

Seasonal Variation in Biodiversity of AM Fungi in *Madhuca Longifolia*

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ABSTRACT

The crucial role of Arbuscular Mycorrhizal (AM) fungi in plant growth and soil health have been well established through observational and evolutionary studies. They support plant growth by enhancing nutrient uptake and improving stress tolerance. The objective of this study was to investigate the seasonal diversity and colonization of AM fungal spores associated with *Madhuca Longifolia* (Indian butter nut tree) roots in Maharashtra Nature Park (MNP), Mumbai. The study was conducted across two seasons- pre-monsoon (April–June) and monsoon (July–September). Soil and root samples were analyzed for spore diversity, root colonization rates, and the impact of environmental factors associated with these seasons on fungal distribution. The results revealed a higher diversity of AM fungal spores during the monsoon season, with the presence of *Aculosporamyriocarpa*, *Glomus*, *Gigaspora*, *Macrocarpum*, and *Clarum* sp. In contrast, only *Aculosporamyriocarpa* and *Glomus* were observed in the pre-monsoon period. Interestingly, despite higher diversity observed in the monsoon season, root colonization was lower (54.8%) compared to the pre-monsoon season (65%). This is most likely due to negative impact of excessive soil moisture on fungal-root interactions. The spores were found to be coated with soil particles, suggesting contamination due to pollution in the habitat. The prevalence of *Aculosporamyriocarpa* and *Glomus* sp. across both seasons indicates their adaptability to the ecological conditions in MNP. In conclusion, this study highlights the seasonal dynamics of AM fungal communities in MNP which may be more adapted to pre-monsoon conditions.

Keywords: Arbuscular Mycorrhizal Fungi, *Madhuca Longifolia*, Root Colonization, Seasonal Variation, Fungal Diversity, Pollution Impact

INTRODUCTION

The Arbuscular Mycorrhizal (AM) fungi form mutualistic associations with the roots of most plant species. Hence, they are an essential component of terrestrial ecosystems. Through their symbiotic association with the roots, AM fungi promotes plant nutrient uptake, while receiving carbohydrates from the host plant. In addition to basic growth and reproduction benefits, AM fungi improve plant tolerance to various abiotic stresses, including drought, heavy metal toxicity, salinity and soil degradation. They also contribute to soil structure maintenance and carbon sequestration[1].

The presence and diversity of AM fungal species in a given environment depend on soil composition, climatic conditions, and host plant species. For this reason, factors such as soil contamination, altered nutrient dynamics, and habitat fragmentation can impact AM fungal diversity and functionality. As a natural response to stress, like all microorganisms, certain AM fungal species exhibit resilience and adaptability which allows them to thrive even in contaminated environments. Understanding their distribution and functional roles can provide valuable insights for ecological conservation and land reclamation efforts[2].

The site selected for this study was Maharashtra Nature Park (MNP). It has a unique ecological history of being transformed from a municipal waste dumping ground, until 1977, into an urban nature park in 1983[3]. In spite of the transformation, the tremendous amount of wastes dumped in this area and the lack of proper waste segregation during its use as a landfill, plants thriving at MNP still face significant challenges due to possible contamination, altered microbial communities, and nutrient imbalances.

Despite these challenges, our previous study on species diversity of AM fungi among selected medicinal plants at MNP showed successful establishment root hyphae and mycorrhiza. This suggests the presence of resilient microbial communities in MNP [4]. The study also showed maximum AM fungal diversity in soil samples of *Madhuca Longifolia* (Indian butter nut tree) among 10 different trees selected. In the present study, we aimed to determine the seasonal variations in AM fungal species diversity and root colonization of *M. longifolia*.

MATERIALS AND METHODS

Study area

The study was conducted at Maharashtra Nature Park (MNP), located in Mumbai. The park is situated in the "H" Block of the Bandra-Kurla Complex (Bandra-Sion Road) along the southern bank of the Mithi River.

Sample collection and processing

Soil samples around the trees of *M.longifolia* were collected from MNP between April and September. The sampling period was categorized into two seasons: Pre-monsoon (April–June) and Monsoon (July–September). The root samples were washed with tap water, cut into 1 cm segments, and preserved in FAA (Formalin-Acetic Acid-Alcohol) solution until use for mycorrhizal colonization studies.

Spore extraction

The method described by Gerdemann and Nicolson [5] was followed for spore extraction. A 10g soil sample was mixed with 1 L of water, and a small pinch of detergent was added to disperse soil aggregates, ensuring a uniform suspension. This step helps in separating spores from the soil matrix. The suspension was sequentially passed through sieves of 500 µm, 250 µm, 150 µm, 75 µm, and 35 µm, allowing water to flow gently for 30 mins to prevent breakage of hyphae and sporocarps. The residues retained on each sieve were carefully collected into beakers containing approximately 100 mL of water. This spore-containing solution was filtered using circular Whatman filter paper, transferred to Petri dishes, and examined under a Motic dissecting microscope for AM fungal spores.

Quantification of spore density

Spores collected from the 10 g soil sample were transferred into five Petri dishes and counted following the method of Gaur and Adholeya[6]. Individual spores were carefully picked up using an injection needle and mounted on glass slides using lactophenol or Polyvinyl Alcohol Lactophenol Glycerol (PVLG). Cover slips were placed over the spores, and the slides were gently heated at 40–50°C to eliminate air bubbles, ensuring clear visibility of spores. The slides were then sealed with DPX to make them semi-permanent.

Root colonization analysis

Root colonization by AM fungi was analyzed using the method described by Phillips and Hayman [7], while the percentage of root colonization was calculated following Read et al. [8]. One cm long root segments were cleaned with 10% KOH for one hour, rinsed with distilled water, and treated with 5N HCl for 3 mins. The roots were then stained overnight with 0.05% trypan blue in lactophenol. The percentage of root colonization was determined using the following formula:

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

Identification of AM Fungi

The identification of AM fungal spores was performed using the online resources 'International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM)' available at www.invam.caf.wvu.edu and 'Mycorrhizal Fungi Database' available at www.zor.zut.edu

RESULTS AND DISCUSSION

The Maharashtra Nature Park is located in an urban setting along the Mithi River in Mumbai. This site is well known as an industrial and domestic dumping ground. The pollution in this region was evident from the spore samples collected as they appeared contaminated with soil particles and debris.

Root colonization and diversity

The diversity and colonization rates of AM fungal spores in roots of *M.longifolia* observed between the pre-monsoon and monsoon seasons are represented in Table 1. Additionally, the seasonal variations are depicted graphically for comparison in Fig. 1. *Aculosporamyriocarpaw* was prevalent across both monsoon and pre-monsoon seasons in roots of *M.longifolia*. In our previous study too, this species was found to be prevalent in roots of all 10 selected medicinal plants in MNP [4]. This highlights its ecological significance at the studied site. *Glomus* species, which was also prevalent in both seasons, are reportedly widespread. Their adaptability to various environmental conditions, soil types, and plant hosts makes them one of the most frequently encountered AM fungi worldwide[9]. Both *Acaulospora* and *Glomus* species produce smaller spores, sporulate profusely and have greater dispersal efficiency within the same habitat and seasonal conditions[10]. The presence of *Gigaspora*, *Macrocarpum*, and *Clarumsp.*, exclusively during the monsoon suggests that they may have specific moisture-dependent sporulation patterns. Previous studies on *Gigasporasp.* have suggested that they produce larger spores, require a longer time to complete their life cycle leading to their relatively lower abundance [11].

imilar studies from India have reported *Glomus* as a dominant species, with *Glomusdeserticola* and *Rhizophagusintraradices* displaying widespread distribution in the mangrove ecosystem of Henry’s Island, Bakkhali, India[9]. In contrast, the dominant endophytic fungi included *Phomopsis* sp. 1 (9.185%) and *Colletotrichumgloeosporioides*in *Madhucaindica*Gmel., isolated from Uttar Pradesh, India. The study further reported monsoon as a favourable condition for AMF diversity as well as colonization rates [12].

Table 1: Seasonal variation in root colonization and AM Fungal spore diversity in *Madhuca Longifolia*

Seasons	Root Colonization	Spore Density /20g of soil	Types of spores observed
Pre-Monsoon	65%	296	<i>Aculosporamyriocarpa</i> , <i>Glomus</i>
Monsoon	54.8%	350	<i>Aculosporamyriocarpa</i> , <i>Glomus</i> , <i>Gigaspora</i> , <i>Macrocarpum</i> , <i>Clarum</i>

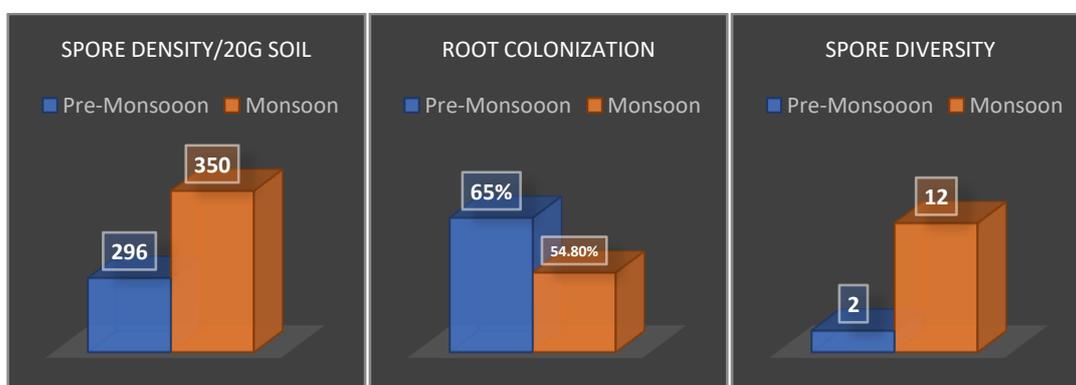


Fig. 1: Comparison of study variables in monsoon and pre-monsoon season

The percentage of root colonization also differed between seasons. Interestingly, as observed in Table 1, no significant correlation was found between spore density and root colonization. This suggests that spore abundance does not necessarily translate to increased root colonization.

Several factors, including soil conditions, host plant compatibility, and microbial competition, may influence the extent of fungal-root interactions. High water content in the soil may reduce oxygen availability, influencing the establishment and penetration of AM fungi within the root system[2].

A study focusing on mycorrhizal fungi dynamics in the Cerrado biome, Brazil also reported higher spore density, mycorrhizal colonization rates, and glomalin content during the dry season compared to the rainy season[13].

The AM fungal diversity observed during the monsoon season may be attributed to factors such as increased soil moisture and nutrient availability. These conditions enhance spore germination and its distribution[1, 2].

Structural characteristics of spores

The structural characteristics of AM fungi associated with roots of *M.longifolia* are represented as Fig. 2. The microscopic analysis of AM fungal spores revealed diverse morphological characteristics. Variations were observed in shape, size, and pigmentation.

They appeared well-formed but exhibited some soil debris and particles. The extensive network of hyphae suggested active colonization, facilitating nutrient exchange between the fungus and the host plant. Arbuscules were well-defined as blue-stained structures confirming intracellular colonization.

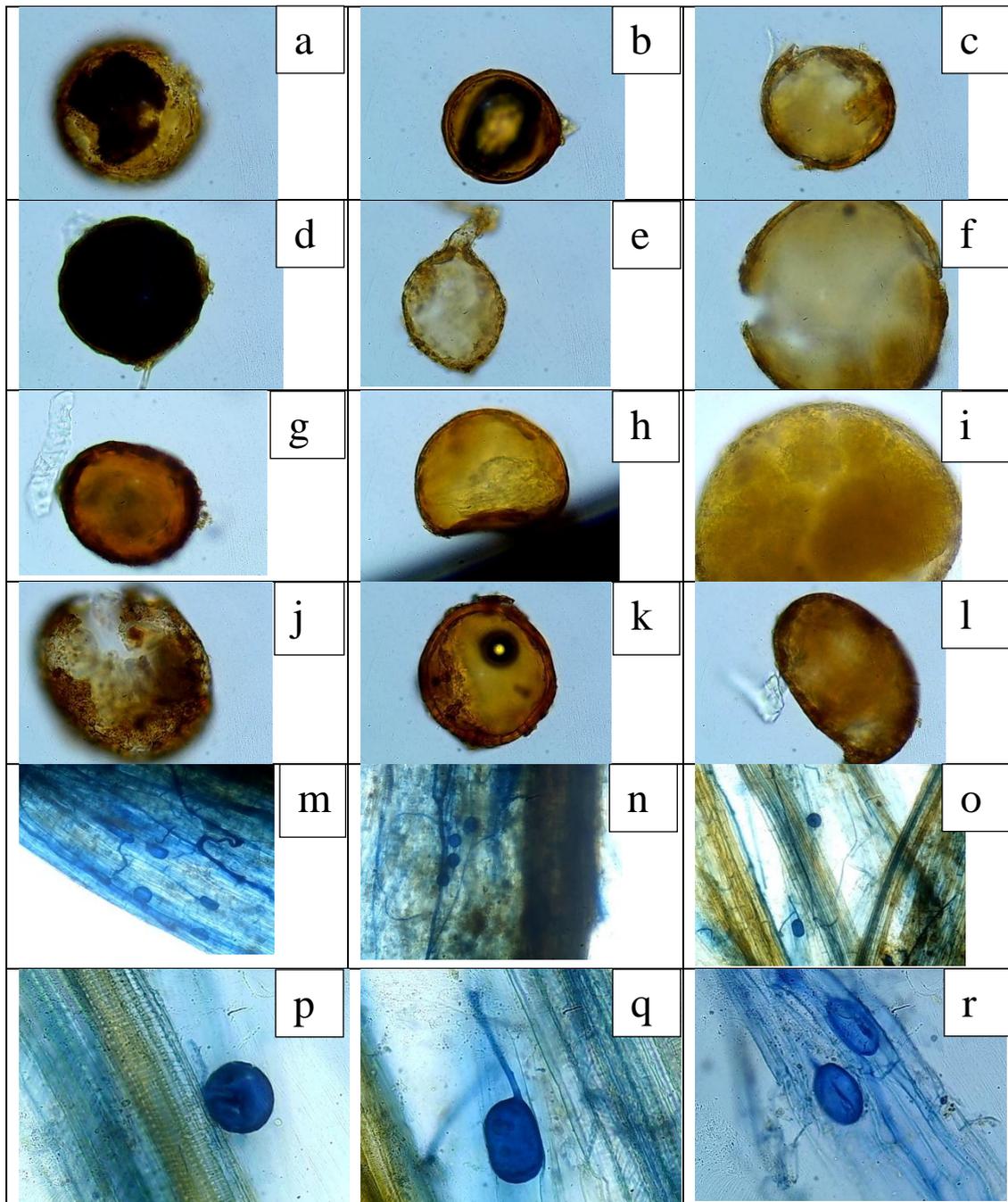


Fig. 2: Structural characteristics of spores associated with roots of *Madhuca Longifolia*

The figure shows spore structures in the soil samples (a to l), hyphae and vesicles (m to o) and Vesicles (p to r)
Aculospora sp.: a, b, c, g, h, i, j, k, l; *Glomus* sp.: e, f; *Gigaspora* sp.: d

CONCLUSION

The proximity of Maharashtra Nature Park to the Mithi River exposes it to heavy metals and other pollutants from the industrial and domestic effluents discarded in it. These pollutants impact AM fungal diversity, viability, and overall ecosystem. This study revealed seasonal variations in AM fungal diversity and root colonization rates in *M. longifolia*. Future studies assessing the impact of soil contamination on AM fungal spore viability and root colonization could provide deeper insights into the role of AM fungi in polluted environments.

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