Universal Impact Factor 0.9285:2012: 1.2210:2013

Index Copernicus
ICV 2011: 5.09
ICV 2012: 6.42
ICV 2013: 15.8

NAAS Rating
2012 : 1.3;2013:2.69

Received on:
30th December 2014

Revised on:
20th January 2015

Accepted on:
20th January 2015

Published on:
1st February 2015

Volume No.
Online & Print
60 (2015)

Page No.
87 to 96

*Life Sciences Leaflets is an international open access print & e journal, peer reviewed, worldwide abstract listed, published every month with ISSN, RNI Free-membership, downloads and access.*

# ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATION IN HOMESTEAD BAMBOO SPECIES OF ASSAM, INDIA

**P. HAZARIKA*, S.C. BISWAS AND D. DUTTA**

**RAIN FOREST RESEARCH INSTITUTE**

**SOTAI, P.B. 136, JORHAT-785 001, ASSAM.**

**Corresponding author's e-mail: hazarikapaug08@gmail.com**

***ABSTRACT:***

Arbuscular mycorrhizal fungi (AMF) association in roots of four bamboo species *Bambusa tulda* Roxb., *B. nutans* Wall ex Munro, *Bambusa pallida* Munro and *B. balcooa* Roxb. grown in homestead area of five different localities of Assam were studied. AM spores were isolated from root rhizosphere soils of four bamboo species and identified tentatively and classified up to the genus level. Soil physico-chemical properties such as soil texture, pH, EC and per cent organic carbon were determined using standard methods. Five genera of AM spores including *Glomus*, *Gigaspora*, *Acaulospora*, *Sclerocystis* and one unidentified species were isolated from rhizosphere of bamboo species. Spores of *Glomus* species were found dominated over others. AM fungi spore number varied between sites and among four bamboo species. Variation in AM root colonization was not as marked as spore number and no correlation could be established in between AM spore and root colonization. Rhizosphere soils of *B. nutans* contained more diverse AM spore types i.e. 9) genera followed by *B. pallida* with 8 genera, 5 genera in *B. tulda* and least in *B. balcooa* with 4 genera. Texture of the soil is sandy loam and loamy. pH of the rhizosphere soils range from 5.0 to 7.3.E.C (µMho) ranges between 0.0918- 0.5529 and % soil organic carbon ranges between 1.81-3.49.

***KEY WORDS:*** *Arbuscular-mycorrhiza, AMF spore, Bamboos, Homestead area, Root colonization.*

## *INTRODUCTION:*

Bamboos are economically important fast growing graminaceous species widely distributed throughout the evergreen and deciduous forest of India with

18 genera and almost 130 species. North Eastern Region of India alone comprises more than 50 per cent of India's total bamboo species (Tiwari, 1993) and habitat of 78 bamboo species of which 42 species are grown in Assam. Bamboo is considered as an important agroforestry crop for the people of northeastern region of India. Now a day, its value is also added as it enables to harness enough importance in regulation of socio-economy of the rural people, employment generation of the educated youths of the region through cultivation of bamboos and establishing different kinds of industries, which have the products of international trade. It has promising scope in restoration and revegetation of eroded and degraded lands; wastelands create after *jhum* cultivation and opencast coalmining. With the increasing demands of Bamboo in upliftment of rural economy, control soil erosion; revegetation in degraded lands etc, it is widely used as ideal plant in afforestration programme of the country. Bamboo is common in almost all homestead area of Assam as plantation crop. In these plantation programmes arbuscular mycorrhiza may play an important role as soil related technology for better management of bamboo cultivation.

*Arbuscular mycorrhizal* fungi (AMF) are ubiquitous in soil. In tropics and subtropics 95 per cent of the forest tree species are Arbuscular mycorrhizal (La Tekon *et. al.*, 1987). AMF may play an important role in vegetation growth in tropical soils where nutrient status is very low (Janos, 1975). The AME association promotes growth of plants by improving uptake of macro- and micronutrient, increasing plant resistance against biotic and abiotic stress, and beneficial alternations of plant growth regulators (Smith & Smith, 1996; Liu & Li, 2000; Govindarajulu *et. al.*, 2005; Kung'u *et. al.*, 2008; Smith & Read, 2008). Studies on the role of AMF in bamboo are very scanty and confined to a very few species only (Gautom and Maitra, 1995; Jha, *et. al.*, 2012; Weixin *et. al.*, 2013). Report on occurrence of AMF are also very few (Gerdemann and Bakshi, 1976; Appaswamy and Ganapati, 1992; Battacharya *et. al.*, 1995 and Ravikumar *et. al.*, 2001). In this study, four economically important bamboo species *i.e. Bambusa tulda* Roxb., *B. nutans* Wall ex Munro., *B. balcooa* Roxb. and *B. pallida* Munro. of five different localities of homestead area of Assam were explored for the occurrence of AMF with an aim to understand natural relationships with the edaphic factors such as soil texture, pH, electrical conductivity and per cent organic carbon and choose priority of these species for successful afforestration programme in degraded land.

## *MATERIALS AND METHODS:*

Five bamboo species i.e. *Bambusa tulda* Roxb., *B. nutans* Wall ex Munro., *B. balcooa* Roxb. and *B. pallida* Munro. of homestead area of Alengmora, Dhekorgarah, Kakojan, Nagajanka and Lichubari of Jorhat District, Assam were selected for the study (Fig. 1). The Jorhat district is located in between 27º35' N to 26º30'N latitude and 93º45'E to 94º30'E Longitude. First two sites are located at a distance of 3 to 7 km from the river Brahmaputra with sandy loam soil. Other three sites are located at a distance of about 25 to 40 km from the Brahamaputra with loamy soil. Soil and root samples were collected from the

rhizosphere of 2-3 years old culms during July-Aug 2005 from clumps not less than 10 years old in plantation sites.

Soil cores were collected at random for each bamboo species from three different culms of same age and mixed thoroughly to have a composite sample. A total of twenty composite soils along with root samples were collected for each of the four bamboo species from each site.

AM fungi spores were isolated from 50ml soil of each composite sample by wet sieving and decanting method (Gerdemann and Nicolson, 1963). 50 ml soil samples were suspended in about 350 ml of water in a 500ml beaker and stirred for 3 to 4 minutes so that all soil particles are suspended. Soil and spore suspension was passed through a brass sieve set of 500, 212, 106 and 53µ mesh size and particles retained on the sieves were carefully transferred to a 100 ml beaker with the help of a wash bottle. This spore suspension was again poured on filer paper that was finally examined and quantified under a stereomicroscope. Apparently healthy spores with regular wall orientations were counted and similar looking spores separated. Isolated AMF spores were examined thoroughly under the stereomicroscope. Similar looking spores were transferred to separate vials, and detailed study for identification and classification was carried out with consultation the taxonomic key in the manual for the identification of AMF by Schenck and Perez (1990).

Roots collected from each composite soil sample were washed with water to remove the adhered materials, cleaned and then kept in a hot air oven at 45 $^{0}$C for 72 h. Such dried roots of the samples were cut into 1 cm pieces, soaked in tap water, and kept in a 10 per cent KOH solution for 24 h. It was then washed in tap water for several times and bleached with 10 per cent $H_2O_2$, slightly acidified with 0.01N HCl at room temperature and stained in 0.05 per cent Trypan blue in lecto-glycerol (Phillips and Hayman, 1970; Kormanik and Mc Graw, 1982). After 24 h the roots were transferred to 50 per cent glycerol and kept until per cent AMF colonization determined. Stained root segments were examined under a compound microscope (Leitz-Laborlux 11 POL compound microscope, (100x) for AMF structures (vesicles, arbuscules, hyphae etc.) and hundred such root segments were examined for per cent AMF colonization. Per cent AMF colonization was quantified by gridline intersect method (Giovannetti and Mosse, 1980). Percentage of root colonization of AMF was calculated from 100 root segments. Microphotographs of AMF structure such as vesicles and arbuscules in stained root segments and spores isolated from different soils were taken.

Per cent AMF colonization of root was calculated by following formula

$$\text{Per cent AMF colonization} = \frac{\text{Number of AMF infected roots}}{\text{Total number of roots observed}} \times 100$$

Soil samples were air-dried and sieved through 2 mm sieve for physicochemical analysis. Soil texture, pH, EC, and per cent organic carbon were determined by following standard methods. Soil texture (hydrometer method, Bouyoucos, 1962), pH (1:2; soil: water); E.C by Calomel Electrode Method and per cent organic carbon by standard Walkley and Black method (1934).

Average, range and standard deviation of per cent AMF colonization and number of AMF spores for different sites were calculated (Table 1 & 2). Using computer statistical package of MS-Excel of Window 2000 all statistical analyses were performed.

## *RESULTS AND DISCUSSION:*

All four bamboo species studied were Arbuscular mycorrhizal. The level of AMF colonization in roots of homestead bamboo species of different sites of Jorhat district was also different (Table 1). Highest percent of root colonization was examined in roots of *B. pallida* and highest spore number was isolated in soils from *B. nutans*. Among these four bamboo species, the roots of *B. pallida* examined highest mean value with 49.6 per cent AMF colonization with a range of 15-76 %. Similarly, second highest mean value of per cent AMF colonization in roots was 45.8 % in *B. nutans* with the range of 16-71 per cent root colonization. Mean value of per cent AMF colonization in roots of *B. balcooa* was 43.2% and which was lowest in *B. tulda* with 41.8 per cent.

The number of AMF spores varied considerably in soils of different sites. AMF spore number $50^{-1}$ml root region soils are presented in (Table 2) and Fig-2 represents the photomicrographs of vesicles and arbuscules in the root cortex of *Bambusa balcooa, B tulta, B. nutans* and *B. Pallida*. Highest number of AMF spores was isolated from the soils of *B. balcooa* with a range of 325-842 spore $50^{-1}$ml soil. However, highest mean value of spore number in $50^{-1}$ml soil was isolated from *B. nutans* with 576 spores, which was followed by *B. balcooa* with 530 AMF spores. The study revealed that rhizosphere soils of *B. pallida* had occurred lowest number of 322 AMF spore $50^{-1}$ml soil and in soils of *B. tulda* it was 399 spores $50^{-1}$ml soil. Das (2010) in a similar study of four bamboo species from northeast India, isolated 166 number of AM fungi spore from $25^{-1}$g rhizosphere soil of *Bambusa tulda*, 481 numbers of AMF spores from $25^{-1}$g rhizosphere soil of *Dendrocalamus hookarii*, 103 number of AMF spores from $25^{-1}$g rhizosphere soil of *D. hamiltonii* and 714 numbers of AMF spores from $25^{-1}$g rhizosphere soil of *Phyllostachys manii* .

A total 9 types of arbuscular mycorrhizal fungi spores were tentatively identified up to the genus level as *Acaulospora, Gigaspora, Glomus* and *Sclerocystis* (Table 3 and Fig-3). The spores of *Acaulospora* and *Glomus* were common to the rhizosphere soils of the all four species of bamboo in every site. Das (2010) also reported dominance of *Acaulospora* and *Glomus* spores while studying the AMF colonization in rhizosphere soil of *B. tulda, Dendrocalamus hookarii D. hamiltonii and Phyllostachys manii* from Northeast India, *Gigaspora* was isolated from *B. tulda* at Dhakorgorah site and *Sclerocystis* was isolated

from *B. nutans* at Kakojan site. Spore diversity was rich in *B. tulda* and *B. pallida* with 7 types of AMF spores. A total number of 6 different types of *Glomus* spores were isolated from rhizosphere soils of *B. pallida*, followed by rhizosphere soils of *B. tulda* with 5 types, followed by *B. nutans* with 4 types of *Glomus* spore and *only* two types of *Glomus* were detected from the rhizosphere soils of *Bambusa balcooa*. Auxiliary cells of *Gigaspora* were abundantly met with the rhizosphere soil samples of *Bambusa balcooa* and *B. nutans* of Lichubari, Jorhat, however no spores were isolated. Highest spore diversity with 7 types of AMF spores were met with *B. tulda* and *B. pallida*. Other two homestead Bamboo species of the district i.e. *B. nutans* was associated with 6 AMF spore types and which was least in *B. balcooa* with 4 spore types.

The physico-chemical properties of rhizospheric soils under different bamboo species of different locations is shown in Table 4. Texture of the soil is sandy loam and loamy. The average pH of the rhizospheric soils was found between range from 5.0 to 7.3. Similarly, E.C (µMho) ranges between 0.0918- 0.5529 and % soil organic carbon ranges between1.81-3.49 indicates that there is a significant difference in soil status amongst bamboo species**.**

## *CONCLUSION:*

All four bamboo species of homesteads are arbuscular mycorrhizal. Variation in AMF diversity in bamboo rhizosphere soils was observed in different sites and bamboo species. This difference may be for their host selectivity. However, more study is required to establish host selectivity of AMF which is questioned by many researchers. Study is also required to investigate mycorrhizal dependency through pot experiments. After evaluation of above parameters priority of these species may be prescribed for successful afforestration programme in degraded land.

## *ACKNOWLEDGEMENTS:*

Authors are very much thankful to the Director, Rain Forest Research Institute, Jorhat, Assam for kind permission, facilities provided and Dr. Y.C. Tripathi, Scientist -E for his encouragement and help to perform the study.

## *REFERENCES:*

Apaswami, T and A. Ganapati 1992 Preliminary survey of vesicular arbuscular mycorrhizal (VAM) association with Bamboo in Western Ghat *BIC India Bulletin* **2**(2) 13-16.

Bhattacharya, P.M., Saha, J., Bannerjee, K., Ali, M and Chaudhuri, S. 1995. Mycorrhizae: Biofertilizers for the Future. In: *Proceedings of the 3rd National Conference on Mycorrhizae*, 13-15 March 1995. pp 17-21.

Biermann, B. and Linderman, R.G. 1981. Quantifying vesicular-arbuscular mycorrhizae: A proposed method towards standardization. *New Phytol.* **87**: 63-67.

Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analysis of soils. *Agron. J.* **54**:464-465.

Das, P. 2010. Arbuscular mycorrhizal fungi and dark septate endophyte colonization in bamboo from Northeast India. *Front. Agric. China*, **4**(3):375-382.

Gautom, S.P and Maitra, A.1995. Mycorrhizae, biofertilizers for the future. In: *Proceedings of the 3rd National Conference on Mycorrhizae*, 13-15 March 1995. pp 400-402.

Gerdemann, J. W and Bakshi, B.K.1976. Endogoneceae of India: two new species. *Trans Br. Mycol. Soc.* **66**: 340-343.

Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.*, **46**: 235-244.

Janos, D.P. 1975. Effect of vesicular-arbuscular mycorrhizae on low land tropical rain forest trees. In: Sanders, F.B., Mossae, B., and P.B. Tinker (Eds.) *Endomycorrhiza*. Academic Press, London and New York. pp. 437-446.

Jha, A., Kumar,A., Saxena, R.K., Kamalvanshi, M and N.Chakravarty 2012. Effect of Arbuscular Mycorrhizal Inoculations on Seedling Growth and Biomass Productivity of Two Bamboo Species *Indian Journal of Microbiology* **52**(2): 281-285.

Kormanik, P.P. and McGraw, A.C. 1982. Quantification of vesicular arbuscular mycorrhizae in plant roots. *In*: N.C.Schenck (eds) *Methods and Principles of Mycorrhizal Research*, pp. 37-45. The American Phytological Society, St Paul Minnesota.

Le Tacon., Carbaya, F.J. and Gur, G.1987. The status of Mycorrhizas in temperate and Tropical Forest. *Symbiosis*, **3**: 179- 206.

Morton, J.B. 1990. Species and clones of arbuscular mycorrhizal fungi (Glomales, Zygomycetes): their role in macro and micro evolutionary process *Mycotaxon*, **37**: 493-515.

Muthukumar T. and Udaiyan K. 2006. Growth of nursery-grown bamboo inoculated with arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in two tropical soil types with and without fertilizer application. *New Forests* **31**: 469–485.

Phillips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular- arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, **55**: 158-161.

Ravikumar, R., Ananthakrishnan, G. Girija,S and Ganapati, A 2001 AM associations of bamboo rhizophere in different study sites of Tamilnadu *Ind. For.* **127**(7) 804-808.

Schenck, N.C. and Perez, Y.1990. *Manual for the identification of VA- mycorrhizae fungi* .2nd edition (INVAM) University of Florida, Gainesville, FL, 245p.

Tewari, D.N. 1989. *Monographs of Bamboo*. International Book Distributor, Dehradun –248006 (India)

Walkley, A. and Black, I.A. 1934. An examination of the different method for determination of soil organic matter and proposed modification of the chromic acid titration method. *Soil. Sci.*, **37**: 29-38.

Weixin J, Guangqian G. and Yulong D. 2013. Influences of arbuscular mycorrhizal fungi on growth and Mineral element absorption of chenglu hybrid bamboo seedlings *Pak. J. Bot*., 45(1): 303-310.

**Table 1 AMF colonization (%) in root region soils of homestead bamboo species of Jorhat District**

| Site | % AM colonization in four Bamboo species | | | |
|---|---|---|---|---|
| | ***Bambusa tulda*** | ***Bambusa nutans*** | ***Bambusa pallida*** | ***Bambusa balcooa*** |
| **Alengmora** | 43.6 | 51.2 | 55.8 | 47.8 |
| **Dhekorgorah** | 45.2 | 52 | 58.4 | 46.4 |
| **Kakojan** | 38.0 | 33.4 | 37.8 | 37.6 |
| **Nagajanka** | 38.2 | 44.2 | 47.8 | 38.6 |
| **Lichubari** | 44.0 | 48.4 | 48.2 | 45.8 |
| **Mean** | 41.8 | 45.8 | 49.6 | 43.2 |
| **Range** | (25-66) | (16-71) | (15-76) | (20-58) |
| **Standard Deviation** | ±10.79 | ±12.84 | ±12.97 | ±9.93 |

**Table 2 AM fungal spore** population of **Bamboo species**

| Site | AM fungal spore number $50^{-1}$ml soil of four Bamboo species | | | |
|---|---|---|---|---|
| | ***Bambusa tulda*** | ***Bambusa nutans*** | ***Bambusa pollida*** | ***Bambusa balcooa*** |
| **Alengmora** | 300 | 647 | 376 | 446 |
| **Dhekorgorah** | 516 | 324 | 402 | 365 |
| **Kakojan** | 477 | 590 | 333 | 816 |
| **Nagajanka** | 486 | 762 | 239 | 521 |
| **Lichubari** | 215 | 557 | 263 | 504 |
| **Mean** | 399 | 576 | 322 | 530 |
| **Range** | (206-692) | (229-782) | (230-537) | (325-842) |
| **Standard Deviation** | ± 151.5 | ± 158.6 | ± 84.24 | ± 162.86 |

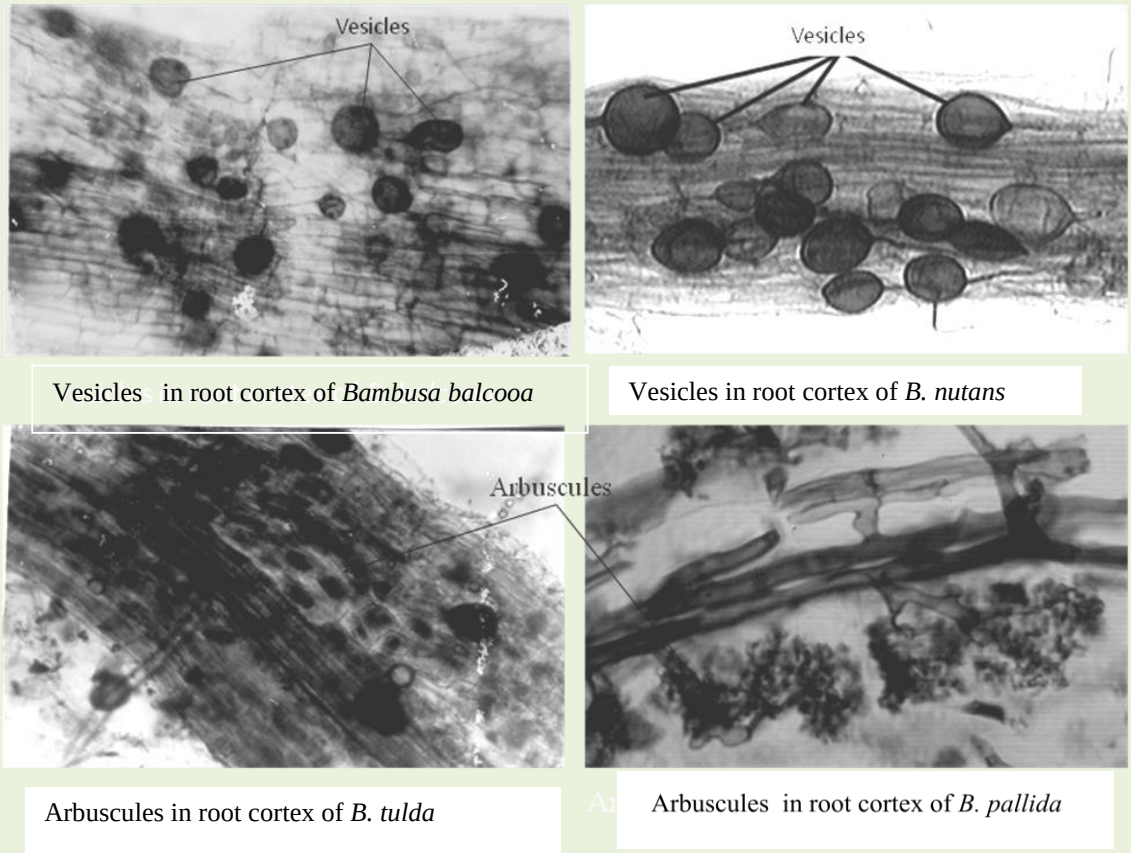
**Table 3 Diversity of AM spore types in four Bamboo species (identified after Morton, J.B.1990)**

| Type of AMF spore | *B. tulda* | *B. nutans* | *B. pallida* | *B. balcooa* |
|---|---|---|---|---|
| *Glomus* type-A | + | ++ | ++ | + |
| *Glomus* type-B | + | + | + | + |
| *Glomus* type-C | + | - | + | - |
| Glomus type-D | ++ | ++ | ++ | ++ |
| *Glomus* type-E | - | +++ | + | - |
| *Glomus* type-F | + | - | + | - |
| *Acaulospora* sp | + | + | + | + |
| *Gigaspora* sp | + | - | - | - |
| *Sclerocystis* sp | - | + | - | - |
| Unidentified | + | - | + | - |

**Table 4 Soil physico-chemical properties of the bamboo rhizospheric soils**

| Site | Soil texture | pH | E.C. (µMho) | % Organic carbon |
|---|---|---|---|---|
| Alengmora | Silt loam | 7.1 | 0.3686 | 2.68 |
| Dhekorgorah | Sandy loam | 7.3 | 0.3686 | 2.68 |
| Kakojan | Sandy loam | 6.0 | 0.0918 | 1.98 |
| Nagajanka | Loamy | 5.0 | 0.5529 | 3.49 |
| Lichubari | Loamy | 5.3 | 0.2765 | 1.81 |


**Fig-1. Location map of study sites of Jorhat District Assam**




Vesicles in root cortex of *Bambusa balcooa*

Vesicles in root cortex of *B. nutans*

Arbuscules in root cortex of *B. tulda*

Arbuscules in root cortex of *B. pallida*

**Fig -2. Photomicrographs of vesicles and arbuscules of arbuscular mycorrhizal fungi in the root cortex of four bamboo species**

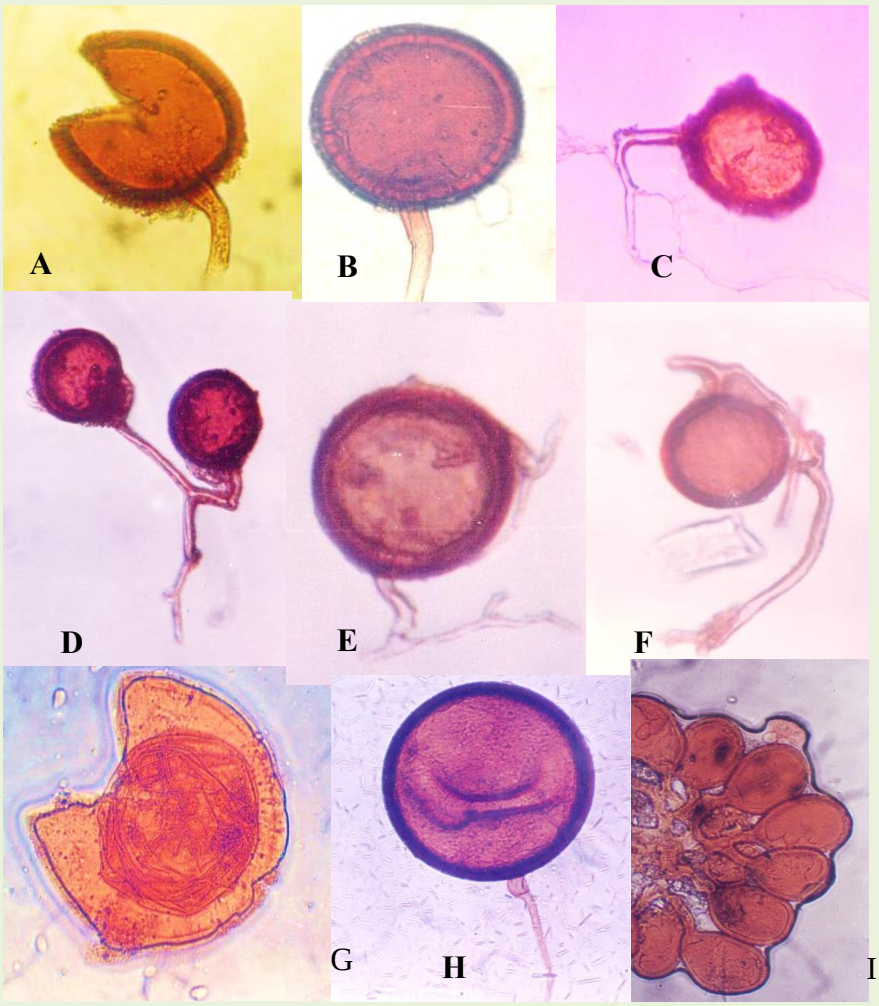



**Fig -3. Photomicrographs of arbuscular mycorrhizal fungi spores isolated from Rhizosphere soils of four bamboo species** (A-F: *Glomus* spp, sp, G – *Acaulospora*, H-*Gigaspora* sp, I- *Sclerocystis sp*,).)