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Full Length Research Paper

Investigation on arbuscular mycorrhizal alliances in some threatened medicinal herbs of Burdwan district, West Bengal, India

Rebeca Ghanta, Sikha Dutta* and Radhanath Mukhopadhyay

Department of Botany, UGC Centre of Advanced Study, The University of Burdwan, Burdwan-713104, West Bengal, India.

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Perseverance of conventional herbal medicines is increasing gradually throughout the world. Considering the growing demand of ethnomedicinal plants in therapeutic treatments to our primary health care system and the role of vesicular arbuscular mycorrhizae (VAM) fungi for enhanced production of active secondary metabolites by the medicinal plants, the present work was undertaken to survey the arbuscular mycorrhizal status in some common medicinal herbs like, *Abrus precatorius*, *Elephantopus scaber*, *Sida rhombifolia* and *Clerodendrum indicum.* As evidenced from Grid-line intersect method, all the plants under investigation were colonized by the vesicular arbuscular mycorrhizal fungi as both the vesicles and arbuscules were present in the roots. Arbuscular frequency was the highest in the roots of *E. scaber* (0.3 to 98.7), followed by *A. precatorius* (5.97 to 61.46) and lowest in *C. indicum* (0.1 to 31.7). Correspondingly, vesicular frequency was the highest in *E. scaber* (0 to 63.33) and lowest in *C. indicum* (31.83 to 40.2). It is apparent from the results that the extent of mycorrhizal colonization varied significantly with the seasonal variations throughout the year. Spore analysis from the rhizosphere soil sample exhibited a great deal of variation in their frequency and occurrence in different seasons of the year and also in their morphological features. The VAM/arbuscular mycorrhizae (AM) fungi observed in the present study mostly belong to the species group of *Glomus*.

Key words: Vesicular arbuscular mycorrhizae (VAM), arbuscular mycorrhizae (AM), *Glomus* species, *Acaulospora scrobiculata*, *Sclerocystis* species, *Abrus precatorius, Elephantopus scaber*, *Sida rhombifolia, Clerodendrum indicum.*

INTRODUCTION

Increased concern about the environmental impact of agrochemicals and high intensity farming, together with examining cost-effective crop production strategies that consign less financial reliance on expensive synthetic inputs, have stimulated the interest in the practical appliance of mycorrhizae and legumes in agriculture. 'Mycorrhiza' is the symbiotic association between soil-born fungi and the roots of higher plants. The word mycorrhiza literally means 'FUNGUS ROOTS' (Mycos = fungus and rrhiza = roots).

*Corresponding author. E-mail: sikha_bu_bot@yahoo.com.

The vesicular-arbuscular mycorrhizae (VAM) are ubiquitous in roots of vascular plants in nature (Harley and Smith, 1983; Powel and Bagyaraj, 1986; Gabor, 1992). The wide distribution of VAM is due to obligate mycotrophy of the plants (Trappe, 1987). It is estimated that about 87% of terrestrial plants form mycorrhizal associations (Stoyke and Currah, 1993) and are known to be well distributed along both the hemispheres. VAM fungi are the most ancient and obligate symbionts (Miller et al., 1999; Mukerji, 1996). They probably form the largest component of all fungal materials within the soil. Plants are infected by VAM fungi from spores naturally present in the soil. Mycorrhizal fungi form mutualistic associations with the roots of terrestrial plants, providing

them with phosphorous and other nutrients in exchange for photosynthates (Allen, 1991; Smith and Read, 1997). This is made possible by the fungal hyphae which extend beyond the immediate area of the root to effectively increase the absorptive area. The practical implications of this are that mycorrhizal plants are more efficient in their use of applied fertilizers, by making fuller use of soil mineral reserves. The small size and hyphal structure allow them to act as microscopic pipelines that can transport carbon and minerals to and away from the plants (Barrow, 2004). They are well adapted to function in lower water potentials and hence can alleviated drought stress even in arid ecosystem (Barrow and Aaltonen, 2001; Griffin, 1997). Traditional herbal medicines are increasingly being used not only by the developing countries, but also by the developed countries in their primary health care system. A bulk of our rural population relies on the drug resources of plant origin. Thus, the cultivation of medicinal plants is increasing steadily to maintain a steady supply and to support their increasing demand. But corresponding research works on the occurrence of VAM fungi and their association in medicinal plants have received very little attention as compared to the studies on forest species and field crops. However, only a few studies have been made in this field. VAM association in three different species of *Cassia* like, *Cassia alata*, *Cassia occidentalis* and *Cassia sophera* and the influence of seasonal variation in their colonization pattern has been investigated by Chatterjee et al. (2010). Studies on mycorrhizal association in the medicinal plants of Azad Jammu and Kashmir have been made by Sidiq (2002). Association of arbuscular mycorrhizal fungi in some medicinal plants like, *Hemidesmus indicus*, *Gymnema sylvestris*, *Andrographis paniculata*, *Andrographis alaba* and *Clerodendrum phlomidis* have been studied by Sampathkumar et al. (2007).

Thus, the purpose of the present study was to investigate the extent of VAM/AM association in some common regionally threatened medicinal plants of Burdwan district, West Bengal, India. In the present study, medicinal plants like, *Abrus precatorius*, *Elephantopus scaber*, *Sida rhombifolia* and *Clerodendrum indicum* were taken into consideration.

Medicinal uses of *E. scaber*

E. scaber L. is a species of flowering plant in the Asteraceae family. The plant has got tremendous medicinal importance. The roots and leaves are used as emollient for dysuria, diarrhoea, dysentery, swellings and stomach pain. It is applied for tooth-ache in powder form with pepper. The leaves are used in applications for eczema and ulcers. The herb is diuretic, laxative, analgesic, alterative, febrifuge, cardiac and brain tonic, used in griping, inflammations and bronchitis. Paste of the whole herb with *Scoparia dulcis* is made into pills and given in the treatment of menorrhagia by the people of Marma. The root is used in fever and to arrest vomiting. The leaves are used in piles. Bruised leaves boiled in coconut oil are applied to ulcers and eczema. The flowers are aphrodisiac, tonic and expectorant, cures biliousness, liver troubles and cough (Ayyanar et al., 2005). Ethanol (50%) extract of the plant showed anticancer activities (Wan Ho et al., 2009). *E. scaber* extract has antimicrobial property (Avani and Neeta, 2005) (Figure 1).

Medicinal uses of *C. indicum*

C. indicum (Linn.) is a member of the family Lamiaceae. The bark of the root contains phenolic glycoside and saponin as active ingredient. Saponin is very helpful as an antihistamine agent thus is very much effective in preventing the bodies or over active reaction of the body towards any external agent entering the body. According to Ayurveda, it is a good anti-inflammatory agent and also helps in the healing of wounds. It improves circulation of blood in the body. Bhangri is also helpful in improving the digestive activities of the body. It acts on respiratory system thus expelling out the excessive mucus in the tract relieving from cough, cold and asthmatic symptoms. It opens the body pores and increases the sweating in the body (Srivastava and Paetel, 2007) (Figure 2).

Medicinal uses of *A. precatorius*

A. precatorius L. belonging to Fabaceae is an important plant having ethno-medicinal importance. In traditional Siddha medicine, the white variety is used to prepare oil that is claimed to be an aphrodisiac. A tea is made from the leaves and used to treat fevers, coughs and colds. Seeds are poisonous and therefore are used after mitigation (Rajaram et al., 1992). Aqueous seed extract of *A. precatorius* has potent role against alcohol induced renal damage and suppresses alcohol-induced renal injury and this effect is related to the attenuation of alcohol-mediated lipid peroxidation of renal parenchyma cells (Ligha et al., 2009) (Figure 3).

Medicinal uses of *S. rhombifolia*

S. rhombifolia plant pacifies vitiated vata, pitta, pain, arthritis, asthma, bronchitis, burning sensation, and urinary retention. The root was reported to contain 450 ppm alkaloids and the presence of ephedrine and saponin, choline, pseudoephedrine, beta-phenethylamine, vascin, hipaphorine and related indole alkaloids (Kuniata and Rapp, 2001). Arrow leaf *Sida* has significant medicinal applications for which it is cultivated throughout India. The pounded leaves are used to relieve swelling,

Figure 1. Plant body of *E. scaber*.

Figure 2. Plant body of *A. precatorius*.

Figure 3. Plant body of *S. rhombifolia*.

the fruits are used to relieve headache, the mucilage is used as an emollient, and the root is used to treat rheumatism (Parrotta, 2001). Australian Aborigines use the herb to treat diarrhea. The leaves are smoked in Mexico and a tea is prepared in India for the stimulation it provides (Shaman Australis Ethnobotanicals, 2002) (Figure 4).

MATERIALS AND METHODS

Plant selected

Some regionally threatened medicinal plants naturally growing in Burdwan, West Bengal, India have been selected for this study which include *A. precatorius, E. scaber*, *S. rhombifolia* and *C. indicum*.

Site of collection

Plant materials were collected from Burdwan and Bankura districts and their adjoining areas.

Identification of the plants

The plants were identified by using published literature and the herbarium of Botany Department, Burdwan University, Burdwan. Voucher specimens of the plants collected were maintained in the Herbarium of Botany Department, University of Burdwan, bearing the voucher specimen numbers Fabaceae: *A. precatorius* L. (BUHA 2011), Asteraceae: *E. scaber* L. (BUHA 2012), Malvaceae: *S. rhombifolia* L. (BUHA 2011) and Lamiaceae: *C. indicum* (Linn) (BUHA 2010) and are now being preserved along with the previous specimens and arranged according to Bentham and Hooker's system of classification.

Collection of root samples

For each species, the feeder roots were collected directly from the plants by digging and tearing the roots up to the base of the main stem.

Maintenance and preservation of roots

The root samples after collection were thoroughly washed in running tap water and rootlets were selected, cut into small pieces and fixed in formaldehyde/acetic acid solution (Johanson, 1940) and were preserved in refrigerator at 4°C temperature.

Collection of soil sample

Soil sample of about 10 g was collected from the root region (rhizosphere) of each of the plant species by digging the soil up to a depth of 10 cm and collected into polythene bags, labeled and stored at 4°C until analysis in refrigerator.

Preparation of root samples

For each specimen, 100 feeder root pieces were thoroughly washed in water and boiled at 95°C temperature for different durations (like 10, 15, 20, 25 and 30 min) in 10% KOH. The segments were washed in distilled water, acidified with 1 (N) HCl and were stained with 0.05% Trypan blue in lactophenol. The excess stain was removed by washing with lactophenol. Root segments were mounted temporarily on slides in acetic acid, glycerol (1:1 V/V) and the edges of the cover slips were sealed with DPX and observed under microscope (Leica, Model no. DMLB 3000).

Assessment of VAM fungal association in roots

The VAM association in the roots of each of the specimens was examined following the method of Phillip and Hayman (1970) and the percentage of mycorrhizal association was calculated.

Collection of mycorrhizal spores from soil samples

At first 10 g, soil sample was taken and dissolved in 100 ml distilled water in a conical flask. The conical flask was then shaken for 30 min after which the flask was kept undisturbed for 30 minutes. The soil particles precipitated at the bottom of the flask and the spores were being floated on the surface of the liquid. Mycorrhizal spores were obtained by wet sieving and decanting technique (Gerdemann

Figure 4. Plant body of *C. indicum*.

and Nicolson, 1963). The solution was then passed through 250, 150, 53 and 45 µm pore size sieve and the spores were collected from the residue of 53 µm sieve. This residue was dissolved in distilled water and filtered. The residue present in the filter paper was taken and mounted on a slide in lactophenol and cotton blue and were examined under microscope (Leica, Model No. DMLB 3000).

Spore count

VAM fungal spores were extracted from three replicates of 50 g soil by wet sieving and decanting technique (Gerdeman and Nicolson, 1963). The decantant were filtered through a filter paper with grid lines. The filter paper was then spread on a glass slide under a dissecting microscope and the number of spores was counted and expressed as spores per 100 g of dry soil.

Identification of VAM fungi

The arbuscular mycorrhizal fungi were identified by using manuals of Trappe (1982), Morton and Benny (1990), Schenck and Perez (1990) and Mukerji (1996).

Statistical methods

All the data were taken in ten replicates and the standard error of mean (SEM) value (±) was calculated. Each of the data was checked for interpretation whether they were statistically significant or not. The data were analyzed by using the statistical method like, analysis of variance (ANOVA), and critical difference (CD) at 5% level was calculated as shown in Table 1.

RESULTS AND DISCUSSION

It was evident from the present study that all the plants under investigation exhibited root colonization by the vesicular arbuscular mycorrhizal fungi as both the vesicles and arbuscules were present in the roots. Arbuscular frequency was the highest in the roots of *E. scaber* (0.3 to 98.7) followed by *A. precatorius* (5.97 to 61.46) and the lowest in *C. indicum* (0.1 to 31.7). Similarly, vesicular frequency was the highest in *E. scaber* (0 to 63.33) and the lowest in *C. indicum* (31.83 to 40.2). It is apparent from the results that the extent of mycorrhizal colonization varied significantly with the seasonal variations throughout the year. Percentage of arbuscules as well as vesicles was maximum in rainy season and minimum in winter in all the plants studied, whereas spore count was maximum in winter except *S. rhombifolia* where it was little bit high during summer than in winter. It is very interesting to note that in *E. scaber* no vesicles and intraradical spores were observed during winter whereas in contrast, intraradical spore count was maximum during winter in the other three species.

The VAM fungi found in this study were identified using standard manual and the synoptic key of Schenck and Perez (1987), Morton and Benny (1990) and Trappe (1982). The VAM/AM fungi recorded in the present study mostly belong to the species group of *Glomus.* The genus includes both sporocarpic and non-sporocarpic species. Those with chlamydospores develop the spores

Table 1. Colonization status of arbuscular mycorrhizal fungi in the roots of threatened medicinal plants.

Plant	Season of collection	Mycorrhizal colonization*			
		Percentage of arbuscules*	Percentage of vesicles*	Percentage of intraradical spores*	Extraradical spores /100 g of soil*
A. precatorius	Winter	5.97 ± 0.13	20.4 ± 2.11	33.349 ± 0.90	420 ± 3.14
	summer	20.4 ± 0.55	10.3 ± 0.90	27.3 ± 1.41	400 ± 2.65
	Rainy	61.46 ± 1.01	44.298 ± 3.05	25.264 ± 1.25	380 ± 1.80
E. scaber	Winter	0.3 ± 0.02	0	0	400 ± 3.11
	summer	45.5 ± 2.26	29.5 ± 2.43	25.1 ± 0.89	310 ± 2.40
	Rainy	98.7 ± 2.81	63.33 ± 2.95	30.33 ± 2.27	300 ± 2.25
S. rhombifolia	Winter	0.3 ± 0.07	26.25 ± 1.00	39.855 ± 1.19	390 ± 1.98
	Summer	35.46 ± 2.73	40 ± 1.23	28.833 ± 0.90	400 ± 3.00
	Rainy	57.9 ± 3.01	51.837 ± 3.11	25.319 ± 1.16	210 ± 2.44
C. indicum	Winter	0.1 ± 0.03	31.83 ± 1.29	12.632 ± 0.55	360 ± 4.17
	Summer	23 ± 1.44	26.9 ± 1.22	27 ± 2.31	310 ± 4.08
	Rainy	31.7 ± 1.66	40.2 ± 3.99	13 ± 0.87	220 ± 1.82

*Data are the mean values of ten replicates; CD at 5% 0.256.

terminally on a single undifferentiated hypha, sometimes in aggregate (Figure 5). The spores are formed at the end of a hypha which may be constricted at the point of attachment to the spore having parallel side walls, or become markedly occluded at the point of attachment to the spores (Figure 5). The spore wall can have one to many layers, without ornamentation (Figures 6 and 7). Germination is either into old subtending hypha or more rarely through the spore wall. Vesicles and arbuscules have been found in the genus *Glomus*. (Figures 5, 6 and 7).

Different species of *Glomus* like *Glomus fasciculatum*, *Glomus mossae*, *Glomus microcarpum*, *Glomus heterosporum*, *Glomus geosporum*, etc., have been recorded. Other genera of VAM/AM fungi obtained in the present study were *Acaulospora scrobiculata*, Figure 7(14) and *Sclerocystis* species, Figure 6 (9).

The variation which was exhibited in root colonization percentage among different species might be due to the effect of rhizosphere soil that favoured the growth of AM fungi. It has been observed by Hayman (1974) and Koske (1981) that VAM colonization percentage remained maximum during summer or spring due to higher photosynthetic activity of the host plant and better symbiosis between the mycorrhizal fungi and the host roots under congenial environment. The gradual increase in the number of spore count in summer than in winter as evidenced in our study corroborate with the findings of some other scientists in several plants like Mason (1964), Hayman (1970), Sutton and Barron (1972) and Saif and Khan (1975).

The presence of mycorrhizal endophytes in plants is influenced by several factors, such as host types, species diversity, degree of stability of habitat, extent of fertilizers usage and edaphic factors like season, soil texture and moisture content (Nicolson, 1960; Raghupathy and Mahadevan, 1991; Muthukumar and Udaiyan, 2000; Gorsi et al., 2002; Wang and Qiu, 2006).

During a survey on AM fungal associations in vascular plants by Bajwa et al. (2001), AM colonization was found to remain stable and it was higher in spring and summer, but maximum during winter. Among the monocots, *Vetiveria zizanioides* showed the lowest but stabilized spore population without any periodic response from spring to autumn which sharply increased to maximum in winter.

These results corroborate with our present findings where AM colonization percentage in the roots of the plants under our study was maximum in winter. It is noteworthy that spore count in the soil was also maximum in winter.

Furthermore, a differential impact of seasonal variation on the formation and development of VAM structures, namely, mycelium, arbuscules and vesicles was observed by Bajawa et al. (2001).

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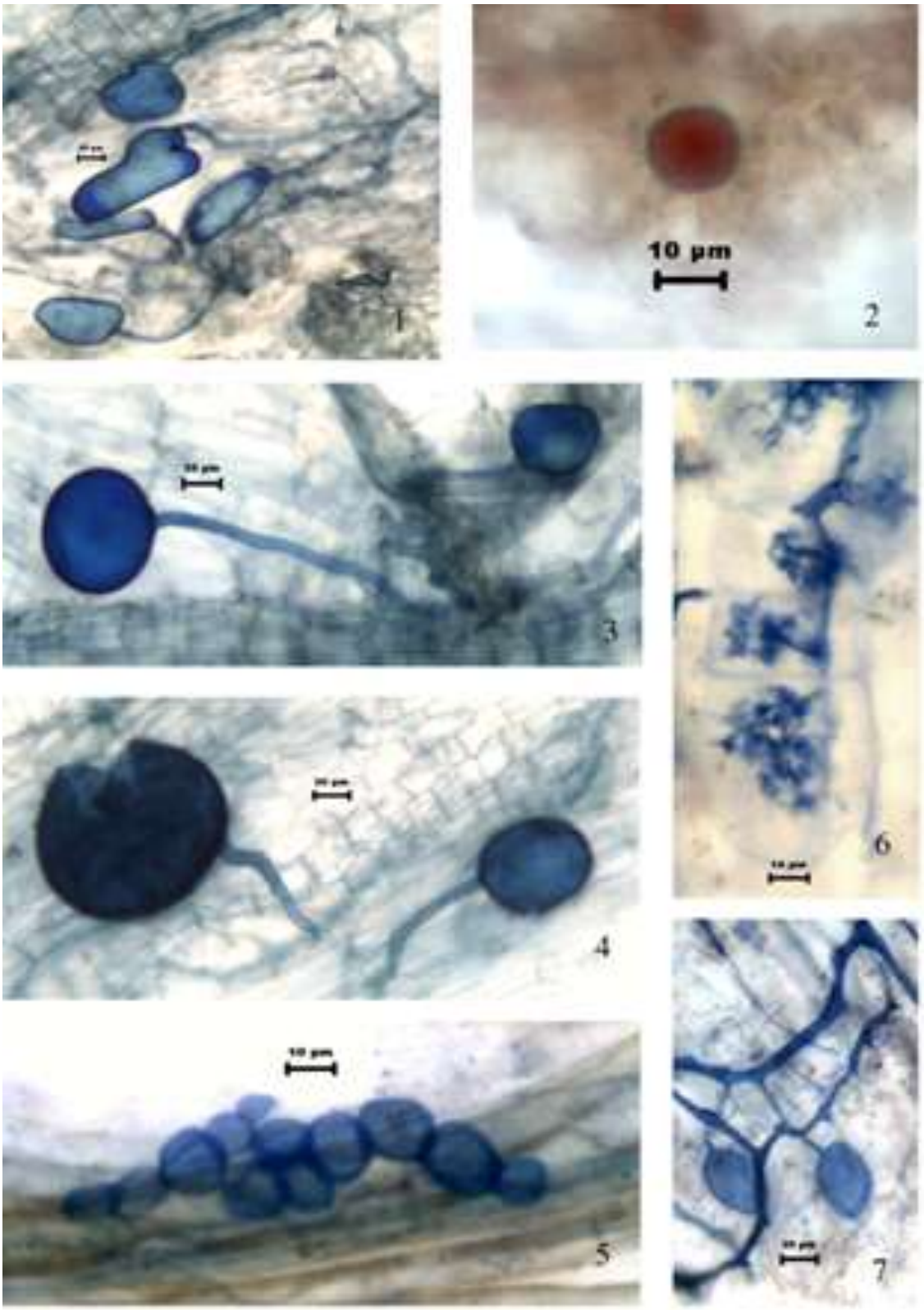

Figure 5. (1) Vesicles of *Glomus fasciculatum* (Thax.) Gerd. and Trapp in the root of *Abrus precatorius*; (2) Chlamydospore of *Glomus geosporum* (Nicol. & Gerd.) Walker in *Abrus precatorius;* (3) Vesicles of *Glomus mossae* (Nicol. & Gerd.) Gerd. & Trappe in the root of *Abrus precatorius*; (4) Zygospore of *Glomus* sp. in *Abrus precatorius*; (5) Intraradical spores of *Glomus aggregatum* Smith & Schenck in *Abrus precatorius*; (6) Arbuscules in *Clerodendrum indicum*; (7) Vesicles of *Glomus fasciculatum* (Thax.) Gerd. and Trapp. in *Clerodendrum indicum.*

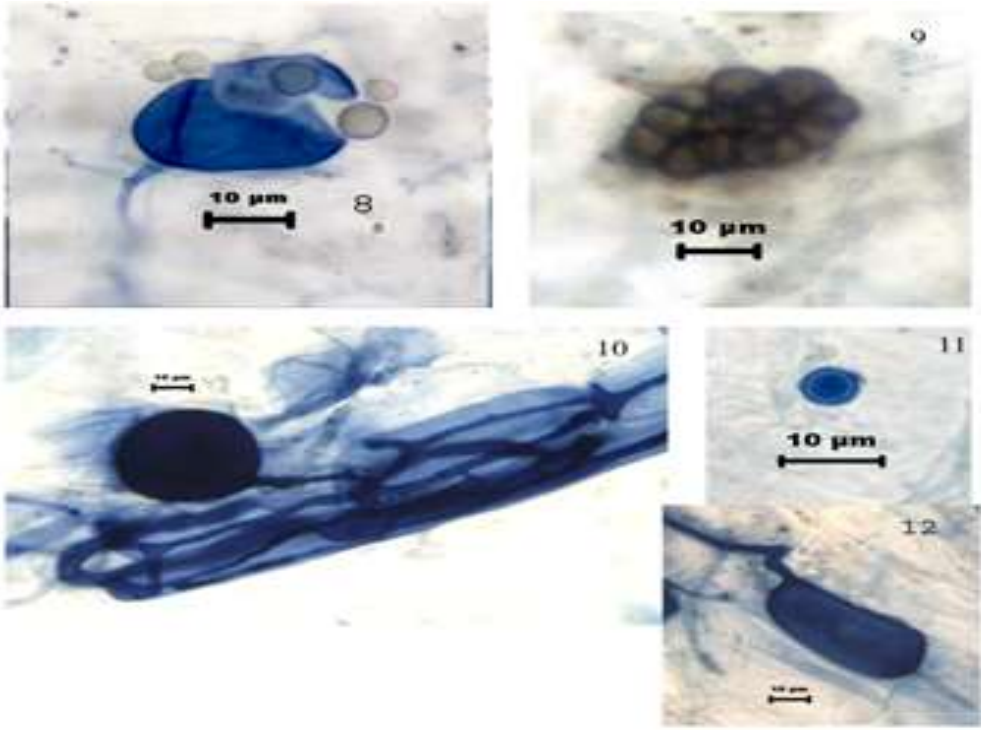

Figure 6. (8) A ruptured vesicle of Glomus *fasciculatum* (Thax.) Gerd. and Trapp. in *Clerodendrum indicum*; (9) Sporocarp of *Sclerocystis* sp. in *Clerodendrum indicum*; (10) Vesicles of *Glomus macrocarpum* Gerd. and Trapp. in the root of *Elephantopus scaber*; (11) Chlamydospore of *Glomus multicaule* Gerd. and Baks. in root of *Elephantopus scaber*; (12) Vesicle of *Glomus* sp. in root of *Elephantopus scaber*.

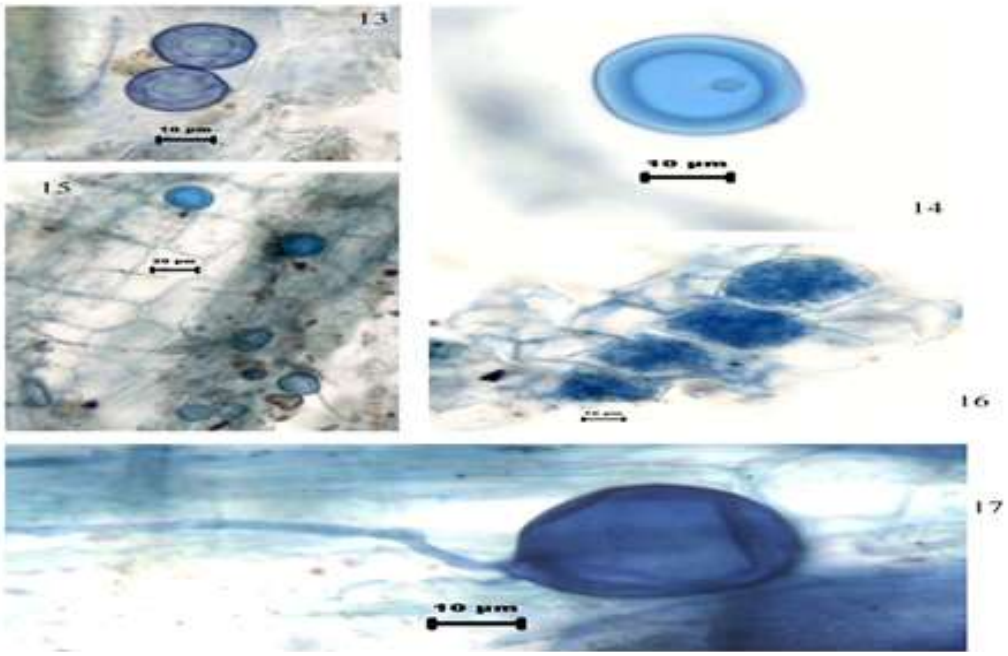

Figure 7. (13) Chlamydospores of *Glomus* sp. in *Elephantopus scaber*; (14) Chlamydospores of *Acaulospora scrobiculata* Trappe in rhizosphere soil of *Elephantopus scaber*; (15) Chlamydospores of *Glomus microaggregatum* Koske, Gemma and Olexia in *Elephantopus scaber*; (16) Arbuscules in the root of *Sida rhombifolia*; (17) Vesicle of *Glomus fasciculatum* (Thax.) Gerd. and Trapp. in the root of *Sida rhombifolia.*

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