clearing and staining techniques, and percent root colonization was assessed by the grid-line intersect method. All studied plant species showed strong arbuscular mycorrhizal association characterized by the presence of hyphae, vesicles, arbuscules, and intra-radical spores. High levels of root colonization (96–100%) indicate a strong symbiotic relationship between these medicinal plants and AMF. The findings highlight the ecological significance of AMF in medicinal plant ecosystems and suggest their potential application in sustainable cultivation practices.

Keywords: Arbuscular Mycorrhizal Fungi, Medicinal Plants, Root Colonization, Melghat, Biofertilizer.

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Introduction:

Melghat, situated in the Satpura hill ranges of Central India, is a biodiversity-rich region characterized by dense forests, varied topography, and diverse microhabitats. This region is well known for its abundance of medicinal plant species traditionally used by indigenous tribal communities for primary healthcare. According to the World Health Organization, nearly 80% of the global population relies on traditional medicine derived from plants.

Medicinal plants such as *Caesalpinia bonduc*, *Clerodendrum serratum*, and *Physalis minima* are widely used in indigenous systems of medicine for treating ailments including fever, inflammation, respiratory disorders, and gastrointestinal problems. However, excessive use of chemical fertilizers and pesticides threatens soil health and the quality of medicinal plant products.

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the roots of nearly 80% of terrestrial plants. These fungi enhance nutrient uptake—especially phosphorus—improve soil structure, increase resistance to biotic and abiotic stresses, and influence the production of secondary metabolites. Understanding the mycorrhizal association of medicinal plants is therefore essential for sustainable cultivation and conservation strategies.

The present study aims to assess the degree of AMF root colonization in selected medicinal plants of the Melghat region using the grid-line intersect method.

Materials And Methods:

Study Area:

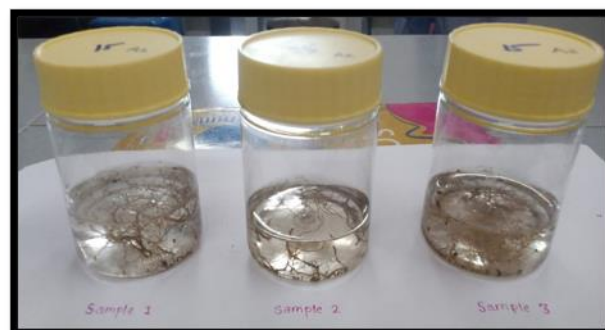
The study was conducted in the Melghat region located in the southwestern Satpura mountain ranges of Maharashtra, India, between latitudes 21°11'–21°46' N and longitudes 77°34'–78°38' E.

Selected Medicinal Plants:

- *Caesalpinia bonduc* (L.) Roxb.
- *Clerodendrum serratum* (L.) Moon
- *Physalis minima* 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) with minor modifications:

1) Clearing (Sathiyadash et al., 2010):

- Fixed roots were washed 4-5 times with tap water to make it free from FAA solution and cut into 1cm long bits.
- The root bits were cleared for 4-5 min in 2.5% KOH at 100°C in test tubes using water bath.



- Then KOH solution containing root pigments was poured off and root bits were rinsed 4-5 times with tap water to remove traces of KOH

2) Bleaching:

In case of highly pigmented roots if they were uncolored with KOH then root bits were bleached using freshly prepared alkaline H₂O₂ solution at room temperature for 10-20 min and then rinsed with tap water for 2-3 times to remove traces of alkaline H₂O₂. [Alkaline H₂O₂ was prepared by adding 30% H₂O₂ (1ml): NH₄OH (1ml): D/W (8ml) – Kaminskyj, 2008]

3) Acidification:

After clearing and bleaching all root bits, were acidified with 2% HCL for 15 min and then HCl was poured off. Finally root bits were not washed with water.

4) Staining (Phillips and Hayman, 1970):

After acidifying, the root bits were covered by adding 0.05% trypan blue staining solution and was kept as it is overnight to get proper staining of AMF structures.

5) Destaining:

- Trypan blue stain was then poured off after 24 hours and root bits were covered with lactoglycerol destaining solution for 1-3 hrs or more depending on intensity of stain gained by each sample.
- Destaining solution removed the excess stain from cortical region of roots which did not have any AMF structures, making

The % root colonization was calculated by the formula:

$$\begin{array}{lcl} \text{\% Infection} & & \text{Total no. of infected roots intersecting grid line X 100} \\ (\% \text{ Root Colonization}) & = & \text{Total no. of roots intersecting grid line} \end{array}$$

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

AMF structures more visible and clearer in other cells. [Lactoglycerol was prepared by adding Lactic acid (40ml): Glycerol (40ml): D/W (20ml) V/V]

6) Mounting:

- Permanent slides were prepared by placing 9-10 properly stained root segments lengthwise with the help of forceps in a thin layer of PVLG mountant on a slide and covered with coverslip by avoiding bubble formation and pressed gently.
- Slides were stored in slide box for further analysis of AMF 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.

Photography:

Photography of both AMF structures in roots isolated from rhizospheric soil was done



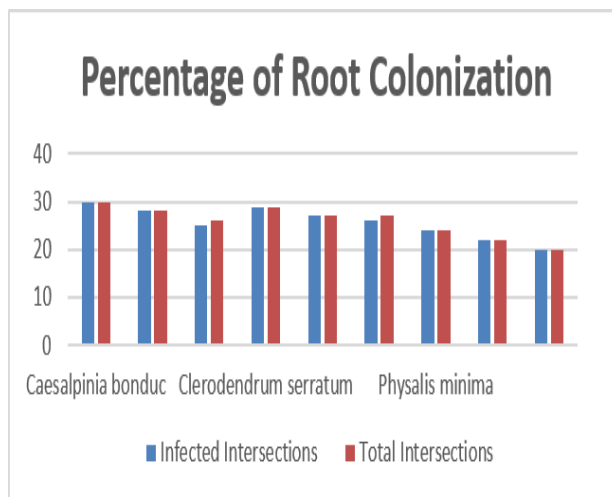
by using Carl Zeiss inverted compound microscope with Tucsen Camera (0.5 MP).

Results And Discussion:

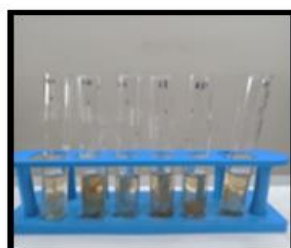
All three medicinal plant species exhibited arbuscular mycorrhizal associations. Microscopic observations confirmed the presence of characteristic AMF structures.

Percentage of Root Colonization:

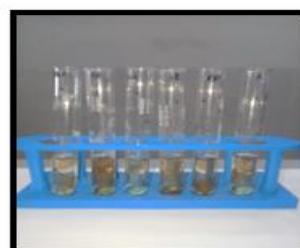
Plant Species	Site	Infected Intersections	Total Intersections
<i>Caesalpinia bonduc</i>	S1	30	30
	S2	28	28
	S3	25	26
<i>Clerodendrum serratum</i>	S1	29	29
	S2	27	27
	S3	26	27
<i>Physalis minima</i>	S1	24	24
	S2	22	22
	S3	20	20



Chemicals



KOH Treatment



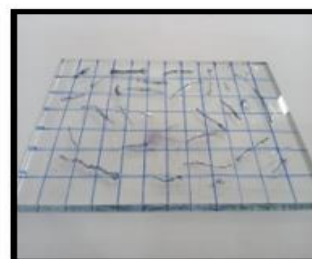
Boiled after KOH Treatment



HCl Treatment



Staining in Trypan Blue



Grid-Line Intersection



Slides of AMF Root Colonization



Qualitative Root Analysis:

Root Samples	Hyphae	Vesicles	Arbuscles	Intra-radicle spore
<i>Caesalpinia bonduc</i> : S1	+++	+++	+++	++
S2	+++	+++	+++	+
S3	+++	+++	+++	++
<i>Clerodendrum serratum</i> : S1	+++	+++	+++	+
S2	+++	+++	+++	+++
S3	+++	+++	++	+
<i>Physalis minima</i> : S1	+++	+++	+++	+
S2	+++	+++	+	++
S3	+++	+++	++	+++

High colonization percentages suggest a strong dependency of these medicinal plants on AMF for nutrient acquisition and overall growth. Similar findings have been reported in other medicinal plant studies, highlighting the ecological importance of mycorrhizal symbiosis.

Conclusion:

The present study confirms that *Caesalpinia bonduc*, *Clerodendrum serratum*, and *Physalis minima* are strongly associated with arbuscular mycorrhizal fungi in the Melghat region. High levels of root colonization indicate the ecological compatibility of these plants with AMF. Incorporation of AMF-based biofertilizers could enhance sustainable cultivation, improve medicinal yield, and reduce dependency on chemical fertilizers. These findings contribute valuable insights into the conservation and eco-friendly cultivation of medicinal plants.

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