Biological Forum — An International Journal, **2**(1): 49-52(2010) **ISSN : 0975-1130**

Study on fungal associates of *Aesculus indica*

Anand Sagar and Rupinder Kaur

Department of Bio-Sciences, Himachal Pradesh University, Summer Hill, Shimla (HP) INDIA

ABSTRACT : Studies conducted to find the fungal associates of *Aesculus indica* revealed the presence of 20 species of fungi belonging to 11 genera *(Absidia, Aspergillus, Cladosporium, Fusarium, Gliocladium, Myrothecium, Oedocephalum, Penicillium,* T*richoderma, Trematostroma* and non sporulating mycelium) from its rhizosphere. Twenty three species of VAM fungal spores belonging to four genera *(Acaulospora, Entrophospora, Gigaspora and Glomus)* were isolated from the mycorrhizosphere soil of this plant. Four species of endophytic fungi were isolated from roots, leaves and bark samples of this plant. Percentage of root infection and percentage of VAM spores isolated from the root adhering soil samples of *Aesculus indica* was found to be 70% and 57% respectively. Further the effect of these fungal associates on the growth and development and artificial regeneration of this threatened plant is being investigated.

Keywords : Mycorrhiza, Rhizosphere, VAM, Endophytes.

INTRODUCTION

The benefits of vesicular arbuscular mycorrhizal (VAM) fungi in forest and agricultural ecosystems are widely recognized. VAM fungi play a key role in uptake and translocation of phosphorus from soil beyond the root zone of absorption through proliferation of their hyphae (Lakshman *et. al., 2006).* The distribution of species of VAM fungi varies with climate and edaphic factors. Similarly presence of fungi in the rhizosphere of plants is useful to plants due to their significance as phosphorus solubilizers and producer of plant growth promoting hormones (Garrett, 1956). One more group of fungi which colonise the aerial tissues of plants without causing any noticeable symptoms is known as endophytic fungi. They represent one of the largest reservoirs of fungal species (Dreyfuss, 1989) and are recognized as a repository of unique bioactive metabolites and anticancer drugs (Li *et. al.,* 1998).

A review of work on microbial associates of different plants revealed that there are many reports of work (Gupta and Mukerji, 2001; Maheshwari;2005; Manoharachary *et al*.2005;) on plants like *Terminalia arjuna* and *Emblica officinalis* (Thapar *et. al.*, 1992), and *Dalbergia ratifolia* (Suryanarayanan and Rajagopal, 2000); *Azadirachata indica* (Rajagopal and Suryanarayanan,2000), *Ocimum* species (Gupta *et. al.,* 2000); Black pepper (Anandraj *et. al.*, 2006); *Santalum album* (Mohan *et*. *al*., 1998) But studies on fungal associates of an important medicinal plant *Aesculus indica* of N.W. Himalayan region remain scarce. Hence the present work was taken on *Aesculus indica* and the results are presented in this communication.

MATERIALS AND METHODS

Aesculus indica Colebr. belongs to family Hippocastanaceae, it is a common tree in Western Himalaya from Nepal westwards, occurring chiefly at 4000-9000 ft. (Troup, 1986). It is mainly distributed in Kashmir, Kullu, Shimla and Chamba in Himachal Pradesh, Tehri,Garhwal and Kumaon in Uttar Pradesh; and Pakistan (Peshawar, Hazara, Baluchistan) (Rastogi and Mehrotra, 1991). Its English names are 'Himalayan Chestnut' and 'Indian Horse Chestnut'.

Seed oil of *Aesculus Indica* exhibit antiseptic activity against human pathogenic bacteria and phytopathogenic fungi (Bakshi, *et. al.*, 1999) In some parts of Himachal Pradesh, the seeds are dried and ground into flour. This flour is bitter and used for making *halwa.* The fruits are used as a medicine for animals as well as for human beings. They are also fed to cattle after steeping them in water. The leaves are lopped and used as a fodder for cattle. The wood is easily worked and used for making water-troughs, packing-cases, tea-boxes, decoration articles, etc. Fruits are given in colic pains and also diuretic. Oil from the seeds is used in rheumatism and roots are used for leucorrhoea. Leaves contain flavones, â- sitosterol, palmitone (Farooq, 2005).

Quantitative estimation of rhizosphere fungi isolated from soil samples

For isolation of soil mycoflora, dilution plate method of Wakesman (1927) and Warcup (1950) was followed. The media used for culturing the rhizosphere fungi was Potato Dextrose Agar (Rawling, 1933). Fungal isolates from the rhizosphere were identified following Nagmani *et. al.*, (2006).

Methodology for VAM Spores Isolation:

"Wet Sieving and Decanting Technique" (Gerdeman and Nicolson, 1963) was used for isolation of VAM spores .Percentage of VAM spores was calculated by screening 100 gm of soil for the presence of these spores. The criteria employed for identification were colour, size, shape, wall characteristics, contents and surface ornamentation of spores, nature of spores, the number and arrangement of spores in sporocarp.VAM fungal spores were identified following Manoharachary (2004) and Trappe(1982).VAM infection in roots was assessed by following the method of Phillips and Hayman (1970). Percent colonization of VAM

was calculated by counting infected and uninfected segments using the formula.

$$\% \text{ of colonization} = \frac{\text{Number of root segments infected by VAM fungi}}{\text{Total number of segments}} \times 100$$

Fungal Endophytes were isolated from the root, leaf and bark samples of *Aesculus Indica* by following three step method of Suryanarayanan and Rajagopal. (2000). These were identified by following Nagmani *et. al.,* (2006).

RESULTS

Rhizosphere fungi Isolated from the root adhering soil samples of *Aesculus indica*

20 species of fungi were isolated from the soil samples collected from the vicinity of roots of *Aesculus indica*. These isolates fall into 11 genera (*i.e., Absidia, Aspergillus, Cladosporium, Cunninghamella, Fusarium, Gliocladium, Myrothecium, Penicillium, Trematostroma* and *Trichoderma)*. One non-sporulating mycelium was also isolated Table 1.

The genus *Aspergillus* was represented by 3 species (*i.e., A. flavus, A. niger and A. versicolor*), the genera *Absidia (A. ramose), Cladosporium (C. oxysporum), Cunninghamella (C. elegans), Gliocladium* sp.1 and *Trematostroma* sp. were represented by one species each. Genus *Fusarium* and *Myrothecium* were represented by 2 species each (*i.e., F. moniliforme, F. solani, M.* roridum and *Myrothecium sp*.). The genus *Trichoderma* was represented by 3 species (*i.e.,* T. *pseudokoningii*, *Trichoderma* sp. and T. *viride*). The genus P*enicillium* was represented by 4 species (*i.e., P. chrysogenum, P.citrinum, P. notatum and P. purpurogenum*). One non-sporulating mycelium was also isolated Table 1.

A comparison of seasonal distribution of these isolates revealed that maximum number of fungi were recorded (8 species each) during rainy season (40%), 7 species in spring season (35%), 6 species in winter season (30%) followed by 5 species (25%) in summer season Table 1.

Table 1 : Comparison of occurrence of Rhizosphere Fungal Species of *Aesculus indica* during different seasons.

Sr.No.	Name of Fungus Isolated	Winter	Spring	Summer	Rainy
1.	*Absidia ramosa*	–	+	–	–
2.	*Aspergillus flavus*	+	–	–	+
3.	*Aspergillus niger*	–	+	+	–
4.	*Aspergillus versicolor*	–	–	–	–
5.	*Cladosporium oxysporum*	–	+	–	–
6.	*Cunninghamella elegans*	–	–	–	+
7.	*Fusarium moniliforme*	–	–	–	+
8.	*Fursarium solani*	–	–	–	+
9.	*Gliocladium* sp. I	+	–	–	–
10.	*Myrothecium roridum*	+	–	–	–
11.	*Myrothecium sp.*	+	–	–	–
12.	Non-sporulating mycelium	+	+	–	–
13.	*Penicillium chrysogenum*	+	+	+	–
14.	*Penicillium citrinum*	–	+	+	–
15.	*Penicillium notatum*	–	–	+	–
16.	*Penicillium purpurogenum*	–	–	–	+
17.	*Trematostroma* sp.	–	–	–	+
18.	*Trichoderma pseudokoninglii*	–	–	–	+
19.	*Trichoderma* sp.	–	+	–	–
20.	*Trichoderma viride*	–	–	+	+
+ = present		30%	35%	25%	40%
= absent					

Further these rhizosphere fungi isolated from the root adhering soil samples of *Aesculus indica* belong to subdivisions Zygomycotina (*Absidia*), Ascomycotina (*Asperigillus, Penicillium),* Basidiomyotina (Non-sporulating) and Deuteromycotina (C*ladosporium, Fusarium, Myrothecium, Trematostroma and Trichoderma, Oedocephalum*).

VAM fungal spores isolated from the root adhering soil samples of *Aesculus indica*

VAM fungal spores isolated from the root adhering soil samples of *Aesculus indica*, belong to 4 genera (*Acaulospora, Entrophospora, Gigaspora* and *Glomus*). Genus *Acaulospora* was represented by 8 species (*i.e., A. appendiculata, A. delicate, A. elegans, A. foveata, A. laevis*

A. mellea, A. micolsonii, and *A. scrobiculata)* the genus *Gigaspora* was represented by 6 species (*i.e., G. albida, G. calospora, G. decipiens, G. gigantea, G. margarita and G. reticulata*) and the genus *Glomus* was represented by 8 species (*i.e., G. candida, G. deserticola, G. fasciculatum, G. formosum, G. interaradices, G. macrocarpum, G. mossea* and *G. reticulatum*). One genus represented by a single species was *Entrophospora* Fig.1-3. Percentage of VAM spores isolated was 70%. Percentage of root colonization was found to be 57%.

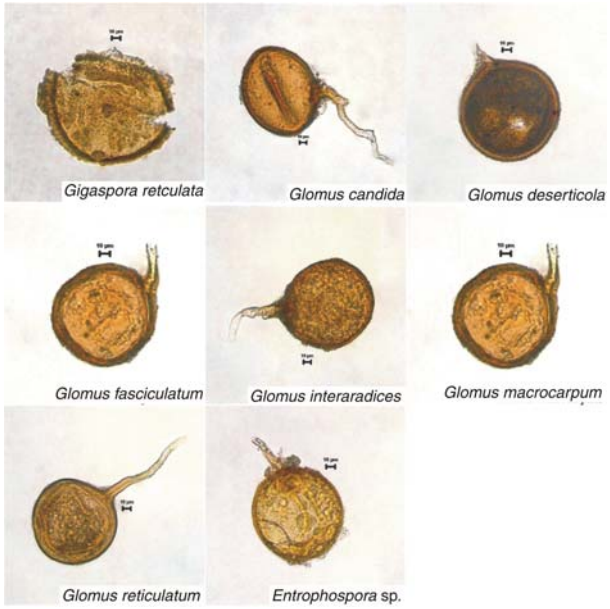

Fig.1.

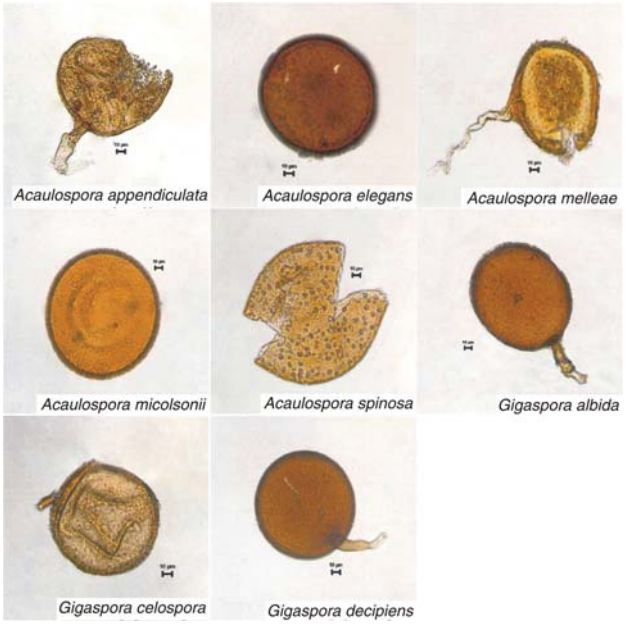

Fig.2.

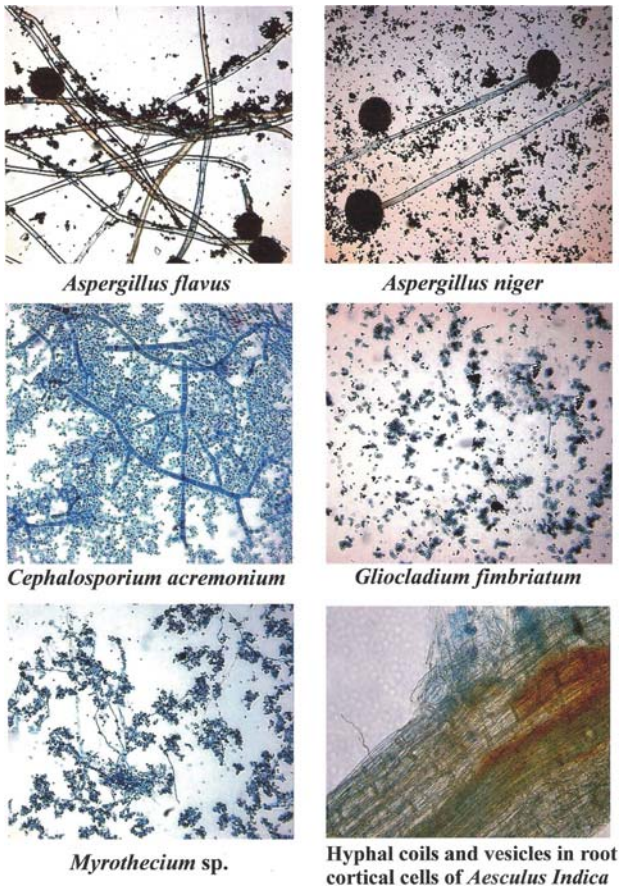

Fig.3.

Endophytic fungal associates of *Aesculus indica*

Five endophytic fungal species belonging to 4 genera were isolated from leaves, bark and roots of *Aesculus indica*. These were *Aspergillus flavus, Aspergillus niger, Cephalosporium acrimonimum, Gliocladium fimbriatum and Myrothecium sp.*

DISCUSSION

Various workers have reported similar fungal genera from the rhizosphere of different plants (Upadhyaya and Rai, 1979; Sagar and Lakhanpal, 1998; Sagar *et. al.,* 2006; Thakur and Sagar, 2007; Thakur, 2008; Chauhan,2009; Sagar and Chauhan, 2009; Sagar and Kaur,2009; Sagar and Kumari,2009). Visser and Parkinson (1975) stated that for a given community only a few fungal species were dominant which may strongly affect the environmental conditions for other species. It was observed that maximum isolated genera belong to subdivision Deuteromycotina which could be attributed to the reason that these "fungi imperfecti" can tolerate wider environmental conditions as compared to the other fungal populations (Behera and Mukherji, 1984). Maximum number of fungi were recorded during the rainy season. Manoharachary (1977) reported a direct correlation of moisture and fungal members of various soils. It was also observed from the present study that mycoflora isolated from the rhizosphere soil did not show any uniform pattern of appearance and distribution. Rangel (1997) has argued that qualitative and quantitative

variations in the rhizosphere mycoflora may be due to different plant species and altered exudation pattern of root system.

In general, findings of the present investigation are in broad agreement with the reports of similar work by the previous workers. Some variations in results can be attributed to change in habitats and climatic factors of the specific region. The study is helpful in gaining preliminary information about the fungal associates and the beneficial potential of these isolates (of rhizosphere and VAM fungi) in the production of nursery seedlings of *Aesculus indica* and secondary metabolites (from enophytes) is being further investigated.

ACKNOWLEDGEMENTS

Authors are grateful to the Chairperson, Department of Biosciences, H.P.U. Shimla for providing Lab. facilities.

REFERENCES

Anandraj, M., Kandiannan, K., Sivaraman, K. and Sharma, Y.R. (2006). Identification of efficient strains of vesicular-arbuscular mycorrhiza for Black Pepper (*Piper nigrum* L.). *In*: Mycorrhiza (Eds. Anil Prakash and V.S. Mehrrotra), Scientific Publishers India., 145-149.

Bakshi, D.N. Guha, Sensharma, P. and Pal, D.C. (1999). A Lexicon of Medicinal Plants In India. **1:** 63-65.

Behera, N. and Mukherji, K.G. (1984). Studies: on soil microfungi in relation to edaphic factors. Act. Bot. Ind. **12:** 153-156.

Chauhan, S. (2009) studies on fungal associates of *Cedrus deodara* and *Quercus leucotriochophora*. M. Phil Dissertation, H.P.U. Shimla

Dreyfuss, M.M. (1989). Microbial diversity and microbial metabolites as sources for new drugs. Princeton Drug Research Symposia, Princeton.

Farooq, S. (2005). 555 Medicinal plants field and laboratory manual (Identification with its phytochemical and *invitro* studies data). International Book Distributors. 259.

Garrett, S.D. (1956). The Biology of Root Infecting Fungi Cambridge University Press, Cambridge.

Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal *Endogone* species extracted from the soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.,* **46:** 235-244.6.

Gupta, M.L., Khalir, A., Pandey. R.S. Singh. H.N. and Kumar, S. (2000). Vesicular arbuscular mycorrhizal fungi associated with *Ocimum* sp. *Herbs, Spices and medicinal Plants*. Vol.7.(abst).

Gupta, R. and Mukerji, K.G. (2001). Fungi as a major group of organisms. In: Microbiol Technology A.P.H. Publishing corporation S, Ansari Road Darya gang, New Delhi.1-6

Lakshman, H.C., Jnchal, R.F. and Mulfa, F.I. (2006). Seasonal fluctuations of arbusculur mycorrhizal fungi on some commonly cultivated crops of Dharwad. *In: Mycorrhiza (Eds.)* A. Parkash and V.S. Mehrotra. Scientific Publishers, Jodhpur. 173-179.

Li, J.Y., Sidhu, R.S., Bollon, A. and Strobel, G.A. (1998). Stimulation of taxol production in liquid cultures of *Pestalatiopsis microspora*. *Mycological. Research.,* **102:** 461-464.

Manoharachary, C. (1977). Microbial ecology of scrub jungle and dry waste soil of Hyderabad. *Proc. Natn. Acad. Sci. Ind.,* **43:** 6-8

Manoharachary, C. (2004). Biodiversity, taxonomy, ecology, conservation and biotechnology of arbuscular mycorhizal fungi. *Indian Phytopathol*, **57:** 01-06.

Manoharachary, C., Sridhar, K., Singh, R., Adnolaaya., A., Suryanarayanan T.S., Rawat., S. and Johri, B.N. (2005). Fungal biodiversity: Distribution, Conservation and Prospecting of Fungi from India. *Cur. Sci*., **89:** 58-71.

Maneshwari, R. (2005). Fungal biology in the 21st century. *Cur. Sci*., **88:** 1406-1418.

Mohan, V., Narayanan, C. and Manocharan, P. (1998). Status of vesicular-arbuscular mycorrhizal (VAM) association of *Santalum album* L. (sandal) in black cotton soils. In: Sandal and its products. Proc. of an international seminar held at Bangalore, India.

Nagmani, A., Kunwar, I.K. and C. (2006). Handbook of Soil Fungi. I.K. International. Publishers. 477.

Phillips, J.M., and Haymann, 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.

Rangel, Z. (1997). Root exudation and mycoflora population in rhizosphere crop genotypes *Plant and Soil*., **196:** 255- 260.

Rastogi, R.P. and B.N. Mehrotra, (1991). Compendium of Indian medicinal plants., **4:** 16-17.

Rawling, T.E. (1933). Phytopathological and botanical research methods. John Wiley and Sons, London.

Rajgopal, K. and Suryanarayanan, T.S. (2000). Isolation of endophytic fungi from leaves of neem (*Azadirachta indica* D.Juss.). *Cur. Sci.,* **78:** 1375-1378.

Sagar, A. and Lakhanpal, T.N. (1998). Studies on mycorrhiza and mycorrhizosphere of *Pinus wallichiana*. J. Hill. Res., **11:** 154-161.

Sagar, A., Raghva, S. and Bhalla, T.C. (2006). Studies on mycoflora of the cold desert area of Himachal Pradesh. *Indian Phytopath*., 59: 507-508.

Sagar, A. and Chauhan, S. (2009). Studies on Fungal Associateds of *Quercus leucotrichophora. J.Pure* and *Appl. Microbiol.*, **3:** 357-362.

Sagar, A. and Kaur, R. (2009). Fungal associates of *Rhododendron arboreum*-A medicinal plant. *Biozone-An international journal of Life Sci*., **1:** 75-84.

Sagar, A. and Kumari, R. (2009). Fungal Associates of *Centella asiatica* and *Ocimum sanctum*. *J. Pure* and *Appl. Microbiol.*, **3:** 243-248.

Suryanarayanan, T.S. and Rajagopal, K. (2000). Fungal endophytes (phellophytes) of some tropical forest trees. *Ind. For.*, **126:** 165-170.

Thapar, H.S., Vijyan, A.K. and Uniyal, K. (1992). Vesicular arbuscular mycorrhizal associations and root colonization in some important species. *Ind. For*. 118: 207-212.

Thakur, M. and Sagar, A. (2007). Fungal associates of *Terminalia chebula, Terminalia bellirica* and *Emblica officinalis. J.Pure* and *Appl. Microbiol*., **1:** 301-306.

Thakur, P. (2008). Fungal Associates of *Ginkgo bioloba* L. and *Platanus orientalis* L. M.Phil Dissertation, H.P.U. Shimla.

Troup, R.S. (1986). The Silviculture of Indian Trees., **1:** 226-229.

Troup, R.S. (1986). The Silviculture of Indian Trees., **2:** 633-635.

Trappe, J.M. (1982). Synoptic keys to the genera and species of Zygomycetous mycorrhizal fungi. *Phytopath.,* **72:** 1102-1109.

Upadhyaya, R.S. and Rai, B. (1979). Ecological survey of Indian soil fungi with special reference to *Aspergillus*, *Penicillium* and *Trichoderma*. *Rev. Ecol. Biol. Soil.*, **16:** 39-40

Visser, S. and Parkinson, D. (1975). Fungal succession in poplar leaf litter. *Can. J. Bot.*, **53:** 507-508.

Wakesman, S.A. (1927). Principles of soil microbiology. Williams and Wilkinson Company Baltimore.

Warcup, J.H. (1950). The soil plate method for isolation of fungi from soil. *Nature*, **166:** 117-118.