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VA MYCORRHIZAL STATUS IN THE AROMATIC PLANTS GROWING UNDER NATURAL OR CULTIVATED CONDITIONS IN AND AROUND ALLAHABAD

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The present study has been undertaken to assess the magnitude of mycorrhization in the aromatic plants of commercial importance growing in and around Allahabad, under natural or cultivated conditions. A total of 15 species of aromatic plants belonging to 10 different genera were collected. Out of which, *Artemisia pallens* Wall. and *Tagetes erecta* L. belonged to family Asteraceae, *Mentha arvensis* DC., *M. piperata* L., *Ocimum basilicum* L. and *O. sanctum L.* to Lamiaceae, *Jasminum roxburghianum* Wallich and *J. sambac* (L.) Aiton to Oleaceae, *Pandanus odoratissimus* L.f. to Pandanaceae, *Cymbopogon flexuosus* Stapf., *C. martinii (Roxb.)* Wats., *C. wintarianus* Jowitt and *Vetiveria zizanioides (L.)* Nash to Poaceae and *Citrus aurantifolia* Swingle and *Murraya koenigii* (L.) Spreng. to Rutaceae. In the present study, all the aromatic plants were found to be mycorrhizal, however, wide variations were observed in the intensity of root infection and sporulation. Percent root bit infection in the aromatic plants ranged from 20 to 72%. Maximum percent root bit infection was recorded in *Tagetes erecta* L. and minimum in *Artemisia pallens* Wall.

Keywords : Aromatic plants; Diversity; Mycorrhizal status; VAM Fungi.

Introduction

Economic significance of the aromatic plants can be traced by the fact that the aroma chemicals contained in them play a vital role in our day to day living as spices, condiments and food flavoring agents, perfumes, *'ittars'* and deodorants, drugs and ointments, gum exudates and oleoresins, antibacterial, germicidal and insecticidal agents *etc.* Because of such vast and varied applications, essential oils are in consistent demand all over the world, especially in developed countries, like EU, USA, China, Japan, East European countries etc.

Application of some native biological input such as arbuscular mycorrhizal (VAM) fungi is relatively a new approach and promising approach for improving the performance of the crops. Plants exhibit considerable dependence on mycorrhizal association for an adequate supply of nutrients and water, enabling them to thrive even under stress conditions. In a number of recent reports it has been well established that the VAM fungi enhance the ability of plants to cope with environmental stresses, generally prevalent in the degraded ecosystems, by providing a number of nutritional and physiological benefits[1-4].

The present study has been undertaken to determine the mycorrhizal dependency of some of the aromatic plants so that they may be exploited to perform well under alkaline/sodic soils of Allahabad, Uttar Pradesh, India.

Material and Methods

Population and diversity of VAM fungi associated with the aromatic plants-A systematic survey of some aromatic plants growing under natural or cultivated conditions in and around Allahabad was undertaken during 2004-2005 to assess the population and diversity of VA mycorrhizal fungi associated with them and to determine the intensity of VA mycorrhizal association in their roots.

Sample Collection: Root samples of the aromatic plants along with their rhizospheric soils were collected. Samples of at least three individuals per plant species were collected and mixed. Samples were brought back to the laboratory and the roots of the plants along with the fine roots present in the rhizospheric soils were washed with tap water and processed for the determination of root colonization. Soil samples were air dried in shade at room temperature and sieved for the estimation of VAM spore population and diversity.

Estimation of VAM Association in the Roots: Intensity of VAM colonization in the root samples was determined by the method given by Philips and Hayman[5]. For quantification of VAM colonization 100 root bits were mounted on slides (10 per slide) in lactophenol and examined under a compound microscope (CH20*i*, Olympus). Percent root bits colonized was calculated and the percent root length colonization was assessed by evaluating the colonized root tissue as a proportion of the total length of observed roots.

Determination of VAM Spore Population: VAM spore population was determined in 20g air-dried soil in triplicates for each sample by wet sieving and decanting method[6]. Soil solution was passed through the sieves of 500μm, 210μm, 150μm, 90μm and 60μm in descending order. VAMF spores were transferred on filter papers, which were counted under a stereoscopic binocular at 20x magnification. Number of spores was expressed as the mean of three replicates.

Identification of VAM Fungi: VAMF spores were mounted in PVLG and PVLG + Melzer's reagent (1:1 v/v) and identified to the species level using the synoptic keys of Trappe[7], Schenck and Perez[8] and INVAM species guide (http//:invam.caf.wvu.edu).

Results and Discussion

In order to assess the magnitude of mycorrhization in the aromatic plants of commercial importance growing in and around Allahabad, under natural or cultivated conditions, a systematic survey was undertaken. A total of 15 species of aromatic plants belonging to 10 different genera were collected. Out of which, *Artemisia pallens* Wall. and *Tagetes erecta* L. belonged to family Asteraceae, *Mentha arvensis* DC., *M. piperata* L., *Ocimum basilicum* L. and *O. sanctum* L. to *Lamiaceae, Jasminum roxburghianum* Wallich and *J. sambac* (L.) Aiton to Oleaceae, *Pandanus odoratissimus* L.*f.* to Pandanaceae, *Cymbopogon flexuosus* Stapf., *C. martinii* (Roxb.) Wats., *C. wintarianus* Jowitt and *Vetiveria zizanioides* (L.) Nash to Poaceae and *Citrus aurantifolia* Swingle and *Murraya koenigii* (L.) Spreng. to Rutaceae.

VAM intensity in these plants was measured in terms of VAM association in their roots and the VAM spore population and diversity in the rhizospheric soils. The data on VAM intensity in the aromatic plants have been represented in Table 1 and diversity of VAM fungi associated with aromatic plants in Table 2.

VAM Association in the Roots of Aromatic Plants : VAM association in the roots of aromatic plants was estimated in terms of percent rootbits infected and percent root length colonized by VAM fungi. All the aromatic plants surveyed were found to be mycorrhizal, however, the extent of mycorrhization and the type of VAM infection varied with the plant species.

Percent rootbit infection in the aromatic plants ranged from 20 to 72%. Maximum percent rootbit infection was recorded in *Tagetes erecta* L. and minimum in *Artemisia pallens* Wall. Similar trend was observed in case of percent root length colonization, which ranged from 8 to 62%. Here also maximum percent root length colonization was recorded in *Tagetes erecta* L. and minimum in *Artemisia pallens* Wall.

Based on the extent of VAM association, aromatic plants were categorized into three different groups. *Cymbopogon flexuosus* Stapf., *Pandanus odoratissimus* L.*f., Tagetes erecta L.* and *Vetiveria zizanioides* (L.) Nash showed high VAM intensity, where percent rootbit infection was more than 60%. *C. martinii* (Roxb.)Wats., *C. wintarianus* Jowitt, *Jasminum roxburghianum* Wallich, *J. sambac* (L.) Aiton. *Mentha arvensis* DC. and *Ocimum sanctum* L. showed moderate VAM intensity, where percent rootbit infection ranged between 40 to 60%. However, *Artemisia pallens* Wall., *Citrus aurantifolia* Swingle, *Mentha piperata* L., *Murraya koenigii* (L.) Spreng. and *Ocimum basilicum* L. showed low VAM intensity, where percent root bit infection was less than 40%.

In case of *Murraya koenigii* (L.) Spreng. only hyphal infection was observed, whereas, in *Artemisia pallens* Wall., *Mentha arvensis* DC. and *Tagetes erecta* L. only vesicular infection was observed. Hyphal as well as vesicular infection was recorded in *Citrus aurantiifolia* Swingle, *Cymbopogon flexuosus* Stapf., *C. martinii* (Roxb.) Wats., *Jasminum roxburghianum* Wallich, *J. sambac* (L.) Aiton, *Mentha piperata* L., *Ocimum basilicum* L., *Ocimum sanctum* L. and *Pandanus odoratissimus* L.*f.* However, in case of *Cymbopogon wintarianus* Jowitt and *Vetiveria zizanioides* (L.) Nash only hyphal, vesicular as well as arbuscular infection was observed (Fig. 4 and 5).

VAM Spore Population and Diversity in the Rhizospheric Soils of the Aromatic Plants : A positive correlation between the VAM association in the roots and the VAM spore population in the rhizospheric soils of the aromatic plants was observed. VAM spore population in the rhizospheric soils of the aromatic plants ranged from 8 to 44 VAM spores/10g air dried soil. Maximum spore population was recorded in *Cymbopogon flexuosus* Stapf. and minimum in *Artemisia pallens* Wall.

A total of 34 species belonging to four different genera of VAM fungi were isolated as the most dominant forms associated with the aromatic plants. *Glomus* was recorded as the most dominant genus with 24 species, *viz. G. aggregatum, G. australe, G. caledonium, G.*

Table 1. Mycorrhizal intensity in the roots and VAM spore population in the rhizospheric soils of aromatic plants growing under natural or cultivated conditions in and around Allahabad.

Aromatic Plants	Mycorrhization			
	VAM Association			VAM Spore Population (/10g air dried soil)
	% Rootbits Infected	% Root Length Colonization	Type of Infection	
Artemisia pallens Wall.	20	8	V	8
Citrus aurantifolia Swingle	38	26	H, V	15
Cymbopogon flexuosus Stapf.	68	55	H, V	44
Cymbopogon martinii (Roxb.) Wats.	46	31	H, V	26
Cymbopogon wintarianus Jowitt	52	40	H, V, A	30
Jasminum roxburghianum Wallich	56	48	H, V	20
Jasminum sambac (L.) Aiton	48	40	H, V	26
Mentha arvensis DC.	48	34	V	24
Mentha piperata L.	33	23	H, V	12
Murraya koenigii (L.) Spreng.	25	18	H	9
Ocimum basilicum L.	34	21	H, V	18
Ocimum sanctum L.	42	32	H, V	27
Pandanus odoratissimus L. f.	67	52	H, V	35
Tagetes erecta L.	72	62	V	38
Vetiveria zizanioides (L.) Nash	70	53	H, V, A	41

H: Hypahae, V: Vesicle, A: Arbuscule

constrictum, G. dimorphicum, G. geosporum, G. intraradices, G. invermaium, G. mosseae, G. tortuosum and 14 unidentified species named *Glomus* sp. AVK1 to AVK14, followed by *Acaulospora* with six species, viz. *A. appendiculata, A. delicata, A. laevis* and three unidentified species named *Acaulospora* sp. AVK1 to AVK3, *Sclerocystis* with three species, *viz. S. pachycaulis, S. sinuosa* and an unidentified species named *Sclerocystis* sp. AVK1, and *Gigaspora* with a single unidentified species named Gigaspora sp. AVK1 (Fig. 1-3).

Glomus mosseae was recorded as the most frequent VAM fungi associated with all the aromatic plants, except *Cymbopogon martinii* (Roxb.) Wats., *C. wintarianus* Jowitt, *Jasminum sambac (L.)* Aiton, *Ocimum basilicum L., Pandanus odoratissimus* L.f. and *Tagetes erecta* L., followed by *G. aggregatum* and *G. invermaium.* In case of genus *Acaulospora, A. laevis* was the most frequent species associated with *Cymbopogon flexuosus* Stapf., *C. martini* (Roxb.) Wats., *Jasminum sambac* (L.) Aiton, *Tagetes erecta* L. and *Vetiveria zizanioides* (L.) Nash. However, in case of genus *Sclerocystis, S. sinuosa* was the most frequent species associated with *Cymbopogon flexuosus* Stapf., *C. wintarianus* Jowitt, *Mentha arvensis* DC. and *Pandanus odoratissimus* L.f.

Likewise the least frequent VAM fungal species among the aromatic plants were *Gigaspora* sp. AVK1 associated with *Murraya koenigii* (L.) Spreng. only, *Glomus* sp. AVK6 associated with *Pandanus odoratissimus* L.f. only and *Sclerocystis* sp. AVK1 associated with *Cymbopogon wintarianus* Jowitt only. However, in case of genus *Acaulospora, Acaulospora* sp. AVK1 associated with *Artemisia pallens* Wall. And *Ocimum basilicum* L. and *Acaulospora* sp. AVK3 associated with *Jasminum sambac* (L.) Aiton and *Pandanus odoratissimus* L.f. were the least frequent species.

In the present study, all the aromatic plants were found to be mycorrhizal, however, wide variations were observed in the intensity of root infection and sporulation[9-10]. These variations in the mycorrhizal status of different plant species could be attributed to several factors related to the host symbiont, soil or environment[11-16]. However, since factors related to soil, symbiont or environment were more or less uniform for all the aromatic plants included in the present study, factors related to host plants seem to be mainly responsible for the variation in mycorrhizal status[17].

A comparison of the mycorrhizal infection and sporulation in different aromatic plants included in the present study shows that a direct correlation between the two was lacking[18]. In a number of plant species, heavy root infection was coupled with low sporulation or a low root infection with heavy sporulation. Only in few cases, the magnitude of the root infection and sporulation was of a similar order, while the root infection is related to the vegetative phase of the endophyte, the sporulation to its

Table 2. Diversity and distribution of dominant VAM fungi in the rhizospheric soils of the aromatic plants growing under natural or cultivated conditions in and around Allahabad.

VAM Fungi/Aromatic Plants	VAM Diversity														
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
Acaulospora appendiculata Sieverding & Schenck	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+
Acaulospora delicata Walker, Pfeiffer & Bloss	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-
Acaulospora laevis Gerdemann & Trappe	-	-	+	+	-	-	+	-	-	-	-	-	-	+	+
Acaulospora sp. AVKA1	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Acaulospora sp. AVKA2	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-
Acaulospora sp. AVKA3	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
Gigaspora sp. AVKGI1	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Glomus aggregatum Schenck & Smith	+	-	-	-	-	+	-	+	+	+	-	-	+	-	-
Glomus australe Berch	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-
Glomus caledonium Nicolson & Gerdemann	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-
Glomus constrictum Trappe	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-
Glomus dimorphicum Boyetchko & Tewari	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
Glomus geosporum (Nicolson & Gerdemann) Walker	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
Glomus intraradices Schenck & Smith	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
Glomus invermaium Hall	-	+	-	-	-	+	-	-	+	-	+	-	-	-	+
Glomus mosseae Nicolson & Gerdemann	+	+	+	-	-	+	-	+	+	+	-	+	-	-	+
Glomus tortuosum Schenck & Smith	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+
Glomus sp. AVKGL1	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Glomus sp. AVKGL2	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Glomus sp. AVKGL3	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Glomus sp. AVKGL4	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Glomus sp. AVKGL5	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-
Glomus sp. AVKGL6	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Glomus sp. AVKGL7	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
Glomus sp. AVKGL8	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
Glomus sp. AVKGL9	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-

(Contd.)

Table 2. Diversity and distribution of dominant VAM fungi in the rhizospheric soils of the aromatic plants growing under natural or cultivated conditions in and around Allahabad

VAM Fungi/Aromatic Plants	VAM Diversity														
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
Glomus sp. AVKGL10	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
Glomus sp. AVKGL11	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
Glomus sp. AVKGL12	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Glomus sp. AVKGL13	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
Glomus sp. AVKGL14	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
Sclerocystis pachycaulis Wu & Chen	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Sclerocystis sinuosa Gerdemann & Bakshi	-	-	+	-	+	-	-	+	-	-	-	-	+	-	-
Sclerocystis sp. AVKS1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

P1: *Artimisia pallens* Wall.
P2: *Citrus aurantifolia* Swingle
P3: *Cymbopogon flexuosus* Stapf.
P4: *Cymbopogon martinii* (Roxb.) Wats.
P5: *Cymbopogon wintarianus* Jowitt
P6: *Jasminum roxburghianum* Wallich
P7: *Jasminum sambac* (L.) Aiton
P8: *Mentha arvensis* DC.
P9: *Mentha piperata* L.
P10: *Murraya koinengii* (L.) Spreng.
P11: *Ocimum basilicum* L.
P12: *Ocimum sanctum* L.
P13: *Pandanus odoratissimum* L.f.
P14: *Tagetus erecta* L.
P15: *Vetiveria zizanioides* (L.) Nash

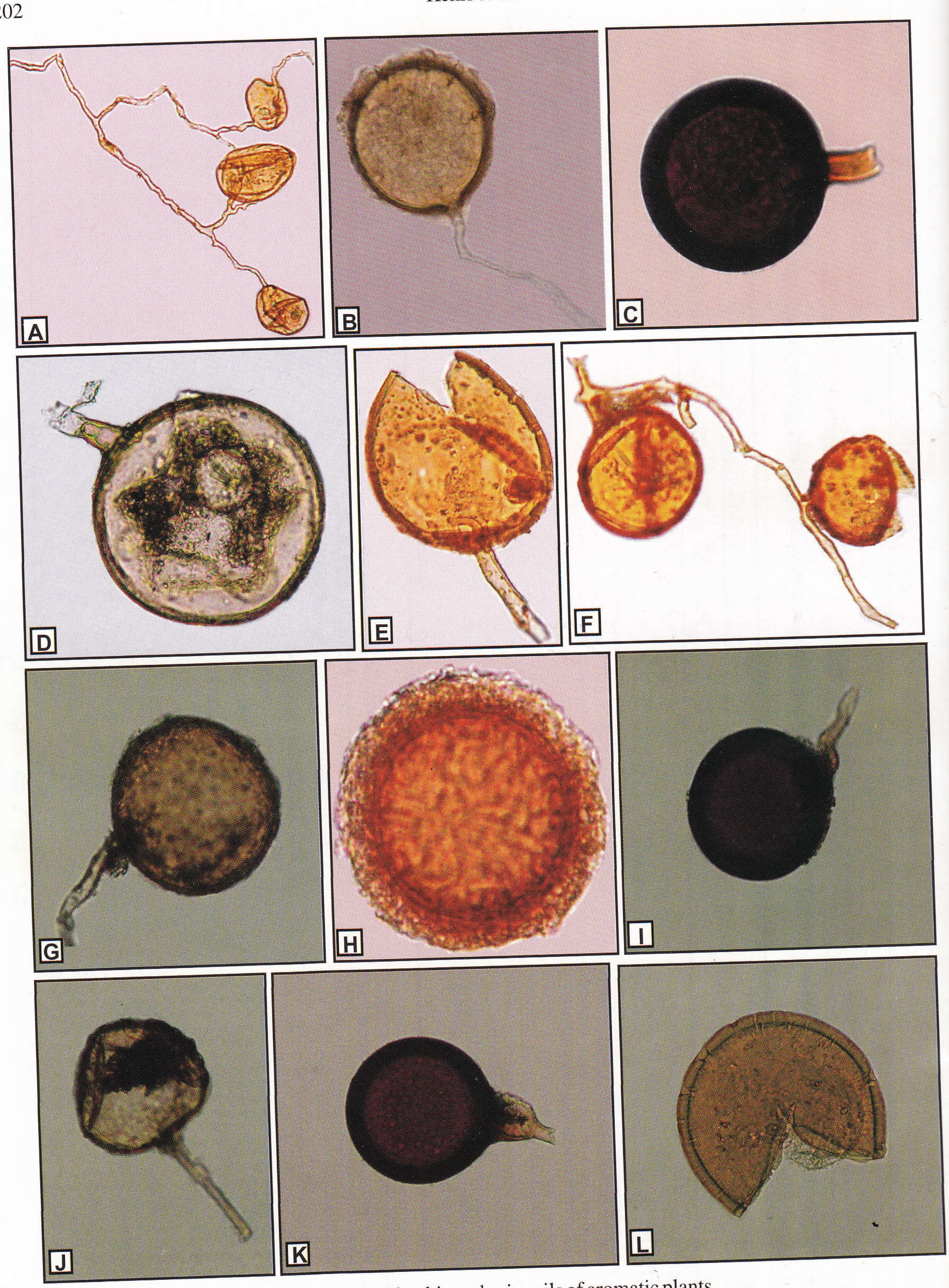


Fig.1. Dominant VAM spores isolated from the rhizospheric soils of aromatic plants. *A. Glomus aggregatum* Schenck & Smith; B. *Glomus mosseae* Nicolson & Gerdemann, C. *Glomus australe* Berch D. *Glomus caledonium* Nicolson & Gedemann; E. *Glomus intraradices* Schenck & Smith; F. *Glomus dimorphicum* Boyetchko & Tewari; G. Glomus sp. AVKGL2; H. *Glomus tortuosum* Schenck & Smith; I. *Glomus* sp. AVKGL1; J. *Glomus* sp. AVKGL4; *K. Glomus* sp. AVKGL3; L. *Acaulospora* sp. AVKA3.

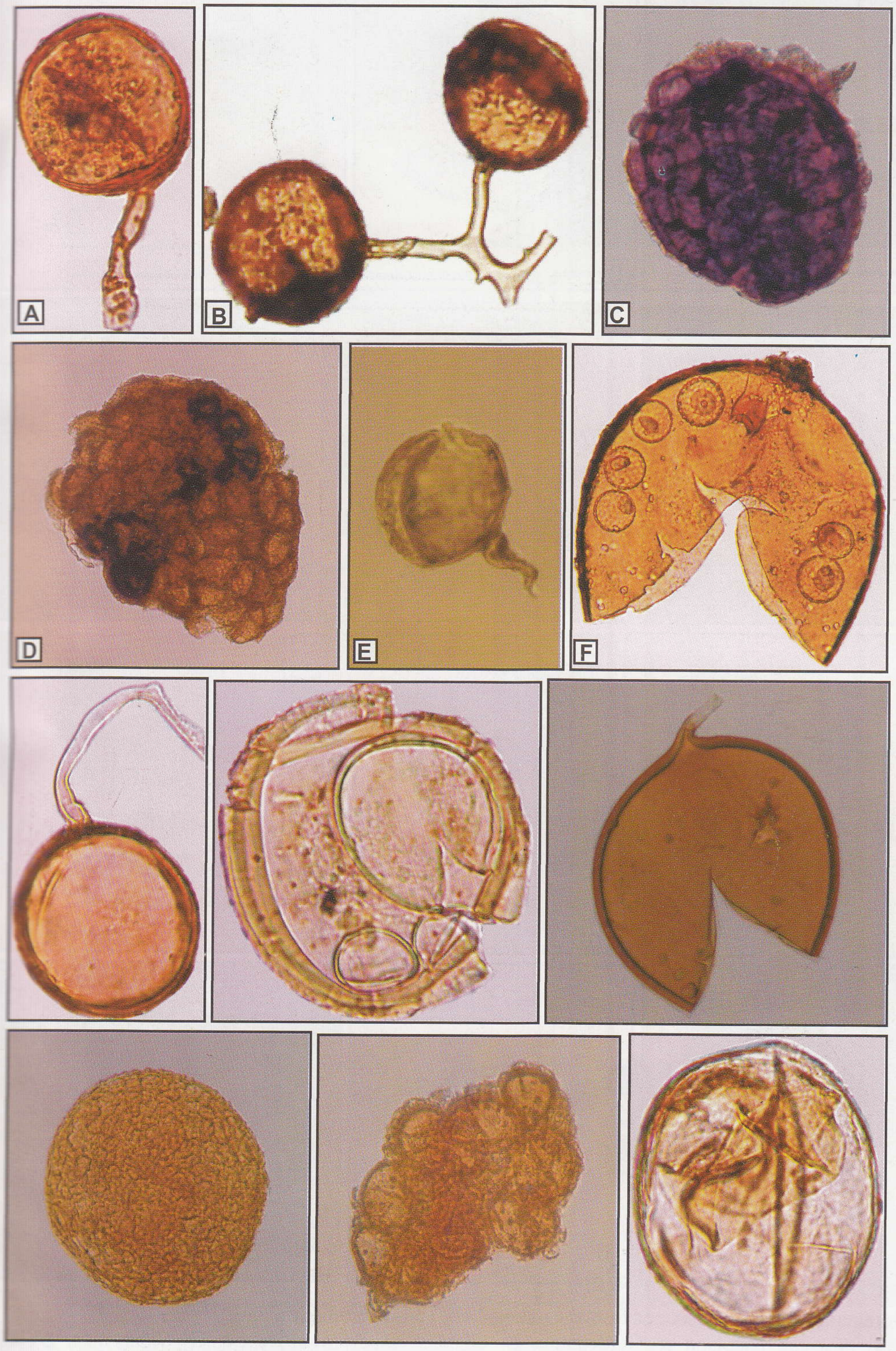
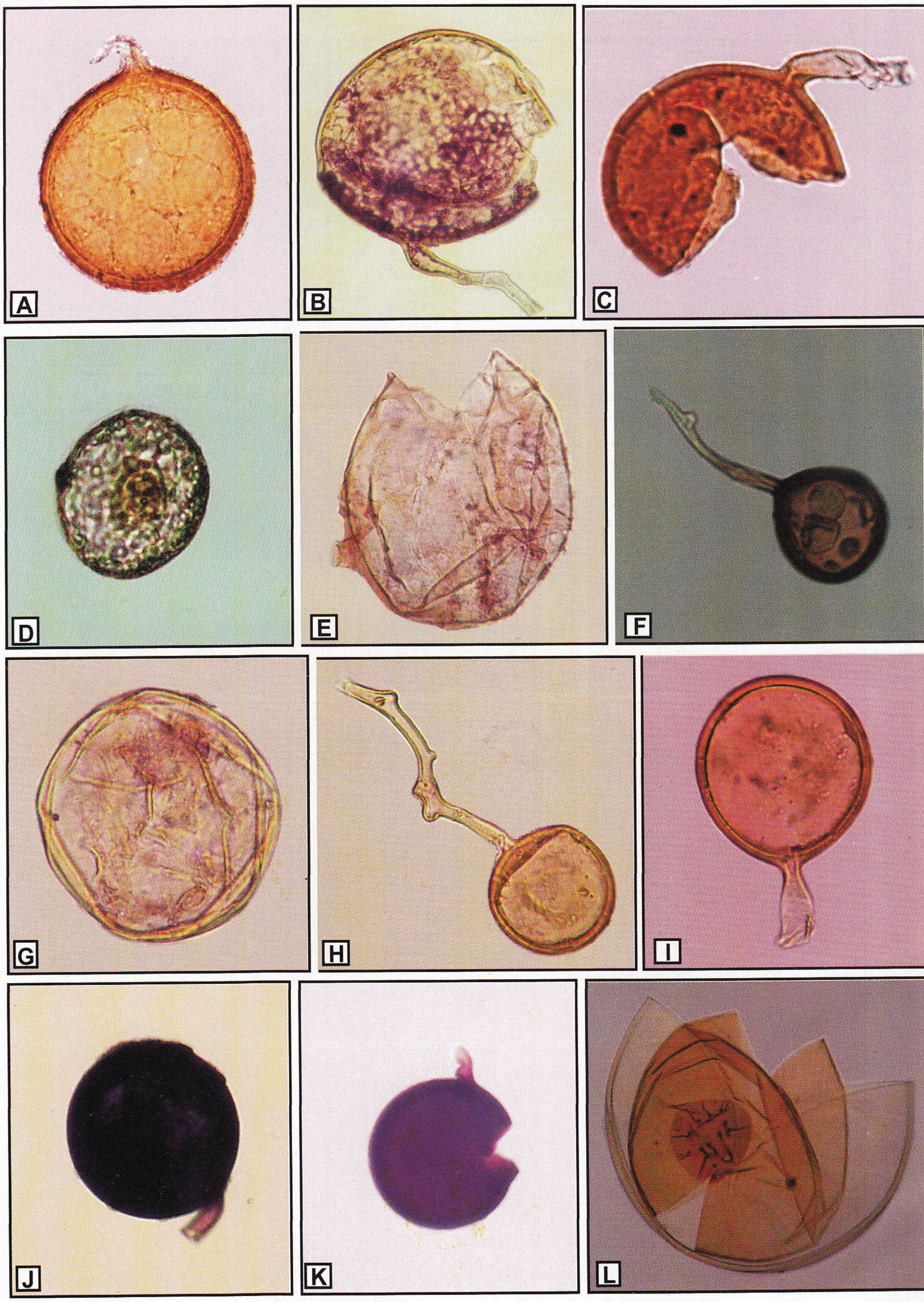


Fig. 2. Dominant VAM spores isolated from the rhizospheric soils of aromatic plants.
A. *Glomus* sp. AVKGL6; B. *Glomus* sp. AVKGL5; C. *Sclerocystis pachycaulis* Wu & Chen; D. *Sclerocystis sinuosa* Gerdemann & Bakshi; E. *Gigaspora* sp. AVKGI1; F. *Glomus* sp. AVKGL7; G. *Glomus* sp. AVKGL8; H. *Acaulospora* sp. AVKA1; I. *Glomus geosporum* (Nicolson & Gerdemann) Walker; J. *Acaulospora appendiculata* Sieverding & Schenck; K. *Sclerocystis* sp. AVKS1; L. *Acaulospora* sp. AVKA2.



Fig, 3. Dominant VAM spores isolated from the rhizospheric soils of aromatic plants.
A. *Glomus mosseae* Nicolson & Gerdemann; B. *Glomus constrictum* Trappe; C. *Glomus* sp. AVKGL9; *Acaulospora appendiculata* Sieverding & Schenck; E. *Glomus* sp. AVKGL12; F. *Glomus* sp. AVKGL10; *Acaulospora delicata;* H. *Glomus* sp. AVKGL11; I. *Glomus invermaium* Hall; J. *Glomus* sp. AVKGL13; K. *Glomus* AVKGL14
L. *Acaulospora laevis* Gerdemann & Trappe.

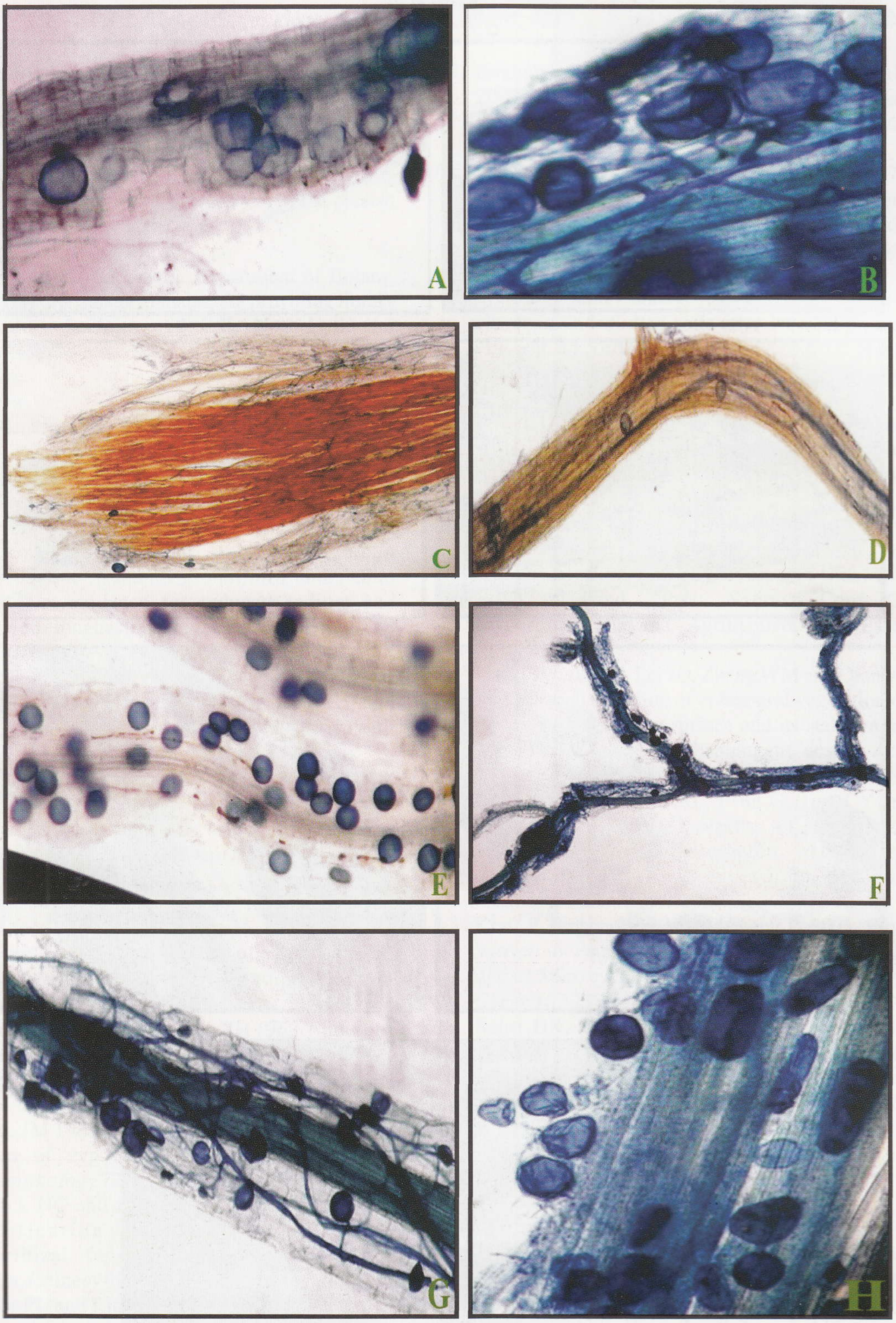
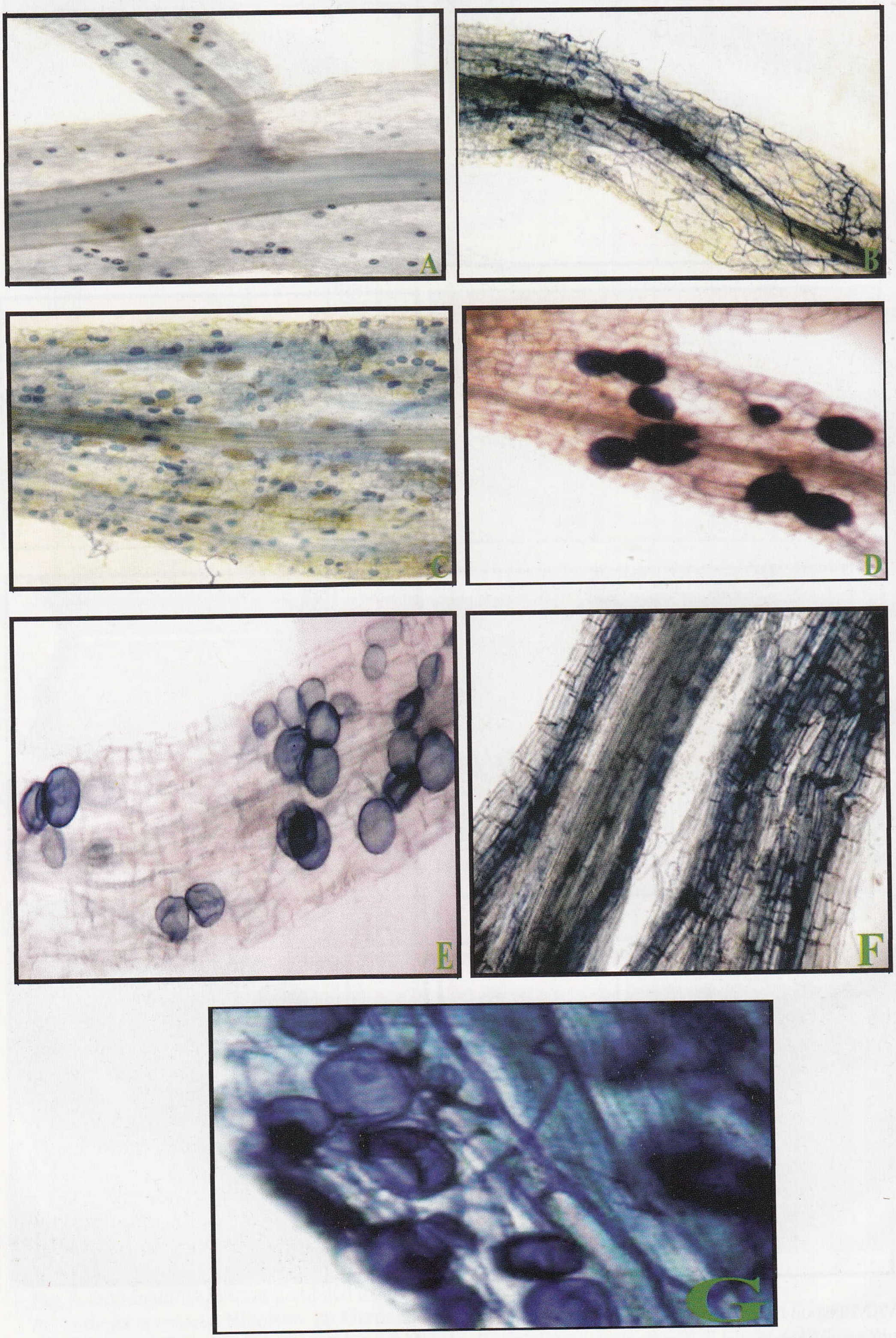


Fig. 4. VAM association in the roots of aromatic plants.
A. *Artemisia pallens* Wall.; B. *Cymbopogon martinii* (Roxb.) Wats.; C. *Jasminum sambac* (L.) Aiton.; D. *Jasminum roxburghianum* Wallich.; E. *Tagetus erecta* L.; F. *Cymbopogon wintarianus* Jowitt.; G. *Cymbopogon flexuosus* Stapf.; H. *Vetiveria zizanioides* (L.) Nash.



Fig,5. VAM association in the roots of aromatic plants.
A. *Mentha arvensis* DC.; B. *Mentha piperata* L.; C. *Pandanus odoratissimum* L.f.; D. *Ocimum sanctum* L; E. *Ocimum basilicum* L.; F. *Murraya koenigii* (L.) Spreng.; G. *Citrus aurantifolia* Swingle.

reproductive potential. Being the divergent phases of the life, the specific requirements for optimum expression of the two are expected to be different. This might have been the probable reason for the lack of a direct correlation between the level of root infection and spore production by the endophyte as observed in the present study[2-3].

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