International Journal of Basic and Applied Biology (IJBAB)
Print ISSN: 2349 – 5820; Online ISSN: 2349 – 5839 ; Volume 2, Number 1; October, 2014 pp. 78 – 82

http://www.krishisanskriti.org/ijbab.html

A.M. Fungal Colonization in Mentha arvensis

[1]Lata Singh, [2]Payal Mago, [3]Vishal Kaushik, [4]Isha Gunwal,

[1, 3] N.R.E.C. College, Khurja, C.C.S. University, Meerut
[2, 4] Sri Aurobindo College, University of Delhi, New Delhi

Abstract: **Mycorrhiza is a mutualistic relationship between the plant roots and fungal hyphae. Arbascular mycorrhizal fungal association in majority of terrestrial plants are universal. A survey of A.M. fungal colonization was done in roots of M. arvensis. It was found that when there is more number of spores in the rhizophere soil, more is the degree of colonisation. In the present study Mentha arvensis showed the total maximum root length colonisation of 90%.**

Keywords: Mycorrhiza, Spore number, Hyphae, Root Colonisation

1. INTRODUCTION

The goal of the ecologist is to study organisms in natural ecosystems without necessarily any desired practical outcome. Although the goals of the ecologist and the agriculturist are thus different, they are often complementary, and research in ecology has great potential to lead to advancement in agriculture. Initial ecological research on arbuscular mycorrhizas led to the realization that the symbiosis was widespread, both in terms of the number of plant species involved, and in terms of the number of ecosystems possessing it. For example, Jones (1924) found that a diversity of soils across the United States supported arbuscular mycorrhizal plants. The autecology of the arbuscular mycorrhizal fungi has been the subject of research for many years. For example, Lohman (1927) investigated the effects of soil pH on mycorrhization, as did Peuss (1958) and Porter et al (1987), among many others. Many have investigated the effects of temperature (Furlan and Fortin 1973; Hayman 1974) and soil moisture (Reid and Bowen 1979) on the symbiosis, but Jones (1924) was probably the first to investigate these relationships. The effects of freezing or drying on survival of the fungi do not appear to have been examined until relatively recently (Jasper et al. 1989; Addy et al. 1994, Kabir et al. 1997; Klironomos et al. 2001). The nature of spore dormancy and the environmental factors that overcome it have been investigated by many authors through the years (Mosse 1959a; Siqueira et al 1985).

Some authors have noted that mycorrhiza inoculum potential varies with soil depth (Schwab and Reeves 1981; Koide and Mooney 1987), but this had already been noted years earlier (Jones 1924). Nicolson (1959) and Mosse (1959a) described in some detail the dimorphic nature of the soil mycelium, and the ecological relevance of this has been discussed by Read (1992). Hyphal anastomoses, which are potentially very important to the ecology of the fungi, were noted early on (Gerdemann 1955b; Mosse 1959b, 1963). Mosse indicated that anastomoses provide the possibility for "hybridization" or exchange of genetic material, which has been confirmed recently (Giovannetti et al. 2001). This provides a means to maintain adaptability and diversity in otherwise apparently asexual fungi. Anastomoses may also permit resource transfer among individual fungi. The fact that different species do not form anastomoses with each other (Giovannetti and Sbrana 2001) indicates that the benefits of the physiological integration among individuals of a single species are not afforded between species. The early observations that plant species differed in their response to mycorrhizal fungi (Lohman 1927; Baylis 1970, 1972b) and that some plant species were nonmycorrhizal, led to the hypothesis that the fungi could help to structure natural plant communities. Indeed, mycorrhizal fungi may influence the course of plant succession (Nicolson 1960; Janos 1980) and the relative competitive abilities of host plants (Crush 1974; Fitter 1977; Hall 1978). In some cases, antagonistic interactions between arbuscular mycorrhizal fungi and some plant species may also serve to exclude these plants from mycorrhizal plant communities (Allen et al. 1989; Francis and Read 1984, 1985). Mycorrhizal fungi may also influence plant communities by affecting species evenness (Grime et al. 1987; O'Connor et al. 2002) or species richness (Gange et al. 1990). Although there is no evidence of strict host-fungus specificity with arbuscular mycorrhizas (but see Helgason et al. 2002), the composition of the mycorrhizal fungal community has the potential to both influence (van der Heijden et al. 1998) and be influenced by (Hetrick and Bloom 1983; Anderson and Liberta 1985; Bever et al. 1996) plant community composition. These interactions clearly have relevance to agroecosystems, particularly where crop rotations or intercropping are involved. Interactions between mycorrhizal fungi and other organisms occur and may influence the function of the fungi. While grazing of mycorrhizal hyphae by fungivorous collembola can reduce host plant P uptake (Warnock et al. 1982; McGonigle and Fitter 1988) collembola may also disseminate mycorrhizal fungal propagules (Klironomos and Moutoglis 1999). Rodents may also be agents of dispersal as Endogone spores were shown to remain viable after passage through their alimentary tracts (Godfrey 1957). Some of the interactions among mycorrhizal fungi and

other soil organisms have been summarized by Azc_n-Aguilar and Barea (1992) and Fitter and Sanders (1992). Electron microscopy has revealed the unexpected presence of large numbers of bacteria-like structures (sometimes referred to as bacteria-like objects or BLOs) within spores and hyphae of arbuscular mycorrhizal fungi (Mosse 1970; MacDonald et al. 1982; Scannerini and Bonfante 1991).

We now know that these truly are bacteria that are apparently obligate symbionts of the fungi. Some of the bacteria are being characterized using DNA-based methods and can represent 40% of total spore DNA (Bianciotto et al. 1996, 2003). Their significance to the biology of the fungus has yet to be worked out. The discovery that benomyl could be effective against arbuscular mycorrhizal fungi led to its use in several ecological studies, particularly by Fitter and his associates (Fitter 1986; Carey et al. 1992; Newsham et al. 1995; Merryweather and Fitter 1996) in which they described the effects of mycorrhizal infection on seed production, and interactions among mycorrhizal fungi and plant pathogenic fungi. Koide and his associates have also shown significant effects of mycorrhizal fungi on plant fitness, both in terms of individual plant fecundity, seed quality, and plant population dynamics (summarized in Koide 2000; Koide and Dickie 2002).

In 1996 Wright and Upadhyaya described the existence of a novel protein produced by arbuscular mycorrhizal fungi. This compound came to be known as glomalin. At least some forms of it appear to be comparatively recalcitrant, thus allowing high concentrations to build up in the soil. Moreover, it may serve a role in soil aggregation and it represents a relatively large pool of carbon and nitrogen (Miller and Jastrow 2000). Thus it is clear that mycorrhizal fungi are important components of natural ecosystems, and that they can have strong influences on plant community composition and ecosystem function. Some of these influences have been summarized by Hart and Klironomos (2002) and by Bever et al. (2002).

2. MATERIAL AND METHOD

Mentha arvensis L.susp.hapocа/yxBriqetvar.p/perscensHolme (Japanese Mint.)

The plant is a downy perennial herb with root-stock creeping (sucker) along or just under the ground surface. It has a rigid branching, pubescent, 60-90 cm tall stem. The leaves are lanceolate to oblong, sharply toothed, 3.7-10.0 cm. Long, sessile or shortly (3-10 cm) petiole, covered with small whitish hair and glandular dots on both surfaces. Cymes of verticitiate purple flowers occur on rather distinct nodes. The plant is perpetuated by vegetative propagation. It is a high chromosome numbered hybrid between M. arvensis L. and M. aquatica L. and is more robust than the parents.

Soil samples were collected from rhizosphere of different members of Lamiaceae during January 2007 to July 2007. In the laboratory the rhizosphere soil was removed after washing the plants thoroughly with water, and the soil samples were analyzed for VA mycorrhizal spore counts and also for identification and taxonomy of the associated AM fungus for two reasons:

To find out whether only one species of AM fungi is responsible for infection or the synergistic influence of more than one species is responsible for infection.

Whether the same species of AM fungi are continuously associated with one host plant or with the seasonal variation, it is replaced by some other species/variety of the same species.

Different methods are known for the extraction of vesicular-arbuscular fungal spores from soil. They are wet sieving and decanting, floatation-adhesion, density gradient centrifugation and differential water/sucrose centrifugation techniques (Gerdemann, 1955; Ohms, 1957; Gerdemann and Nicolson, 1963; Mosse and Jones, 1968; Sutton and Barron, 1972; Furlan and Fortin, 1975; Alien et at, 1979; Mertz el al., 1979; David C. lanson and Michael F. Alien, 1986). The efficiency of the separating techniques are limited by the texture of the soil samples used. The method used here in this study for sampling soils was wet sieving and decanting (Gerdemann, 1955; Gerdemann and Nicolson, 1963). This method has been slightly modified by Smith and Skipper (1979). The method is as follows:

In a beaker, 20 g. of air dried soil was taken to which 500 ml of water was added. The resulting suspension was stirred for about 30 minutes, followed by five minutes settling time. Thereafter soil solution was passed through the sieves of different mesh numbers (100, 200, and 300 in the increasing order). The contents of all the mesh numbers were washed individually and suspended in water in separate beakers. AM fungal spores have a strong tendency to stick to the glass surfaces, so soil solution in a beaker was gradually stirred to remove them from glass surface, and to allow them float on the surface of water. This solution was immediately poured onto the filter paper (Whatman No. 1) and was spread in Petridish. The spores were then observed under a dissecting binocular microscope.

The spores were readily seen among the soil particles by their characteristic hyaline to coloured subtending hyphae, spore wall and size. These spores were picked up with a needle on slide. Microscopic examination was done by mounting the spores in Lactophenol. However for permanent preparation mounting was done in Hoyer's medium which preserved the features of spores in their original form. Finally the slides were sealed with quick fix; since it is transparent the original colour of spore was also preserved.

Spore counting was done by taking 10 gms. of soil sample. To facilitate accuracy in counting, semicircles were made on a dry filter paper and each semicircle was numbered as 1,2,3,4 and so on. The counting was done in each numbered, semicircle and noted separately. Spore count of one soil sample was recorded on the average of 3-5 samples of the respective examined under binocular microscope.
Spore count is denoted in the tabular form.

3. RESULT

Mentha arvensis –

In *M. viridis* the maximum % colonization was 87.7% reached on 15th April 2007 with maximum spore count *258* in the month of May shows the diameter of hyphae in the outer cortex is 5 um, and in inner cortex is 3um. Intercellular and parallel hyphae were present. Aspersoria were also observed.

Looped and coiled hyphae also were not observed. H and Y hyphal junction were also seen while S shaped junctions were not seen .Vesicles were present in abundance (nearly 40), they are circular to oval in shape. Arbuscules were also present intercellularly nearly 2-5 in number relates the root % colonization and spore count with various edaphic factors. Like the results observed in M. viridis in M. arvensis also it was found that as the Ph value rises to slight alkaline range the no.of spores decreases. It is also seen that more the soil moisture less is the no. of spores i.e. soil moisture is inversely related to spore count.

As the root % colonization increases the soil available Phosphorus also increases but as the plants grows older available phosphorus decreases slightly. With the increase in the age of the plant soil organic natter was also found to increase. In *Mentha arvensis*, the infection pattern was very similar to that of M. viridis except that in the former, the internal parallel hyphae were also observed. In M. arvensis the root is densly colonized by external AM hyphae which also shows Y shaped connections. Number of vesicles per mm. of root segments were less as, compared to M. arvensis Stelar infection was also observed in this plant.

In *Mentha spicata* external hyphae branches extensively on root surface and form not very distinct appresoria before entering into the root. No parallel internal hyphae was observed and stele showed dense colonization of AM fungal hyphae. Arbuscules are more as compared to vesicles. Vesicles are round to oval in shape and internal hyphae show. projections.

In *M .citrata* external hyphae showed distinct appresorium. Stele as well as the cortex was densely colonized by vesicles and arbuscules . Arbuscules are less in number as compared to vesicles, internal hyphae show loops as well as coils.

4. DISCUSSION

It is observed in all the plants that after inoculating the soil with AM inoculums the initial rhizospere spore count was hige while the root % colonization was low but as the plants grew older the rooy % colonization increases and spore count decreases . At the same time with the root colonization reaches its maximum the spore count was also found to increase.
The spores found in the rhizosphere soil were of *Glomus fasiculatum and Glomus fugianum.*

5. TABLES

Table – 1 : Percent colonized roots and AM fungal spore number in rhizoshpere soil of *Mentha arvensis* at 15 days intervals.

Month of Collection	Percent colonization	AM fungal spore number
1st January 2007	63.24	134
15th January 2007	67.77	111
1st February 2007	69.75	127
15th February 2007	75.80	94
1st March 2007	78.35	98
15th March 2007	79.35	128
1st April 2007	83.75	130
15th April 2007	87.37	146
1st May 2007	90.30	153
15 May 2007	82.50	258
1st June 2007	79.00	235

Table - 2: Percent colonized roots and AM fungal spore number in rhizosphere soil of *Mentha arvensis* in relation to various soil ecological factors.

Percent colonization	AM fungal spore number	Soil pH	Soil moisture (%)	Soil available phosphorus (mg/gram)	Soil organic matter (%)

63.24	134	7.80	25.20	0.300	0.32
67.77	111	7.51	32.65	0.304	0.38
69.75	127	7.15	20.80	0.350	0.37
75.80	94	8.60	58.67	0.316	0.41
78.35	98	8.61	69.46	0.304	0.44
79.35	128	7.35	46.87	0.392	0.28
83.75	130	7.92	52.98	0.496	0.36
87.37	146	7.64	65.55,	0.419,	0.57,
90.30	153	8.30	30.63	0.496	1.01

Table – 3: Anatomy of the Association of host and endopyte

	Mentha arvensis
Diameter of the hyphae in the outer cortes (um)	5
Diameter of the hyphae in the inner cortes (um)	3.5
Hyphae intercellular and parallel	+
Appressoria formed	+
Looped hyphae present	-
Coiled hyphae present	-
Vesicle present or not	+
Vesicle abundance (Number / mm of infected root)	100
Vesicle shape	Circular
Arbuscule abundance (Number / mm of infected root)	2-5
Types of hyphal junctions present	
H	+
Y	+
S	-

6. PHOTOS

PHOTO 1:

Mentha arvensis growing in experimental plots

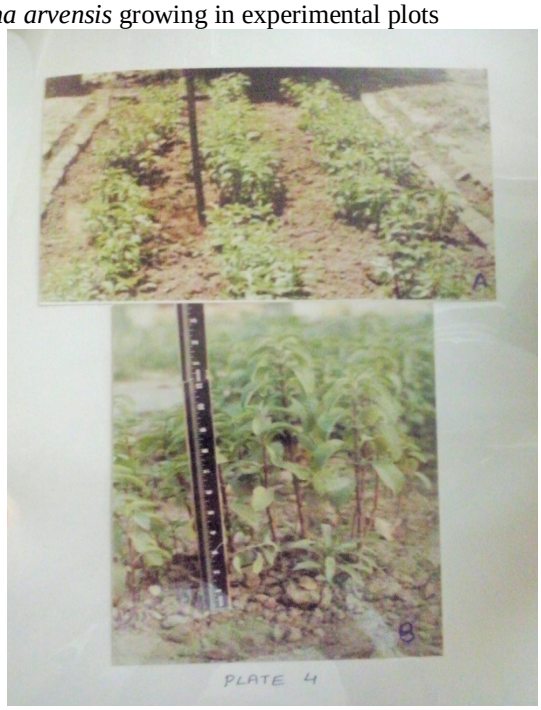
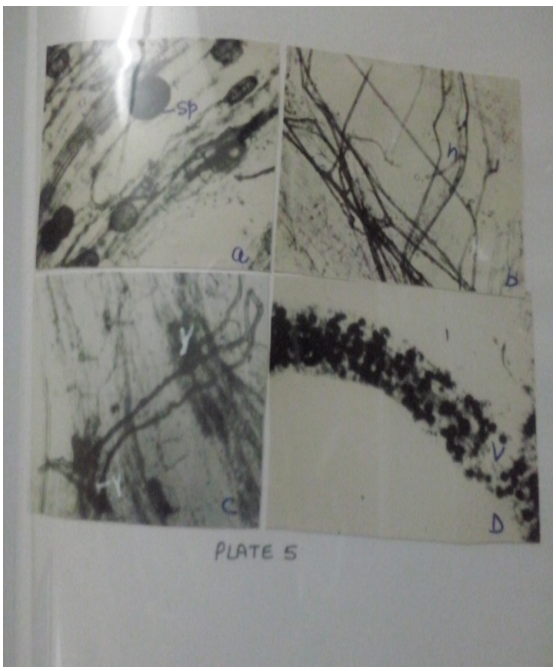


PHOTO 2:

AM coloniation in *Mentha arvensis*

A) Spores germinating on the surface of the hyphae
B) Parallel hyphae showing H shaped connection (h)
C) Thick internal hyphae showing Y shaped connection(y)
D) Root showing abundant vesicles(v)



7. ACKNOWLEDGEMENT

The authors are thankful to the department of botany, university of delhi, for providing necessary laboratory facilities.

8. REFERENCE

[1] **Adholeya A** (2003) Commercial production of AMF through industrial mode and its large scale application. In: Abstracts, 4th International Conference on Mycorrhizae. Montreal, Canada, p 240

[2] **Barker, S. J. & Tagu, D**. (2000). The roles of auxins and cytokinins in mycorrhizal symbioses. Journal of Plant Growth Regulation 19, 144–154.

[3] **Carafa A., Duckett J.G, Ligrone R.** (2003) Subterranean gametophytic axes in the primitive liverwort Haplomitrium harbour a unique type of endophytic association with aseptate fungi. New Phytol 160:185–197

[4] **Dalpe´, Y.&Declerck, S.** (2002). Development of Acaulospora rehmii spore and hyphal swellings under root-organ culture. Mycologia 94, 850–855.

[5] **Elmer WH** (2002) Influence of formononetin and NaCl on mycorrhizal colonization and fusarium crown and root rot of asparagus. Plant Dis 86:1318–1324

[6] **Fitter A.H.,** (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperature deciduous woodland. J Ecol 90:371–384

[7] **Gao C.,** (2000) Takakiaceae. In: Yunnanica F (ed) Kunming Institute of Botany, Chinese Academy of Sciences , vol. 17, Bryophyta: Hepaticae, Anthocerotae. Science, Beijing, pp 1–2

[8] **Harrington, T. J. & Mitchell, D. T.** (2002). Colonization of root systems of Carex flacca and C. pilulifera by Cortinarius (Dermocybe) cinnamomeus. Mycological Research 106, 452–459.

[9] **Janouskova M., Pavlikova D., Macek T., Vosatka M.,** (2005) Arbuscular mycorrhiza decreases cadmium phytoextraction by transgenic tobacco with inserted metallothionein. Plant Soil 272:29–40

[10] **Jarup L.,** (2003) Hazards of heavy metal contamination. Br Med Bull 68:167–182

[11] **Kerp H, Trewin NH, Hass H** (2004) New gametophytes from the Early Devonian Rhynie chert. Trans R Soc Edinb Earth Sci 94:411–428

[12] **Lanfranco L, Bolchi A, Ros EC, Ottonello S, Bonfante P** (2002) Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. Plant Physiol 130:58–67

[13] **Maldonado-Mendoza I.,E, Dewbre G.R, Harrison M.J.,** (2001) A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus Glomus intraradices is regulated in response to phosphate in the environment. Mol Plant Microbe **NEHLS, U., MIKOLAJEWSKI, S., MAGEL, E. & HAMPP, R.** (2001). Carbohydrate metabolism in ectomycorrhizas : gene expression, monosaccharide transport and metabolic control. New Phytologist 150, 533–541

[14] **Ouziad F., Hildebrandt U., Schmelzer E., Bothe H.,** (2005) Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. J Plant Physiol 162:634–649

[15] **Paracer, S. & Ahmadjian, V.** (2000). Symbiosis an Introduction to Biological Interactions. Oxford University Press, Oxford.

[16] **Qiu Y-L, Lee J** (2000) Transition to a land flora: a molecular phylogenetic perspective. J Phycol 36:799–802

[17] **Rasmussen, H. N.** (2002). Recent developments in the study of orchid mycorrhiza. Plant and Soil 244, 149–163.

[18] Sch_ßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

[19] **Taylor TN, Kerp H, Hass H** (2005) Life history biology of early land plants: deciphering the gametophyte phase. Proc Natl Acad Sci U S A 102:5892–5897

[20] **VAN DER HEIJDEN, E. W.** (2001). Differential benefits of arbuscular mycorrhizal and ectomycorrhizal infection of Salix repens. Mycorrhiza 10, 185–193

[21] **Weisburger JH (2002)** Comments on the history and importance of aromatic and heterocyclic amines in public health. Mutat Res 506–507: 9–20

[22] **YU, T. E. J.-C., EGGER, K.N. & PETERSON, R. L.** (2001). Ectendomycorrhizal associations – characteristics and functions. Mycorrhiza 11, 167–177.

[23] **Zhou, Z.H., Miwa, M. and Hogetsu, T.** (2001) Polymorphism of simple sequence repeats reveals gene ¯ow within and between ectomycorrhizal Suillus grevillei populations. New Phytologist 149, 339±348.