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MYCORRHIZAL RICHNES S IN *TINOSPORA CORDIFOLIA*

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ABSTRACT

Tinospora cordifolia, commonly known as Giloy or Amrita or even heart-leaved moonseed is known to be a great immunity booster, and is used in Ayurvedic medicines. It belongs to family Menispermaceae. The present study was carried out to investigate the Arbuscular Mycorrhizal Fungi (AMF) associated with the roots of this plant and if this plant has any affinity to the AMF. Trap culture was established to study the different species of Arbuscular Mycorrhizal Fungi. Spore density was found to be 1600±24 spores /100g soil. A total of 19 species of Arbuscular Mycorrhizal Fungi were isolated. Nine species of *Glomus*, five species of *Acaulospora*, one species of *Gigaspora*, one species of *Ambispora* and one species of *Rhizophagous irregularis* were identified. There were some unidentified species of *Acaulospora*. The root colonization by the fungi was 96% and the fungal structures observed were fungal hyphae, arbuscules, vesicles, pelotons and intraradical spores. Dark Septate Endophytic Fungi were also observed in the roots.

KEYWORDS: Arbuscular Mycorrhizal Fungi, *Tinospora cordifolia*, *Glomus*, *Acaulospora*, Medicinal plants. Immunity booster.

INTRODUCTION

Tinospora cordifolia is commonly used in ayurvedic medicines for numerous aliments like fever, diabetes, jaundice, anaemia, polyuria and skin diseases. It has hypoglycemic, antipyretic, anti-allergic, Antineoplastic, anti-inflammatory, anti-oxidant, and immune-modulatory properties. Due to these medicinal qualities of Giloy, it has become greatly popular for consumption during Covid times. Arbuscular Mycorrhizal Fungi (AMF) contribute greatly to crop productivity and ecosystem sustainability in new plant production strategies (Gianinazzi et al.2010) and are essential for the sustainable management of agricultural ecosystems (Smith and Read,2008). They promote the accumulation of effective ingredients of medicinal plants, which has become a hot area of research (Zeng Yet.al. 2013). The increasing use and demand for *Tinospora cordifolia* created inquisitiveness to study the Mycorrhizal diversity of the plant. Trap cultures have been widely used to access AMF diversity and isolate indigenous fungi (Patrícia Lopes Leal et.al, 2009). Therefore, the Arbuscular Mycorrhiza Fungi was studied by establishing trap culture according to Rodrigues & Muthukumar, 2009.

MATERIALS AND METHODS

Trap Culture: The trap culture was set up in April 2020 as follows: a) A mixture of sterilized soil and sand in the ratio of 2:1 was taken. b) This mixture was filled in 30cm diameter pots which were sterilized by wiping with alcohol. c) *Tinospora* was planted along with the roots and rhizosphere soil collected from the Rewari Khera village, district Jhajjar of Haryana state, India. d) The pots were watered twice a week for 7 months without adding any nutrient medium. e) After 7 months the plants were uprooted in November 2020 and root colonization checked in the roots. f) The soil from the pots was dried and Mycorrhizal Spore density checked in the rhizosphere soil.

Spore extraction: The soil samples were subjected to wet-sieving and decanting technique of Gerdemann and Nicolson, 1963 for the isolation of spores in 100g soil. The experiment was replicated three times and the data presented as mean of three replicates. The isolated spores were observed under Motic Stereozoom microscope for the spore count. The spore number was counted by Gaur and Adholeya method, 1994 in 10cm diameter petridishes with a gridline of 1cm. The spores were picked up with needle, mounted in polyvinyl lactoglycerol and observed under compound microscope

(Labomed-LX300) and photographs taken by Dewinter camera.

Taxonomic identification: Morphological Identification of spores up to species level was made using the identification manual (Rodrigues & Muthukumar, 2009) and species descriptions provided by https://invam.wvu.edu/ and http://www.zor.zut.edu.pl/Glomeromycota.html.

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

$$\text{Percent colonization} = \frac{\text{Total number of colonized root segments}}{\text{Total number of root segments examined}} \times 100$$

RESULTS AND DISCUSSION

Table 1: Status of AM Fungi Colonization in the Trap Culture of *Tinospora cordifolia.*

Mean Spore Density	% Root Colonization	Mycorrhizal Species isolated & identified	AM Structures observed in the Roots
1600±24 spores /100g soil	96%	***Glomus*** *Glomus macrocarpum,Glomus constrictum Glomus multicaule, Glomus claroideum Glomus gibbosum, Glomus minutum, Glomus badium, Glomus callosum, Glomus geosporum* ***Rhizophagus irregularis*** ***Acaulospora*** *Acaulospora scrobiculata, Acaulospora polonica, Acaulospora myriocarpa, Acaulospora bireticulata Acaulospora sps1* ***Ambispora appendicula*** ***Gigaspora sps***	Arbuscules, Hyphae with H & Y connections Linear (Arum type) and coiled hyphae (Paris type) vesicles - elliptical & oval Intraradical spores

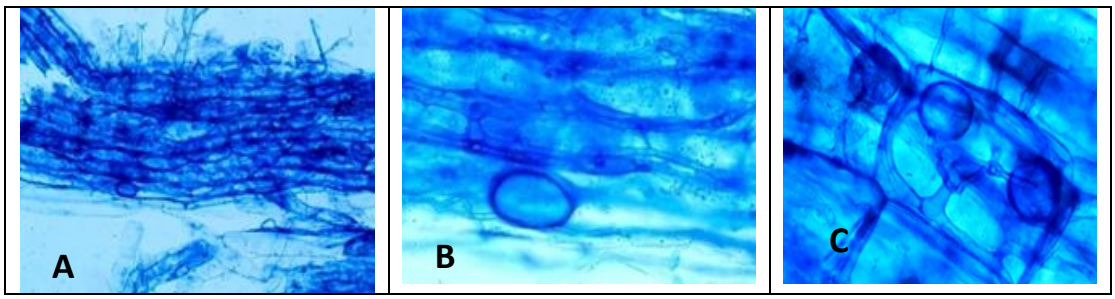

Figure 1: A. Linear type of hyphae B. H-connection and elliptic vesicle C. Pelotons and oval vesicles.

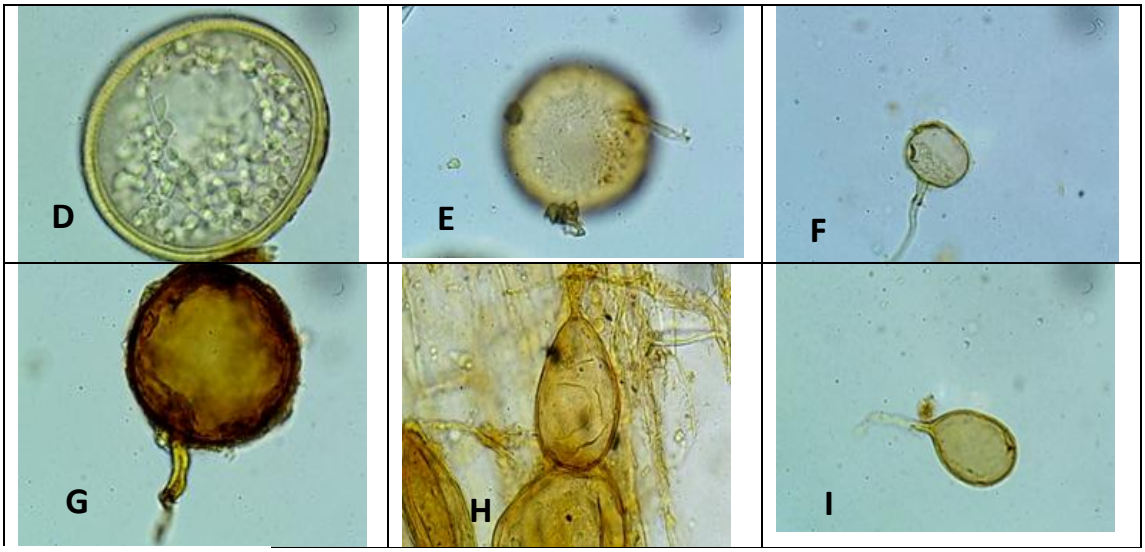

Figure 2: ***Glomus Species:*** **A.** ***Glomus macrocarpum*** **B.** ***Glomus constrictum*** **C.** ***Glomus multicaule*** **D.** ***Glomus claroideum*** **E.** ***Glomus gibbosum*** **F.** ***Glomus minutum*** **G.** ***Glomus badium*** **H.** ***Glomus callosum*** **I.** ***Glomus geosporum.***

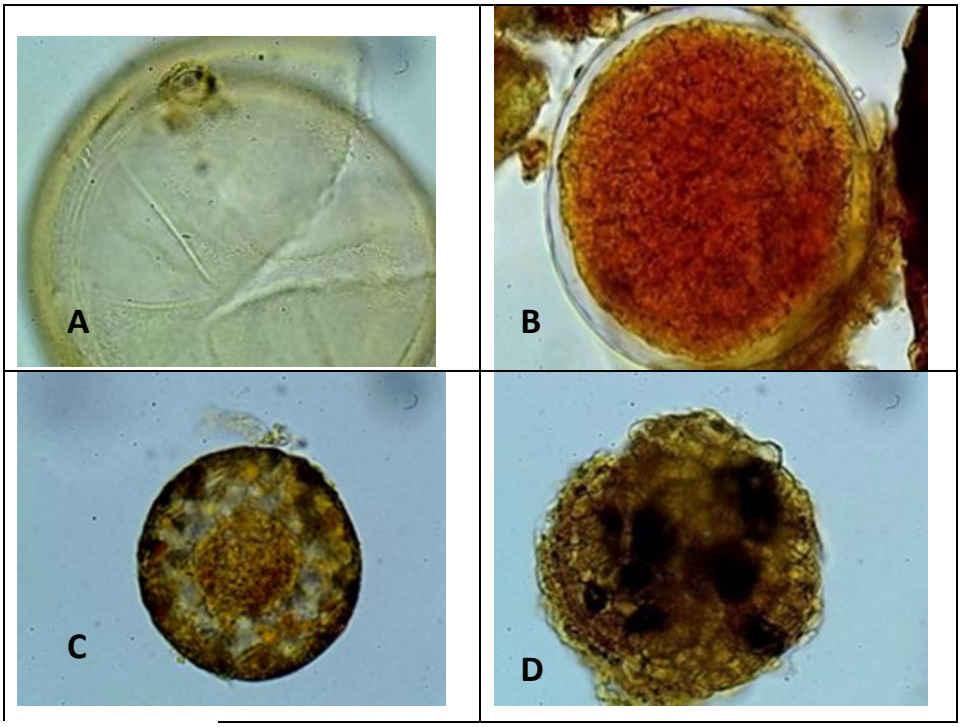

Figure 3: ***Acaulospora species*** **A.** ***Acaulospora spinosa*** **B.** ***Acaulospora polonica*** **C.** ***Acaulospora myriocarpa*** **D.** ***Acaulospora bireticulata.***

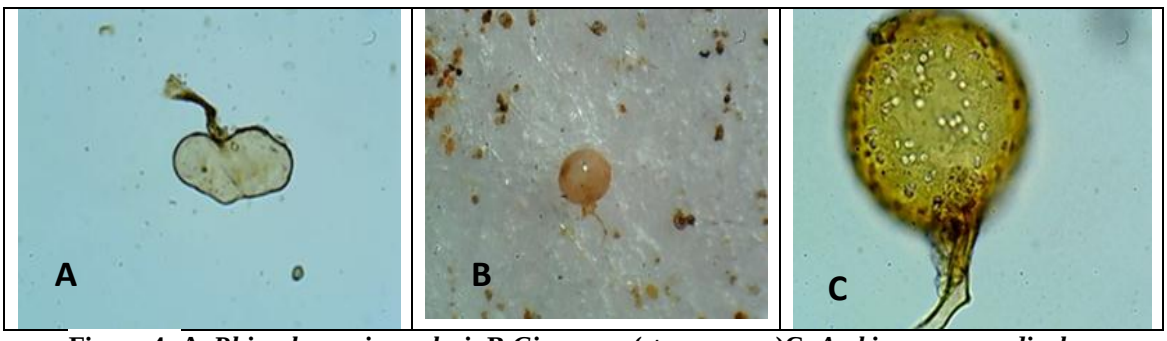

Figure 4: A. ***Rhizophagus irregularis*** **B.*Gigaspora*** **(stereozoom)C.** ***Ambispora appendicular.***

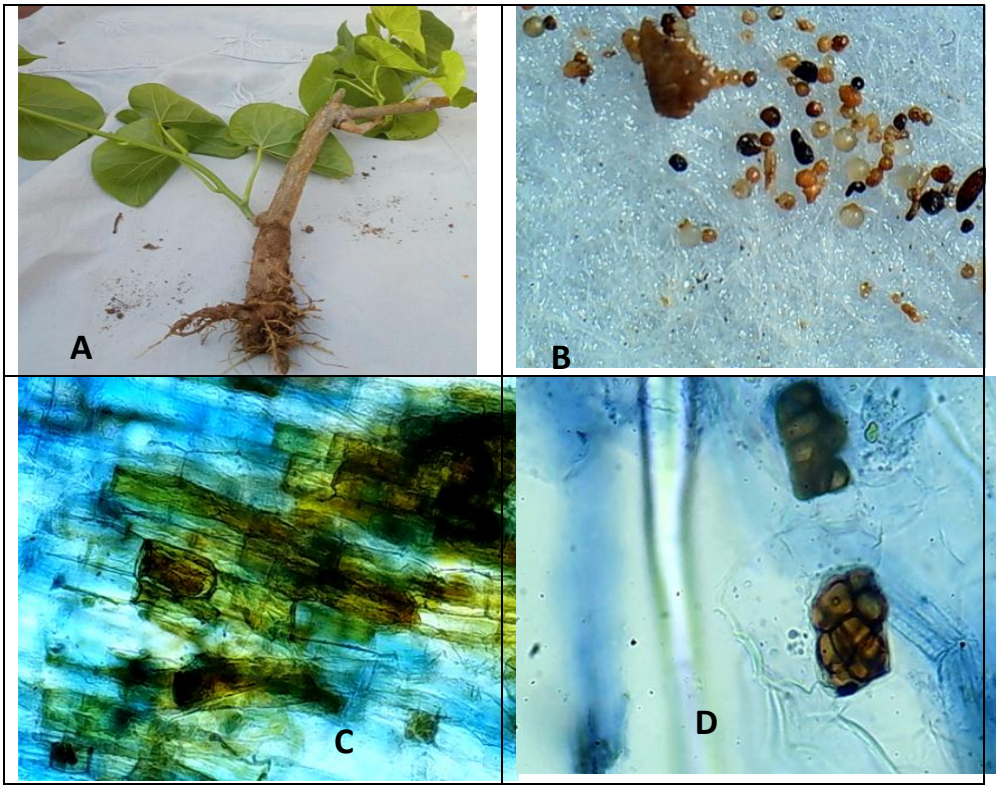

Figure 5: A. *Tinospora* showing roots B. Spores on filter paper C. & D. DSEF.

The results are shown in the table 1. The study revealed that spore number was very high in trap culture (1600±24 spores /100g soil) after a period of 7 months. The high spore count can be attributed to the trap culture, as the dormant species sometimes sporulate in trap cultures (Muthukumar and Udaiyan, 2002). The roots of the plant are shallow and very scanty (Fig5A). 17 Arbuscular Mycorrhizal morphotypes were recovered, out of which, 9 were *Glomus*, 5 *Acaulospora*, 1 *Ambispora*, 1 *Gigaspora* and 1 belonged to *Rhizophagous* (Figure 2,3,4.). Species of *Acaulospora* and *Gigaspora* could not be identified upto species level. The species are listed in the Table1.Genus *Glomus* dominates with 9 species followed by *Acaulospora* with 5 species. *Acaulospora* and *Glomus* species sporulate profusely in shorter span of time due to small size of their spores than *Gigaspora* and *Scutellospora* (Nandakwang.P *et.al*., 2008). *Glomus* and *Acaulospora* also show a high adaptive value.

The percentage of root colonization was found to be 96%. Sarawade et.al, 2011 and Rajkumar et al, 2012 received 92% root colonization in *Tinospora cordifolia.* The photographs of the roots showing infection are shown in the Figure1.The hyphal structures showed both densely and lightly stained hyphae, thin and thick hyphae, coiled hyphae within the cells (pelotons). The hyphae were both Arum type (Linear) and Paris type (Coiled). H connections, Y connections and intraradical spores were clearly seen. Vesicles were spherical, oval as well as elliptical type. Since the roots showed all types of Arbuscular Mycorrhizal Fungal structures, it confirms the presence of different species of AM Fungi. According to Brundrett.M, 2004, Hyphae produced by *Glomus* are relatively straight, ramify along the root cortex 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. Vesicles are oval, which usually form between root cortex cells. Intraradical spores in Glomaceae are usually globose, subglobose to elliptical. Hyphae produced by *Acaulospora* are thin walled, often stain weakly. Intraradical spores in *Acaulospora* are pleomorphic, knobby and stain lightly in trypan blue. Hyphae of *Gigaspora* and *Scutellospora* are thick walled and stain darkly.

Dark Septate Endophytic Fungi (DSEF) were also observed as shown in Figure 5-C,D. They belong to ascomycetes fungi and help the plants in uptake of nutrients and growth of the plant (Chao He et. al., 2019). Mishra.et.al, 2012 identified 1151fungal endophytes from leaf, stem, petiole and root of *Tinospora cordifolia.* Endophytes act as reservoirs of novel bioactive secondary metabolites, such as alkaloids, phenolic acids, quinones, steroids, saponins, tannins, and terpenoids that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties.

CONCLUSION

From the study it can be concluded that *Tinospora cordifolia* plant has good affinity for AM fungi and it is polysporal. Mycorrhization not only affects plant growth but also affects secondary metabolism eliciting positive changes in the medicinal compounds (Rapparini et al.,

2007; Chaudhary et al.,2008). Hence the data of AM species will be useful to further researchers.

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