

Original Research Article

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Diversity of Arbuscular Mycorrhizal Fungi Associated with *Smilax perfoliata* Lour

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ABSTRACT

Smilax perfoliata Lour is a traditionally used medicinal plant by many tribes of North-east India. There is no report of AM fungal diversity of this medicinally important plant. The present study is aimed to assess the AM fungal diversity in *Smilax perfoliata* Lour. Soil sample was collected from Manas National Park, Assam, India. The studied soil sample was found slightly alkaline in nature (pH: 7.8). The percent root colonization was recorded 82.98±1.01 %. AM Fungal spore density in the soil sample of *Smilax perfoliata* Lour was 147 spores 20gm⁻¹ and species richness was 23. *Rhizophagus fasciculatus* and *Glomus citricola* showed highest relative abundance (6.45%). Eighteen AM fungal taxa were recorded from trap culture. *Glomus citricola* showed highest relative abundance (12.84%) in trap culture. Nine AM fungal taxa viz. *Acaulospora denticulata*, *Glomus* sp.4 *G. pellucidum*, *G. tortuosum*, *Rhizophagus diaphanum*, *Ambispora fecundispora*, *Gigaspora rosea* and *Sclerocystis* sp.1. were recorded in trap culture that was not observed in original soil sample. *Glomus* was the dominant genus during the present investigation. The monocultures of *Rhizophagus fasciculatus*, *Glomus aggregatum*, *G. citricola*, *Funneliformis geosporum* and *Claroideoglomus etunicatum* were successfully raised. The root colonization and spore density were highest in monoculture of *F. geosporum* and lowest in case of *C. etunicatum*. The present investigation provides knowledge and understanding about the presence and diversity of AM fungi in *Smilax perfoliata* Lour. This also provides good possibilities for further studies and utilization of AM fungi.

Keywords

Smilax perfoliata Lour; AM fungi, Root colonization, Spore density, Monosporic culture.

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Introduction

Mycorrhiza is a mutualistic symbiotic relationship between the root system of higher plants and fungal hyphae. The fungal network increases the capacity of plant to absorb more water and nutrients. This process helps in growth and favours rapid development of roots and plants. Arbuscular mycorrhiza (AM) is the most ancient type of mycorrhizal association between land plants and fungi of the phylum Glomeromycota (Tapadar *et al.*,

2016b; Schüßler *et al.*, 2001). AM fungi colonize the terrestrial plants of most ecosystem and forms functional links between soil and plants (Smith and Read, 2008). They facilitate inter plant transfer of nutrients, modify plant water relations and thereby improve stability of plant community (Smith and Read, 1997). It also plays an important role in stability of soil structure and water retention by the production of sticky

proteinaceous substances known as glomalin (Bedini *et al.*, 2009). The endosymbiotic AM Fungi develop some special structures called vesicles and arbuscules, within the root cells. Vesicles are thick-walled, spherical to oval shaped structures born on the tip of the hyphae for storage purpose either in intercellular spaces or in the cortical cells of the root. Arbuscules are the dichotomously branched structures, which are formed inside the parenchyma cells for nutrient exchange with the plant (Gianinazzi-Pearson, 1996). Arbuscules are the sites of exchange for phosphorus, carbon, water and other nutrients. The populations of AM Fungi is greatest in plant communities with high diversity such as tropical rainforests and temperate grasslands where they have many potential host plants and can take advantage of their ability to colonize a broad host range.

There have been many research works carried out throughout the world to understand the interactions between AM Fungi and plants (Li *et al.*, 2013; Stein *et al.*, 2009; van der Heijden and Horton, 2009; Jordan and Huerd, 2008), and to understand the functionality of AM fungi as influenced by variations in environmental conditions, especially in their natural environment (Likar *et al.*, 2009). AM Fungi shows different responses on root colonization intensity and spore population depending on the host species or environmental factors (Likar *et al.*, 2008; Li *et al.*, 2005). Reports suggest that AM Fungi contribute to plant diversity, community structure and ecosystem sustainability (Grime *et al.*, 1987; van der Heijden *et al.*, 2008). In different medicinal plants, the occurrence of AM Fungi have been studied previously by many researchers (Tapadar *et al.*, 2016b; Muthukumar and Udaiyan, 2000; Selvoraj, Murugan and Bhaskaran, 2001; Rani and Bhaduria, 2001). Discoveries of medicinal research lead to know that the plant contained abundant of principle active metabolites

which can be used to cure diseases. These plants have good application in herbal, pharmaceutical, agricultural sectors. AM Fungi affects secondary metabolism and active ingredient production of plants (Lingua *et al.*, 2013).

Smilax perfoliata Lour is also a traditionally used medicinal plant by many tribes of North-east India. Smilax is a large genus belongs to Smilacaceae family. The genus includes both deciduous and evergreen species. It is distributed in tropical, subtropical and temperate regions of the world. Stem and root of *Smilax perfoliata* Lour is used as anticancer, anti-dysenteric and in urinary complaints (Pawer *et al.*, 2011). It is also used as a blood purifier (Buragohain, 2011). Zhasa *et al.*, (2015) reported ethnomedicinal use of Leaves and roots of *Smilax perfoliata* as demulcent, depurative, diaphoretic, diuretic, parasiticide, stimulant and used as a tonic. Hence, the present study is aimed to assess the AM fungal diversity in medicinally important *Smilax perfoliata* Lour. This is the first report of AM fungal diversity of this medicinally important plant.

Materials and Methods

Sampling site and collection of soil and root samples

The sampling site was located in Manas National Park, Assam in India. The study site was located in the Himalayan foothills, it is contiguous with the Royal Manas National Park in Bhutan. The geographical coordinates of the study sites are 26^o43'N and 90^o56'E. *Smilax perfoliata* Lour, a medicinally important plant was selected for the present investigation (Photo plate 1). Rhizospheric soil samples of the selected plant *Smilax perfoliata* Lour were aseptically collected from a depth of <20 cm using V-shaped technique (Bashan and Wolowelsky, 1987).

The fine roots of the plant were also collected during the sampling. Sampling was carried out in triplicates. The soil and root samples of a single plant were collected separately in polypropylene bags and transported to the laboratory on the same day of collection. Samples were kept at 4 °C in the laboratory for further analysis.

Soil physicochemical characteristics

Fresh air dried soil samples were used for physicochemical analysis. Soil pH was determined using electronic digital pH meter (eco Testr pH1) in soil/water suspension, 1:5, w/v. Soil moisture content (MC) was determined as used by Tapadar and Jha (2016a). Walky and Black's (1934) titrimetric chromic acid wet oxidation method was used for the determination of soil organic carbon (C_{org}) content. Bray's method (1945) was adopted for the determination of available phosphorus (P) concentration, while available nitrogen (N) concentration was estimated by the alkaline potassium permanganate method of Subbiah and Asija (1956). Potassium (K) concentration was estimated using flame photometric method (Toth and Prince, 1949).

AM fungal root colonization

AM fungal root colonization of fine roots were assessed by following the method of Kormanik and McGraw (1982). Roots of 1 cm length were mixed with KOH (10 %) and autoclaved at 15 lb inch⁻² pressure for 5 minutes. The coloured root segments were bleached with freshly prepared alkaline H₂O₂ and were acidified with 1% HCl. The acidified root segments were stained with 0.05 % (W/V) trypan blue. The stained roots were destained in lactic acid and mounted on a slide using lactophenol and examined under light microscope. The root colonization (RC) percentage was calculated by following the formula: RC (%) = (total no. of colonized root segments/total no. of examined root

segments) × 100.

AM fungal spore isolation

Spores of arbuscular mycorrhizal fungi were extracted from soil samples using slightly modified combination of wet sieving and decanting method (Gerdemann and Nicolson, 1963).

Twenty gram air-dried soil was suspended in 1000 ml of water and shaken for 10 mins and kept undisturbed for 1 h in order to allow heavier particles to settle down. The soil suspension was decanted through a series of sieves of different sizes i.e. 250 µm (1st), 125 µm (2nd), 60 µm (3rd) and 37 µm (4th).

The seivings retained on the 2nd, 3rd and 4th sieves were suspended in minimal amount of water and filtered using filter paper. Filter papers were transferred to petridishes and spores were counted and sorted based on their size, shape, and colour under stereomicroscope (Labomed CZM4 model; 10X×4X) using bamboo needle (15 cm long). The morphologically identical spores were used together (20-25 nos.) for preparation of diagnostic slide using polyvinyl-lactoglycerol (PVLG) or PVLG+Melzer's reagent (1:1 ratio) as mountants and observed under compound microscope (Labomed ATC2000 model;10X×40X magnification) using the method of Schenck and Perez (1990).

Identification of AM spores were done based on size, shape, colour, wall structure, surface ornamentation, hyphal attachments and presence or absence of bulbous suspensor using online species descriptions of INVAM (International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi; <http://invam.caf.wvu.edu>), <http://www.amf-phylogeny.com>, <http://indexfungorum.org> and published original species descriptions.

Trap culture

Trap cultures were established from fresh soil samples to isolate more spores. *Allium cepa* and *Zea mays* were used alternatively as trap plants. The experiment was conducted under normal conditions. The root colonization and spore density were studied after 2 month of establishment of trap culture.

Monosporic culture of AMF

For establishment of monosporic culture, soil and sand in 1:1 ratio was sterilized twice separately with a time gap of 24 h in polypropylene bags at 15 lb inch⁻² pressure for 20 min. Five maize seeds were allowed to germinate in a medium sized plastic pot filled with sterilized soil and sand (1:1) mixture. After germination seedlings were thinned to 3 per pot. The roots of maize seedling were inoculated with a healthy single spore. Establishment of root infection was confirmed after 30 days by examining root pieces for colonization. Upon confirmation, successful cultures (soil and sand mixture with root pieces) were transferred to big pots (2.5 Kg). The spores of each isolate were identified and maintained for further investigation.

Statistical analysis

Relative abundance (RA) of AM fungal species was studied as described by Dandan and Zhiwei (2007).

Results and Discussion

Phycochemical properties of soil

The examined physicochemical properties were pH, moisture content (MC), C_{org} (%), Av. N₂ (Kg/ha), Av. P₂O₅ (Kg/ha) and Av. K₂O (Kg/ha). The studied soil sample was found to be slightly alkaline with p^H 7.8. The C_{org} concentration was found high (4.07 %).

The available concentration of NPK were 1354.15 (Kg/ha), 106.44 (Kg/ha) and 356.16 (Kg/ha) respectively for nitrogen, phosphorus and potassium.

AM fungal root colonization

The roots of *Smilax perfoliata* Lour were colonized by AM fungi. The colonization in the form of arbuscles, vesicles, hyphae and hyphal coils were observed during the investigation. The percent root colonization was recorded 82.98±1.01 %. The percentage of hyphae, vesicle and arbuscule structures observed in colonized roots were respectively 29.9%, 52.06% and 18.04%.

AM fungal spore density and composition

AM Fungal spore density in the soil sample of *Smilax perfoliata* Lour was recorded 147 spores 20gm⁻¹. A total 23 AM fungal taxa were identified based on their morphological characteristics (Table 1). Some spores could not be identified due to lack of proper spore structures and low number.

Among the identified taxa, 16 (69.57%) were identified at the species level, while the remaining 7 (30.43%) were identified at the generic level. Out of the 23 identified AM fungal taxa, 16 belonged to Glomeraceae, 3 belonged to Acaulosporaceae, 2 belonged to Gigasporaceae and 2 belonged to Claroideoglomeraceae. Three generic taxa (*Funneliformis*, *Glomus*, *Rhizophagus*) were identified within Glomeraceae; 1 generic taxa identified each within Acaulosporaceae (*Acaulospora*), Gigasporaceae (*Scutellospora*) and Claroideoglomeraceae (*Claroideoglomus*). *Rhizophagus fasciculatus* and *Glomus citricola* showed highest relative abundance (6.45%) followed by *Glomus aureum*, *Rhizophagus clarus* and *Glomus aggregatum* (5.79%), *Funneliformis badium*, *F. caledonium* (5.11%), *G. sp.3* (5.01%),

Claroideoglosum etunicatum, *F. geosporum*, *C. claroideum*, *G.* sp.1, *Acaulospora scrobiculata* (4.43%), *G. multicaule*, *G. reticulatum*, *G. hoi*, *R. intraradices*, *G.* sp.2 (3.75%), *G. canadense*, *A.* sp.1, *A.* sp.2, *Scutellospora* sp.2 (3.07%) and *S.* sp.1 (2.39%) (Fig. 1).

Trap culture

The percentage of AM fungal root colonization was recorded 52% and 56% respectively in maize and onion trap plant. The root colonization of onion plants was more as compared to roots of maize plants. The AM fungal spore density in soil of trap culture was found 45 spore 20gm⁻¹ soil. Eighteen AM fungal taxa were recorded from trap culture (Table 2). *Glomus citricola* showed highest relative abundance (12.84%) followed by *Rhizophagus fasciculatus* (10.62%), *Acaulospora scrobiculata*, *Funneliformis geosporum*, *Glomus aggregatum* (8.39%), *Ambispora jimgerdemannii* and *G. pellucidum* (7.28%), *Acaulospora denticulata* (6.17%), *A.* sp.1, *G.* sp.4 and *G. aureum* (5.06%), *G. badium*, *G. tortuosum* and *Gigaspora rosea* (3.95%), *R.*

diaphanum, *Sclerocystis* sp. and *Scutellospora* sp.1 (2.84%). Nine AM fungal taxa viz. *Acaulospora gerdemanni*, *A. denticulata*, *Glomus* sp.4, *G. pellucidum*, *G. tortuosum*, *Rhizophagus diaphanum*, *Ambispora fecundispora*, *A. jimgerdemannii*, *Gigaspora rosea* and *Sclerocystis* sp.1 were recorded in trap culture that was not observed in original soil sample.

Monosporic culture

There were five monosporic culture of AM fungi successfully developed. Root colonization test was also carried out for confirmation of the success of root infection. Subsequently, spores of monosporic culture were extracted and verified. The results of monosporic culture are presented in table 3. The monocultures of *Rhizophagus fasciculatus*, *Glomus aggregatum*, *G. citricola*, *Funneliformis geosporum* and *Claroideoglosum etunicatum* were successfully raised. The root colonization and spore density were highest in case of *F. geosporum* and lowest in case of *C. etunicatum*.

Plate.1 *Smilax perfoliata* Lour. Plant



Plate.2 AM fungal morphotypes isolated from the rhizospheric soils of *Smilax perfoliata* Lour. (a: *Rhizophagus fasciculatus*; b: *Glomus citricola*; c: *G. hoi*; d: *G. geosporum*; e: *Glomus* sp.; f: *Sclerocystis* sp.; g: *Funneliformis badium*; h: *G. reticulatum*; i: *Glomus* sp.)

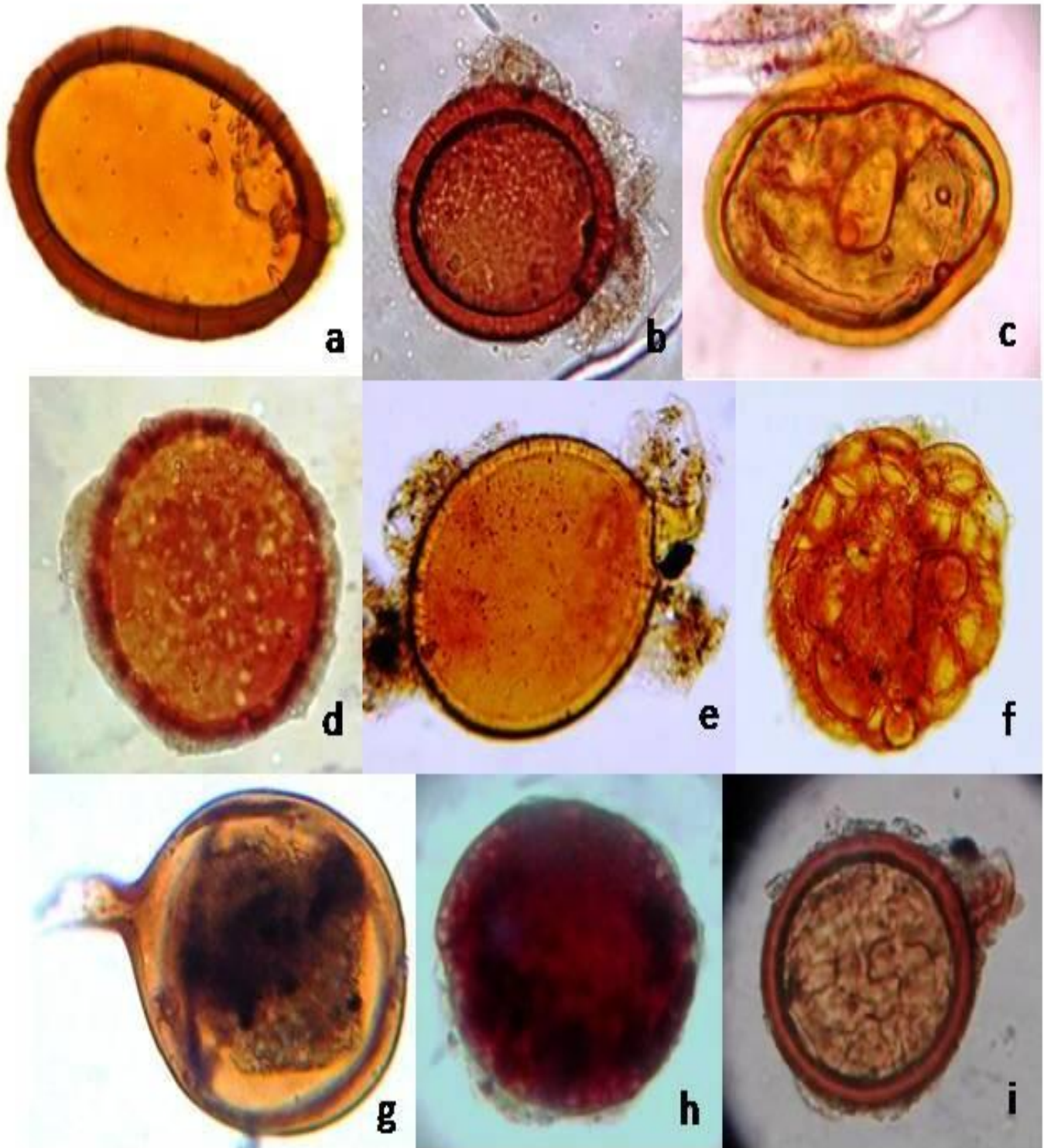


Table.1 AM fungal composition in *Smilax perfoliata* Lour.

AM Fungal Taxa	Relative abundance (%)
<i>Funneliformis badium</i> Oehl, D. Redecker & Sieverd.	5.11
<i>F. geosporum</i> C.Walker	4.43
<i>F. caledonium</i> (T.H. Nicolson & Gerd.) Trappe & Gerd.	5.11
<i>Glomus aureum</i> Oehl & Sieverd	5.79
<i>G. multicaule</i> Gerd.& B.K. Bakshi	3.75
<i>G. reticulatum</i>	3.75
<i>G. aggregatum</i> N.C. Schenck & G.S. Sm.	5.79
<i>G. citricola</i> D.Z. Tang & M. Zang	6.45
<i>G. hoi</i> S.M. Berch & Trappe	3.75
<i>G. canadense</i> (Thaxt.) Trappe & Gerd.	3.07
<i>G. sp.1</i>	4.43
<i>G. sp.2</i>	3.75
<i>G. sp.3</i>	5.01
<i>Rhizophagus fasciculatus</i> (Thaxt.) Gerd. & Trappe	6.45
<i>R. intraradices</i> N.C.Schenck & G.S. Sm.	3.75
<i>R. clarus</i> T.H. Nicolson & N.C. Schenck	5.79
<i>Claroideoglomus etunicatum</i> W.N. Becker & Gerd.	4.43
<i>C. claroideum</i> D.D. Mill. & C. Walker	4.43
<i>Acaulospora scrobiculata</i> Trappe	4.43
<i>A. sp.1</i>	3.07
<i>A. sp.2</i>	3.07
<i>Scutellospora sp.1</i>	2.39
<i>S. sp.2</i>	3.07

Table.2 AM fungal composition in trap culture

AMF	Relative abundance (%)
<i>Funneliformis geosporum</i> C.Walker	8.39
<i>F. badium</i> Oehl, Redecker & Sieverd	3.95
<i>Glomus citricola</i> D.Z. Tang & M. Zang	12.84
<i>G. aggregatum</i> N.C. Schenck & G.S. SM	8.39
<i>G. aureum</i> Oehl & Sieverd	5.06
<i>G. pellucidum</i> Mc Gee & Pattinson	7.28
<i>G. tortuosum</i> N.C. Schenck & G.S. Sm.	3.95
<i>G. sp.4</i>	5.06
<i>Rhizophagus fasciculatus</i> Gerd & Trappe	10.62
<i>R. diaphanum</i> J.B. Morton & C. Walker	2.84
<i>Sclerocystis sp. 1</i>	2.84
<i>Gigaspora rosea</i> T.H. Nicolson & N.C. Schenck	3.95
<i>Scutellospora sp.1</i>	2.84
<i>Acaulospora scrobiculata</i> Trappe	8.39
<i>A. denticulata</i> Sieverd. & S. Toro	6.17
<i>A. sp.1</i>	5.06
<i>Ambispora fecundispora</i> N.C. Schenck & G.S. Sm.	6.17
<i>A. jimgerdemannii</i> N.C. Schenck& T.H. Nicolson	7.28

Table.3 Root colonization (%) and Diversity measurement of AM Fungi community in monoculture of AM Fungi

AM Fungi	Root colonization (%)	Spore density (50gm ⁻¹)
<i>Rhizophagus fasciculatus</i> (Thaxt.) Gerd. & Trappe	46	80
<i>Glomus aggregatum</i> N.C. Schenck & G.S. SM	59	75
<i>Glomus citricola</i> D.Z. Tang & M. Zang	59	75
<i>Funneliformis geosporum</i> C.Walker	62	85
<i>Claroideoglomus etunicatum</i> W.N. Becker & Gerd	40	40

The present study provided the status of AM fungal diversity in *Smilax perfoliata* Lour. The AM fungal population and diversity status in the soil is dependent on the factors viz. climate, soil physicochemical properties and the host plant (Hayman 1982; Brundett 2009; Dehne *et al.*, 1987). It was reported that abiotic factors are more important (Panwar and Tarafdar 2006) in the distribution of AM fungi. The physicochemical results of studied soil sample indicated that the soil was alkaline (pH 7.8). Alkalinity of soils is primarily caused due to the presence of soil minerals producing carbonate upon weathering (Wang *et al.*, 2015). It was reported that the soil moisture influenced the AM fungal root colonization (Lingfei *et al.*, 2005). Jha *et al.*, (1992) reported positive correlation between AM fungal root colonization and soil moisture. The root colonization percentage and AM fungal spore density of *Smilax perfoliata* Lour plant was high suggesting high degree of infection. Total 23 different AM fungal taxa were found from the soil sample. In trap culture, total 18 AM fungal taxa were isolated. In trap culture, additional species were isolated viz. *Acaulospora denticulata*, *Glomus nigrum*, *G. pellucidum*, *G. tortuosum*, *Rhizophagus diaphanum*, *Ambispora fecundispora*, *A. jimgerdemannii*, *Gigaspora rosea* and *Sclerocystis* sp.1 that were not recorded from field soil sample of *Smilax perfoliata* Lour.

Glomus was the dominant genus during the present investigation. It was isolated in more

numbers as compared to the other genus in both field and trap soil samples of *Smilax perfoliata* Lour. It was reported that the *Glomus* is the dominant genus of various natural ecosystems (Li *et al.*, 2009; Muthukumar and Udaiyan 2000; Zhao *et al.*, 2001; Guaderramma and Alveraz-Sanchez 1999; Hijri *et al.*, 2006). Species of the genus *Glomus* has more tolerant to various disturbances and agricultural practices. Singh *et al.*, (2008) observed strong dominance of *Glomus* and moderate numbers of *Acaulospora*, *Gigaspora* and *Scutellospora* in cultivated and natural tea bushes. It was reported also that various disturbance alter species composition of AM fungi, including loss of large-spored Gigasporaceae and an increase in smaller *Glomus* species (Johnson, 1993).

Five monocultures of *Rhizophagus fasciculatus*, *Glomus aggregatum*, *Glomus citricola*, *Funneliformis geosporum* and *Claroideoglomus etunicatum* were successfully developed. These monosporic cultures of AMF can be used in applied field.

Thus, further studies should focus on establishment monoculture of these AM fungal species, which have a significant use as inoculants for nursery seedlings and trees of the region. The present investigation provides knowledge and understanding about the presence and diversity of AMF in *Smilax perfoliata* Lour. and good possibilities for further studies and utilization of AM fungi.

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