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Structural diversity of AM Fungi in the roots of *Lantana camara* and *Stachytarpheta indica*

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ABSTRACT

The structural diversity of AM Fungi in the roots of *Lantana camara* and *Stachytarpheta indica* was studied. Soil samples and the roots were collected from approximately20 cm below the soil surface of the two plants present in the NES Ratnam College campus, Bhandup (West), Mumbai, Maharashtra, India . The plants were sown approximately a year ago in the college garden. Both the plants are perennial shrubs and nectar plants for several species of butterflies belonging to family verbenaceae. The AM Fungal spore density was checked in the soil samples and the spore types were identified. The spore density in *Stachytarpheta indica* was 91.34 ± 6.5 and in *Lantana camara* it was 122.67 ± 9.45. *Lantana camara* showed dominance of *Glomus* species spores where as *Stachytarpheta indica* showed dominance of *Acaulospora* species spores in the rhizosphere soil. Hence a thorough study of the AM structures found in the roots was done. The anatomical study of roots revealed that *Lantana camara* showed 95 % root colonization with thin darkly stained hyphae and oval vesicles where as *Stachytarpheta indica* showed 90% root colonization, thick, lightly stained, coiled hyphae with arbuscules and vesicles with oil globules.

Key words: AM Fungi, *Lantana camara, Stachytarpheta indica, Glomus, Acaulospora.*

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) belonging to the phylum Glomeromycota are important soil organisms that form mutualistic associations with plants, and which are involved in the uptake and transport of mineral nutrients to plant roots (Barea *et al*. 2002). Up to 90 % of analysed plant species are able to form this symbiosis (Smith &

Read 1997). AMF ubiquitous presence and their taxonomic, genetic and functional diversity are directly related to plant and soil processes and therefore there is an increasing interest in the assessment of the biodiversity and functions of AMF communities.

Diversity of AMF species is measured mainly by extracting, counting and identifying their field collected asexual spores, the fungal propagule that possess morphological characters to define species in this group of organisms although molecular techniques have been revealed as useful tool for characterization and identification of AMF. Hyphae within root also show different structures. Gallaud (1905) observed that VAM associations in different species formed two distinctive morphology types, which he named the *Arum* and *Paris*s eries after host plants. **Linear** (*Arum*) series associations hyphae proliferate in the cortex by growing longitudinally between host cells. This occurs because hyphae grow through longitudinal intercellular air spaces that are present. **Coiling** (*Paris*) series hyphae spread by forming coils within cells because there are no continuous longitudinal air spaces. **Arbuscules** (tree shaped structures) Arbuscules are intricately branched haustoria that are formed within a root cortex cell. Arbuscules are considered the major site of exchange between the fungus and host. Arbuscules are short-lived and begin to collapse after a few days, but hyphae and vesicles can remain in roots for months or years. **Vesicles** develop to accumulate storage products in many VAM associations. Vesicles are initiated soon after the first arbuscules, but continue to develop when the arbuscules senesce. Vesicles are hyphal swellings in the root cortex that contain lipids and cytoplasm. These may be inter- or intracellular in active mycorrhizae (Bago *et al*., 1998). *Gigaspora, and scutellospora* do not form vesicles within the roots .

Mycorrhizas produced by *Glomus* show relatively straight hyphae that ramify along the root cortex (if root anatomy permits), often producing "H" branches which result in simultaneous growth in 2 directions. Staining of these hyphae is usually relatively dark, arbuscules can be dense and compact. Oval vesicles, which usually form between root cortex cells, are present in many cases. These vesicles persist in roots and often develop thickened and/or multi-layered walls. Intraradical spores in Glomaceae are usually globose, subglobose to elliptical.

Mycorrhizas produced by *Acaulospora* show hyphae that are more irregularly branched, looped or coiled than for *Glomus*. Internal hyphae are thin walled, often stain weakly and thus may be very hard to see, but may be visible due to rows of lipid droplets. Intracellular oil-filled vesicles, that are initially rectangular, but often become irregularly lobed due to expansion into adjacent cells, are a characteristic feature. Intraradical spores in *Acaulospora* are pleomorphic, knobby and stain lightly in trypan blue.(https://mycorrhizas.info/vam.html).

MATERIAL AND METHODS

Soil sampling : Root samples and rhizosphere soil of *Lantana camara* and *Stachytarpheta indica* was collected from NES Ratnam College campus, Bhandup (West), Mumbai, Maharashtra, India. Soil sample upto20 cm depth was collected.

Spore extraction (Gerdeman and Nicolson,1963)
The soil samples were subjected to wet-sieving and decanting technique for the isolation of spores. The isolated spores were picked up with the needle under a dissecting microscope and were mounted in polyvinyl lactoglycerol and observed under compound microscope. The spore number was counted by Gaur and Adholeya method ,1994.

Taxonomic identification of spores was done by descriptions provided by the www.invam.caf.wvu.edu. andwww.zor.zut.edu.

Root Colonization of AM Fungi (Philips and Hayman,1970**)**
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 cleared with 5N HCl for 3min, and stained with 0.05% trypan blue in

Lactophenol and percentage of root colonization was calculated by Read et.al,1976.

RESULTS AND DISCUSSION

Root Colonization in *Lantana camara* & *Stachytarpheta indica*

The mycorrhizal root colonization was 95% in *Lantana camara*. Themycorrhizal structures present in the roots included mycelium, vesicles and arbuscules. Mycelia of various type like Y-shaped, H-shaped and parallel mycelia were seen. Vesicles were of elliptical shape. The hyphae were of arum type (Linear). Other endophytes were not observed. The photographs of the roots are shown in Figure 1. The mycorrhizal root colonization was 90% in *Stachytarpheta indica*. Themycorrhizal structures observed were coiled, lightly stained mycelium, oval vesicles with prominent oil globules and highly coiled arbuscules. The photographs of the roots are shown in Figure 2.

Species Composition in *Lantana camara* & *Stachytarpheta indica*

The AM spore density was 122.67± 9.45/10g soil in *Lantana camara* and 91.34 ± 6.5/10g soil in *Stachytarpheta indica*. Two genera were identified, *Glomus* and *Acaulospora*. The spores were identified on the basis of their morphological characteristics. The dominant species in *Lantana*was *Glomus* (6 species) and few spores of *Acaulospora* (3 species) were also identified(Figure1).The dominant species in *Stachytarpheta* was *Acaulopspora* (8 species) and *Glomus* were less in number (3 species) (Figure 2).

AM Fungi have been described as keystone mutualists in ecosystems due to their unique position at the root-soil interface (Aditya Kumar et.al 2010).The present study was carried out to study the structural diversity of AM Fungi associated with the roots of *Lantana camara* and *Stachytarpheta indica*. The above results of *Lantana* showing darkly stained, thin walled hyphae which show H-shaped connections are produced by *Glomus* species is confirmed from the website Mycorrhizal associations (Brundrett *et al*. 1985, Brundrett & Kendrick 1988, Brundrett *et al*. 1996, https://mycorrhizas.info/vam.html). The structures seen in the roots of *Stachytarpheta* are lightly stained mycelium, coiled arbuscules and oval vesicles with oil globules are produced by *Acaulospora* species is also confirmed from the website of Mycorrhizal associations. They have also described the similar structures produced by *Acaulospora* species. From the above study differences in the structures of mycelium between *Glomus* and *Acaulospora* was confirmed. Since *Lantana* and *Stachytarpheta* are ecologically important plants, the knowledge of mycorrhizal status will be of immense importance to the researchers.

Table 1: AM Fungal status of *Lantana camara and Stachytarpheta indica*

Plant name	Mean Spore Density	% Root Colonization	Mycorrhizal Spores isolated	AM Structures Observed		
				Arbuscules	Vesicles	Hyphae
Lantana camara	122.67 ± 9.45	95%	*Glomus* (6 Species) *Acaulospora* (3Species)	+ Arum type	+++ Oval, Spherical	+++
Stachytarpheta indica	91.34 ± 6.5	90%	*Glomus* (3 Species) *Acaulospora* (8 Species)	+++ Coiled type	++ Oval with oil globules	+++

+Poor, ++Moderate, +++Good, +++ Excellent, - Absent

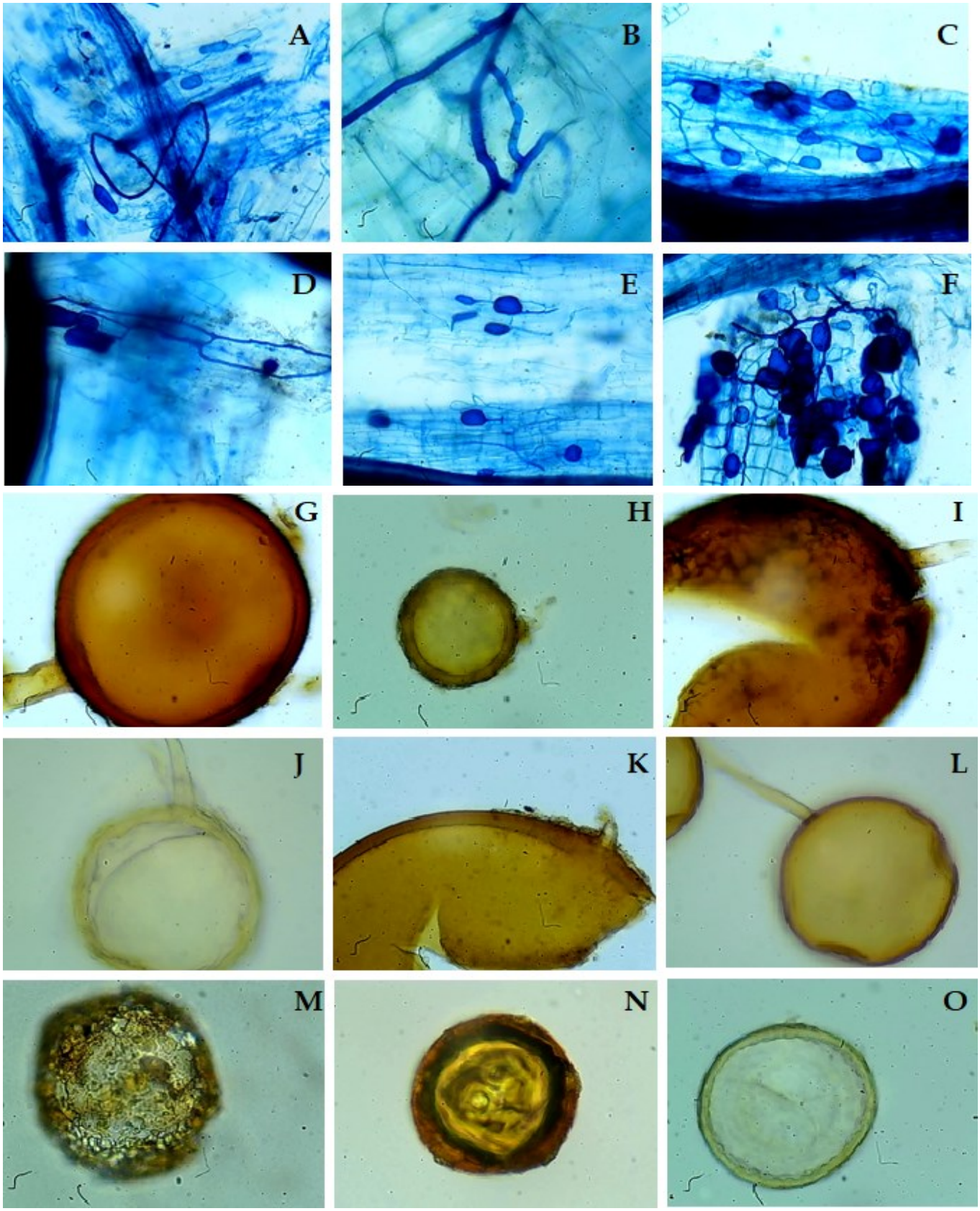

Fig. 1. A-F :*Lantana camara* root showing linear type hyphae with H shaped hyphal connections, oval & spherical vesicles,Spore types in rhizosphere soil **G - L** : *Glomus* species, **M - N** : *Acaulospora* species **O** : *Glomus* species

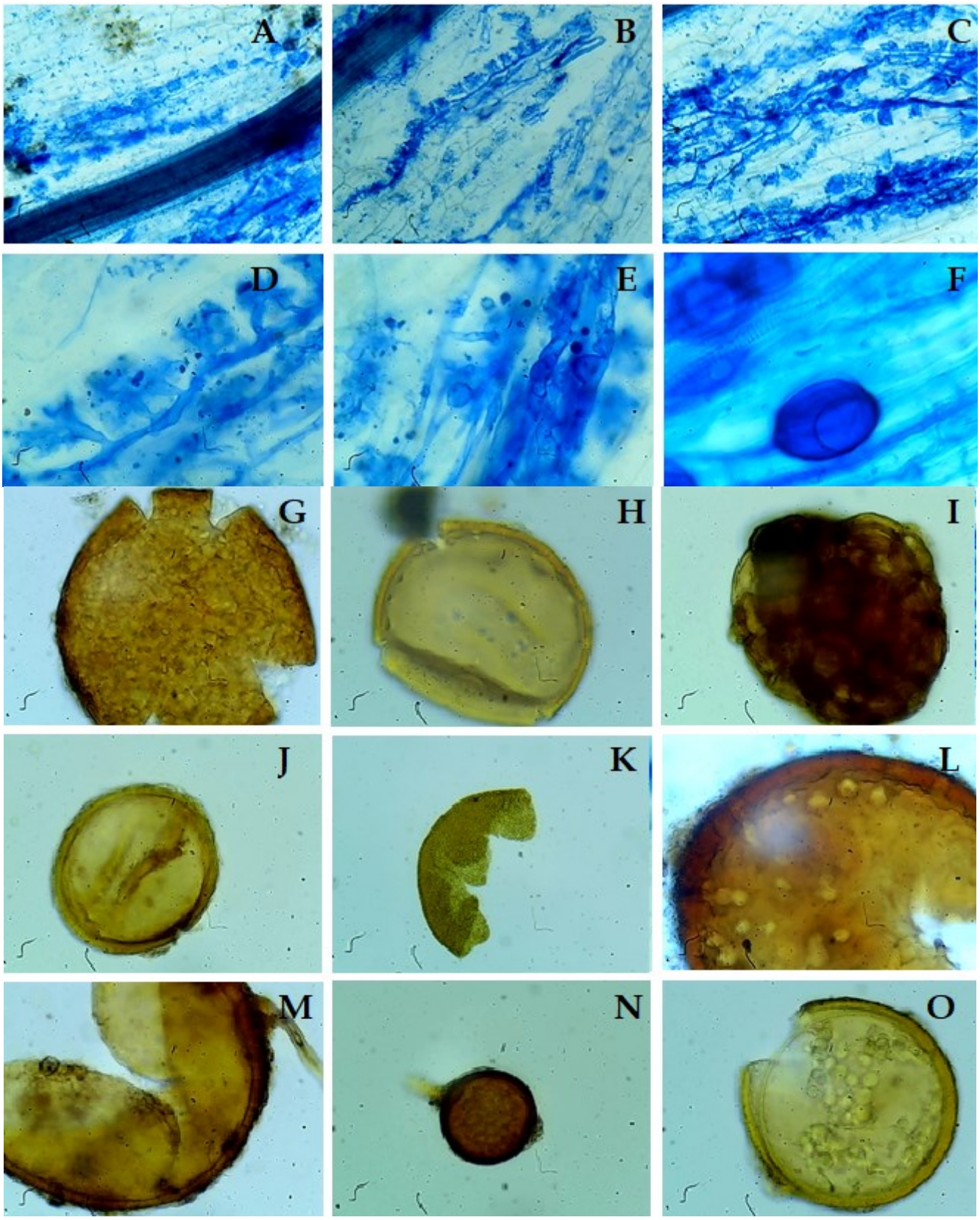

Fig 2. A-F : *Stachytarpheta indica* root showing hyphae with prominent arbuscules, hyphae lightly stained, oval vesicles with oil globules, Spore types in rhizosphere soil G - L : *Acaulospora* species M - O : *Glomus*species,

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Conflicts of interest: The authors stated that no conflicts of interest.

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