

Arbuscular Mycorrhizal (AM) diversity in some threatened North West Himalayan flora of Kinnaur

Vaneet Jishtu¹, Rupam Kapoor², Joginder Singh³ and T. N. Lakhanpal³

¹Himalayan Forest Research Institute, Conifer Campus, Panthaghati, SHIMLA - 171013 (HP)

²Department of Botany, University of Delhi, Delhi - 110007

³Department of Bio Sciences, HP University, Shimla (HP)

Corresponding author Email: jishtu@yahoo.com

(Submitted on May 25, 2019; Accepted on June 28, 2019)

ABSTRACT

The AM associations are integral symbiotic associations of both wild and cultivated plants. It has been widely investigated in the cultivated plants but less so in the wild plants. In this article the AM diversity in some very important and threatened wild plants of the tribal belt of Kinnaur in Himachal Pradesh is being reported for the first time. The study area has a vast wealth of medicinal, aromatic and other economically important plants. With the upsurge in tourism and increasing developmental projects like the Hydro electric dams, etc the floral diversity and their associated mycorrhizal diversity has come under great threat. It is in this context that along with floristics, investigations of AM fungi has been undertaken on the following threatened plants, majority of which are endemic to the region. Of late, much attention has been paid to the use of AM fungi in the establishment of forests and improvement of soil fertility. The plants investigated are: *Acer caesium*, *Abies spectabilis*, *Betula utilis*, *Juglans regia*, *Rhododendron campanulatum*, *Quercus ilex*, *Hippophae tibetana*, *Sophora mollis*, *Elaeagnus umbellata*, *Rheum australe*, *Ribes alpestre*, *Juniperus communis*, *Piptanthes nepalensis*, *Saussurea costus* and *Fritillaria roylei*. The reported three genera are *Acaulospora*, *Gigaspora* and *Glomus* and in these three genera 13 species of AM fungi have been reported and illustrated. The genus *Glomus* is the most predominant with 10 species.

Key Words: AM Fungi, VAM, threatened, reforestation, floristics

INTRODUCTION

Mycorrhiza, meaning 'fungus-roots', is a symbiotic association between plants and fungi that colonize the cortical tissue of roots during periods of active plant growth (Frank, 1885). The vast majority (95%) of plant species form one or the other type of mycorrhizae (Trappe, 1977). There are seven types of mycorrhizae, which are associated with different groups of plants. These are ecto, ectendo, endo, arbutoid, ericoid, monotropoid and orchidaceous type. Out of the seven mycorrhizal types, endomycorrhiza or Vesicular Arbuscular Mycorrhiza (VAM) or the Arbuscular Mycorrhiza (AM) is most predominant being associated with almost 90% of the plant species.

The term Vesicular Arbuscular Mycorrhiza (VAM) was originally applied to symbiotic associations formed by all fungi in the *Glomales*, but because a major subclass lacks the ability to form vesicles in roots; Arbuscular Mycorrhiza (AM) is now the preferred acronym. The Arbuscular Mycorrhizae (AM) form highly branched 'arbuscules' (the term literally means little trees) within root cortical cells. Other structures produced by some AM fungi include, 'vesicles'- thin-walled, lipid-filled structures that usually form in intercellular spaces, such AM fungi are called as VAM. The hyphae of AM fungi are usually recognizably distinct from other kinds of soil fungi. Their reproductive spores can be formed either in the root or more commonly in the soil. The AM type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody plants. Major AM plant families include *Polygonaceae*, *Urticaceae*, *Poaceae*, *Fabaceae*, *Taxodiaceae*, *Taxaceae*, *Cupressaceae*, *Aceraceae*, *Juglandaceae*, *Podocarpaceae*, *Casuarinaceae* and the Pteridophytes. Plants with rare AM associations include *Caryophyllaceae*, *Brassicaceae*, *Chenopodiaceae* and *Cyperaceae*. Their distribution is widespread and they have been reported in plants growing in Arctic, temperate and tropical regions. Their habitat is also very diverse, they have

been reported from sand dunes, coal mines and aquatic environments (Bagyaraj, 2011). They are distributed in about 1000 genera of plants in 200 families. They have at least 300,000 receptive hosts in the world flora, for about 220 species of AM fungi (Bagyaraj, 2015). The fungi that form AM are currently all classified in the order *Glomales*, which is further divided into suborders based on the presence or absence of vesicles (Morton and Benny, 1990).

Arbuscules are primary structures involved in the bi-directional transfer of nutrients between fungal symbionts and host plants (Cox and Tinker, 1976). The hyphae literally form a bridge that connects the plant root with large areas of soil and serves as a pipeline to funnel nutrients back to the plant. In return, the plant must supply the VAM fungi with carbon for its growth and energy requirements. This plant-fungal relationship is an elegant association and its development is evidently regulated by several factors. Different soil bacteria enhance the promotion of root colonization by the VAM fungi. Plant produced discharges (exudates) sent out through their roots that contain specific compounds activating the VAMF to stimulate the hyphal growth.

The symbiotic association increases the uptake of certain nutrients, particularly P, Cu and Zn, by the plant, due to exploration by the external hyphae of the soil beyond the root hair and depletion zones. VAM are known to increase tolerance to heavy metals, saline soils and drought (Michelson and Rosendahl, 1990); decrease transplant shock (Sylvia *et al.*, 1993) and inhibit fungal pathogens (Garcia *et al.*, 1988; Jalali and Chand, 1988; Marx, 1973); increase resistance against nematodes (Sikora and Schönback, 1975); and weeds (Jordan *et al.*, 2000); increase uptake of water (Dudderidge *et al.*, 1980) and drought resistance of young seedlings (Parke *et al.*, 1983). They help the plants to tolerate cold (Harley and Smith, 1983) as well as high temperature (Marx and Bryan, 1971) and also provide tolerance to soil

(Barea, 1991) and to heavy metal toxicity (Henning, 1993).

AM association in plants is known to help in increased growth in several crops like: grapes, soybean, potatoes, onion, cowpea, apple, raspberry, strawberrry, *Andropogon gerardii*, *Salvia officinalis*, *Thymus vulgaris*, cacti (Possingham and Obbink, 1971; Ross, 1971; Graham et al, 2001; Jain and Sethi, 1988; Ikombi *et al.*, 1991; Granger *et al.*, 1983, Gnewkow and Marschneri, 1989; Gianinazzi *et al.*, 2002; Niemi and Vestberg, 1992; Hetrick *et al.*, 1986; Camprabi *et al.*, 1992; Jose *et al.*, 1990).

Recently much attention has been paid to the use of AM fungi in reinstatement of forest and improvement of soil fertility. They are considered as an important biological tool for balancing soil nutrients, nutrient loss and the sustainability of forest ecosystems (Chamola *et al.*, 1999; Giri *et al.*, 2003; Cavagnaro *et al.*, 2015). They have potential use in reclamation and revegetation of wastelands due to their potential for increasing growth, survival, and biomass production under conditions of environmental stress (Giri *et al.*, 2007; Kaur and Mukerji, 1999). The role of mycorrhizal fungi in the improvement of quality of planting stock is well recognised (Mukerji *et al.*, 1996; Dixon *et al.*, 1997; Chen *et al.*, 2018) and their practical application in stressed conditions can lead to successful afforestation and restoration (Barr, 2010) programmes and eco-restoration of degraded areas (Palmer, *et al.*, 1997; Alexander *et al.*, 1992; Al-Karaki, 2013; Manaut *et al.*, 2015; Asmelash *et al.*, 2016; Sharma and Jha, 2017).

Much work has been done on the cultivated plants of economic importance and their mycorrhizal association. But there is practically no work on mycorrhizal association of wild plants. Further, there is literally no work undertaken on the unique flora found in the North West Himalayan tribal belts. Hence, in the present study the mycorrhizal association of some important wild plants of Baspa valley of Kinnaur has been investigated to ascertain the type and extent of associations.

MATERIAL AND METHODS

Location and Scope: The area explored during the present study falls in district Kinnaur of Himachal Pradesh. Kinnaur previously formed Chini tehsil of Mahasu district and came into being as an independent tribal district during 1960. During pre-independence times, Kinnaur was a part of the erstwhile Bushahr State. The district derives its name from 'Kannaura' or 'Kinnara', the original inhabitants of the region, which have been listed as a scheduled tribe. The Kinnaur district also forms international border with Tibet and commands a special place in Himachal Pradesh because of its unique culture, history and geographical features.

The specific area of study investigated in the present study is called Baspa Valley, popularly referred to as the Sangla Valley following the name of main village. Baspa valley derives its name from the river Baspa and forms one of the major valleys in the district. The study area lies on both sides of Baspa River and extends from Karchum (1,700 m), near its confluence with river Satluj to Ranikanda (4,200 m), about 15 kms upstream of Chitkul, the last village in the valley. As far as

altitudinal range of the study area is concerned, an upper altitudinal benchmark of 4,750 m above mean sea level at Rupin Pass has been maintained. The geological limits of the area lie between 31° 15' to 31° 36' North latitude and 78° 10' to 78 ° 31' East longitudes. Nestled in the interior, but awesomely majestic Himalayas, this valley is related to the epic Mahabharata. So secluded is the region that, the Pandavas are believed to have spent the last years of their exile here. Today, however, the valley is better recognized as heaven for the outgoing, adventurous type people and is definitely a trekker's paradise.

Topographic Features: The Baspa River with a total length of about 72 Kms, broadly follows a south-east to north-west course in the study area and divides the valley into north-east and south-west facing slopes. In general, the topography of the study area ranges from flat valleys to gentle to precipitous slopes, scree slopes, glacial moraines and lofty peaks. The upper part of the valley is surrounded by barren ridges, whereas the lower regions are flatter with plenty of green pastures and cultivated lands. The rise in altitude is more abrupt along the right bank of the river than along the left. It is due to the reason that the high Kinner Kailash ridge-forming boundary of the valley rises from the basin of the Baspa river over a very short horizontal distance. The side valleys along left bank of Baspa River are fairly deep but cascading streams in these valleys also form gorges resulting in steepness of slopes. General elevation of the valley ranges from about 1,700 m at the confluence of river Baspa with Satluj to more than 6,000 m along the Kinner Kailash range along the right bank of the river Baspa. There are various passes that link the valley to other areas.

Climate: Due to its geographical location, the climate of the region, in general, differs from the climate in the adjacent Shimla and Lahaul and Spiti districts of the state. It has a long winter from October to May and a short summer from June to September; April to May is spring and September to October is autumn. Therefore, the short mild summers, brief light monsoons in the mid valley, extreme cold arid conditions bereft of monsoons in the upper part of the valley and prolonged winters with moderate to heavy snowfall characterize the general climate of the area.

Collection of VAM samples from the soil: Soil was collected by digging around the plant selected. In case of herbs the collection was made by digging out the entire plant and collection of the soil attached to its fine roots. In case of trees the soil attached to the root hairs was collected. About, 100 gms of soil was taken as sample for analysis in the laboratory.

Methodology for isolation of AM spores from the soil: To isolate AM spores from the soil, modified method of wet sieving and decanting technique (Gerdemann and Nicolson, 1963) was used in the present study. In this technique about 10 g of air-dried soil sample was placed in beaker containing 500 ml of water. The soil suspension was stirred for 5 min. The coarse particles were allowed to settle down. Thereafter soil suspension was passed through stacked sieves of different mesh number (100, 200, and 300) in the increasing order. Contents of the beaker were decanted through the sieves.

Since AM fungal spores are lighter, they float on the surface of water. The spore suspension was immediately filtered through Whatman filter paper No.-1. The AM fungal spores form a distinct ring on the filter paper. The filter paper containing spores is spread on a Petri-plate for observation under binocular microscope. Spores were easily distinguished from soil particles by their characteristic hyaline to coloured subtending hyphae.

For identification the spores were transferred on a slide with the help of a needle and observed under compound microscope. Later, all slides were observed carefully under oil immersion for segregation and identification into genera and species. The standard criteria followed for identification e.g. colour, size, shape, wall characteristics, contents and surface ornamentations of the spores, nature of spore, the number and arrangement of the spores in sporocarps and the presence or absence of peridium for the sporocarps were carefully recorded. (Hall, 1984, 1987; Morton, 1988, 2002; Raman and Kumar, 1988; Schenck and Perez, 1987; Trappe, 1982; Walker, 1981).

RESULTS AND DISCUSSION

Enumeration of VAM Fungi: The plants selected for mycorrhizal association were short listed after analyzing the general flora of the valley (Jishtu, 2005). A tentative list of plants was worked out and then after repetitive consultations with the local inhabitants, a list of 15 plants was finalized which are listed in **table-1**. Care was taken to include those species that were endangered/ threatened or locally important.

The endangered/ threatened plants that were short listed are *Acer caesium*, *Abies spectabilis*, *Betula utilis*, *Juglans regia*, *Rhododendron campanulatum*, *Quercus ilex*, *Hippophae tibetana*, *Sophora mollis*, *Elaeagnus umbellata*, *Rheum australe*, *Ribes alpestre*, *Juniperus communis*, *Piptanthes nepalensis*, *Saussurea costus* and *Fritillaria roylei* (**Plate - 1**). Threat status has been considered as per the Conservation Assessment and Management Prioritisation (CAMP) Workshop (Shimla, 2010) and IUCN (ver. 3.1) Red List Data (Zhang *et al.*, 2011; Goraya *et al.*, 2013; Saha *et al.*, 2015; Rivers and Allen, 2017; Rankau *et al.*, 2017; Chen *et al.*, 2018).

As it emerges from the present study and the similar studies by other workers elsewhere, *G. mosseae*, is the most predominant AM fungus. That is why this species has been extensively used for mass propagation and enhanced yield of oats, barley, clovers, potatoes, alfa-alfa, onions, etc., (Muromtsev *et al.*, 1990; McArthur and Knowles, 1993; IJdo *et al.*, 2011). The results regarding the mycorrhizal associations depict that the genus *Glomus* has a dominant association in the plants of the valley, being associated with all the plants examined for AM. Further, among the genus *Glomus*, the species *G. mosseae* is the most prevalent in its occurrence. It is in agreement with other similar works on mycorrhizae carried out elsewhere (Pindi *et al.*, 2008; Manoharachary *et al.*, 2008; Bagyaraj, 1991). Khaliel (1988) reported *G. mosseae* to be the more dominant species in the sand humus, in Riyadh.

Such diversity studies on AM fungi are required if these are to be used for nursery inoculations because studies have shown dependency of plants on mycorrhiza, though the plants greatly differ in their needs on mycorrhizal infection. Selection of efficient strain can result only from exhaustive surveys. The efficient AM strains have been shown to enhance growth of several forest tree species and medicinal plants or plantation crops (Tilak *et al.*, 2010; Bhagyaraj, 2011; Lakshmipathy *et al.*, 2000; Manoharachary and Reddy, 1995). Of late the role of AM fungi is being emphasized for the conservation of endangered plants (Evelin *et al.*, 2019).

In the present study, the AM spores isolated and identified from the threatened plants are, represented only by three genera, viz., *Acaulospora*, *Gigaspora* and *Glomus*. The salient features of these genera are as follows:

***Acaulospora*:** It has azygospore type of spores, which is formed within a lateral swelling of the sporogenous saccule. This genus is known to form vesicular arbuscular mycorrhiza produced singly in soil or in sporocarps. Presently, 28 species belong to this genus. Spores are globose, subglobose or ellipsoidae with oil contents.

***Gigaspora*:** These are azygospores borne on a tip of a bulbous hypha. They produce large spores in soil. About 8 species belong to this genus.

***Glomus*:** Presently 77 species are known in this genus. Spores are formed in compact sporocarps in loose clusters in small fascicles or as single spore in soil.

Descriptions of the Species: The AM fungi isolated from the selected plants of the valley are represented by the following species.

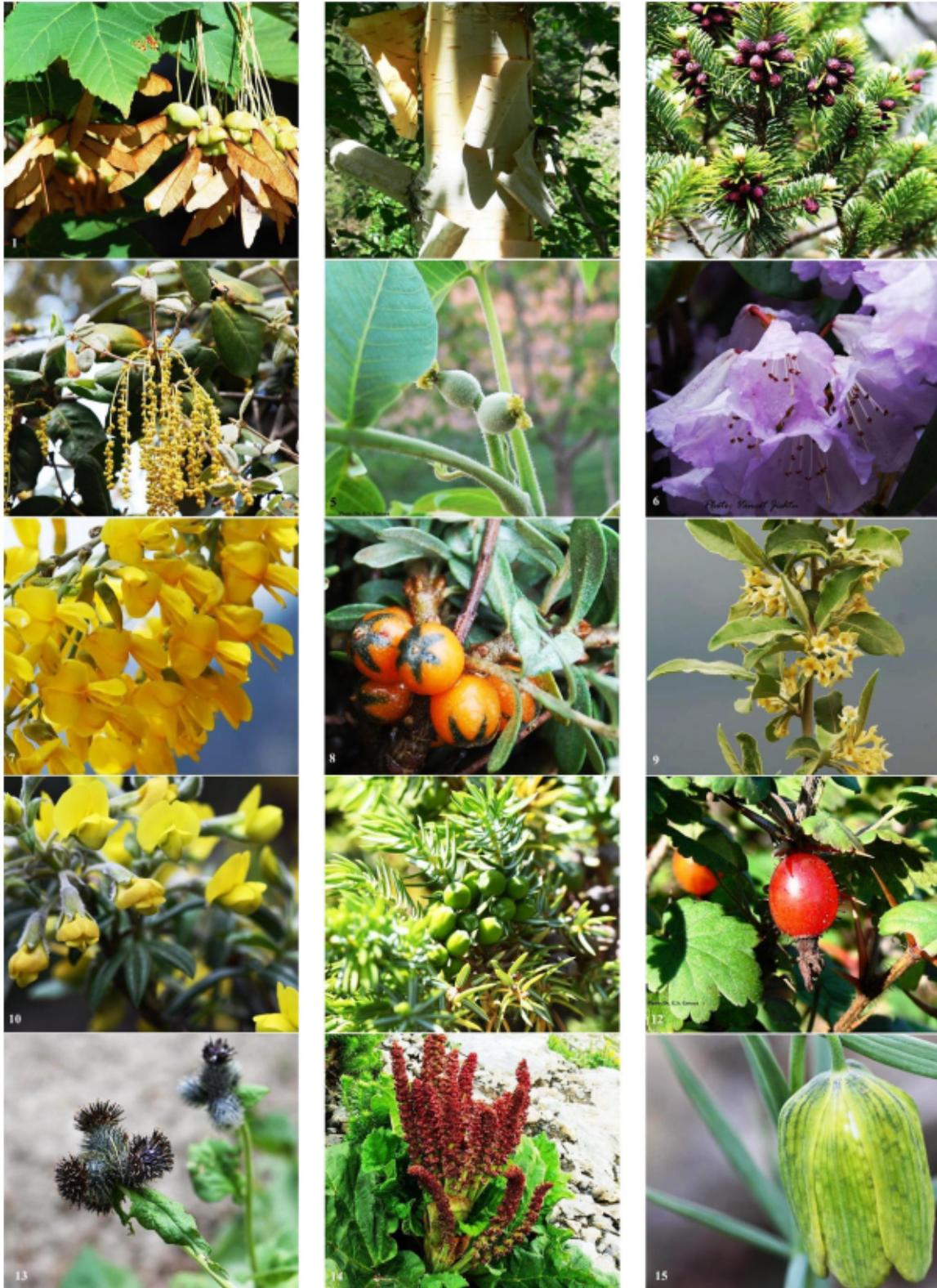
Acaulospora foveata Trappe and Janos: Chlamydospores formed singly, globose to ellipsoidal measuring 182 - 30 (-410) x 115 - 350 (-480) μm , yellow-brown to light reddish-brown, turning red-brown to brown black at maturity; surface uniformly pitted. Outer spore wall yellowish or reddish-brown with an adherent but separable hyaline inner wall; spore contents of small hyaline guttules.

Acaulospora laevis Gerdemann and Trappe: Spores formed singly, globose to sub-globose, ellipsoid or reniform, irregular, dull yellow, turning deep yellow-brown to red, 140-220 μm in diameter. Spore wall continuous, three layered with an outer rigid yellow-brown to reddish-brown with two hyaline inner membranes; spore contents globose to almost polygonal (reticulate).

Gigaspora albida Schenck and Smith: Azygospores formed singly in soil; spherical, colour dull white with light greenish yellow, 232 - 252 x 235 - 250 μm . Spore wall continuous; outer being thin. A bulbous suspensor, separating it from the spore contents is seen. Azygospore attached to a single, hyaline to yellow, bulbous suspensor, attached to septate hyphae with fine hyphal branches.

Gigaspora calospora Nicolson and Gerdemann: Azygospores formed solitary in soil with colour ranging from pale yellow to greenish yellow; spore wall thick, perforated, 2-layered with the in-between space being hyaline yellow.

Plate-I



1. *Acer caesium* Wall. ex Brandis; 2. *Betula utilis* D. Don; 3. *Abies spectabilis* (D. Don) Mirb.; 4. *Quercus ilex* L.; 5. *Juglans regia* L.; 6. *Rhododendron campanulatum* D. Don; 7. *Sophora mollis* (Royle) Baker; 8. *Hippophae tibetana* Schtdl.; 9. *Elaeagnus umbelata* Thumb.; 10. *Piptanthus nepalensis* (Hook.) D. Don; 11. *Juniperum communis* L.; 12. *Ribes alpestre* Wall. ex Decne.; 13. *Saussurea costus* (Falc.) Lipsch.; 14. *Rheum australe* D. Don.; 15. *Fritillaria roylei* Hk.

Table 1: Selected plant *taxa* with the associated AM species

S. No.	Plant Species (Family)	Threat Status	Mycorrhizal Spores
1.	<i>Rhododendron campanulatum</i> D. Don (Ericaceae)	VU	<i>Gigaspora colkospora</i> , <i>Glomus macrocarpum</i> , <i>G. constrictum</i> , <i>Glomus</i> spp.
2.	<i>Quercus ilex</i> L. (Fagaceae)	LC	<i>Glomus multisubtansum</i> , <i>G. macrocarpum</i> , <i>Gigaspora albida</i> , <i>Glomus entunicatum</i> ,
3.	<i>Sophora mollis</i> (Royle) Baker (Fabaceae)	Endemic; populations in decline	<i>Acaulospora laevis</i> , <i>Glomus constrictum</i> , <i>G. macrocarpum</i> , <i>G. mosseae</i> , <i>G. macrocarpum</i> .
4.	<i>Elaeagnus umbellata</i> Thunb. (Elaeagnaceae)	Endemic; populations in decline	<i>Glomus fasciculatum</i> , <i>G. mosseae</i> .
5.	<i>Juniperus communis</i> L. (Cupressaceae)	VU	<i>Gigaspora albida</i> , <i>Glomus mosseae</i> , <i>G. fasciculatum</i>
6.	<i>Betula utilis</i> D. Don (Betulaceae)	EN	<i>Glomus aggregatum</i> , <i>G. mosseae</i> , <i>G. macrocarpum</i> .
7.	<i>Piptanthus nepalensis</i> (Hook.) D. Don (Fabaceae)	Endemic; populations in decline	<i>Gigaspora albida</i> , <i>G. constrictum</i> , <i>G. fasciculatum</i> , <i>G. intrasadies</i> .
8.	<i>Abies spectabilis</i> (D. Don) Mirb. (Pinaceae)	NT	<i>Glomus mosseae</i> , <i>G. fasciculatum</i> , <i>G. fecundisporum</i> .
9.	<i>Acer caesium</i> Wall. ex Brandis; (Aceraceae)	LC	<i>Acaulospora laevis</i> , <i>Glomus constrictum</i> , <i>G. macrocarpum</i> , <i>G. mosseae</i> , <i>Gigaspora gigantea</i> .
10.	<i>Saussurea costus</i> (Falc.) Lipsch. (Asteraceae)	CR	<i>Acaulospora foveolata</i> , <i>Glomus mosseae</i> , <i>Glomus</i> spp.
11.	<i>Hippophae tibetana</i> Schldtl. (Elaeagnaceae)	Endemic; restricted distribution	<i>Gigaspora albida</i> , <i>Glomus mosseae</i> , <i>G. fasciculatum</i>
12.	<i>Juglans regia</i> L. (Juglandaceae)	LC	<i>Glomus constrictum</i> , <i>G. fasciculatum</i> , <i>G. aggregatum</i> .
13.	<i>Fritillaria roylei</i> Hook. (Liliaceae)	EN	<i>Gigaspora albida</i> , <i>Glomus aggregatum</i> , <i>G. fasciculatum</i>
14.	<i>Ribes alpestre</i> Wall. ex Decne.; (Grossularaceae)	Endemic to the region	<i>Acaulospora foveolata</i> , <i>Glomus mosseae</i> , <i>G. constrictum</i> ,
15.	<i>Rheum australe</i> D. Don (Polygonaceae)	VU	<i>Glomus aggregatum</i> , <i>G. mosseae</i> , <i>Glomus</i> spp.

CR=Critically Endangered; EN= Endangered; NT=Near Threatened; VU=Vulnerable; LC=Least Concern
Threat status as per CAMP Workshop (Shimla, 2010) and IUCN 3.1 (Zhang *et al.*, 2011; Goraya *et al.*, 2013; Saha *et al.*, 2015; Rivers and Allen, 2017; Rankau *et al.*, 2017; Chen *et al.*, 2018)

Spore contents are hyaline, vacuolated and reticulated with a suspensor like cell attached, being smooth, hyaline to light brown.

Gigaspora gigantea Nicolson and Gerdemann: Azygospores formed solitary in soil; spherical, ellipsoidal, cylindrical to irregular; colour bright yellow with greenish tinge, 183 - 500 X 291 - 812 µm. Outer spore wall thin, tightly covering a thick walled, continuous endospore; a single bulbous suspensor, with slender hyphae, extending from the suspensor to the base of the spore.

Glomus aggregatum Schenck and Smith: Chlamydospores formed in loose clusters, being globose, yellow. Spore surface smooth; wall 1-2 layered; outer thick and light coloured, inner layer thin. Spore contents white to hyaline globules. Hyphal envelope absent; pore at spore wall closed by inner wall septum.

Glomus albidum Schenck and Smith: Chlamydospores globose to sub globose, white to yellow, 150 - 182 µm, in diameter. Spore surface coarse rough; wall 2 layered of equal thickness. Spore contents white to hyaline globules. Hyphal envelope absent; pore at spore wall closed by presence of septum.

Glomus constrictum Trappe: Chlamydospores globose to sub globose, yellow to brown, 142 - 180 µm, in diameter. Spore surface smooth; wall 2 layered of equal thickness.

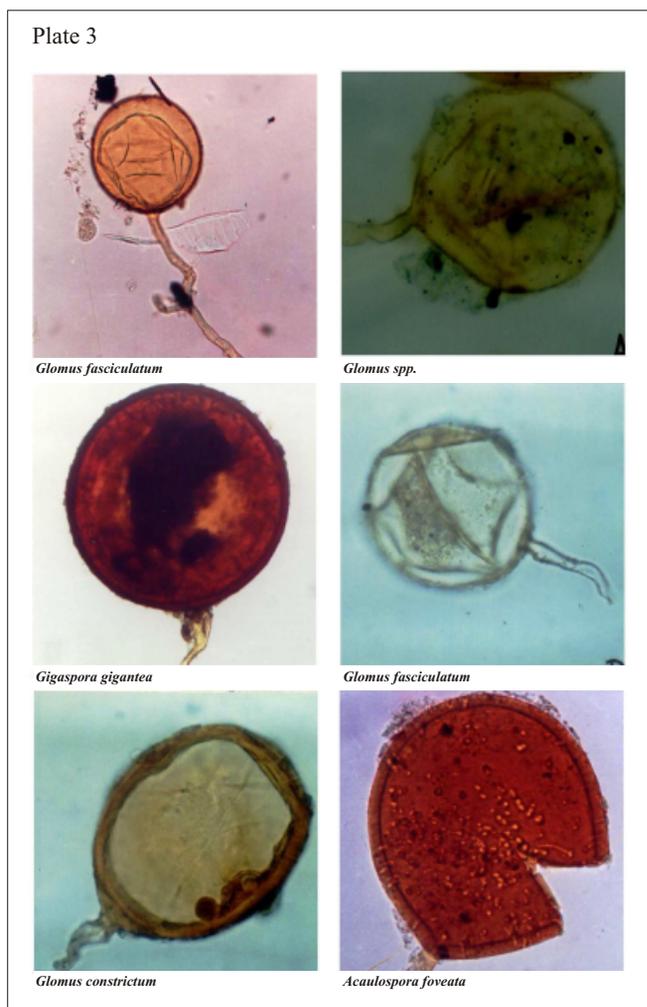
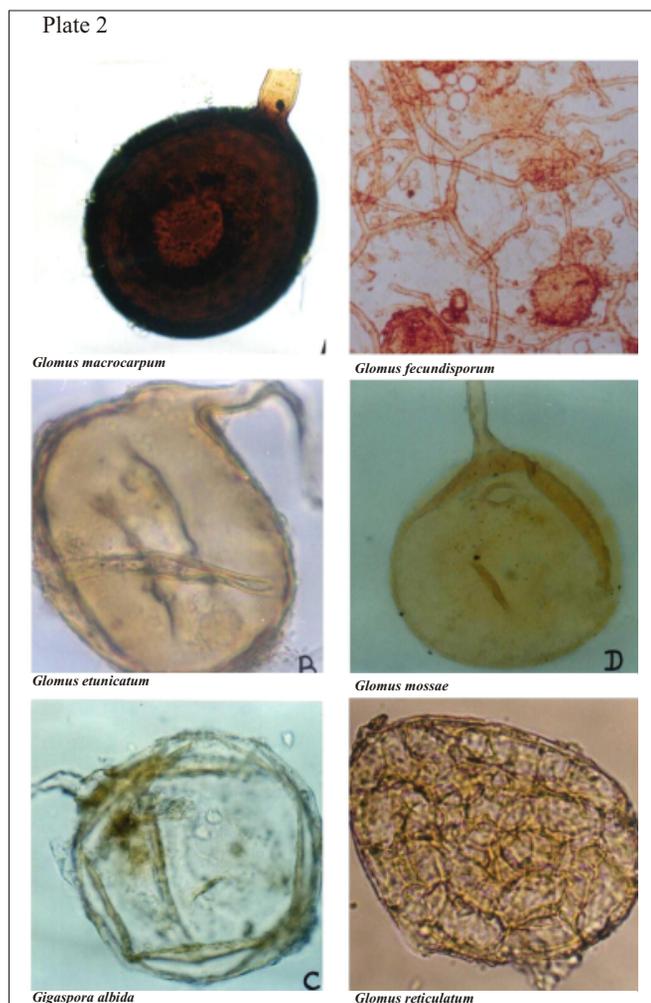
Spore contents bright - yellow globules. Sporogenous hypha one, cylindrical, contracted at the points of attachment, first straight and then recurved. Pore at spore wall closed by presence of septum. Sporocarp not observed.

Glomus etunicatum Becker and Gerdemann: Chlamydospores globose to sub globose, dirty yellow 118 - 120 µm in diameter. Spore surface smooth; wall 2 layered, outer thin hyaline and inner thick. Spore contents hyaline globules. Sporogenous hyphae one, cylindrical. Pore at spore wall closed by presence of septum. Sporocarp absent.

Glomus intraradices Schenck and Smith: Chlamydospores single or in clusters, globose to sub globose, yellow to brown, 140 - 162 µm, in diameter. Spore surface smooth or rough; wall 2 or 3 layered, outer hyaline yellow, inner walls darker than outer. Spore contents orange to brown globules. Sporogenous hyphae one, cylindrical. Pore at spore wall occluded by spore thickening, which forms a tubaeform juncture at attachment of hypha.

Glomus fecundisporum Schenck and Smith: Chlamydospores formed singly or in loose clusters, being globose, elongate to irregular. Spore wall yellow brown to dark brown, rough with adhering debris; inner and outer walls of approximately equal thickness. Spore contents sub hyaline to grey- white. Extra mycelial hyphae with outer surface.

Glomus fasciculatum Thaxter, *sensu* Gerd: Chlamydospores



borne, irregularly globose, 86 μm , - 112.8 μm . Spore wall relatively thick, pale yellow brown, hyphae attached. Spore contents sub hyaline to grey- white. Extra mycelial hyphae with outer surface. Spores contain numerous fat globules, tending to be irregular in shape.

Glomus macrocarpum Gerdemann and Trappe: Chlamydo spores single or in loose clusters, globose to sub globose, yellow to dark-brown, 140 - 190 μm , in diameter. Spore surface smooth or slightly rough; wall 2 layered. Spore contents yellow to brown hyaline globules. Sporogenous hyphae one, flared towards the point of attachment. Pore at spore wall occluded by spore thickening or plug. Sporocarp not observed.

Glomus mossae Nicolson and Gerdemann: Chlamydo spores yellow to brown, 105 - 310 x 110 - 305 μm , globose to ovoid, somewhat irregular with one funnel shaped base; divided from subtending hyphae by a recurved septum; walls thick with white or hyaline outer membrane and a thick brownish-yellow inner layer.

Glomus multisubtensum Mukerji *et. al.*: Chlamydo spores single or in loose clusters, globose to sub globose, light to dark-brown, 120 - 180 μm in diameter. Hyphal envelope absent. Spore surface smooth or slightly rough; wall with 2 inseparable layers, outer thick, brown and inner layer pale

yellow. Sporogenous hyphae 2 to many, always arise from one end of the spore, hyaline, pale yellow, cylindrical. Pore at spore wall closed by septum. Sporocarp not observed.

The results regarding the mycorrhizal associations are given in **table - 1**. From this table it is clear that *Glomus mosseae* is the dominant species in the plants of the Baspa valley. The different *Glomus* species are *Glomus aggregatum*, *G. constrictum*, *G. fasciculatum*, *G. fecundisporum*, *G. macrocarpum*, *G. multisubtansum* and *G. entunicatum* (**Plate 2 & 3**). The other AM genera and species associated with the plants were *Gigaspora albida*, *G. gigantea*, *G. constrictum*, *G. colkospora*, *G. fasciculatum* and *G. intrasides*, *Acaulospora laevis* and *A. foveata*. In a recent study by Banta *et al.*, (2018) on the diversity of AM spores in the rhizospheric soils of the cold desert areas of Kinnaur (HP) it was observed that *Glomus*, *Acaulospora* and *Gigaspora* were the predominant AM genera. Among the *Glomus* species, *G. macropoda* was the most dominant species, followed by *G. geosporum* and *G. mosseae*.

Earlier workers with different plant species have reported similar AM associations, dominant being the genus *Glomus*; and *G. mosseae*, being the most predominant AM fungus here and elsewhere which has also been extensively used for mass propagation and enhanced yield of oats, barley, clovers,

potatoes, alfa-alfa, onions, lettuce, etc., (Alexander *et al.*, 1989; Muromtsev *et al.*, 1990; McArthur and Knowles, 1993; Akhtar and Abdullah, 2014; Garmendia and Mangas, 2014; Vani *et al.*, 2014) and other globally important food security crops (Ceballos *et al.*, 2013). Of late, these AM inoculations have also been found profitable in plant production at a large agricultural scale (Chen *et al.*, 2018).

REFERENCES

- Akhtar, Mohd Sayeed and Abdullah, Siti 2014. Mass Production Techniques of Arbuscular Mycorrhizal Fungi: Major Advantages and Disadvantages: A Review. *Biosciences Biotechnology Research Asia* **11**: 1199-1204. 10.13005/bbra/1506.
- Alexander, I., Norani, A. and Lee, S.S. 1992. The role of mycorrhizas in the regeneration of some Malaysian forest trees. *Phil. Trans. R. Soc., Lond. B* **335**: 379-388.
- Alexander, T., Toth, R., Meier, R., and Weber, H.C. 1989. Dynamics of arbuscule development and degeneration in onion, bean, and tomato with reference to vesicular-arbuscular mycorrhizae in grasses. *Can. J. Bot.-Rev. Can. Bot.* **67**: 2505-2513. doi: 10.1139/b89-320.
- Al-Karaki, G.N. 2013. "The role of mycorrhiza in the reclamation of degraded lands in arid environments." In: *Developments in Soil Classification, Land Use Planning and Policy Implications: Innovative Thinking of Soil Inventory for Land Use Planning and Management of Land Resources* (Eds.: Shahid, S.A., Taha, F.K. and Abdelfattah, M.A.). Dordrecht: Springer Science+Business Media, 823-836.
- Asmelash Fisseha, Bekele Tamrat and Birhane Emiru 2016. The Potential Role of Arbuscular Mycorrhizal Fungi in the Restoration of Degraded Lands. *Frontiers in Microbiology* **7**. DOI=10.3389/fmicb.2016.01095.
- Bagyaraj, D.J. 1991. Ecology of vesicular arbuscular mycorrhizae. In: *Hand Book of Applied. Mycology* Vol. I (Eds.: Arora, D. K., Rai, B., Mukerji, K. G., and Knudsen, G. R., Marcel Dekker), New York, pp: 3-34.
- Bagyaraj, D.J. 2011. *Microbial Biotechnology for Sustainable Agriculture, Horticulture and Forestry*. New India Publishing Agency, New Delhi.
- Bhagyaraj, D.J. 2015. Status paper on arbuscular mycorrhizal fungi. In: *Advances in mycorrhiza and useful microbes in forestry*. (Eds.: Harsh, N.S.K. and Kumar Ashwani). Greenfield Publ. Dehradun, India; pp: 21-37.
- Banta, Ayush, Bhattacharya, Sujata and Chauhan, Vijay 2018. Diversity of fungal spores associated with Rhizospheric soil of *Juniper Communis*, *Juniper Recurva* and *Juniper Macropoda* of cold deserts of Himachal Pradesh. *Int. J. Pharma. Bio. Sci.* **9** (2): (B) 273-280.
- Barea, J.M. 1991. Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. *Adv. Soil Sci.* **15**: 140.
- Barr, J. 2010. Restoration of plant communities in The Netherlands through the application of arbuscular mycorrhizal fungi. *Symbiosis* **52**: 8794. 10.1007/s13199-010-0105-z.
- Camprabi, A., Estuan, V. and Calvet, C. 1992. Effect of aromatic plants and species on vesicular arbuscular mycorrhizal establishment in *Pistacia terebinthus*. *Plant Soil* **139**: 299-301.
- Cavagnaro, T.R, Bender, S.F, Asghari, H.R and van der Heijden, M.G.A. 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends Plant Sci.* **20**: 283290. doi: 10.1016/j.tplants.2015.03.004.
- Ceballos, I., Ruiz, M., Fernández, C., Peña, R., Rodríguez, A. and Sanders I.R. 2013. The *in vitro* mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the globally important food security crop cassava. *PLoS ONE* **8**: e70633. 10.1371/journal.pone.0070633.
- Chamola, B.P., Giri, B., Mukerji, K.G. 1999. Vesicular Arbuscular Mycorrhizal Fungi: Biofertilizer for the Future. In: *From Ethnomycology to Fungal Biotechnology* (Eds.: Singh, J. and Aneja, K.R.). Springer, Boston, MA.
- Chen Min, Arato Miguel, Borghi Lorenzo, Nouri Eva and Reinhardt Didier 2018. Beneficial Services of Arbuscular Mycorrhizal Fungi From Ecology to Application. *Frontiers in Plant Science*, **9**.
- Chen, Y., Gibbs, D. and Oldfield, S 2018. *Acer caesium*. The IUCN Red List of Threatened Species 2018: e.T62937A3116837. <http://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T62937A3116837.en>.
- Cox, G. and Tinker, P.B. 1976. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas -I. The arbuscule and phosphorus transfer: A quantitative ultrastructural study. *New Phytol.* **77**: 371-378.
- Dixon, R.K., Mukerji, K.G., Chamola, B.P. and Kaushik, A. 1997. Vesicular arbuscular mycorrhizal in relation to Forests in Arid Land. *Ann. For.* **5**: 1-9
- Dudderidge, J.A., Malibari, A.S. and Read, D.J. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. *Nature* (London), **287**: 834-836.
- Evelin, Heikham, Sharma, Esha and Kapoor, Rupam 2019. Arbuscular Mycorrhizal Fungi: Potential Role in Conservation of Endangered Plants. In: *Plant Reproductive Biology and Conservation* (Eds.) IK International Publishing House Pvt Ltd.
- Frank, A.B. 1885. Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. *Berichte der Deutschen Botanischen*

- Gesellschaft* **3**: 128-145.
- García-Olmedo, F., Molina, A., Alamillo, J.M., and Rodríguez Palenzuela, P. 1998. Plant defense peptides. *Biopolymers* **47**:479-491.
- Garmendia, I. and Mangas, V.J. 2014. Comparative study of substrate-based and commercial formulations of arbuscular mycorrhizal fungi in romaine lettuce subjected to salt stress. *J. Plant Nutr.* **37**: 17171731. 10.1080/01904167.2014.889149.
- 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.
- Gianinazzi, S., Schuepp, H., Barea, J.M. and Haselwandter, K. 2002. *Mycorrhizal Technology in Agriculture: From Genes to Bioproducts*. Springer, Switzerland.
- Giri, B., Kapoor, R. and Mukerji, K.G. 2003. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrition of *Acacia auriculiformis*. *Biol. Fert. Soils* **38**: 176-180.
- Giri, B., Kapoor, R. and Mukerji, K.G. 2007. Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues, *Microbial Ecol.* **54**: 753-760.
- Gnewkow, M.A. and Marschner, H. 1989. Role of VA mycorrhiza in growth and mineral nutrition of apple (*Malus pumila* var. *domesticata*) rootstock cuttings. *Plant and Soil* **119**: 285-293.
- Goraya, G.S., Jishtu, V., Rawat, G.S., Ved, D.K. 2013. Wild medicinal plants of Himachal Pradesh: An assessment for their conservation status and management prioritisation. Himachal Pradesh Forest Department, Shimla, Himachal Pradesh, India.
- Graham, R.D., Welch, R.M. and Bouis, H.E. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives and knowledge gaps. *Advances in Agronomy*, p. 77-142. Academic Press.
- Granger, R.L., Planchette, C. and Fortin, J.A. 1983. Effect of a vesicular-arbuscular (VA) endomycorrhizal fungus (*Glomus epigaeum*) on the growth and leaf mineral content of two apple clones propagated in vitro. *Can. J. Plant Sci.* **63**:551-555.
- Hall, I.R. 1984. Taxonomy of V.A. Mycorrhizal Fungi. In: *V.A. Mycorrhiza* (Eds.: Powell, C.L. and Bagyaraj, D.J.). CRC Press, Boca Raton, Florida, pp: 57-94.
- Hall, I.R. 1987. Taxonomy and identification of vesicular arbuscular mycorrhizal fungi. *Z. Agew. Bot.* **61**: 145-152.
- Harley, J.L. and Smith, S.E. 1983. *Mycorrhizal Symbiosis*. Academic Press, London, UK, 483 pp.
- Henning, K., 1993. Response of roots to heavy metal toxicity. *Env. Exploratory Bot.* **33**: 99-119.
- Hetrick, B.A.D, Kitt, D.G. and Wilson, G.T. 1986. The influence of phosphorus fertilisation, drought, fungal species and non soil on mycorrhizal growth response in tall grass prairie plants. *Canadian Journal of Botany* **64**:1199-1203.
- IJdo, M., Cranenbrouck, S. and Declerck, S. 2011. Methods for large-scale production of AM fungi: past, present, and future. *Mycorrhiza* **21**: 1. <https://doi.org/10.1007/s00572-010-0337-z>.
- Ikombu, B.M., Edwards, D.G. and Asher, C.J. 1991. The role of VAM in the phosphorous nutrition of cowpea (*Vigna unguilata* L. Wald). *Aust. J.Agr. Res.* **42**: 129-138.
- Jain, R.K. and Sethi, C.L. 1988. Influence of endomycorrhizal fungi *Glomus fasciculatum* and *G. epigaeus* on penetration and development of *Heterodera cajanion* cowpea. *Indian J. Nematol.* **18**: 89-93.
- Jalali, B.L. and Chand, H. 1988. Role of VAM in Biological Control of Plant Diseases. In: *Mycorrhizae for Green Asia* (Eds.: Mahadevan, A., Raman, N. and Natarajan, K.). Madras Express Service, India, pp: 209-215.
- Jishtu, V. 2005. *Studies on the Floristic and Associated Mycorrhiza of Baspas Valley in Kinnaur, Himachal Pradesh*. Ph.D. Thesis, FRI, Deemed University, Dehra Dun. 393 pp.
- Jordan, N.R., Zhang and Huerd 2000. Arbuscular-mycorrhizal fungi: Potential roles in weed management. *Weed Research* **40** (5), 397-410. <https://doi.org/10.1046/j.1365-3180.2000.00207.x>
- Jose, M., Neeraj, A.S., Kumar, R. and Verma, A. 1990. VAM fungi from the rhizospheres of desert cacti. Proc. Of the Natl. Con. on Mycorrhiza Haryana Agrl. Univ., Hissar.
- Kaur, M. and Mukerji, K.G. 1999. The Application of Vesicular Arbuscular Mycorrhizal Fungi in Afforestation. In: *From Ethnomycology to Fungal Biotechnology* (Eds.: Singh, J. and Aneja, K.R.). Springer, Boston, MA.
- Khaliel, A.S. 1988. Incidence of VAM in some desert plants and correlation with edaphic factors. In: *Mycorrhizae for Green Asia. First Asian Conference on Mycorrhizae* (Eds.: Mahadevan, A., Raman, N. and Natarajan, K.). Univ. of Madras, Madras, pp: 56-59.
- Lakshmipathy, R., Balakrishna, A.N., Bagyaraj, D.J. and Kumar, D.P. 2000. Symbiotic response of cashew root stock to different VAM fungi. *The Cashew* **14**: 20-24.
- Manaut, N., Sanguin, H., Ouahmane, L., Bressan, M., Thioulouse, J. and Baudoin, E. 2015. Potentialities

- of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. *Ecol. Eng.* **79**: 113-119. 10.1016/j.ecoleng.2015.03.007
- Manoharachary, C. and Reddy, D. J. M. 1995. Role of vesicular arbuscular mycorrhizal fungi in forestry. In: *Mycorrhizae: Biofertilizer for the Future*, (Eds.: Adholeya, A. and Singh, S.). Tata Energy Research Institute, New Delhi, India, pp: 297-302.
- Manoharachary, C., Swarupa, Rani, S. and Kunwar, I.K. 2008. Arbuscular mycorrhizal fungi associated with some *Apocynaceae*. *Indian Journal of Mycology and Plant Pathology* **38**: 91-92.
- Marx, D.H. 1973. Mycorrhizae and feeder root diseases. In: *Ectomycorrhizae: their Ecology and Physiology* (Ed.. Marks, G.C. and Kozlowski, T.T.), pp. 351-382. Academic Press, New York.
- Marx, D.H. and Bryan, W.C. 1971. Influence of Ectomycorrhizae on Survival and Growth of Aseptic Seedlings of Loblolly Pine at High Temperature. *Forest Science* **17** (1): 37-41.
- McArthur, D.A.J. and N.R. Knowles 1993. Influence of vesicular arbuscular mycorrhizal fungi on the response of potato to phosphorus deficiency. *Plant Physiol.* **101**: 147-160.
- Michelson, A. and Rosendahl, S. 1990. The effect of V.A. mycorrhizal fungi, phosphorus and drought stress on the growth of *Acacia nilotica* and *Leucaena leucocephala* seedlings. *Plant and Soil* **124**: 713.
- Morton, J.B. 1988. Taxonomy of VAM fungi, classification, nomenclature and identification. *Mycotaxon* **32**: 267-324.
- Morton, J.B. 2002. *International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi*. West Virginia University.
- Morton, J.B. and Benny, G.L. 1990. Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*): a new order, *Glomales*, two new suborders, *Glomineae* and *Gigasporineae*, and two new families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of *Glomaceae*. *Mycotaxon* **37**: 471-491
- Mukerji, K.G., Chamola, B.P., Kaushik, A., Sarwar, N. and Dixon, R. 1996. Vesicular arbuscular mycorrhiza: potential biofertilizer for nursery raised multipurpose tree species in tropical soils, *Ann. For.* **4**: 12-20.
- Muromtsev, G.S., Marshunova, G.K. and Yakobi, L.M. 1990. Efficiency of crop inoculation with endomycorrhizal fungi. *Agric. Ecosyst. Environment* **29**: 1-4.
- Niemi, M. and Vestberg, M. 1992. Inoculation of commercially grown strawberry with mycorrhizal fungi. *Plant Soil* **144**: 133-142.
- Palmer M.A., Ambrose R.F., Poff, N.L. 1997. Ecological theory and community restoration ecology. *Restor. Ecol.* **5**: 291-300. 10.1046/j.1526-100X.1997.00543
- Parke, J.L., Linderman, R.G. and Trappe, J.M. 1983. Effects of forest litter of mycorrhiza development and growth of Douglas-fir and western red cedar seedlings. *Can. J. For. Res.* **13** 666-671.
- Pindi, P.K., Reddy, S.R. and Reddy, S.M. 2008. Distribution of VAM fungi in four soils of T.S. with reference to some agroforestry tree species. *J. Basic Applied Mycol.* **7**: 14-17.
- Possingham, J.V. and Groot Obbink, J. 1971. Endotrophic mycorrhiza and the nutrition of grape vines. *Vitis* **10**: 120-130.
- Raman, N. and Mohan Kumar, V. 1988. *Techniques in Mycorrhizal Research*. University of Madras, Madras. 279p.
- Rankau, H., M'SOU, S., Barstow, M., Harvey-Brown, Y. and Martin, G. 2017. *Quercus ilex*. The IUCN Red List of Threatened Species 2017: e.T62537A3116134. <http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T62537A3116134.en>.
- Rivers, M.C. and Allen, D.J. 2017. *Juglans regia*. The IUCN Red List of Threatened Species 2017: e.T63495A61526700. <http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T63495A61526700.en>.
- Ross, J.P. 1971. Effect of phosphate fertilization as yield of Mycorrhizal and non Mycorrhizal soybeans. *Phytopathol* **61**: 1400-1403.
- Saha, D., Ved, D., Ravikumar, K. and Haridasan, K. 2015. *Saussurea costus*. The IUCN Red List of Threatened Species 2015: e.T50126641A50131430. <http://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T50126641A50131430.en>.
- Schenck, N.C. and Perez, Y. 1987. *Manual for the identification of VA-mycorrhizal fungi*. University of Florida, Gainesville, FL. 245 p.
- Sharma, Bidisha and Jha, Dhruva Kumar 2017. The Role Played by Mycorrhizal Fungi in Ecorestoration. In: *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration* (Eds.: Varma, A., Prasad, R. and Tuteja, N.). Springer International Publishing, pp 435-449.
- Sikora, R. A. and Schönbeck, F. 1975. Effect of vesicular-arbuscular mycorrhizae (*Endogone mosseae*) on the population dynamics of the rootknot nematodes *Meloidogyne incognita* and *Meloidogyne hapla*. In 'Proceedings VIII international congress on plant protection'. pp. 158166. (Moscow, Russia).
- Sylvia, D.M., A.G. Jarstfer and M. Vosatka 1993. Comparisons of vesicular-arbuscular mycorrhizal species and inocula formulations in a commercial nursery and on diverse Florida beaches, *Biology and Fertility of Soils* **16** (1993): 139-144.

- Tilak, K. V. B. R., Pal, K. K. and Dey, R. 2010. *Microbes for sustainable Agriculture*. IK International Publ. House, New Delhi.
- Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. *Ann. Rev. Phytopath.* **15**: 203-222.
- Trappe, P.B. 1982. Mycorrhizas. The Present Position. Transactions 12th Intl. Cong. *Soil Science*, New Delhi. Pp.155-166.
- Vani, S., Motha, H., Amballa and Bhumi, N.R. 2014. Arbuscular mycorrhizal fungi associated with rhizosphere soils of brinjal cultivated in Andhra Pradesh, India. *Int. J. Curr. Appl. Sci.* **3** (5): 519-529.
- Walker, C. 1981. *Acaulospora spinosa* sp. nov. with a key to the species of *Acaulospora*. *Mycotoxan.* **12**: 512-521.
- Zhang, D., Rushforth, K. and Katsuki, T. 2011. *Abies spectabilis*. The IUCN Red List of Threatened Species 2011: e.T42300A10686224. <http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T42300A10686224.en>.