Monthly variation in percent root colonization and spore number in *Ocimum sanctum and Mentha arvensis*

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Abstract

Mycorrhiza is a mutualistic relationship between plant roots and fungal hyphae. Arbuscular mycorrhizal (AM) fungal associations in majority of terrestrial plants are universal. The present studies enlist the prevalence of AM fungal colonization in *Oscimum sanctum and Mentha arvensis*. Field studies were undertaken to screen AM fungal (AMF) association and isolate interesting AM fungal spores. Ten to fifteen plants have screened for AMF which were grown in experimental plots in Department of Botany, Sri Aurobindo college, Delhi University during August 2012 to August 2013. Altogether five indigenous AM fungal spores are recovered from this study. There was variation in the mycorrhizal colonization and spore number. Therefore, the present study revealed that the genus *Glomus* was more predominant than others and *Acuolospora* was least amongst the recovered AMF spores. The presence of fungal components in the roots and the spore number in the soil had brought possible mycorrhizal association with varied per cent of colonization in *Ocimum sanctum and Menthe arvensis.*

Keywords: Hyphae, Mycorrhiza, Root colonization, Spore number

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1. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are important component of rhizosphere microbial communities in natural ecosystems, forming symbiotic associations with the majority of land plant roots (15). It has been proved that arbuscular mycorrhizae (AM) can be found in almost all sorts of soils in different tropical, mild and cold habitats (18). The fungal hyphae spread into the soil from host plant roots and improve the efficiency of nutrient uptake, such as immobile phosphate ions (12, 13). Today, mycorrhizal symbiosis are found associated with more than 90% of terrestrial plants, distributed in all climates and ecosystems regardless of soil type, vegetation and environmental conditions (10). Colonization is restricted to root cortex and does not enter the vascular cylinder. In natural communities, approximately 80% of higher plants are obligatorily dependent on fungal associates and 18% typically non mycorrhizal (17). This is in contrast to the antagonistic interactions of plants and pathogenic fungi, with defense mechanism of Arbuscular mycorrhizal fungal relationship with plants which can increase the growth of plants by enhancing phosphate uptake mainly and perhaps the other minerals such as K, Fe, Cu, Ca and Zn (8).

2. MATERIAL AND METHOD

2.1 Study area and sample collection

Some plants of *Ocimum sanctum and Mentha arvensis* were grown under natural conditions in experimental plots of Department of Botany, Shri Aurobindo college, Delhi University and screened for the presence of arbuscular mycorrhiza in fine root segments at regular intervals. Rhizosphere soil was also collected every month to investigate the distribution of AM fungi in soil. Soil and plant samples were collected from surface to 30 cm depth. Root samples were fixed in FAA (100 ml alcohol, 100 ml distilled water, 13 ml formalin and 5 ml acetic acid) solution. They were transferred to laboratory and the fine roots in each sample were selected, removed and rinsed with tap water carefully for determination of root colonization. The soil samples

then were air dried in the shade at laboratory temperature for spore counting.

2.2 Analysis of Rhizosphere soil

Soil samples were collected from rhizosphere of *O. sanctum* and *M. arvensis* plants during August 2012 to August 2013. In the laboratory the rhizosphere soil was removed after washing the plants thoroughly with water, and the soil samples were analyzed for AM spore counts.

2.3 Counting of AMF spores

Spores were extracted from 10 g rhizospheric soil of each sample by wet sieving followed by floatation centrifugation in 50% sucrose (3). The spores were collected on a grid pattern filter paper and washed with distilled water to spread spores evenly over the entire grid. They were counted under a stereoscopic microscope (40×). The number of spores was expressed as the mean of three replicates.

2.4 Root colonization

Plants of *O. sanctum* and *M. arvensis* were harvested at regular intervals from experimental plots during August 2012 to August 2013. Minimum of ten to fifteen plants were collected at random at one time. Roots were rinsed with distilled water, cleared by 10% KOH, 30-45 min at 90°C and acidified in 1% HCl for 5-10 min. Then they were stained using Trypan Blue (0.05% in lacto-phenol) for 10 min. They were left in lacto-phenol at 90°C for 45 min for elimination of undesired dye particles. For quantification of AMF colonization, 70 one cm sections were selected randomly and left them on slides under microscope (80×) and percentage root colonization (PRC) was calculated according to *Phillips and Hayman (9)* procedure.

3. RESULT AND DISCUSSION

During the present investigation it was found that the degree of AM formation varied in all the plants studied. The colonization was higher in *Mentha arvensis* as compared to *Ocimum sanctum* **(Table 1 & 2).**The results of this investigation clearly show that all the plants of *Oscimum* and *Mentha* examined were mycorrhizal their type being arbuscular. In *Mentha arvensis* the cleared roots showed dense mycorrhizal colonization. Thick external hyphae, forming distinct appressoria at their entry points into the root tissue **(Plate II)**. The internal hyphae showed differentiation into intercellular arbuscules and intercellular vesicles. There are no projections present on the internal hyphae. In *Ocimum sanctum* the infection pattern was very similar to that of M. arvensis except that in the former the internal parallel hyphae was also observed. Root is densly colonized by external AM hyphae which showed thick external hyphae, forming distinct appressoria at their entry points into the root tissue and it also shows Y shaped connections. Stelar infection was also observed in this plant **(Plate III).** The experimental plots had an average 110 spores/5 g. of soil. The characteristic spores present in the soil samples were those of *Glomus mosseae, G. fasiculatum, G.macrocarpum, G.constrictum, G. fugianum and Acaulospora laevis.* **(Plate I)**

AM is prevalent in the field crops under optimal conditions (4,5,6,16).The present study confirms formation of similar arbuscular mycorrrhizae in field grown *Mentha arvensis and Ocimum sanctum* under the tropical soil conditions in India. Nearlly all the plants species and mycorrhizal infection ranging from 12% to 83% of their fine root length **(Table 1&2)**. This is in accordance with the results obtained by *Jagpal & Mukerji (7).*The endomycorrhizal colonization results from three simultaneous processes viz. root growth, formation of fungal entry points on the root surface and growth of fungal hyphae along the length of the interior of the root (15). Some plants become heavily colonized with the arbuscular mycorrhizae eg. Maize, onion, and clover, others become moderately infected under same conditions eg. Tomato (4) and yet others usually develop little infection eg. Rye grass. The host plant itself affects successful entry by the host endophytes. These endophytes spread much faster in some plants than in others and thus their final colonization level vary considerably in different hosts. But even within optimum host-endophyte combinations 100% infection is never achieved for eg. Strongly mycorrhizal plants like onion grown in pots, often reached a maximum of 80% of the total root length colonized (2). In the present study *Mentha arvensis* showed the total maximum root length colonization of 82%. The colonization by AM fungi was maximum during the month of July and August and minimum in Jan in *M. arvensis.* Although no definite pattern of colonization was observed during different months **(Table 1).** As compared to *M. arvensis* in *Ocimum sanctum* the highest level of colonization was observed in the month of May and minimum colonization in the month of August **(Table 2).** Further, the results indicate a definite correlation between level of mycorrhizal infection in the roots and the spore count of the soil around them. The more is the number of spores in the rhizosphere soil, the more is the degree of colonization.

Safir (11) observed similar pattern in the variation in number of spores in the rhizosphere of certain crops. He reported an initial decrease followed by a steady increase until harvest. Here, a routine monthly

examination to follow the structural development of endomycorrhizae in the host plant has shown that AM fungi reproduce only in association with the host roots. Senescing roots showed the release of thick walled resting spores into soil by decay of dead host root cells. Here, the results indicate a marked variation in the number of spores in different soil samples. The spore number/ 5 gm. Soil ranged from 94-278.

4. TABLES AND FIGURES

Month of Collection	% root Colonization	Spore number
October,2012	63.24	134
November,2012	67.77	111
December,2012	48.45	147
January,2013	12.12	94
February,2013	40.00	98
March,2013	48.97	128
April,2013	52.68	100
May,2013	68.00	120
June,2013	68.00	153
July,2013	82.50	258
August,2013	79.00	196

Table 1- Per cent colonized roots and AM fungal spore spore number in rhizosphere soil of *Mentha arvensis* at regular monthly interval.

Month of Collection	% root Colonization	Spore number
October,2012	52.45	138
November,2012	60.00	191
December,20112	16.00	132
January,2013	30.58	168
February,2013	32.48	153
March,2013	40.37	180
April,2013	44.20	99
May,2013	52.96	110
June,2013	51.00	140
July,2013	58.76	147
August,2012	76.47	278

Table 2- Per cent colonized roots and AM fungal number in rhizosphere soil of *Ocimum sanctum* at monthly interval.

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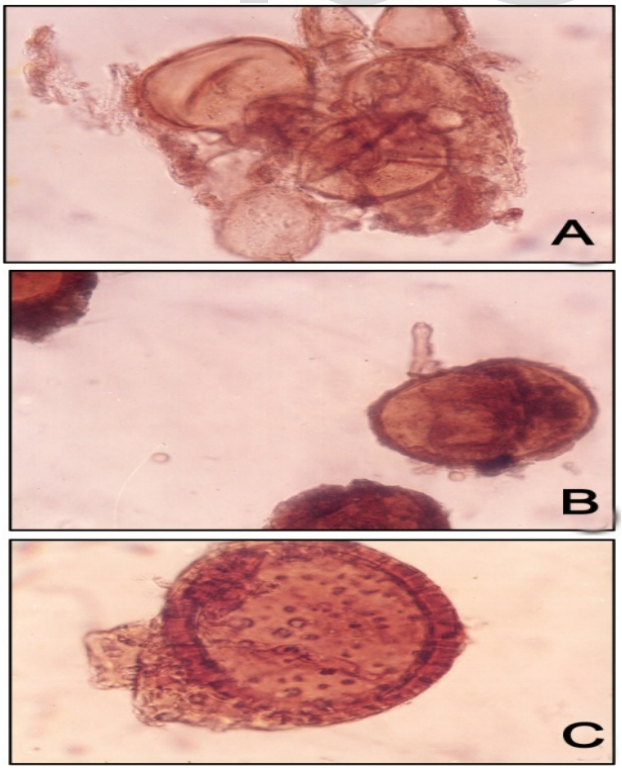

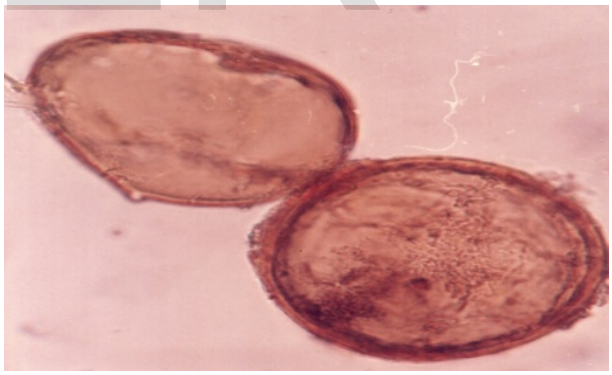

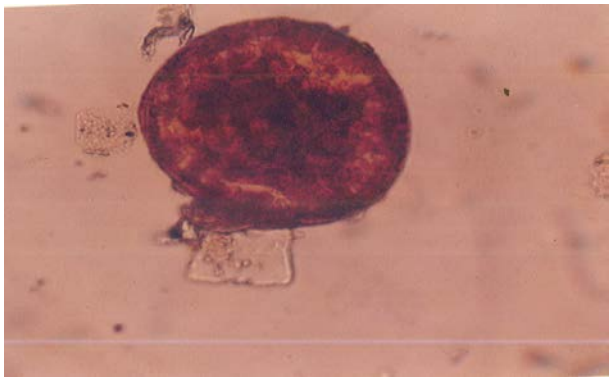

Plate I – (A-E) Showing some important arbuscular mycorrhizal fungal spores recovered from rhizosphere of *Ocimum sanctum and Mentha arvensis.*

A. Cluster of spores of *Glomus fugianum (Trappe and Gerd.)*
B. Spore of *Glomus constrictum*
C. Spore of *Glomus macrocarpum (Tul. And Tub.) Giorn*
D. Spore of *Glomus fasciculatum (Thaxter sensu Gerd.) Gerd. And Trappe*
E. Spore of *Acaulospora leavis (Gerdemann and Trappe)*

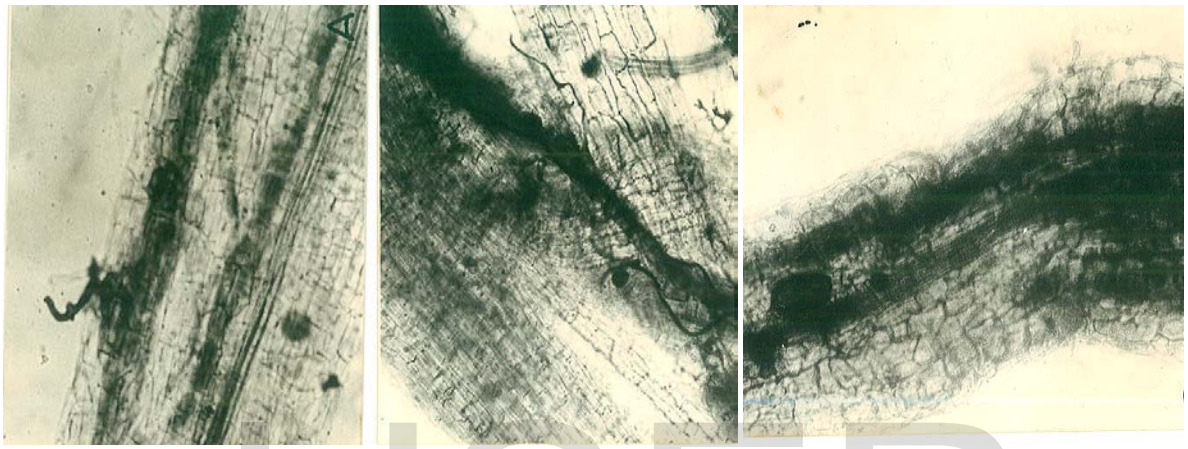

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Plate II - Showing arbuscular mycorrhizal fungal components in macerated root sections of *Mentha arvensis*

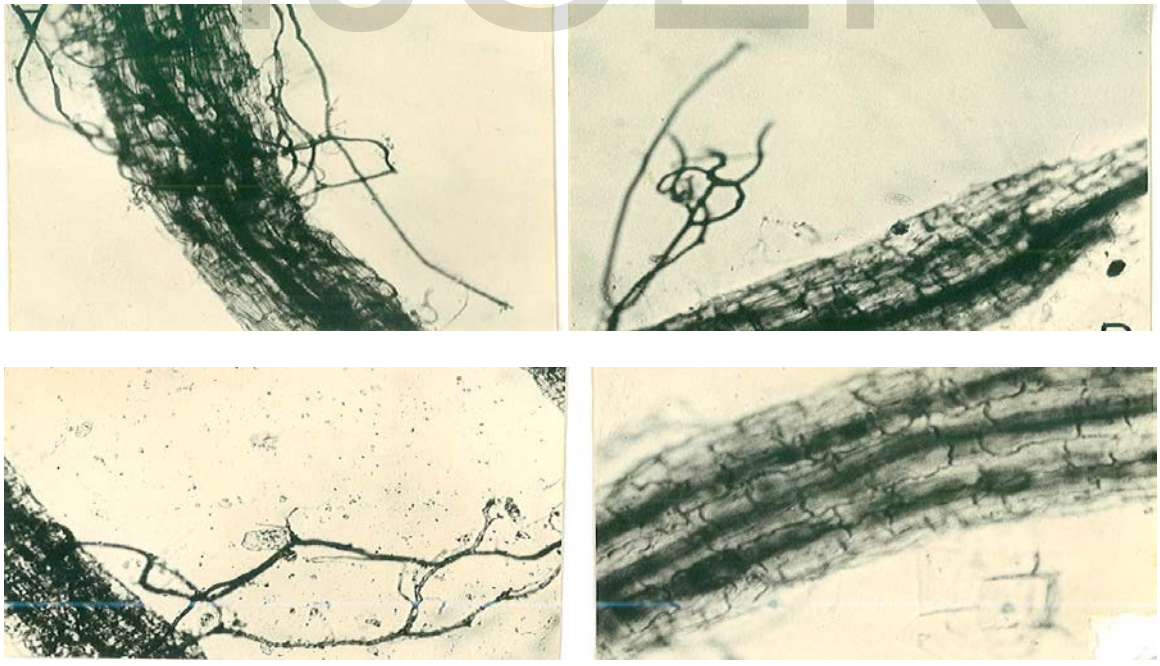

Plate III - Showing arbuscular mycorrhizal fungal components in macerated root sections of *Osimum sanctum*

5. CONCLUSION

The present work deals with the abundance of AM fungi in two plants species i.e *Mentha arvensis* and *Ocimum sanctum* of family *Labiatae* and its relationship with spore number. During the present investigation it was found that the degree of AM formation varied in all the plant studied. The colonization was higher in *Mentha arvensis* as compared to *Ocimum sanctum*. Results indicate a definite correlation between level of mycorrhizal infection in the roots and the spore counts of the soils around them. The more is the number of spores in the rhizosphere soil, the more is the degree of colonization.

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