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Percentage Colonisation Roots and AM Fungal Spore Number In Rhizoshpere Soil of Mentha Spicata at 15 Days Intervals

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Abstract: **Here, a routine 15 day interval examination was done to follow the structural development of endomycorrhizae in the host plant. Results have shown that VAM fungi reproduce only in association with the host roots. Senescing roots showed the release of thick walled resting spores into soil by the decay of dead host root cells. In *Mentha spicta* after inoculating the soil with AM inoculums the initial rhizospere spore count was high while the root % colonization was low but as the plants grew older the root % 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.**

Keywords: Endomycorrhizae, Spores, Rhizosphere, Inoculums.

1. INTRODUCTION

Mycorrhizal plants can successfully tolerate severe drought stress by improving the water relationships of host plant, by increasing hydraulic conductivity (Boyer, 1969; Hardie, 1985; Hardie and Leyton, 1981: Krishna and Bagyaraj, 1984; Nelson and Safir, 1982; Safir et al., 1971).

Mosse and Hayman (1971) observed that mycorrhizal onions did not wilt when transplanted, but that non-mycorrhizal plants did. The problem was first investigated systematically by Safir et al. (1971, 1972) and they concluded that the effect was probably due to improved nutrition. The ability of avocado seedlings to survive transplanting injury better when mycorrhizal, is attributed to improved water absorption (Menge et al., 1978b). The better recovery from water stress of mycorrhizal seedlings of rough lemon (Citrus jambhiri), was apparently related to effects on stomatal regulation (Levy and Kirkun, 1980) using the grass Bouteloua gracilis and Glomus fasciculatum. Onion plants colonised by Glomus etunicatus were more drought resistant than uninfected plants when exposed to several cycles of soil water stress (Nelsen and Safir, 1982). This was attributed to the ability of the mycorrhizal fungus to maintain adequate phosphorous nutrition in the onions when low soil moisture decreased the mobility of phosphate ions.

Many reports summarize the comparative water relations of AM and nonAM plants (Augé 2001). However, the influence of AM symbiosis on the water relations and moisture retention properties of soils remains largely unstudied. Fungi may be the most effective soil organisms in stabilizing soil structure (McCalla 1946; Swaby 1949; Foster 1994), and AM fungi often comprise the largest portion of the soil microbial biomass (Hayman 1978). Fungal hyphae, especially those of AM fungi, grow into the soil matrix to create the skeletal structure that holds primary soil particles together via physical entanglement. They create conditions conducive to formation of microaggregates, and they chemically enmesh and stabilize microaggregates and smaller macroaggregates into macroaggregate structures (Miller and Jastrow 2000). Rillig 2004, reported, AM fungi produce copious amounts of the glycoprotein glomalin.

Although soil aggregation is a complex hierarchical process (Tisdall and Oades 1982), concentration of glomalin is tightly correlated with aggregate stability across many soils (Wright and Upadhyaya 1998; Rillig 2004). AM symbiosis has been linked to changes in soil structure in both pot and field experiments (Schreiner and Bethlenfalvay 1995; Rillig 2004). Soil structure refers to pore space as well as to aggregates, and the number and dimension of the pore spaces between soil particles are important in functional considerations of soil structure, especially from the standpoint of soil water relations (Hamblin 1985). The moisture characteristic of a soil depends on the size and distribution of its pores, or void space (Hamblin 1985). Because AM fungi affect soil structure, it seems logical to suspect that AM colonization of a soil might affect its moisture retention properties and, in turn, the behaviors of plants growing in the soil, particularly when soil is relatively dry.

2. MATERIAL AND METHOD

The vam fungal inoculum already grown in glass house (method is discussed below) was spread in the field. Two days after spreading the inoculum rhizomes of the experimental plants were sown and after a month of sowing the roots were taken at 15 days interval for six months and screened for the presence of arbuscular mycorrhizae fungi in fine root segments. Rhizosphere soil was also collected every month to investigate the distribution of VAM fungi in soil. These soil samples were also used for analysis of soil ecological factors.. It is indigenous species of northern England. The plant is now grown practically all over the world. Like other mints it is also perennial, propagated by suckers from which 30-60 cm. erect ascending branches arise. Leaves are sessile or nearly so, smooth, lanceolate, or ovate-lanceolate, sharply serrate, smooth above or glandular below, acute apex and up to 6.5 cm. long. The leaves possess a characteristic odour and pungent taste, lacking cooling after-effect in contrast to that of peppermint. This species is very variable in morphological characters and is often erroneously named as M. viridis. It is hybrid between M. rotund ifolia L. and M. longifolia Huds. Its chromosome numbers also varies from 36, 48 to 84 (in 2n).

Gerdemann (1955) cleared the roots by boiling in 0.01% acid fuchsin in saturated chloral hydrate. However an improved technique of clearing and staining was described by Phillips and Hayman (197O), that proved to be the most successful one. Moreover in this study a modified Phillips and Hayman's (1970) technique was used.

For the present study the mycorrhizal .status of plants were analysed by: (a) For rough calculation of percentage of infection, the technique of Nicolson (1955) was employed. The numbers of root segments infected by VAM fungi and uninfected were recorded in this technique and the percentage root infection was then determined as follows:

$$\% \text{ root infection} = \frac{\text{Number of infected segments}}{\text{Total No. of segments examined}} \times 100$$

3. RESULT

1. Relationship between % root colonization and spore count
2. Anatomy of the association of host and endophyte.
3. Relationship between % root colonization and spore count and various edaphic factors.

***Mentha spicata* –** In *M. spicata* the maximum % colonization was 90 % reached on 1st of May 2007 with maximum spore count 235 in the month of july.

The diameter of hyphae in the outer cortex is 5 um, and in inner cortex is 3um. Intercellular and parallel hyphae were present. Appressoria 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 The root % colonization and spore count with various edaphic factors. Like the results observed in M. viridis and in M. arvensis in M. spicata also it was found that as the ph value rises to slight alkaline range the no. of spores decreases.

It is also observed that the soil moisture is not related to spore count.

As the root % colonization increases with the age of the plant 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 but as the plant grows older organic matter decreases slightly.

4. DISCUSSION

A routine 15 day intervel examination was done to follow the structural development of endomycorrhizae in the host plant. Results have shown that VAM fungi reproduce only in association with the host roots. Senescing roots showed the release of thick walled resting spores into soil by the decay of dead host root cells.

It was observed that in all the plants after inoculating the soil with AM inoculums the initial rhizospere spore count was high while the root % colonization was low but as the plants grew older the root % 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. This may be because initially the soil was inoculated with inoculum

rich in AM spores that is why rhizosphere showed high spore count but as the spores come in contact with roots they germinate and infect the root thus the spore count

decreases initially. Later on with age the root becomes heavily colonized and again the spore count increases with increase in colonization.

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.

5. TABLES

Table – 1: Per cent colonized roots and AM fungal Table – 2: Anatomy of the Association of host of spore number in rhizosphere soil of *Mentha spicata* *Andopyte.* at 15 days intervals.

Month of Collection	Percent colonization	AM fungal spore number
1st January 2007	32.8	200
15th January 2007	35.38	182
1st February 2007	47.34	127
15th February 2007	63.38	86
1st March 2007	75.35	80
15th March 2007	89.35	95
1st April 2007	83.75	139
15th April 2007	87.37	156
1st May 2007	90.30	153
15 May 2007	82.50	228
1st June 2007	79.00	235

	Mentha spicata
Diameter of the hyphae in the outer cortes (um)	5
Diameter of the hyphae in the inner cortes (um)	2.5
Hyphae intercellular and parallel	-
Appressoria formed	-
Looped hyphae present	-
Coiled hyphae present	+
Vesicle present or not	+
Vesicle abundance (Number / mm of infected root)	15
Vesicle shape	Circular
Arbuscule abundance (Number / mm of infected root)	5-10
Types of hyphal junctions present	
H	-
Y	+

Table - 3: Percent colonized roots and AM fungal spore number in rhizosphere soil of *Mentha spicata* 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 (%)
32.8	200	7.00	50.60	0.10	1.5
35.38	182	7.15	52.76	0.11	1.9
47.34	127	7.05	50.20	0.15	1.7
63.38	86	7.02	48.79	0.19	1.7
75.35	80	8.00	56.64	0.24	1.9
89.35	95	8.15	56.802	0.92	1.9
83.75	139	7.90	82.940	0.96	1.3
87.37	156	7.44	85.55,	0.90	1.6
90.30	153	7.30	90.53	0.86	1.2
82.50	228	7.06	90.74	0.86	1.7
79.00	235	7.50	81.40	0.80	1.2

6. PHOTOS

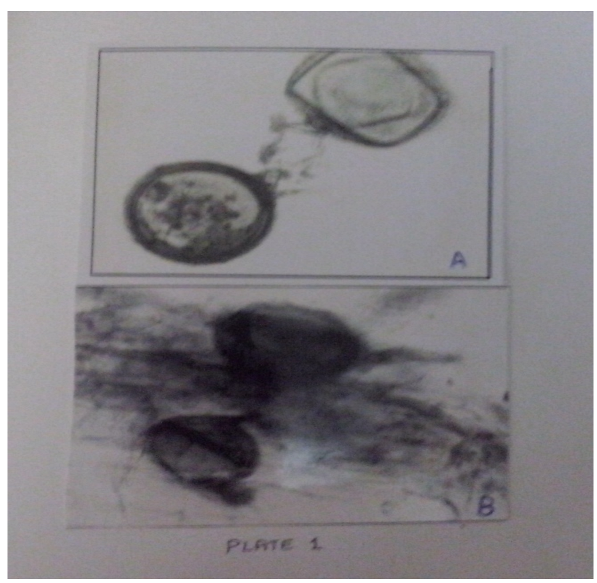

Photo 1:

A) Spores of *Glomus fasiculatum* isolated from the rhizosphere soil.
B) Spores of *Glomus fugianum* isolated from the rhizosphere soil.

Photo 2:
Mentha spicata growing in experimental plots.

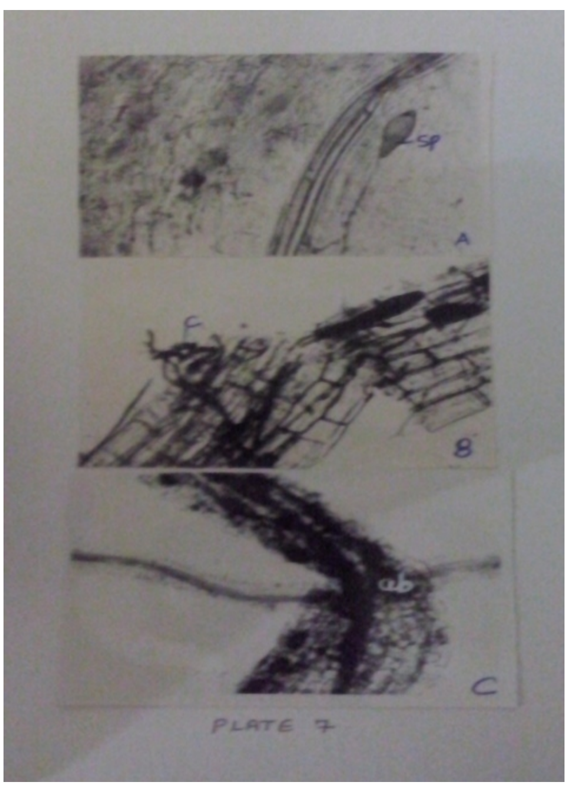

Photo 3:
AM colonisation in *Mentha spicata*

A) Elliptical spore germinating on the root surface (sp)
B) Root showing coiled hyphae (c)
C) Root showing arbuccules (ab

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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.