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Harnessing the Richness of Arbuscular Mycorrhizal Fungal Root Colonization in Some Medicinal Plants from the Melghat Region, Amravati (M.S.)

M. M. Malviya and MaggirwarR. C.
Mycorrhizal Bio-technology Lab,
P.G. Department of Botany,
Shri Shivaji Science College,
Amravati, Maharashtra,
monikamalviya9496@gmail.com and dr.krekha@yahoo.com

ABSTRACT

Asia, endowed with a vast array of medicinal plant species, serves as a leading global supplier of these valuable resources. India, recognized as one of the most bio diverse countries in Asia. It is a home to a remarkable diversity of ecosystems and medicinal plant species. In India, Melghat nestled in the Satpuda hill ranges of Central India stands out as a region of immense ecological wealth. Its pristine, forests, coupled with a unique confluence of ecosystems, render it a crucial reservoir of medicinal undisturbed plants. As global agricultural practices shift towards sustainability, there is a growing focus on reducing the use of chemical fertilizers and pesticides. In this context, Arbuscular Mycorrhizal Fungi (AMF) have emerged as pivotal components in this transition. These symbiotic fungi play an instrumental role in enhancing plant growth and soil fertility by improving nutrient uptake, supporting overall plant development.

Consequently, the objective of this research was to investigate the extent of root colonization by AMF in selected medicinal plant species like *Caesalpinia bonduc*(L.) Roxb., *Clerodendron serratum*(L.) Moon.and *Physalis communis*L.from the Melghat region. The grid line intersect method was employed to quantify root colonization. Hyphae, vesicles and Arbuscules were observed and their frequency was recorded. It was found that out of these three plant species the *Clerodendron serratum*(L.) was highly colonized by AMF. The findings of this study pave the way for several potential avenues of further research and application. Firstly, the identification and mass propagation of native AMF species can be expanded to assess their effects on a wider range of medicinal plants, including rare or endangered species. Exploring the interactions between AMF and different plant species will provide deeper insights into their specificity, efficacy, and potential for enhancing the cultivation of a diverse array of medicinal plants.

Keywords: Melghat Region, Medicinal Plants, AMF, Root Colonization.

INTRODUCTION

On the Asian level, the medicinal biodiversity in the Melghat region contributes to India's standing as one of the richest countries in terms of medicinal plants. India is home to over 7500 plant species that have been traditionally used in medicine, especially in Ayurveda, Unani, and Siddha systems of medicine. Melghat, being part of the Satpura hills, adds significantly to the Indian subcontinent's biodiversity and plays a role in the conservation of medicinal plant species that have a wider application in traditional Asian herbal medicine.Efforts to sustainably manage these resources can further bolster India's role as a hub for medicinal plant research and ethnobotany in Asia(Tiwari *et. al.*, 2013).

As the world moves towards more sustainable agricultural practices, the urgent need to reduce chemical fertilizers and pesticides both of which degrade soil health and disrupt ecosystems has never been clearer.Arbuscular Mycorrhizal Fungi (AMF), nature's unsung heroes in the quest for healthier, more resilient farming systems. These remarkable fungi are stepping up as key players in this agricultural revolution, offering a natural, eco-friendly solution to nourish soils, boost plant growth, and safeguard the environment all without the need for harmful chemicals. Beyond their role in conventional crops, AMF also play a crucial part in enhancing the growth of medicinal plants. These plants, often requiring delicate care to maintain their therapeutic properties, benefit greatly from the nutrient-boosting power of AMF. By promoting healthier root systems and increasing nutrient uptake, AMF help cultivate high-quality medicinal plants, ensuring they thrive without the need for synthetic fertilizers or pesticides ultimately supporting both human health and ecological balance. (Hart M. & Forsythe T., 2015).This type

of mycorrhiza is typically found in a wide variety of plants, including medicinal plants, food crops, and trees. (Smith, S. E., & Read, D. J., 2008)

The aim of this research was to study percent of root colonization of Arbuscular Mycorrhizal Fungi (AMF) of some medicinal plants by grid line intersect method.

MATERIAL AND METHODS

Area of Study

Melghat in the South-western Satpura mountain ranges. Melghat means 'meeting of the ghats'. It stretches from south to north between latitudes 21 0 -11' and 21 0 - 46' north and from west to east between longitude 78 0 - 38' and 77 0 -34' east. Melghat is in Amravati District in the Indian state of Maharashtra.

Selected medicinal plants are:

**Caesalpinia bonduc*(L.) Roxb. * *Clerodendron serratum*(L.) Moon. **Physalis communis*L.

Collection of Root samples

The root samples were collected from different regions of Melghat. The collected fibrous root samples of selected medicinal plants from different sites were washed gently to free it from soil particles and were fixed in FAA solution (5: 5: 90 v/v) in labelled containers. All root samples were stored in refrigerator at 4^0C for further processing.

Estimation of root colonization

The root samples were stained according to the procedure given by Phillips and Hayman (1970) and permanent slides were prepared for assessment of mycorrhizal colonization. Degree of mycorrhizal colonization was estimated using Grid line intersect method (Giovannetti and Mosse, 1980)

The root samples were stained by making some modifications in existing techniques given as

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 100ºC 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 uncleared with KOH then root bits were bleached using freshly prepared alkaline H_2O_2 solution at room temperature for 10-20 min and then rinsed with tap water for 2-3 times to remove traces of alkaline H_2O_2. [Alkaline H_2O_2 was prepared by adding 30% H_2O_2 (1ml): NH_4OH (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.

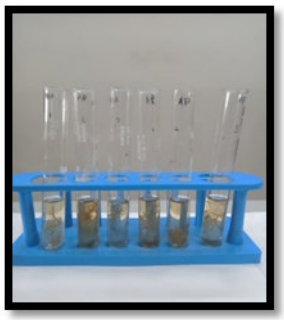
• Destaining solution removed the excess stain from cortical region of roots which did not have any AMF structures, making AMF structures more visible and clearer in other cells. [Lactoglycerol was prepared by adding Lactic acid (40ml): Glycerol (40ml): D/W (20ml) V/V]

6) Mounting

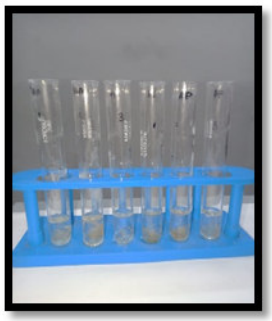
• Permanent slides were prepared by placing 9-10 properly stained root segments lengthwise with the help of forceps in a thin layer of PVLG mountant on a slide and covered with coverslip by avoiding bubble formation and pressed gently.

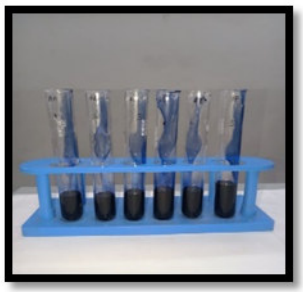
• Slides were stored in slide box for further analysis of AMF and photography.


Chemicals


Root in KOH

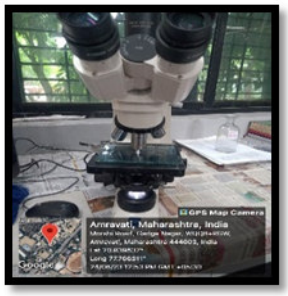

Root After Boiling in KOH


Roots in HCL


Roots In Trypan Blue


Grid-line Intersection


Slides of Roots



Observation of Root Colonization

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

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

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).

RESULT AND DISCUSSION

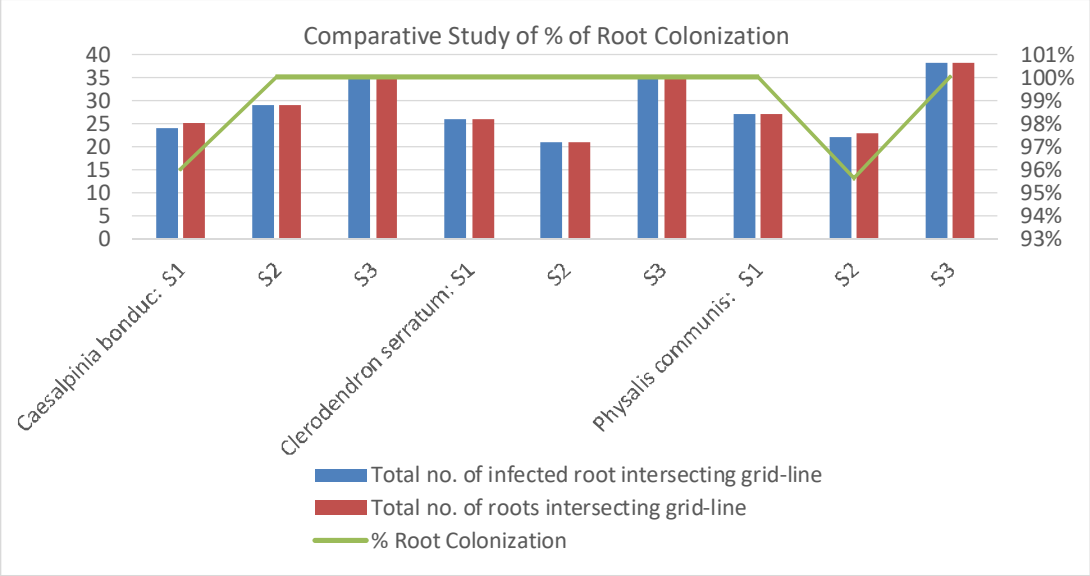
All the samples studied exhibited AM fungal association. The root association was characterized by the presence of hyphal, arbuscular and vesicular characteristic features. The % of root colonization in *Caesalpinia bonduc*(L.) Roxb.from the sample -1(S1) was found to be 96%, Sample-2(S2) was 100% and from Sample-3 (S3) was 100%. The % of root colonization in *Clerodendron serratum*(L.) Moon.from the sample -1(S1) was found to be 100%, Sample-2(S2) was 100% and from Sample-3 (S3) was 100%. The % of root colonization in *Physalis*

*communis*L.from the sample -1(S1) was found to be 100%, from Sample-2(S2) was 95.66% and from Sample-3 (S3) was 100%. Maggirwar *et. al*., (2013) also reported the % root colonization in *Dioscorea* species from the site S1 was found to be 90.32%, S2 site 77.27% and from S3 site 75%.

The current study showed that AMF had a good symbiotic association with the selected medicinal plant species. Occurrence of these association was detected microscopically. The present study confirms that *Caesalpinia bonduc*(L.) Roxb., *Clerodendron serratum*(L.) Moon.and*Physalis communis*L.having rich colonization of AMF.It was found that out of these three plant species the *Clerodendron serratum*(L.) was highly colonized by AMF.

Percentage of Root Colonization:

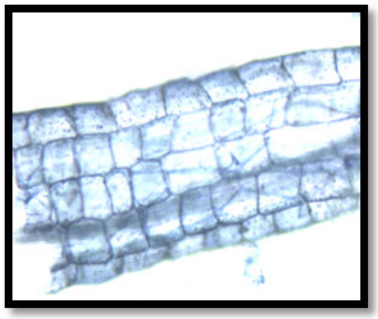
Root Samples	Total no. of infected root intersecting grid-line	Total no. of roots intersecting grid-line	% Root Colonization
Caesalpinia bonduc: S1	24	25	96%
S2	29	29	100%
S3	35	35	100%
*Clerodendron serratum:*S1	26	26	100%
S2	21	21	100%
S3	35	35	100%
Physalis communis: S1	27	27	100%
S2	22	23	95.66%
S3	38	38	100%

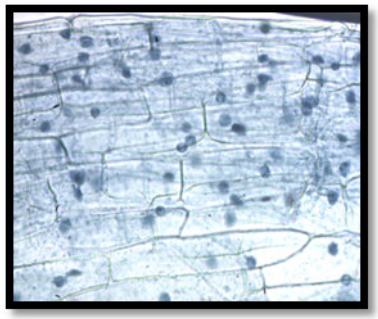


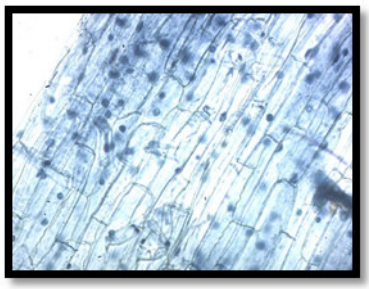
Qualitative Root Analysis-

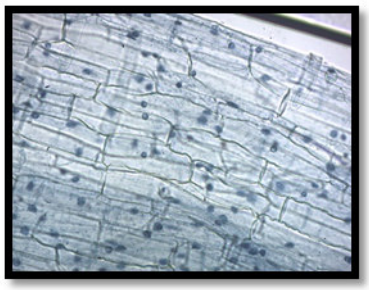
Root Samples	Hyphae	Vesicle	Arbuscle	Intra-radicle spore
Caesalpinia bonduc: S1	+++	+++	+++	+++
S2	+++	+++	+++	+++
S3	+++	++	++	-
*Clerodendron serratum:*S1	+++	+++	+++	++
S2	+++	+++	+++	+
S3	+++	+++	+++	+++
Physalis communis: S1	+++	++	-	++
S2	+++	+++	+++	++
S3	+++	++	+++	+++


H- Shaped Hyphae


H- Shaped HyphaeY-Shaped Hyphae




Vesicles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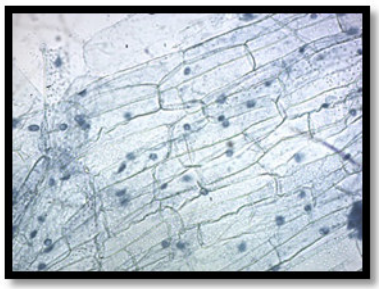
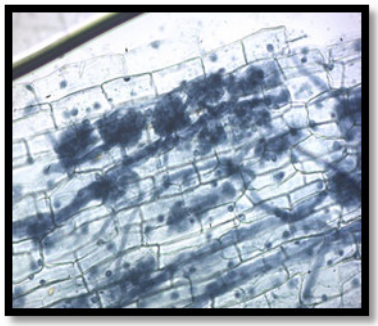
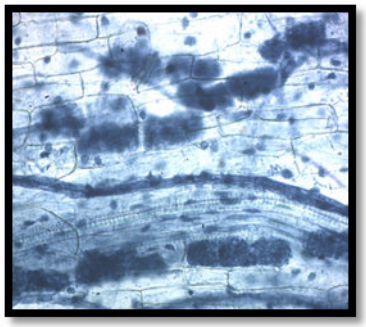
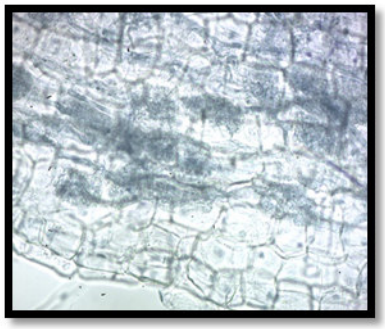
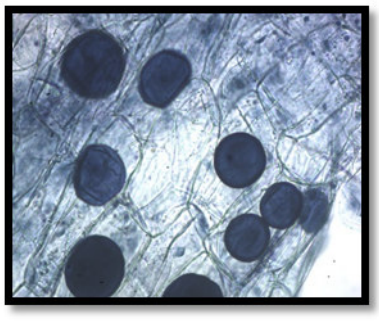
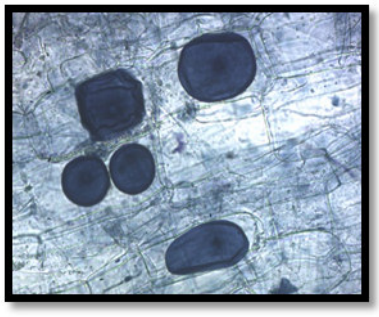

Vesicles

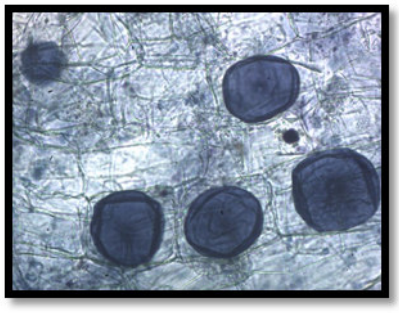

Arbuscule


Arbuscule


Arbuscule


Intra-radical Spore


Intra-radical Spore


Intra-radical Spore

Present investigation delves into the spatial aspects of root colonization, providing detailed insights into the extent and distribution of fungal structures within the root system. Environmental factors are considered, offering a comprehensive understanding of the nuanced interplay. The findings enhance cultivation practices for selected medicinal plants and contribute valuable knowledge to the broader ecological implications of mycorrhizal associations in medicinal plant ecosystems.

CONCLUSION

The present study demonstrates a strong and consistent association between arbuscular mycorrhizal fungi (AMF) and the selected medicinal plant species: *Caesalpinia bonduc*, *Clerodendron serratum*, and *Physalis communis*. All samples examined displayed clear evidence of AMF colonization, with characteristic hyphal, arbuscular, and vesicular structures identified microscopically. Root colonization rates varied across samples, with *Caesalpinia bonduc* showing near-complete colonization (96% to 100%), *Clerodendron serratum* exhibiting consistent 100% colonization across all samples, and *Physalis communis* demonstrating high colonization rates (95.66% to 100%).It was found that out of these three plant species the *Clerodendron serratum*(L.) was highly colonized by AMF. Overall, this study underscores the importance of AMF symbiosis for the growth and health of medicinal plants, highlighting the potential for enhancing cultivation practices through mycorrhizal associations. The observed high levels of AMF colonization suggest a strong ecological relationship, which may contribute to improved plant resilience and nutrient uptake. Selected medicinal plants often rely on secondary metabolites for their therapeutic properties, future studies could explore how AMF colonization affects the production of bioactive compounds in these plants.

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