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Characterization of arbuscular mycorrhizal fungi in apple (*Malus domestica* Borkh) growing area in Kashmir Himalaya (India): A case study of Bandipora district

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Rhizosphere soil samples and root pieces of 3 year old apple trees (var. Red delicious) were collected from 10 villages of district Bandipora of Kashmir Himalaya. Samples were processed for isolation of arbuscular mycorrhizal spores and their identification. On the basis of various morphological characters such as spore shape, colour, size, hyphal colour and with the help of International Collection of Vesicular-Arbuscular Mycorrhizal (INVAM), the isolated genera were identified as *Acaulospora, Scutellospora*, *Gigaspora,* and *Glomus*. *Glomus* spores were more predominant in the district followed by *Acaulospora*. Spores of *Gigaspora* were larger in diameter while as *Glomus* spores were smaller. *Glomus* species showed higher root colonization from Markondal followed by *Acaulospora* species from Gorura, *Gigaspora* species from Ajas and *Scutellospora* species from Sangri. The highest biological activity was due to adequate application of organic manures in the soil and also due to application of fertilizers.

Key words: Arbuscular mycorrhizal fungi (AMF), apple, *Acaulospora*, *Scutellospora*, *Gigaspora*, *Glomus*, root colonization, Kashmir.

INTRODUCTION

Apple (*Malus domestica* Borkh) is considered as one of the most important and widely grown fruits in temperate zones of the world with regard to its acreage, production, economic returns and high nutritive value. Apple is the fourth widely produced fruit in the world after banana, orange and grapes. Indian apple production averages nearly 1.4 million making it the sixth largest apple producer in the world (Satish et al., 2006) and second in Asia (Deepa, 2008). In India, apple is mainly grown in three mountainous states of North India, viz. Himachal Pradesh, Jammu and Kashmir and Uttarakhand where they are typically grown at an altitude of 4000 to 11000 ft.

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Jammu and Kashmir and Himachal Pradesh have almost equal area under apple, but later has the highest average yield and accounts for 67% of the total apple production (Masoodi, 2003) and 50% of its export in the country, earning a substantial foreign exchange. Productivity is much higher than national average of 6.86 tons/ha. India annually exports apple worth Rs. 400 million (Nearly US $ 10 million) out of which apples worth Rs. 200 million come from Kashmir. Moreover, it provides job opportunity to 1.2 million people directly or indirectly. In horticulture sector, the largest area of 43.53% is occupied by apple out of total area under fruit and 65.46% out of fresh fruit area (Anonymous, 2007), thereby making it the largest contributor to the state GDP among the horticulture produce.

Bandipora is a newly carved district from erstwhile Baramulla district. The district is surrounded by Himalayan Mountains having district Kargil on north, Kupwara in west, Baramulla in south and Ganderbal in east. The district is situated between 34°25´ and 34°42´ N Latitude and 74°39´ and 74°65´ E longitude. It has unique agro climatic conditions of low temperature even during summer months. The main fruit crop of the district is apple. Out of the total area of 140156 hactare under apple in Kashmir district Bandipora covers area of 5605 hactare (Anonymous, 2013). Bandipora is known for the better quality apple in the valley.

Phosphorus which is the second primary nutrient after nitrogen has a direct effect on yield and tree health. It is also important in determining fruit size, firmness, colour and storage potential. It increases the potential of fruiting by increasing the number of flower clusters, their intensity and the level of fruit set. But, phosphorus is an extremely immobile element in soils and even if it was added to soil in soluble form, it becomes immobilized as organic phosphorus, calcium phosphates, or other fixed forms (Jackson and Mason, 1984). AM-fungi are known to be effective in increasing phosphorus uptake in many crops, particularly in low phosphorus soils (Osonubi et al., 1991). They are associated with improved growth of many plant species due to increased nutrient uptake, production of growth promoting substances, tolerance to drought and synergistic interaction with other beneficial soil micro-organisms such as N-fixers and P-solubilizers (Sreenivasa and Bagyaraj, 1989). Arbuscular mycorrhizal fungi play an important role in sustainable agriculture as well as agricultural ecosystem management. The important genera of endomycorrhizal fungi reported so far are *Acaulospora, Entrophosphora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* (Morton, 1988). All these fungi are obligately associated with plant roots and develop symbiotic relationship with their hosts. Different species can be differentiated on the basis of sporocarp size, spore dimension, presence/absence of hyphal mantles, spore ornamentation (warts, wrinkles, pits, reticulum, spines, etc.), spore walls, spore content, hyphal attachment, manner of spore germination, histochemical reaction, etc. (Schenck and Smith, 1982). Keeping in view the importance of arbuscular mycorrhizal fungi in phosphorus solubilization and very little work having been done on arbuscular mycorrhizae with respect to apple, the present investigation was taken up with the objective of isolation and purification of arbuscular mycorrhizal spores from the rhizospheric soil of apple, morphological characterization of the spores upto generic level and their root colonization studies.

MATERIALS AND METHODS

Study area

Bandipora is situated on the banks of the Wular, the largest fresh-water lake in Asia, with geographic coordinates of 34.5052° N, 74.6869° E. In Bandipora district, apple production has swelled to 69,147 MT in 2014-15 from 65,102 MT in 2013-14. Similarly, the area under crop extended from 5,605 to 5,840 hectares in one year and thus contributes a lot in horticulture sector of Jammu and Kashmir. Ten villages (Arigam, Ajas, Asham, Gorura, Nadihal, Naidkhai, Sumbal, Markondal, Sangri, Watlab) were selected from district Bandipora. From each village three orchards were randomly chosen and from each orchard five rhizosphere soil samples were drawn which were composited into one representative sample. Most of the orchardists followed the pesticide schedule as per Department of Horticulture, Government of Jammu and Kashmir (Anonymous, 2015).

Isolation and purification of arbuscular mycorrhizal spores

Rhizosphere soil samples were collected from the feeder roots on all sides of the canopy of the tree (fruiting stage). The soil samples taken from the rhizosphere of apparently healthy apple trees were collected in June 2014. The soil samples (about 250 g each) were immediately brought to the laboratory, air-dried and processed for AM isolation using wet sieving and decantation method (Gerdemann and Nicolson, 1963). Counting of spores was done under microscope Olympus CH20i with magnification of 10×40.

Measurement of available sulphur, soil organic carbon, available phosphorus, available potassium

Available sulphur, soil organic carbon, available phosphorus, available potassium were studied as per Chesnin and Yein (1951), Walkley and Black (1934), Olsen et al. (1954), and Stanford and English (1949), respectively. Soil used during the present study contained 1.74% organic carbon, 357.43 kg/ha available nitrogen, 17.05 kg/ha phosphorus, 12.02 kg/ha available sulphur and 185.38 kg/ha available potassium and all these were in medium range.

Identification

The arbuscular mycorrhizal fungal species were morphologically identified on the basis of spore size, spore colour and spore wall up to the genera level as per guidelines by INVAM (http://invam.caf.wvu.edu).

Root colonization studies of the isolated spores

The isolated spores were further purified and mass multiplied on

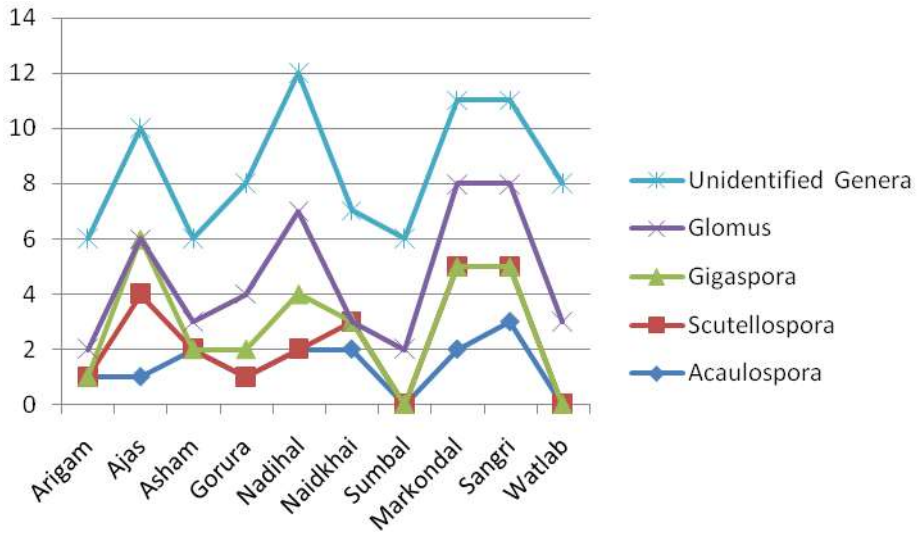

Figure 1. Isolation of arbuscular mycorrhizal spores from rhizospheric soil of apple from different locations of district Bandipora.

maize. Surface sterilized healthy maize seeds, pre-germinated in Petri plates under aseptic conditions, were sown in polythene bags containing sterilized soil + sand mixture (1:2 w/w). These bags were aseptically inoculated with identical AM spores at 5 cm depth (Jackson, 1973). The bags were kept in a greenhouse at 25±3°C and irrigated with sterile water. The plants were uprooted after 45 days. The roots were collected, washed with sterile water to remove adhering soil debris and observed for mycorrhizal infection. The infectivity was proved by noticing the presence of hartig net, vesicles, asbuscules or hyphae of endophytes on roots.

For estimating mycorrhizal root colonization, the root samples were collected and washed carefully to remove the adhering debris. The tertiary roots were cut into small pieces of approximately 1 cm length and subjected to differential staining as described by Phillips and Hayman (1970). The estimation of mycorrhizal infection in roots was made by visual observation (Giovannetti and Mosse, 1980). A randomly selected aliquot of stained root segments, suspended in water, was spread in a Petridish viewed under a dissecting microscope at a magnification of 10 and 40×. In case of AM colonization, root segments containing vesicles and arbuscules of endophyte and number of mycorrhizal short roots were considered infected as suggested by Beckjord et al. (1984).

Per cent mycorrhizal infection = Number of infected root segments / Total number of segments examined × 100

The data recorded during the investigation was statistically analyzed with the help of Pearson correlation (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Morphological characterization of arbuscular mycorrhizal spores

Spore morphology and wall characteristics were considered for the identification of arbuscular mycorrhizal fungi. The spores isolated from soils of district Bandipora were identified upto generic level using bibliographies provided by Walker et al. (2007). Four types of genera were isolated. The genera isolated were *Acaulospora, Scutellospora*, *Gigaspora,* and *Glomus*. There were 3 to 6 unidentified spores per gram from all studied locations (Figure 1).

Acaulospora spores were present singly in the soil and develop laterally on the neck of asporiferous saccule. Spores were light orange to yellowish brown (Table 1 and Figures 4, 5, 6 and 7), globose to sub-globose in shape and 150 to 210 µm in diameter. These spores were triple layered with L1 which forms the spore surface light yellow to apricot yellow in colour and 0.7 to 2.0 µm in thickness. L2 was laminate and light orange to yellowish brown, 6.8 to 7.4 µm in thickness. L3 was laminate, hyaline, 0.8 to 1.6 µm in thickness and usually tightly adherent to L2. Similar observations have been reported by others also (Walker et al., 2007; Sharma et al., 2009). *Gigaspora* spore wall consisted of a permanent outer layer enclosing a laminate layer, each with different properties that distinguish species (e.g. color, thickness, etc). Our observations corroborate with those of Koske (1987) and Bentivenga and Morton (1995). *Scutellospora* spores were with or without ornamentations. Spores consisted of a bilayered spore wall and two bilayered flexible inner walls. Thin-walled auxillary cells with smooth to knobby surfaces were produced on hyphae in the soil near the root surface and were also reported by Schenck and Perez (1990). The spore colour of the species of *Glomus* was of wide range. It varied from red-brown to almost black or straw to dark orange but most were yellow brown in colour. Spores possessed globose to sub-globose shape, about 40 to 120 µm in size. Spore wall consisted of three layers (L1, L2 and L3). Our findings corroborate with those of many other workers (Koske, 1984;

Table 1. Morphological features of isolated genera of AM fungi.

Genera	Spore size (µm diameter)	Spore shape	Spore colour	Spore wall	Hyphal colour
Acaulospora	115-170	Globose to sub globose	Yellow brown to dark brown	Three layered (L1, L2 and L3)	Grey white
Gigaspora	200-300	Globose to sub globose	White to cream usually a rose pink tint.	Bilayered layered (L1 and L2)	Orange brown
Scutellospora	100-170	Sub globose to ellipsoid to oblong	Cream to yellow or pale orange brown to dark orange brown	Bilayered spore wall (L1 and L2)	Hyaline to orange white.
Glomus	40-120	Globose to ellipsoid	Red brown to almost black most are yellow brown	Three layered (L1, L2 and L3)	Hyaline to yellowish.

Table 2. Correlation between spore population and other studied parameters of district Bandipora.

Parameter	Spore population
Spore population	1
Organic carbon	0.752*
Available nitrogen	0.626
Available phosphorus	-0.543*
Available sulphur	0.561
Available potassium	0.599
Root colonization	0.613*

*****Correlation is significant at the 0.05 level.

Koske and Gemma, 1990).

There was no evidence of any ectomycorrhizal association with apple roots, and this corroborates with the findings of Greene et al. (1982). *Glomus* species was common and made up for more than 75% of total isolates followed by *Acaulospora, Gigaspora* and *Scutellospora.* Dominancy of *Glomus* in the present study is in agreement with the findings of many other workers (Mridha and Dhar, 2007; Burni et al., 2009; Sharma et al., 2009). The predominance of *Glomus* spp*.* under varying soil conditions might be due to the fact that they are widely adaptable to the varied soil conditions and survive in acidic as well as in alkaline soils (Pande and Tarafdar, 2004).

Root colonization studies of arbuscular mycorrhizal fungi

In the current study, the AM colonization in the apple roots from Bandipora district varied between 62.04 and 81.13% (Figure 2, 3, 8, 9, 10 and 11). The results are in conformity with the Kandula et al., (2006) who also observed higher colonization in the apple roots and confirmed the ubiquitous nature of AMF spores. The highest root colonization was recorded in response to the inoculation with *Glomus* spp. (81.13%) followed by *Acaulospora* species (75.34%), *Gigaspora* species (73.13%) and *Scutellospora* species (70.00%). Similar results were reported by some workers (Hosamani et al., 2004; Smith and Read, 2008).

Results of the present study indicate that the nutrient contents of the soils played a significant role in occurrence of different species of arbuscular mycorrhizal fungi and it is evident from the Perusal of the data presented in Table 2 which revealed that AM spore population of district Bandipora was positively and significantly correlated with organic carbon (r=0.752*). The results are in conformity with those of Lipinski et al. (2003) who also reported a significant positive correlation between soil organic carbon and AM spore population. Negative relationship of AM spore population with available phosphorus (-0.543*) content in the soil was also reported. Stribley et al. (1980) who also observed that infection developing under conditions of high phosphorus availability may function parasitically without making any beneficial contribution to plant nutrient supply. Our findings corroborate with those of Graham and Timmer (1984) and Wu et al. (2006). There was a significant correlation between AM spore population and root colonization (r=0.613*) in district Bandipora. Kumar et al. (2013) also found a significant positive correlation between mycorrhizal spores and colonization. Yang et al. (2010) found a positive correlation between and mycorrhizal colonization and spores. These results are also supported by Li et al. (2009). Since the climatic conditions of the study area fall under temperate zone which are conducive to the mycorrhizal development, it is possible that concentration of such propagules may be higher (Akhter, 2005).

Moreover, influence of apple roots through their exudates cannot be ruled out which needs further studies.

Conflicts of interests

The authors have not declared any conflict of interests.

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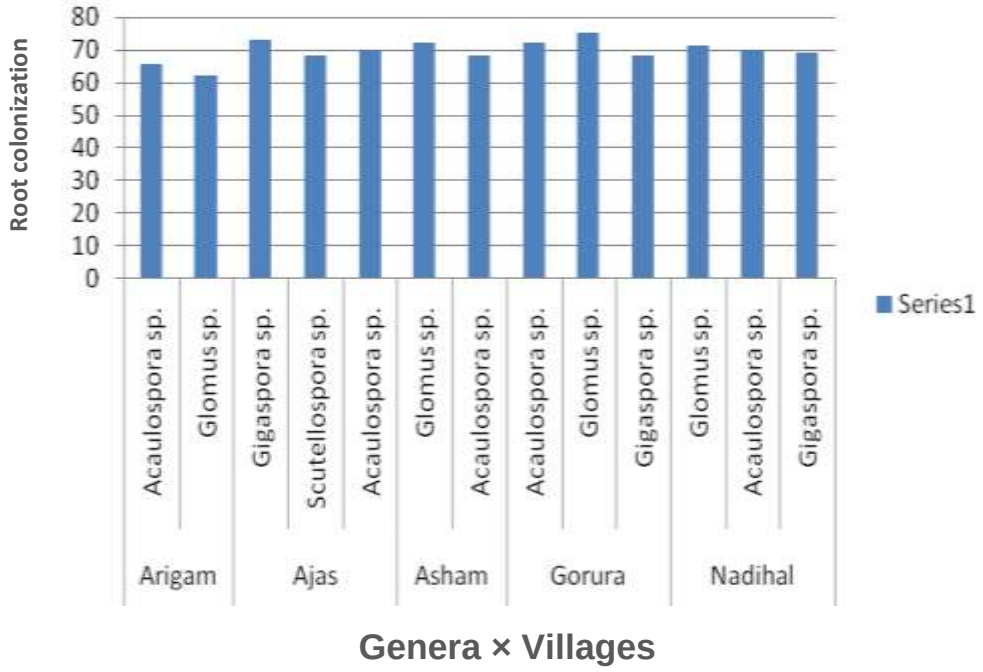

Figure 2. *In-vitro* root colonization by AM fungal spores isolated from district Bandipora.

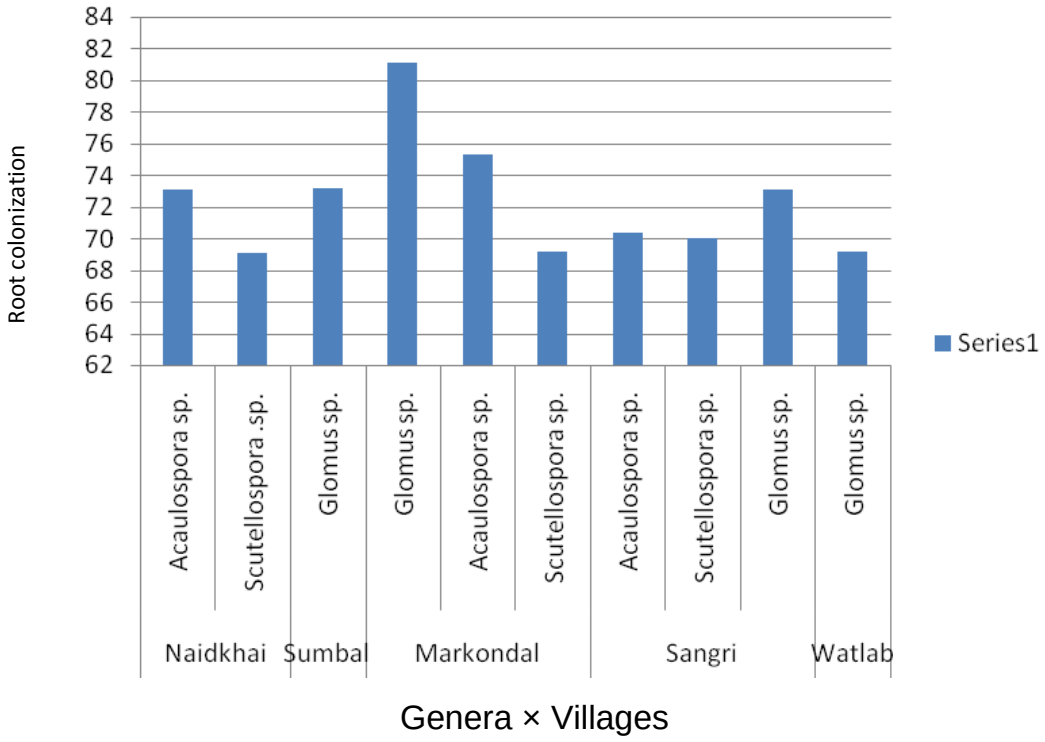

Figure 3. *In-vitro* root colonization by AM fungal spores isolated from district Bandipora.

University of Agricultural Science and Technology of Kashmir for providing laboratory facilities; the faculty for rendering there kind help and support; and help rendered by the orchardists during collection-cum-surveys trips is

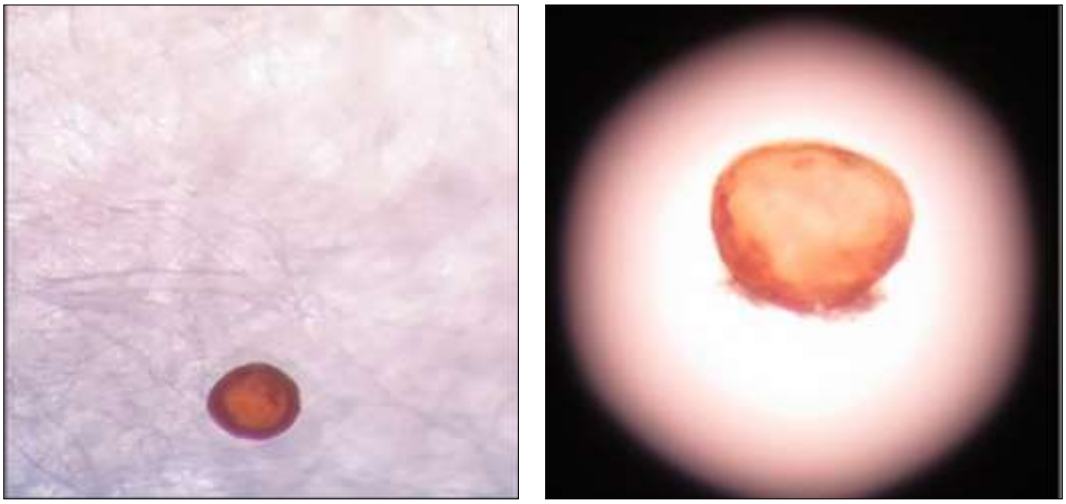

Figure 4. Spores of the genus *Acaulospora*.

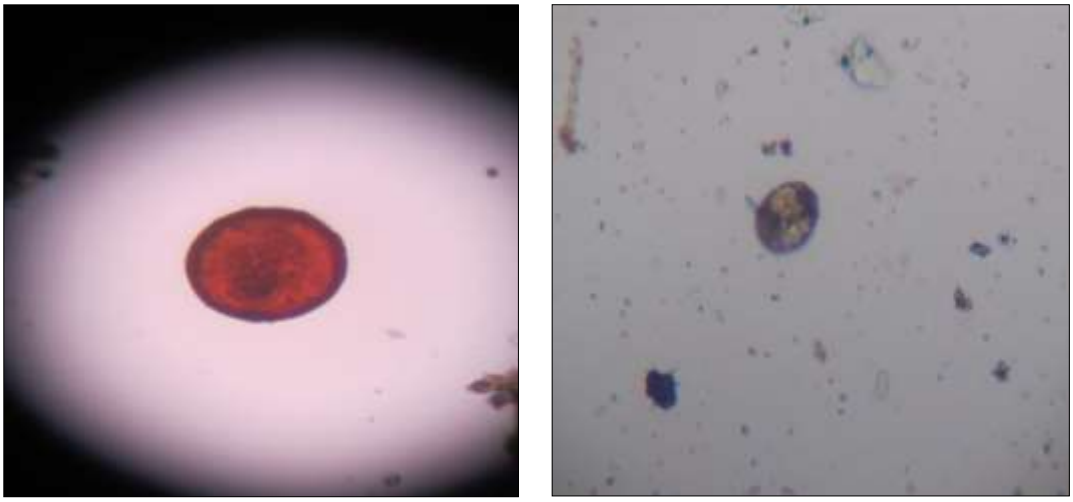

Figure 5. Spores of the genus *Glomus*.

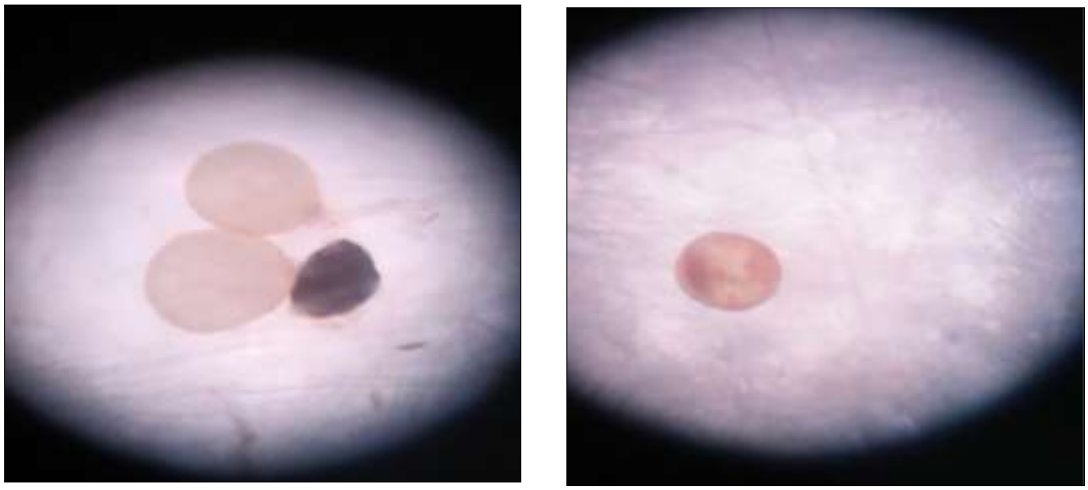

Figure 6. Spores of the genus *Gigaspora*.

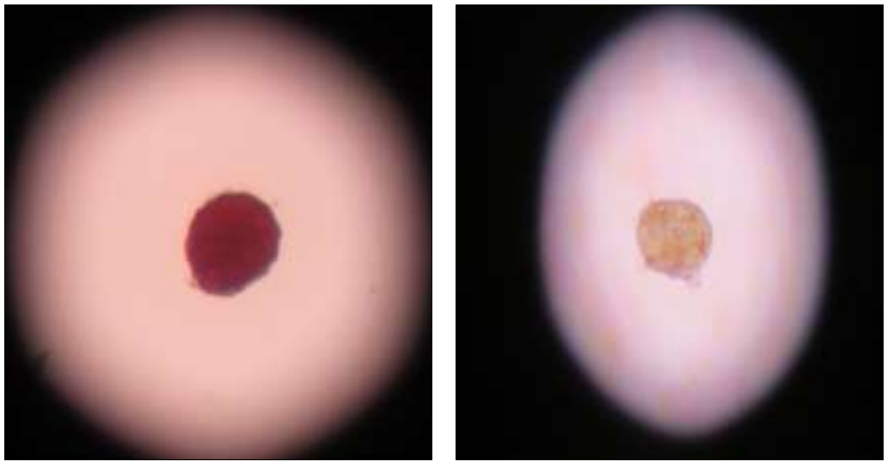

Figure 7. Spores of the genus *Scutellospora*.

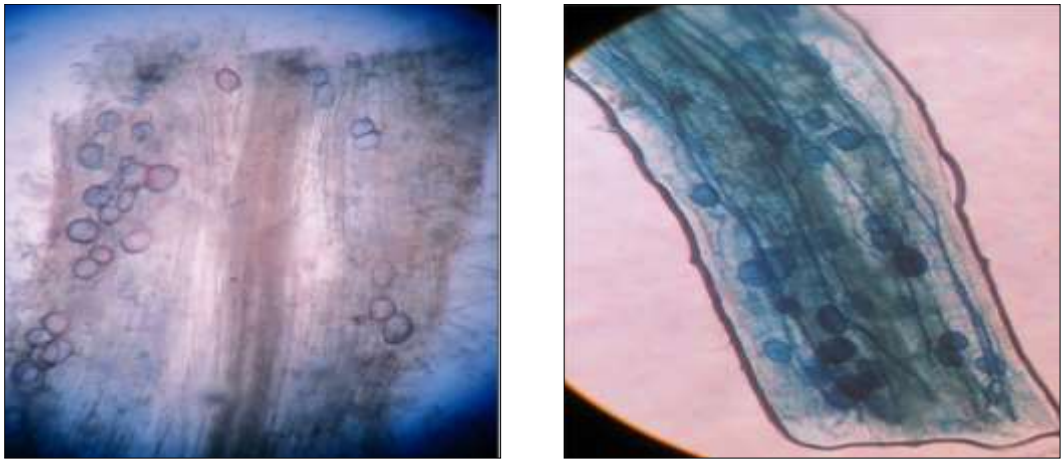

Figure 8. Root colonisation of the genus *Acaulospora*.

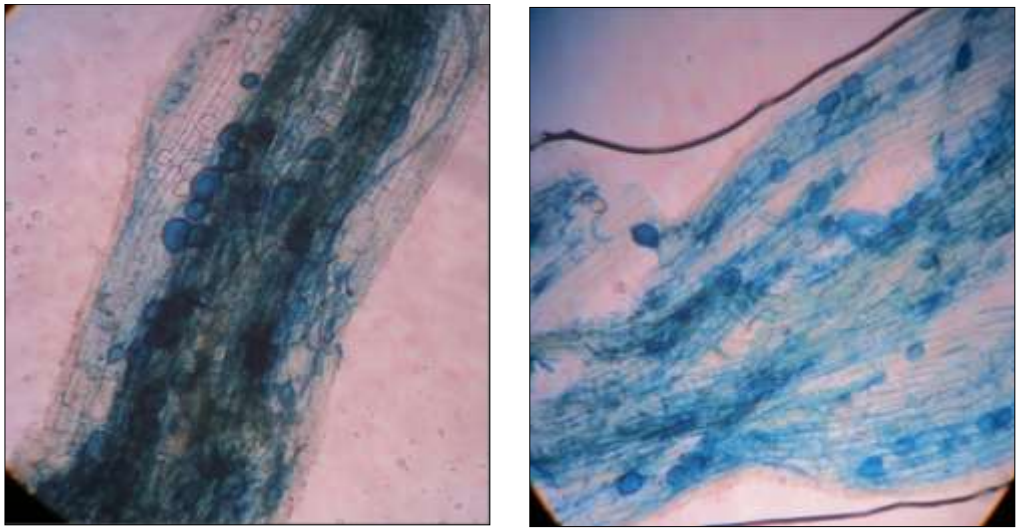

Figure 9. Root colonisation of the genus *Scutellospora*.

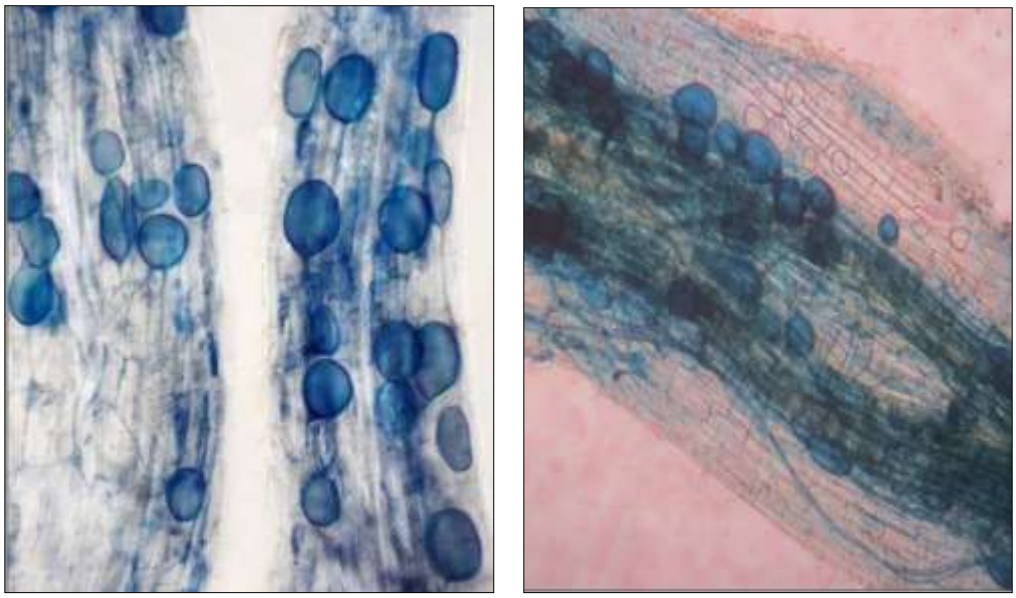

Figure 10. Root colonisation of the genus *Glomus*.

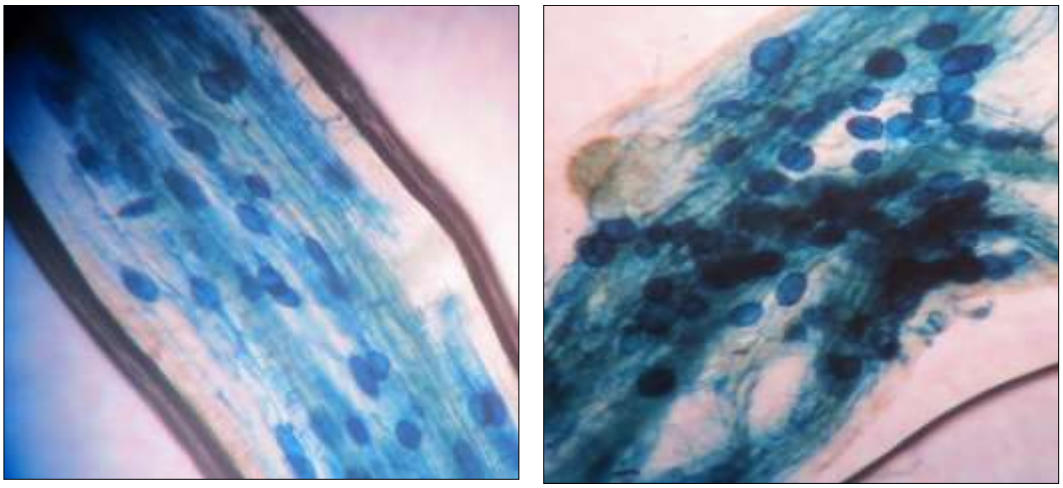

Figure 11. Root colonisation of the genus *Gigaspora*.

highly put on record.

REFERENCES

Akhter F (2005). Studies on relationship between fruit yield and quality with soil and leaf nutrient content in apple orchards of Zangier block of district Baramulla Kashmir. University of Kashmir Digital Repository.

Anonymous (2007). Area and Production of main Fruits in Jammu and Kashmir State. Department of Horticulture, Government of Jammu and Kashmir, Srinagar, J&K.

Anonymous (2013). Area and Production of main Fruits in Jammu and Kashmir State. Department of Horticulture, Government of Jammu and Kashmir, Srinagar, J&K.

Anonymous (2015).Area and Production of main Fruits in Jammu and Kashmir State. Department of Horticulture, Government of Jammu and Kashmir, Srinagar, J&K.

Beckjord PR, McIntosh MS, Hacskaylo E, Melhuish JH (1984). Inoculation of Loblolly Pine Seedlings at Planting with Basidiospores of Ectomycorrhizal Fungi in Chip Form. Research Note No. 324, Forest Service, North-Eastern Forest Experiment Station, Florida, USA, P 25.

Bentivenga SP, Morton JB (1995). A monograph of the genus Gigaspora, incorporating developmental patterns of morphological characters. Mycologia 87:720-732.

Burni T, Iftikhar S, Jabeen M, Zainab SB (2009). Diversity of VA (Vesicular Arbuscular) fungi in some weeds of cauliflower fields of Peshawar, Pakistan. Pak. J. Plant Sci. 15(1):59-67.

Chesnin L, Yien CH (1951). Turbidimetric determination of available sulphur. Proc. Soil Sci. Soc. Am. 15:149-151.

Deepa D (2008) Indian product, “Brief India fresh fruit sector”, Holy Higgins US Embassy.

Gerdemann JW, Nicolson TW (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting method. Trans. Br. Mycol. Soc. 46:235-245.
Giovannetti M, Mosse B (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489-509.
Gomez KA, Gomez AA (1984). Statistical procedure for Agricultural Research An international Rice Research Institute Book. John Wiley and sons, 2nd edition 190.
Graham JH, Timmer LW (1984). Vesicular-arbuscular mycorrhizal development and growth response of rough lemon in soil and soilless media: Effects of phosphorus sources. J. Am. Soc. Hortic. Sci. 109:118-121.
Greene DW, Manning WJ, Cooley DR (1982) Effect of the ectomycorrhizal fungus Pisolithustinctorius and auxin rooting formulations on growth of 'Cortland' apple trees. Hortic. Sci. 17:655-656.
Hosamani PA, Lakshman HC, Kadam LB, Kiran P (2004) Screening of Arbuscular mycorrhizal fungi and their comparative efficacy on Withaniasomnifera Dunl. Nat. Environ. Pollut. Technol. 3(2):201-204.
INVAM website (2005). http://invam.caf.wvu.Edu/fungi/taxonomy/classification.html.
Jackson ML (1973) Soil Chemical Analysis. Prentice Hall India, New Delhi, India, P 498.
Jackson RM, Mason PA (1984). Mycorrhiza. Edward Arnold, Ltd., London: 60. ISBN 0-7131- 2876-3.
Kandula K, Jones E, Stewart A, Horner J (2006). Colonisaton of apple roots by arbuscular mycorrhiza in specific apple replant disease affected soil. New Zealand Plant Prot. 59:92-96.
Koske RE (1984). Spores of VAM fungi inside spores of VAM fungi. Mycologia 76:853-862
Koske RE (1987). Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. Mycologia 79:55-68.
Koske RE, Gemma JN (1990) VA mycorrhizae in strand vegetation of Hawaii: evidence for long-distance codispersal of plants and fungi. Am. J. Bot. 77:466-474.
Kumar S, Chaudhuri S, Maiti SK (2013). Soil dehydrogenase enzyme activity, in Natural and mine soil - a review. Middle-East J. Sci. Res. 13(7):898-906.
Li L, Li T, Zhang Y, Zhao Z (2009). Molecular diversity of arbuscular mycorrhizal fungi and their distribution patterns related to host-plants and habitats in a hot and arid ecosystem, southwest China. FEMS Microbiol. Ecol. 71:418-427.
Lipinski W, Terelak H, Motowicka T (2003). Suggestion for liming values of sulphatesulphur content in mineral soils for fertilization advisory needs. Soil Sci. Annu. 54(3):79-84.
Masoodi MA (2003). Agriculture in Jammu and Kashmir a perspective. Mohisarw Book Series Srinagar P 195.
Morton JB (1988). Taxonomy of VAM fungi, classification, nomenclature and identification. Mycotaxon 32:267-324.
Mridha M, Dhar P (2007). Biodiversity of Arbuscular Mycorrhizal colonization and spore population in different Agroforestry trees and crop species growing in Dianajpur Bangladesh. J. Forest. Res. 18(2):91-96.
Olsen SR, Cole CV, Watanabe FS, Dean LA (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dept. Agric. Circ. P 939.
Osonubi O, Mulongoy K, Awotoye O, Atayese M, Okali U (1991). Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings. Plant Soil 136:131-143.
Pande M, Tarafdar JF (2004) Arbuscular Mycorrhizal fungal diversity in Neem based Agroforestry Systems in Rajistan. Appl. Soil Ecol. 26:233-241.
Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:150-161.
Satish YD, Maurice L, Barry K (2006) Prospects for India's Emerging Apple Market, USDA 8.
Schenck NC, Parez Y (1990). Manual for the identification of VA mycorrhizal fungi INVAM, University of Florida, Gainesville USA 1-283.
Schenck NC, Smith GS (1982). Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. Mycologia 77:566-574.
Sharma D, Kapoor R, Bhaytnagar AR (2009). Differential growth response of Curculigoorchoides to native AMF communities varying in number and fungal components. Euro. J. Soil Biol. 45(4):328-333.
Smith SE, Read DJ (2008). Mycorrhizal Symbioses. Academic Press, London, U.K. Assessment of Mycorrhizal Frequency in the Roots of Fruit Plants Using Different Dyes.
Sreenivasa MN, Bagyaraj DJ (1989). Use of pesticide for mass production of vesicular-arbuscular mycorrhizal inoculum. Plant Soil 119:127-132.
Stanford S, English (1949). Use of the flame photometer in rapid soil tests of potassium and calcium. Agron. J. 41:446-447.
Stribley DP, Tinker PB, Rayner JH (1980). Relation of internal phosphorus concentration and plant weight in plants infected by vesicular arbuscular mycorrhizae. New Phytol. 86:261-266.
Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Nishmura I, Schubler A (2007). Molecular phylogeny and new taxa in the *Archaesporales* (*Gloremycota*): *Ambiospora feennicagen sp.*nov., *Ambisporaceae* fam nov., and emendation of *archaeospora* and *Archaesporaceae*. Mycol. Res.111:137-153.
Walkley A, Black IA (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29-38.
Wu Q, Xia R, Zou Y (2006). Arbuscular mycorrhizal fungal growth on citrus roots and its correlations with soil available phosphorus content and phosphatase activity. Ying Yong Sheng Tai Xue Bao 17:685-690.
Yang RY, Zan ST, Tang JJ, Chen X, Zhang Q (2010) Variation in community structure of arbuscular mycorrhizal fungi associated with a Cu tolerant plant- *Elsholtzia splendens*. Appl. Soil Ecol. 44:191-197.