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AM Fungal Diversity in selected medicinal trees of Sanjay Gandhi National Park, Borivali, Mumbai, India

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ABSTRACT

The AM fungal composition, diversity & distribution was studied in five trees (Sapindus *trifoliatus*, *Psidium gujava, Citrus limonia, Aegle marmelos and Bauhinia variegata*) of medicinal importance from Thane region of Sanjay Gandhi National Park, Mumbai, India. AM colonization ranged from 75% to 85%. AM spore density ranged from 58±5 to 75± 7 in the rhizospheric soils. Based on morphological characters 24 Arbuscular Mycorrhizal Fungal species belonging to five genera were isolated and identified. Out of the five trees,*Citrus limonia* showed maximum species richness followed by *Bauhinia variegata* and *Aegle marmelos.* The isolation frequency of *Acaulospora* and *Glomus* was 100%. *Ambispor*a and *Scutellospora* were less frequently found.

Key words: AM Fungi, Sanjay Gandhi National Park, *Glomus, Acaulospora, Gigaspora.*

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) are associated with about 80% of the plant families in the world (Giovannetti and Sbrana. 1998). The AM Fungi are known to enhance plant growth, primarily through increased phosphorus absorption. AM Fungi are ubiquitous and thus are a part of every cultivated or natural ecosystem, whether in tropical, temperate or desert regions and regardless of crop and cropping system. This suggest that AM Fungi play a critical role in plant survival and species diversity in many natural ecosystems. Occurrence of microorganisms, especially vesicular arbuscular mycorrhizal fungi, in medicinal plants has been shown to improve the status of both water and nutrients in host plants. AMF are known to benefit plant establishment by increasing resistance to environmental stresses, enhancing plant nutrient acquisition, water relations, disease resistance and improving soil quality (Smith and Read, 2008).

This study evaluates the status of AM Fungi in five medicinal trees viz. *Aegle marmelos* (Bel), *Bauhinia variegata* (Kachnar), *Citrus limon*ia (Lemon),

Psidium guajava (Guava) and *Sapindus trifoliatus* (Soapnut). The medicinal plants were collected from Sanjay Gandhi National Park, Borivali, Mumbai, Maharashtra state and were examined for the root colonization and diversity of AM Fungal spores in their rhizosphere soils.

MATERIAL AND METHODS

Study area:

The samples were collected from **t**he Borivali National Park, officially known as the Sanjay Gandhi National Park, a unique National Park in that it lies within the borders of Mumbai city. The park lies on the northern fringes of suburban Mumbai of Maharashtra state in India. The park has a rich flora and fauna. The plants were selected from Thane region of the National park. The topography of this region is uneven.

Soil sampling: The samples were collected between October 2016 and March 2017. Root samples and rhizosphere soil of the medicinal plants were collected from National Park, Borivali, Mumbai and preserved in sterile polythene bags and stored in refrigerator at 4 ^{0}C until use. Soil sample up to 20 cm depth was collected. Root samples were cut into 1cm bits and preserved in FAA until use for mycorrhizal colonization.

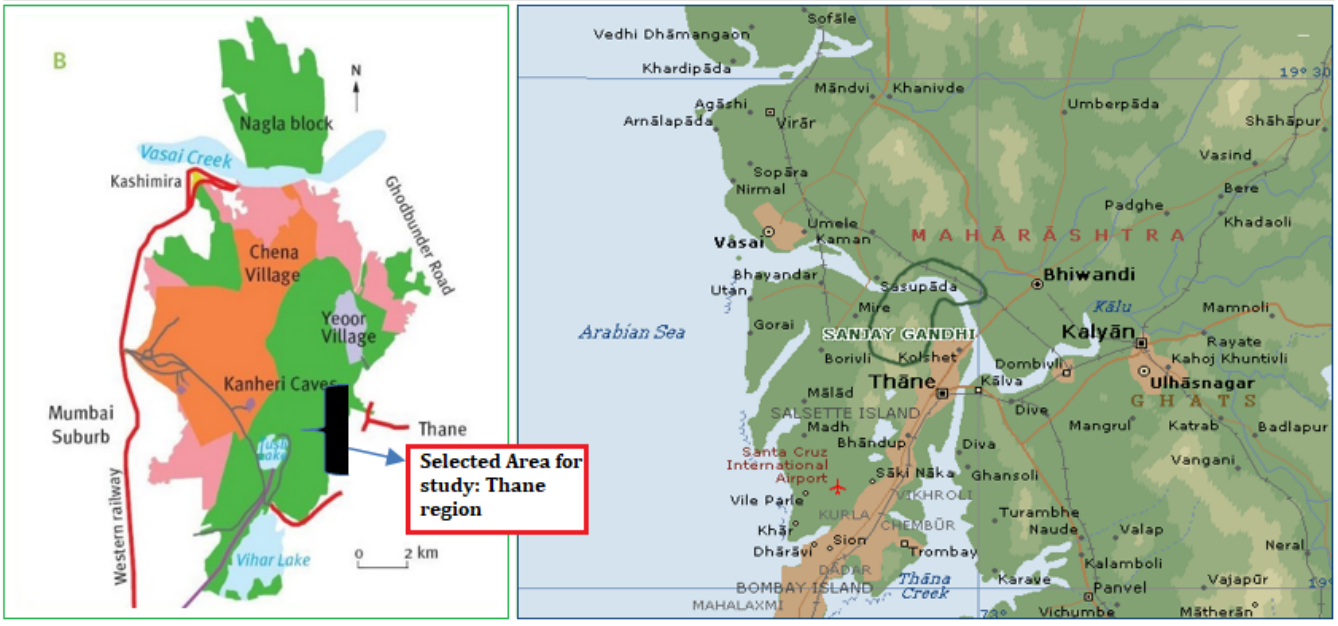
Spore extraction was done by Gerdeman and Nicolson method, 1963. 10gm of soil sample was taken and mixed with one litre of warm water, a pinch of detergent added to it and left for half an hour until all the aggregates dispersed to leave a uniform suspension. The detergent washes away the soil particles attached to the spores.The suspension is passed through 500 µm, 250 µm, 150 µm, 75 µm and 35 µm sieves and the tap water allowed to flow for half an hour mildly so that the hyphae and sporocarps do not break apart. The residues in the respective sieves were collected in beakers carefully with approximately 100ml of water. This water which contains spores, sporocarps are filtered on the circular whatman filter paper, taken in petridishes and observed under motic dissecting microscope for AM fungal spores.

Spore Quantification

Spore Density

Spores in the 10gm of soil sieve were collected in the five petridishes and the spore number was counted by Gaur and Adholeya method, 1994. Then the spores were picked up using an injection needle. The spores were mounted on clear glass slides using lacto phenol or polyvinyl alcohol lacto phenol Glycerol (PVLG) and covered with cover slips.

Map of Sanjay Gandhi National Park Mumbai, Maharashtra.	Map of Maharashtra, India, showing Mumbai region



Source of Map : Google images

The slides are heated at 40-50 ^{0}C temperature so that the air bubbles are removed and the spores appear clear. This is then sealed with DPX which makes the slides semi-permanent.

Root Colonization of AM Fungi was done by Philips and Hayman method, 1970 and percentage of root colonization was calculated by Read et.al, 1976.
Root samples were subjected to root clearing and staining technique in which the root samples were cut into 1cm bits and then cleared with 10% KOH for one hour, rinsed with distilled water and kept in 5N HCl for 3min, and stained with 0.05% trypan blue in Lactophenol. This was kept overnight and percentage of root colonization was calculated by Read et.al,1976.

Ten root pieces of 1cm were mounted on slides, a drop of lactophenol was put on the slide and covered with cover slip and gently pressed This was observed under Labomed compound microscope to check the root colonization by AMF. The root colonization percentage was calculated by the following formulae:

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

Species Richness is the number of identified AMF species per soil sample.

Isolation frequency is defined as the percentage of soil samples in which a species occurred, which revealed extent of distribution of given AMF species in an ecosystem (Kavitha and Nelson, 2013).

$$\text{IF (Isolation Frequency)} = \frac{\text{The number soil samples in which AMF species occurred}}{\text{The total number of soil}} \times 100$$

Identification of VAM fungi
The identification of arbuscular mycorrhizal fungal spores was done based on spore colour, size, hyphal attachment, wall layers and germination shield using websites www.invam.caf.wvu.edu. and www.zor.zut.edu.

RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi have been described as 'keystone mutualists' in ecosystems due to their unique position at the root-soil interface (Aditya kumar et al. 2010).

Most of the naturally growing plants are mycorrhizal and they depend on these symbionts for their nutritional and physiological needs. The objective of the study was to find out diversity of AM Fungi, species richness and isolation frequency of different genera and species from Sanjay Gandhi National Park as there are no reports till now from this area.

Twenty four Morphotypes belonging to five genera viz., *Glomus* (11 species), *Acaulospora* (9 species), *Gigaspora* (1 species) and *Scutellospora* (1 species) were isolated and identified from the rhizosphere soil of five trees having medicinal importance from the Sanjay Gandhi

Table 1: Root Colonization, Spore density, Species richness & Spore types in the trees

S. No.	Name of the Plant	Family	Root colonization (%)	Spore Density /10g soil	Species Richness	Mycorrhizal Spore types
1	*Sapindus trifoliatus*	Sapindaceae	75%	50±6	5	*Acaulospora sps 1., Acaulospora mellea, Glomus albidum, Glomus intraradices, Gigaspora albida*
2	*Psidium gujava*	Myrtaceae	80%	60±5	4	*Acaulospora mellea, Gigaspora albida, Glomus macrocarpum, Scutellospora persica*
3	*Citrus limonia*	Rutaceae	85%	58±5	11	*Acaulospora scrobiculata, Acaulospora myriocarpa, Acaulospora koskei, Acaulospora soloidea, Ambispora appendicula, Ambispora callosa, Glomus callosum, Glomus fasciculatum, Glomus constrictum, Glomus melanosperma, Gigaspora albida*
4	*Aegle marmelos*	Rutaceae	80%	69±12	6	*Acaulospora myriocarpa, Acaulospora foveata, Acaulospora scrobiculata, Acaulospora cavernata, Glomus clavisporum, Glomus melanosporum*
5	*Bauhinia purpurea*	Leguminosae	85%	75± 7	6	*Acaulospora sps 1., Acaulospora myriocarpa, Acaulospora rehmii, Glomus fuegianum, Glomus badium, Glomus sps, Gigaspora albida*

Table 2: Isolation Frequency of AM Fungal species in the rhizosphere of the trees

S. No	Name of the AM Species	Isolation Frequency %	S. No	Name of the AM Species	Isolation Frequency %
1	*Acaulospora sp.,*	40	13	*Glomus intraradices*	20
2	*Acaulospora mellea*	40	14	*Glomus macrocarpum*	20
3	*Acaulospora scrobiculata*	40	15	*Glomus callosum*	20
4	*Acaulospora myriocarpa*	60	16	*Glomus fasciculatum,*	20
5	*Acaulospora koskei*	20	17	*Glomus constrictum,*	20
6	*Acaulospora soloidea*	20	18	*Glomus melanosperma*	40
7	*Acaulospora rehmii,*	20	19	*Glomus fuegianum*	20
8	*Acaulospora foveata*	20	20	*Glomus badium*	20
9	*Acaulospora cavernata*	20	21	*Glomus sps*	20
10	*Ambispora appendicula,*	20	22	*Glomus clavisporum*	20
11	*Ambispora callosa*	20	23	*Gigaspora albida*	80
12	*Glomus albidum*	20	24	*Scutellospora persica*	20

Table 3: Isolation Frequency of AM Fungal Genera in the rhizosphere of the trees

S. No	Name of the AM Genera	Isolation Frequency %
1	*Acaulospora*	100
2	*Ambispora*	20
3	*Glomus*	100
4	*Gigaspora*	80
5	*Scutellospora*	20

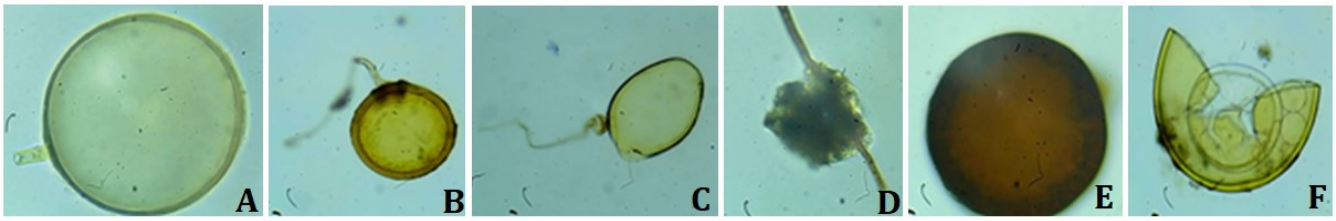


Figure 1:AM Fungal Spore types in rhizosphere soil of *Sapindus trifoliatus*
A: *Glomus albidum;* B: *Glomus intraradices;* C: *Gigaspora albida;* D: Auxillary Cells of *Gigaspora;* E : *Acaulospora sps.;* F: *Acaulospora mellea*

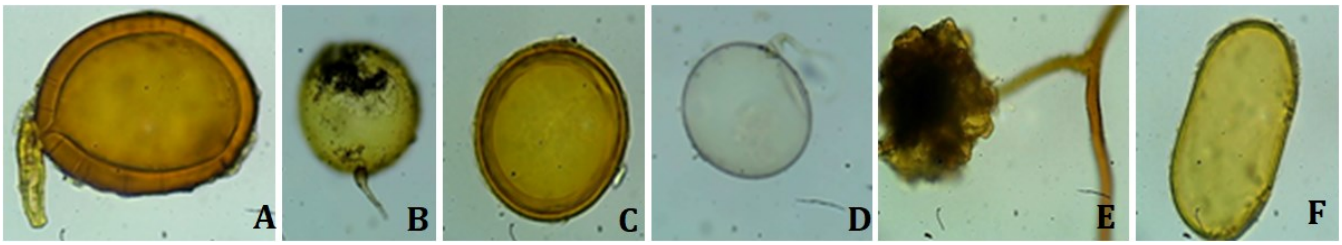


Figure 2: AM Fungal Spore types in rhizosphere soil of *Psidium gujava*
A: *Glomus macrocarpum,* B: *Scutellospora persica,* C: *Acualopspora mellea* D: *Gigaspora albida,* E *Auxillary cells of Gigaspora*, F:

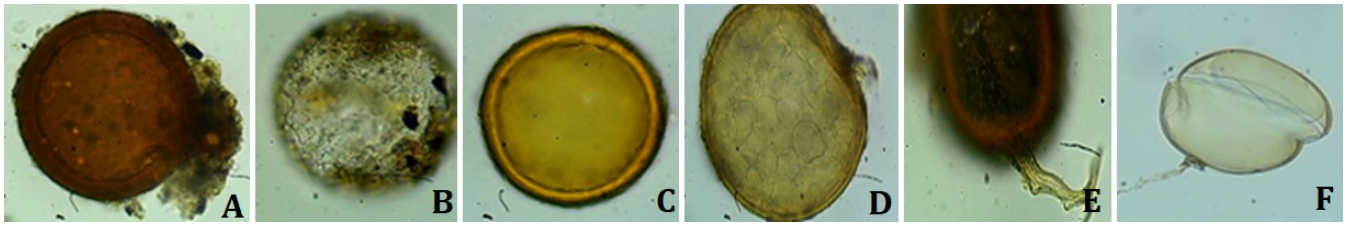


Figure 3: AM Fungal Spore types in rhizosphere soil of *Bauhinia variegata*
A: *Glomus badium,* B: *Acaulospora myriocarpa,* C: *Acaulospora rehmii,* D: *Glomus fuegianum,* E: *Glomus sp*, F: *Gigaspora albida*

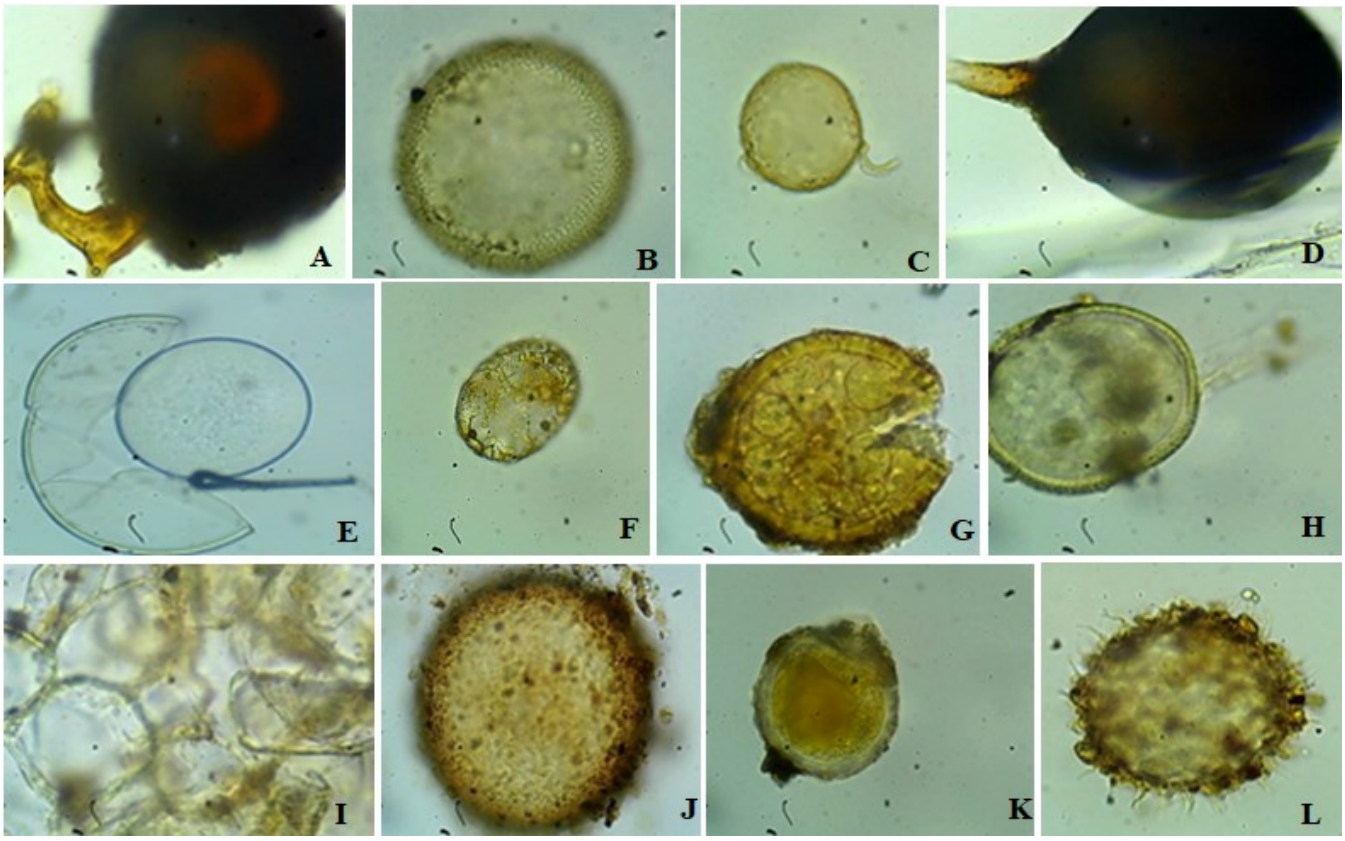


Figure 4: AM Fungal Spore types in rhizosphere soil of *Citrus limonia*
A: *Glomus constrictum*, B: *Acaulospora scrobiculata*, C: *Glomus fasciculatum*, D: *Glomus* E: *Gigaspora albida* F: *Acaulospora myriocarpa*, G: *Acaulospora koskei*, H: *Ambispora appendicula*, I: Sporocarp of *Ambispora*, J: *Ambispora callosal*, K: *Glomus callosum*, L : *Acaulospora soloidea*

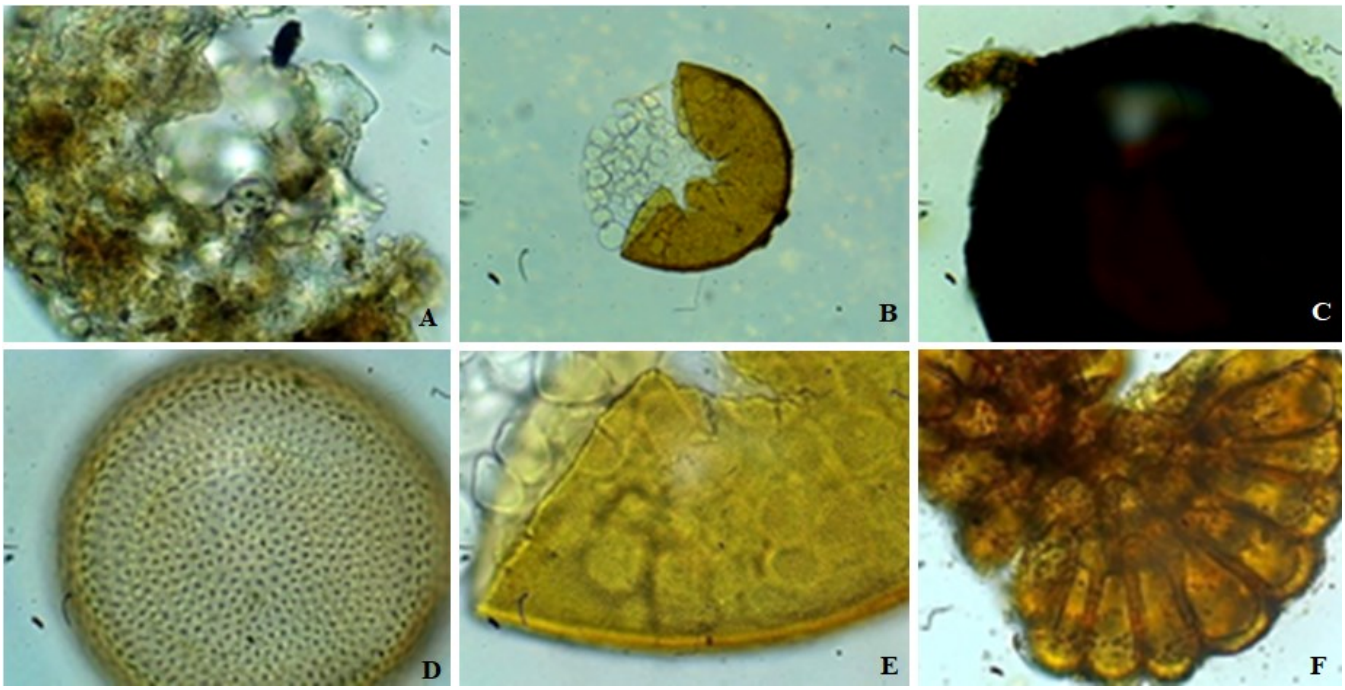


Figure 5: AM Fungal Spore types in rhizosphere soil of *Aegle marmelos*
A: *Acaulosporamyriocarpa*, B: *Acaulospora foveata*, C: *Glomus melanosporum*, D*: Acaulospora scrobiculata*, E: *Acaulospora cavernata*, F: *Glomus clavisporum-Sporocarp*

significant observation. Highest spore density was found in *Bauhinia purpurea* (75± 7 spores / 10g of soil) followed by *Aegle marmelos, Psidium gujava, Citrus limonia and Sapindus trifoliatus* whose spore density varied from 50±6 to 69±12/10g of soil respectively.

Percent root colonization of AM Fungi varied from 75% to 85% in the five plants. This indicates a very good association of AM Fungi with the host plants. Mycorrhizal structures observed were hyphae, arbuscules and vesicles in the roots.

Species richness was highest in *Citrus limonia* (11 species) followed by *Aegle marmelos* (6 species) and *Bauhinia purpurea* (6 species). Lowest species richness was shown by *Psidium gujava* which harbored 4 species of AM Fungi. (Table 1) (Figure 1 to 5). Isolation frequency of *Acaulospora* and *Glomus* was highest. Among species, *Acualospora myriocarpa* and *Gigaspora albida* showed 80% isolation frequency.

No significant correlation was found between spore density and root colonization. Similar results were observed by Rajkumar *et al,* 2012 from the western ghats of India, where similar soil type is found. Dominance of genus *Glomus* from medicinal plants has been reported earlier by Selvaraj et al. (2001). Muthukumar et al. (2001) reported 35 arbuscular mycorrhizal fungal species from 329 medicinal plant species from Western Ghats.

No host specificity was observed between AM Fungi and the plants. In conclusion, the present work revealed significant diversity of AM Fungi in the five medicinally important trees.

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Competing interests

The authors declare that they have no competing interests.

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