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# Biodiversity of AM fungi in selected medicinal plants of Maharashtra Nature Park, Mumbai

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**Abstract**
The objective of the study was to find out biodiversity of AM fungi associated with selected medicinal plants in Maharashtra Nature Park, which was a dumping ground of Mumbai city almost four decades ago. The ten plants selected for the study were *Bombax ceiba, Bauhinia variegata, Madhuca longifolia, Piper longum, Asparagus racemosus, Ocimum sanctum, Plectranthus amboinicus, Bryophyllum pinnatum, Costus igneus* and *Justicia adhatoda.* Root zone soil and roots were collected from a depth of 20cm during the period between March 2019 and June 2019.
The soil and roots were screened for Arbuscular Mycorrhizal (AM) spore count and root colonization. Highest spore density was found in *Madhuca indica* (296 spores/20 g of soil), while minimum spore density was found in *Asparagus racemosus* (42 spores/20 g of soil). The dominant AM genera found were *Acaulospora* and *Glomus. Gigaspora* was found in two plants. Isolation Frequency of *Acaulospora* was 90% and that of *Glomus* was 70%. Spore density found was highly variable in the ten plants.
Maximum root colonization (100%) was found in *Bryophyllum pinnatum* while minimum root colonization of 30% was found in *Piper longum.* Root colonization in the roots was in the form of arbuscules, vesicles, spores (intra radical and extra radical spores), hyphae and sporocarps. Another type of potentially beneficial fungi, namely dark septate endophytic fungi (DSEF) was observed associated with the roots of *Asparagus racemosus.*

**Keywords:** Am fungi, Maharashtra Nature Park, medicinal plants

## Introduction
Fungi are the largest biotic community in India after insects (Manoharachary *et. al*., 2005) [7]. AM fungi form symbiotic association with roots of terrestrial plants. As a result of this association the plants are able to absorb the nutrients more efficiently and fungus gets a source of carbon for its survival and sporulation (Smith *et. al.* 1994; Bonfante and Genre 2010) [15, 2]. AM fungi help plants to mobilise phosphorus present in soil, enhance plant tolerance to a variety of stresses including nutrients, drought, metal toxicity, salinity and pathogens all of which may affect plant success in a contaminated or polluted soil. It also helps in maintaining soil structure and carbon sequestration in soil (Smith and Smith 2011) [14]. The area under study was Maharashtra Nature Park, Mumbai which was earlier a dumping ground for municipal waste till 1977. No segregation method for waste was followed. It was developed as Nature Park with help of World Wide Fund for Nature from year 1983 onwards. Since no work was carried out previously, this study was an attempt to evaluate the status of AM fungi in 10 plants of Maharashtra Nature Park, viz, *Bombax ceiba, Bauhinia variegata, Madhuca longifolia, Piper longum, Asparagus racemosus,, Ocimum sanctum, Plectranthus amboinicus, Bryophyllum pinnatum, Costus igneus,* and *Justicia adhatoda.* Biodiversity of AM fungi in a reclaimed urban forest would help us to enlist the AM species which can successfully survive under such stress conditions prevailing thereon. Such data may be beneficial in land reclamation programmes.

**Study area:** The area chosen for the study was Maharashtra Nature Park situated in, Mumbai. It is situated in the "H" Block of Bandra-Kurla Complex (Bandra-Sion Road) on the Southern bank of Mithi River.

**Sample Collection:** Sample collection was carried out between March 2019 and June 2019. Root zone soil up to a depth of 20 cm was collected in a sterile polythene ziplock bags. Soil was stored in refrigerator at 4°C until use. The collected root samples were cut into 1 cm long pieces after a wash with tap water.
These were preserved in FAA until use for mycorrhizal colonization study.

## Materials and Methods
**Spore Extraction:** Gerdeman and Nicolson method (1963), was followed for extraction of spores. Twenty grams of root zone soil sample was taken and mixed with 1L of water. One small pinch of detergent was added so that the soil aggregates disperse to form uniform solution. This helps to segregate soil from spore. The suspension was passed through 500µm, 250µm, 150µm, 75µm, 35µm sieves and the water was allowed to flow for half an hour mildly so that hyphae and sporocarp do not break. The residue in the respective sieves were collected in beakers carefully with approximately 100ml of water. This water which contained spores and sporocarps was filtered on a circular Whatman filter paper, taken in petri dish and observed under Motic dissecting microscope for AM fungal spores.

**Spore Quantification**

Spore Density-Spores in the 20g of soil were collected in five petri dishes and the spore number was counted by the method of Gaur and Adholeya, (1994) [4]. The spores were picked up using needle. The spores were mounted on glass slide in PolyVinyl Lacto Glycerol (PVLG) and covered with cover slips. The slides were heated at 40-50°C temperature so that the air bubbles are removed and the spores appear clear. This is then sealed with Dibutyl Pthalate Xylene (DPX) which makes the slide semi-permanent.

**Root colonization** of AM fungi was studied by (Philips and Hayman method, 1970) and percentage of root colonization was calculated by (Read *et.al.*, 1976) [13].

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

Isolation frequency was calculated as the percentage of soil sample in which a species occurred, which revealed the extent of distribution of given AMF species in an ecosystem.

$$Isolation Frequency = \frac{No. of soil samples in which AMF species occured}{Total number of soil samples studied} X\ 100$$

Identification of AMF based on morphological characters: The spores were identified on the morphological characters including attachment of hyphae, ornamentation of wall and colour of spore. The identification of arbuscular mycorrhizal fungal spores was done with the help of websites www.invam.caf.wvu.edu and www.zor.zut.edu.

**Results and Discussion**

Maharashtra Nature Park is a reclaimed area in the heart of Mumbai city, developed on a dumping ground with the objective of making an urban forest. The flora above the ground took time to establish itself due to presence of untreated and non-segregated municipal waste. Our interest was to study the microflora found below the ground, especially AM Fungal diversity. Ten plants were randomly selected which included herbs (*Ocimum sanctum*, *Justicia adhatoda*, *Plectranthus amboinicus*, *Costus igneus*, *Bryophyllum pinnatum*, *Asparagus racemosus)* Climber *(Piper*) and Trees *( Bombax, Madhuca and Bauhinia).*

Highest spore density was found in *Madhuca indica*(296 spores/20 g of soil), followed by O*cimum sanctum* (274 spores/ 20 g of soil), *Bombax* (138 spores/20 g of soil), *Piper longum* (128 spores/20 g of soil), *Bryophyllum pinnatum* ( 90 spores/ 20 g of soil), *Costus igneus (* 87 spores/20 g of soil), *Bauhinia variegate ( 82* spores/ 20 g of soil) and *Justicia adhatoda (38* spores/20 g of soil). (Table. 1) The AMF species identified in the plants are mentioned in the Table 1. Maximum Species richness was observed in *Bombax ceiba*

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

| Sr. No | Name of Plant | Family | % Root Colonisation | Species Richness | Spore density /20 g | AMF associated |
|---|---|---|---|---|---|---|
| 1 | *Plectranthus amboinicus* | Lamiaceae | 80% | 3 | 110 | *Aculosporasps, Gigaspora,Glomus* |
| 2 | *Bryophyllum pinnatum* | Crassulaceae | 100% | 2 | 90 | *A.scrobiculata, Glomus* |
| 3 | *Costus igneus* | Costaceae | 82% | 2 | 87 | *Gigaspora, Glomus verruculosum* |
| 4 | *Bauhinia variegata* | Fabaceae | 60% | 1 | 82 | *A. myriocarpa* |
| 5 | *Madhuca longifolia* | Sapotaceae | 65% | 2 | 296 | *A. myriocarpa, Glomus* |
| 6 | *Piper longum* | Piperaceae | 30% | 3 | 128 | *Glomus sps., Glomus macrocarpum, Acaulospora* |
| 7 | *Bombax ceiba* | Malvaceae | 40% | 6 | 138 | *A. myriocarpa, A. spinosa, Glomus macrocarpum, Acaulospo rarehmii* |
| 8 | *Asparagus racemosus* | Asparagaceae | 50% | 1 | 42 | *A. myriocarpa* |
| 9 | *Ocimum sanctum* | Lamiaceae | 80% | 3 | 274 | *A. myriocarpa, Gigaspora macrocarpum, Glomus,* |
| 10 | *Justicia adhatoda* | Acanthaceae | 34% | 3 | 38 | *A. myriocarpa, A. rehmii Glomus multicaule,* |

Percent colonization varied from 30% in *Piper* to 100% in *Bryophyllum pinnatum.* (Table 1) Spore density was found to be highly variable among the plants selected and was comparatively low compared to the similar kind of work carried out by (Chahar and Belose, 2018) [3]. They obtained 75±7 spores per 10g of soil in *Bauhinia variegate* as compared to ours, 41 spores per 10g of soil. Spore production of AMF is known to vary greatly in different ecosystems, and it is influenced by an array of factors such as environment, host, and fungus, and spore density tends to increase during root inactivity or senescence (Muthukumar et al. 2003) [8]. This could be the reason for high spore density in *Ocimum sanctum*. Zhao *et. al.* (2001) [16] suggested that the uneven spatial distribution of AMF spores and the complex structure of the underground root component could be major factors affecting spore density of AMF. A total of 14 species belonging to three genera (*Acaulospora*, *Glomus* and *Gigaspora*) were observed in the 10 plants selected. The dominant AM species observed in all the plants screened was *Acaulospora myriocarpa*. (Table 1). This may be because *Acaulospora* and *Glomus* species sporulate profusely in shorter span of time due to smaller size of their spores than *Gigaspora* and *Scutellospora* in the same habitat and time frame (Hepper, 1984; Bever *et. al.* 1996; Patipan Nandakwang *et.al*., 2008) [1, 6, 9]. Also, *Glomus* species is considered as cosmopolitan AMF species in many ecosystems.

**Table 2:** Isolation Frequency of AM Fungal Genera in the rhizosphere soils

| S.No | AM Genera | Isolation Frequency % |
|---|---|---|
| 1 | *Acaulospora* | 90 |
| 2 | *Glomus* | 70 |
| 3 | *Gigaspora* | 30 |

Occurrence of other AMF genera was less in this study. (Biermann and Linderman, 1983) suggested that *Gigasporaceae* are capable of propagation only with viable spores or from an intact mycelium whereas *Glomeraceae* are capable of colonizing even with fragments of mycelium. Perhaps due to this, low occurrence of *Gigaspora* was observed in our study. Furthermore, *Scutellospora* and

*Gigaspora* produce large spores and these require a longer period to develop as compared to the small-spore species (Hepper, 1984) [6]. The structures observed in the roots confirmed root colonization were 'H' and 'V' shaped hyphae which stained dark blue with trypan blue. Intraradical and extraradical spores, elliptical vesicles and arbuscules were also observed. The roots of *Asparagus* showed presence of sclerotia of dark septate endophytic fungi. No significant correlation was found between spore density and root colonization (Fig. 1). Similar results were observed in AMF from the western ghats of India (Rajkumar *et al.* 2012) [11].

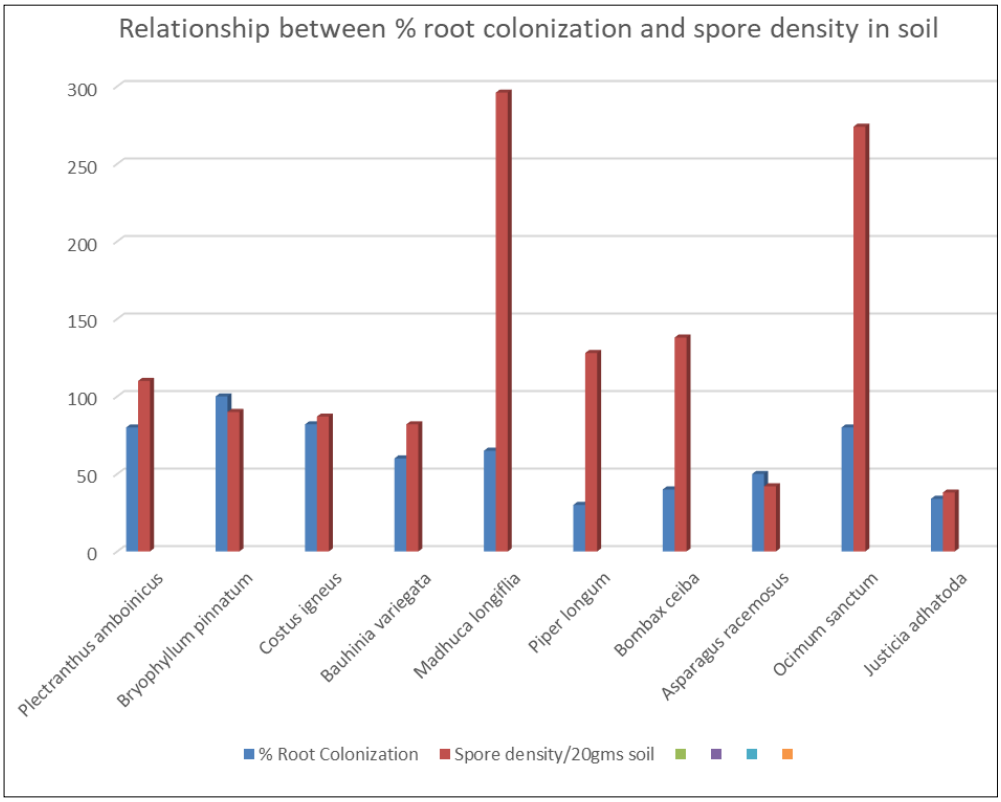
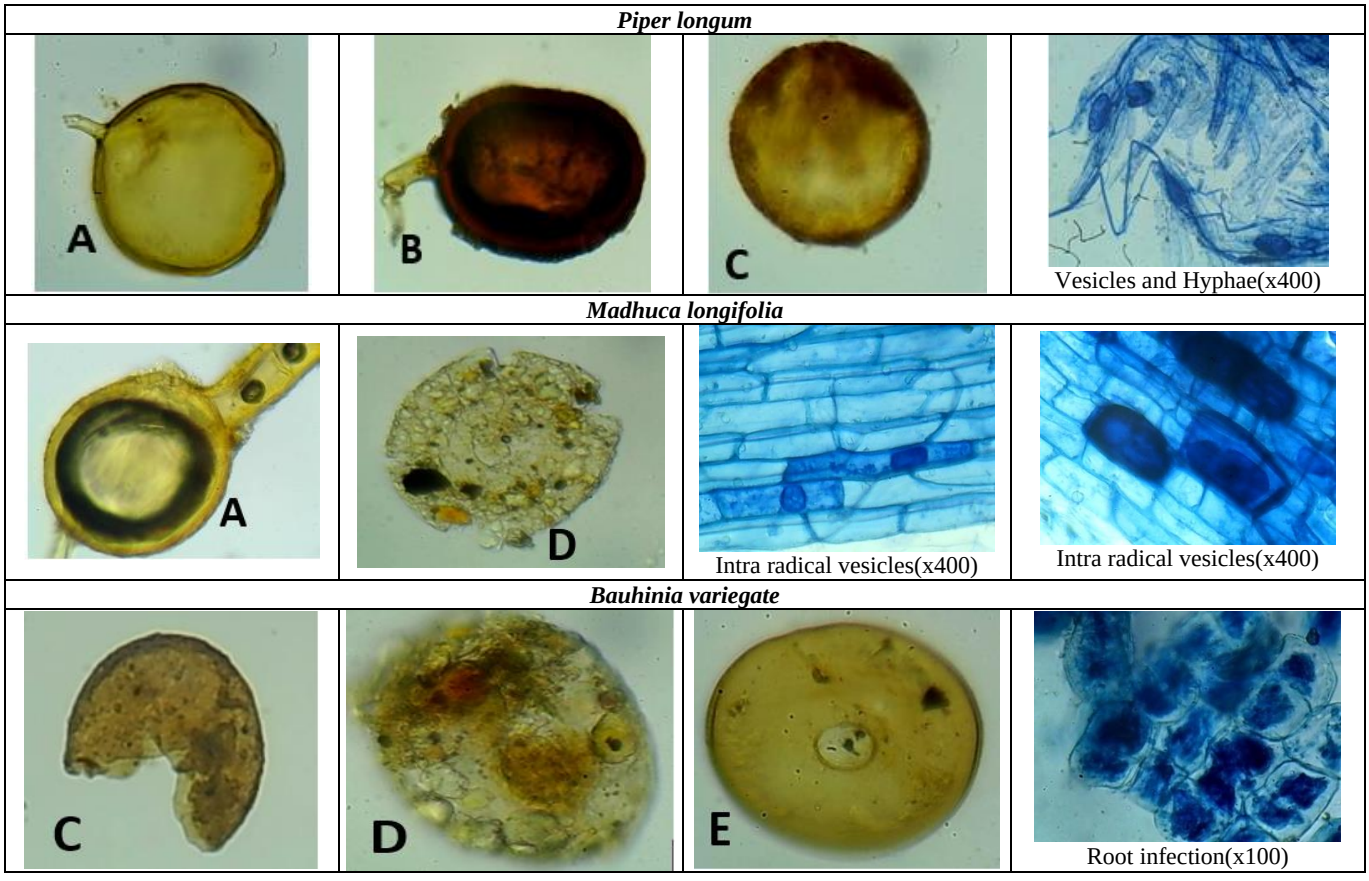



**Fig 1:** Relationship between percentage root colonization and spore density in soil.



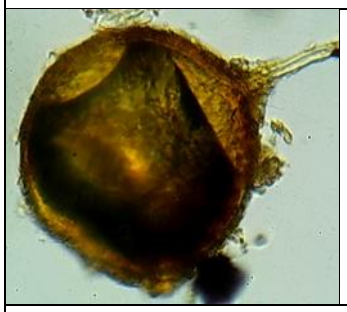
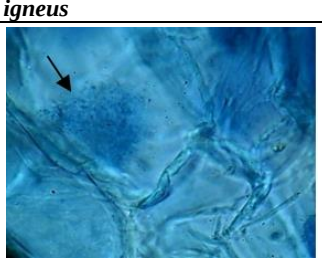
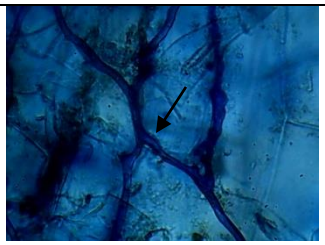
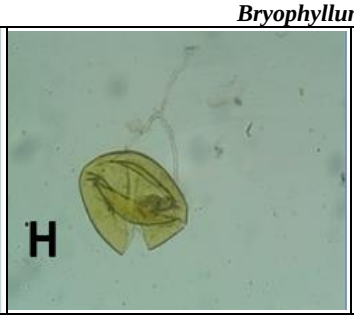
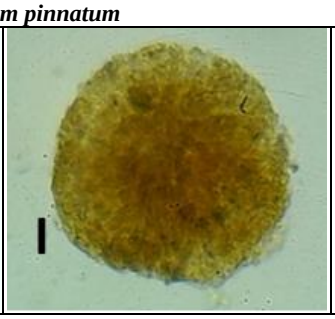
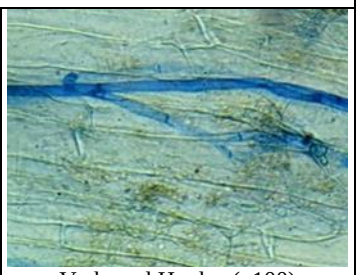
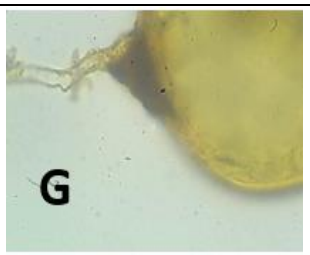
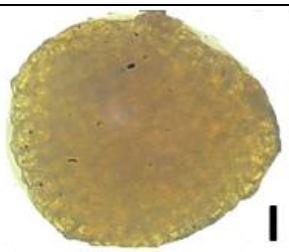
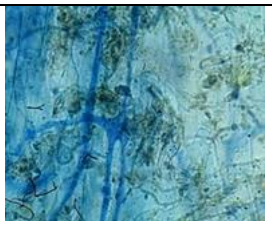
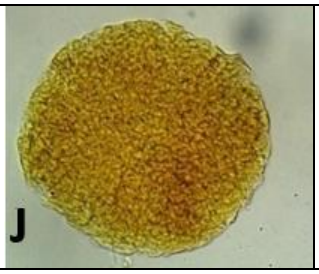
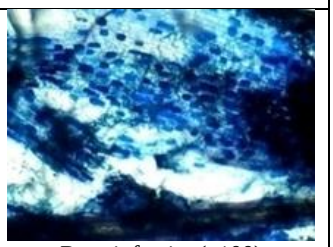
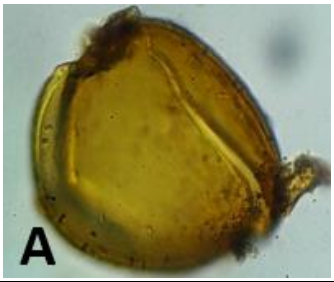
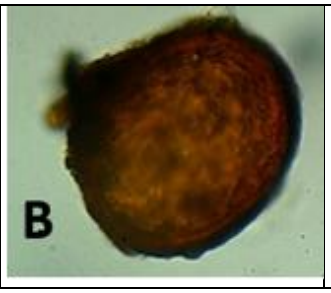
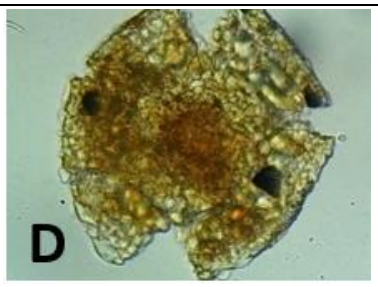
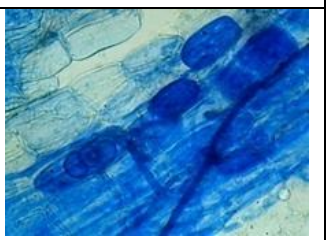
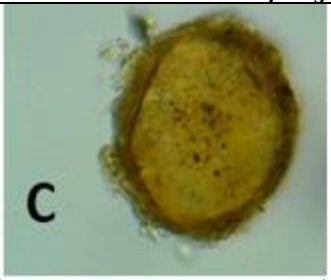
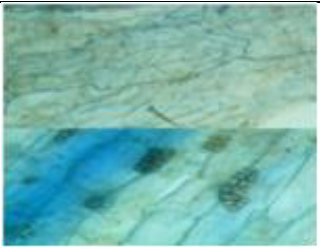
| *Costus igneus* | | | |
|---|---|---|---|
|  |   |  Root infection showing arbuscules(x400) |  Root infection showing H-Shaped Hyphae(x400) |
| ***Bryophyllum pinnatum*** | | | |
|   |   |   |  V-shaped Hyphae(x100) |

**Plate-1**

| ***Plectranthusambonicus*** | | | |
|---|---|---|---|
|   |   |   |  Root showing H-hyphae (x100) |
| ***Justicia adhathoda*** | | | |
|   |   |   |  Root infection(x100) |
| ***Ocimum sanctum*** | | | |
|   |   |   |  Root Infection showing spore within spore(x400) |
| ***Asparagus racemosus*** | | | |
|   |   |   |  Hyphae and Endophytes (x100 |

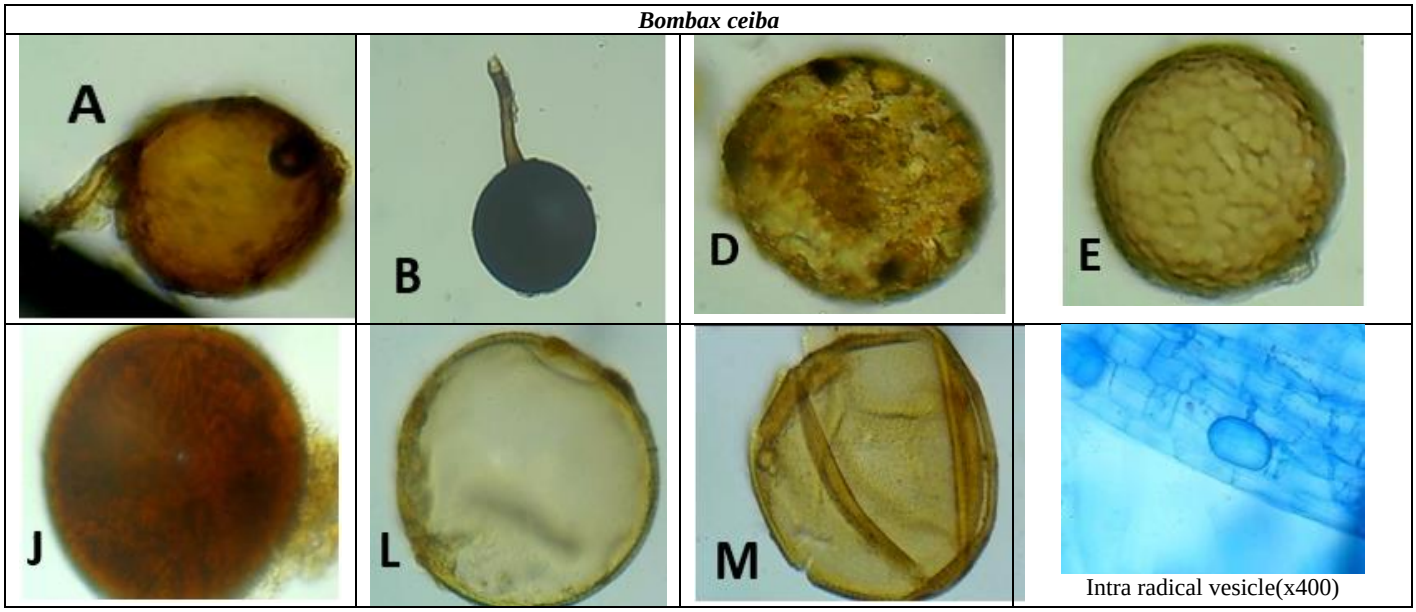



**Plate 2**

## Abbrevation Key

**Table 3**

| **A: *Glomus sp.*** | **B: *Glomus macrocarpum*** | **C: *Aculospora sp.*** | **D:*Aculospora myriocarpa*** |
|---|---|---|---|
| E: Unidentified | F:*Glomus verruculosum* | G: *Gigaspora albida* | H: Scrobiculata sp. |
| I:*Aculospora bireticulata* | J:*Aculospora rehmii* | K:*Glomus multicaule* | L:*Aculospora scrobiculata* |
| | M:*Aculospora spinosa* | | |

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## Conflict of Interest
The corresponding author on behalf of author declares no potential conflict of interest.

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