Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
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http://ansinet.com

Asian Journal of Plant Sciences
Volume 1 Number 4: 383-387, 2002

Studies on Mycorrhizal Association in Some Medicinal Plants of Azad Jammu and Kashmir

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Abstract: Seventy six medicinal plants were investigated for vesicular-arbuscular mycorrhizal (VAM) association in a survey of the Azad Jammu and Kashmir. Vesicular-arbuscular mycorrhiza was found to be of universal occurrence in all plants located at different habitats. Great variations were found in the VAM infection percentage and the extent of hyphal infection. Plants at vegetative stage exhibited more VAM infection percentage compared to those at flowering and fruiting stages. Herbaceous plants showed more infections in comparison with the shrubby and woody plants. The extent of root colonization by VA endohphytes varied with the soil type and plant species. Endophytes other than VA were also recorded.

Key words: VAM infections, vesicular-arbuscular mycorrhizae, medicinal plants, Azad Kashmir

Introduction

The vesicular-arbuscular mycorrhizae are ubiquitous in roots of vascular plants in nature. (Harley and Smith, 1983; Powel and Bagyaraj, 1986; Gabor, 1992). They are common and wide spread among the plants of great economic importance growing on most of the soils (Nicolson, 1959). This wide distribution of VAM is due to obligate mycotrophy of the plants (Trappe, 1987). Indigenous nature to soil and distribution patterns of Endogonaceous fungi. The VAM fungi helps the plant in uptake of P, N, Zn, K, Cu, Sr, S and other mineral elements (Powel and Bagyaraj, 1984). The benefit of VAM fungi is due to an increased zone of nutrient depletion by mycorrhizal roots as compared to non-mycorrhizal ones (Owusu Bennoach and Wild, 1979).

The state of Azad Jammu and Kashmir is very rich in medicinal and aromatic plants. These medicinal plants occur naturally and most of them propagate vegetatively by under ground rhizomes, stems, bulbs or corms. This herbal wealth is being used not only by developing countries but also by developed countries for their health care systems. A bulk of our rural population relies on drug resources of plant origin. Locally collected plants are sold, where they are exploited commercially for preparation of medicines. The medicinal plants from some areas of Azad Jammu and Kashmir have been described by Haq and Hussain (1995), Bukhari (1996), Dastagir (2001), Shahzad (2001), Gorsi and Shahzad (2002).

However, no survey has so far been conducted on the mycorrhizal association of medicinal plants of Azad Kashmir. Therefore, an intensive survey of different areas of Azad Jammu and Kashmir was conducted in a preliminary attempt to observe the mycorrhizal associations of these plants.

Materials and Methods

The medicinal plant species were sampled from different areas of Azad Jammu and Kashmir during the period 1999-2000. The areas of sampling included Bhimber, Chapper, Chikar, Chinari, Dhir Kot, Dinour, Leepa, Muzaffarabad, Pattika, Mirpur, Kotli, Punjeri, Afzalpur, Sudhen Gali, Bagh, Hevali and Nikyal. The plants were carefully dug up along with their rhizospheric soil in triplicate and transported to the laboratory in polythene bags. Plant roots were gently washed under tap water to remove soil particles. These roots were fixed in FA (formalin, acetic acid, rectified spirit, 5:5:90 by volume). These roots were cleared in 10% KOH by simmering at 90 °C for 1 hour. The KOH cleared roots were then stained in lactophenol following the procedure of Phillips and Hayman (1970). Stained roots were cut up into 1cm long pieces. Ten such stained root pieces were mounted in lactic acid on a microscope slide and were studied for VA mycorrhizal infections under the microscope. The extent of VA infections was calculated 100 cm^{-1} of roots.

Plants were pressed in presser papers and were dried carefully. The collected plants were identified with the help of available literature (Nasir and Ali, 1970-1987). Further identification was done by comparing the collected plant specimens with the Herbarium specimens available at the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad.

Results and Discussion

All medicinal plants in the present survey were found to be VA mycorrhizal (Table 1), despite the fact that they have an active principle in them, which is responsible for their medicinal value. Many of the medicinal plants have their active principle in under ground plant parts e.g., *Arisaema jacquemontii* and *Valeriana wallichii* (Table 1). Most probably, the mycorrhizal fungi can resist the presence of such compounds which the other common fungi can not. Earlier, the presence of VA mycorrhizal fungi has been observed in medicinal rhizomes (Iqbal and Nasim, 1986) and medicinal bulbs (Iqbal and Firdaus-e-Bareen, 1986).

The extent of root colonization by VA endophytes varied with the soil types and plant species. The percentage of VAM infections in the investigated plant species ranged from 10 to 98. Septate and aseptate hyphae of *Atropa belladona* forming coils (Fig. 1) and clamp -connections of *Solanum surattense* (Fig. 2) were observed in the root samples. Extent of aseptate hyphal infections varied from 11.17 to 95.57 100 cm^{-1}, while the range of septate hyphal infections was 1.02 to 87.05 100 cm^{-1}. Aseptate hyphae were counted more than septate hyphae. Profusely spread hyphae with average diameter ranging from 1-26 μ were noticed in most of the root samples. Two types of appressoria, dichotomously branched and single stranded with characteristic pegs entering the root cortex were also observed in *Ajuga bracteosa* (Fig. 3). Plants at vegetative stage were noted with higher VAM infection percentage than at flowering and fruiting stages. Vesicular and arbuscular infections were predominant at vegetative stage, while flowering with vesicles only which after fructification give rise to spores. Higher number of spores and low extent of hyphae were observed in plants at fruiting stage. Moreover herbaceous plants exhibited more VA mycorrhizal infection than shrubby and woody plants. Plants of woody nature restrict mycorrhizal infection frequently to herbaceous stages. This is probably due to the incorporation of mycorrhizal infection during more active stage, the herbaceous stage.

Vesicular infections ranging from 10 to 96% was of common occurrence, whereas arbuscular infection ranged from 10 to 60% and was less frequently observed. Vesicles of various dimensions (60-900 μ^2) and shapes i.e., spherical; *Datura alba* (Fig. 11) oval; *Indigofera trifoliata* (Fig. 8) rounded, oval-lobed, elongated; *Ocimum basilicum* (Fig. 4) dumbell-shaped and V-shaped were found in the root cortical cells. In some cases vesicles and spores of the genus *Glomus*, the most frequent VA mycorrhizal associate and most common of soil borne fungi were found intermixed within root cells. Terminal *Eucalyptus* sp. (Fig. 5) and intercalary vesicles containing oil droplets were frequently observed in many samples. Dichotomously branched arbuscules were seen in *Xanthium strumarium* (Fig. 6) and in some other plants. Collapsed network of arbuscules was also found in the root cortical cells.

Table 1: Vesicular arbuscular mycorrhizal status of some medicinal plants of Azad Kashmir

						Vesicular arbuscular mycorrhizal infection				
									Extent of hype 100 cm^{-1}	
Plant species	Family	Locality	Age	Habit	Soil type	General VAM infection %	Vesicular infection%	Arbuscular infection%	Aseptate hyphae	septate hyphae
Acacia modesta Wall.	Mimosaceae	Bhimber	Veg.	Tree	Sandy loam	40	20	40	37.25	7.55
			F1.	Tree	Sandy loam	60	60	0	10.22	44.11
Acacia nilotica (L.) Delile.	Mimosaceae	Bhimber	Fl.	Tree	Loamy sand	30	0	30	64.45	0
Achyranthes aspera L.	Amaranthaceae	Bhimber	Fr.	Herb	Sandy loam	30	30	0	80.45	0
Adiantum capillus-veneris L.	Pteridaceae	Bagh	Fr.	Herb	Silty clay	30	30	20	0	25.75
Adiantum venustum D.Don.	Pteridaceae	Bagh	Veg.	Herb	Silty clay	20	20	0	44.25	0
Ajuga bracteosa Wall ex Bth.	Lamiaceae	Muzaffarabad	Veg.	Herb	Loamy silt	80	80	0	55.08	18.23
Albizia lebbeck (L.) Bth.	Mimosaceae	Bhimber	Veg.	Tree	Loamy sand	20	0	20	11.27	63.10
Aloe barbadensis Mill.	Liliaceae	Muzaffarabad	Veg.	Shrub	Sandy	80	30	80	14.07	81.55
Amaranthus spinosus L.	Amaranthaceae	Bhimber	F1.	Herb	Loamy sand	60	60	0	17.55	62.84
Anagallis arvensis. L.	Primulaceae	Muzaffarabad	F1.	Herb	Loamy clay	60	60	0	55.85	10.58
Anethum graveolens. L.	Umbelliferae	Bhimber	F1.	Herb	Clayey	60	60	0	40.50	0
Arisaema jacquemontii Blume.	Araceae	Sudhan Galli	Veg.	Herb	Clayey	80	80	0	85.95	7.05
Artemisia gmelinii	Asteraceae	Dinour	F1.	Herb	Loamy snad	20	20	0	65.17	0
A. Maritima L.	Asteraceae	Chinari	Veg.	Herb	Silty clay	80	30	80	95.33	10.58
A. scoparia Waldst.	Asteraceae	Bhimber	Fr.	Herb	Sandy loam	80	80	30	17.55	88.05
Asparagus racemosus Willd.	Liliaceae	Kongra	Veg.	Climber	Sandy loam	40	30	40	19.55	66.35
Atropa belladonna L.	Solanaceae	Leepa	F1.	Herb	Clay	70	70	0	30.44	68.25
Bauhinia variegata L.	Caesalpiniaceae	Muzaffarabad	F1.	Tree	Sandy loam	40	40	20	55.17	11.18
			Fr.	Tree	Sandy loam	10	10	0	0	0
Butae monosperma (Lam.)Taub.	Papolionaceae	Bhimber	Veg.	Tree	Sandy	50	50	0	65.17	0.0
			F1.	Tree	Sandy	70	10	0	13.14	33.44
Canabis sativa L.	Cannabinaceae	Muzaffarabad	Veg.	Shrub	Silty clay	90	90	40	88.05	27.55
Citrullus colocynthis (L.) schrad.	Cucurbitaceae	Bhimber	F1.	Tree	Loamy silt	20	10	20	11.17	0
Coriandrum sativum L.	Umbelliferae	Muzaffarabad	F1.	Herb	Clay	80	80	0	12.25	0
Crotolaria juncea L.	Papilionaceae	Bhimber	Veg.	Tree	Loamy silt	30	30	10	14.25	38.10
Cymbopogon citratus Stapf.	Gramineae	Muzaffarabad	Veg.	Grass	Clayey	50	50	0	0	37.82
C. Jawarancusa (Jones). Schult.	Gramineae	Muzaffarabad	F1.	Grass	Clayey	20	20	0	42.52	15.05
Dalbergia sissoo Roxb.	Papilionaceae	Bhimber	Veg.	Tree	Sand	60	60	10	57.15	11.02
Datura alba Nees.	Solanaceae	Bhimber	Fr.	Shrub	Sandy loam	98	98	20	95.17	0
Daucus carota L.	Umbelliferae	Bhimber	Veg.	Herb	Silty clay	30	30	10	20.85	45.02
Eucalyptus sp.	Myrtaceae	Muzaffarbad	Veg.	Tree	Silty soil	20	20	0	47.55	0
Euphorbia helioscopia L.	Euphorbiaceae	Muzaffarbad	F1.	Herb	Siltyc laom	30	0	10	18.89	0
E. Prostrata Ait.	Euphorbiaceae	Muzaffarbad	F1.	Herb	Silty soil	97	97	0	28.23	9.85
Fumaria indica (Hausskn) Pugsley.	Fumariaceae	Muzaffarbad	Fr.	Herb	Silty clay	97	0	0	25.05	45.55
Hibisucus esculentum L.	Malvaceae	Muzaffarbad	Fr.	Herb	Loamy sand	97	97	0	95.05	0
Hedera helix Auct.	Araliaceae	Bhimber	Veg.	Climber	Loamy	90	90	60	75.33	12.19
Hypericum perforatum L.	Hypericaceae	Sudhen Galli	F1.	Herb	Clayey	60	10	60	95.50	0
Impatiens balsmina L.	Balsaminaceae	Sudhen Galli	F1.	Herb	Clayey	20	0	20	48.99	20.00
Indigofera trifoliata L.	Papilionaceae	Sudhen Galli	F1.	Shrub	Clayey	80	80	30	33.05	0
Ipomoea botatis (L.) Poir.	Convolvulaceae	Chikar	Veg.	Climber	Clayey	97	60	90	72.46	10.70
I. corneae	Convolvulaceae	Afzalpur	F1.	Shrub	Loamy sand	98	60	94	55.05	19.45
Justicia adhatoda L.	Acanthaceae	Phakroot	F1.	Herb	Clayey	80	80	0	22.40	51.07
Lathyrus aphaca Linn.	Papilionaceae	Muzaffarabad	F1.	Herb	Loamy soil	94	94	60	82.00	0
Launaea nudiculis Hook.	Compositae	Kotli	F1.	Herb	Sandy loam	70	70	20	0	26.33
Lens asculenta Moench.	Papilionaceae	Bhimber	F1.	Herb	Lomay	93	30	93	67.50	7.88
Melia azedarach L.	Meliaceae	Bhimber	Veg.	Tree	Siltyclay	80	80	0	77.55	17.15
Mentha longifolia (L.) Huds.	Labiatae	Muzaffarabad	Veg.	Herb	Clayey	40	40	20	11.19	63.44
Mentha piperita L.	Labiatae	Muzaffarabad	F1.	Herb	Sandy	70	70	30	0	37.10
Ocimum basilicum L.	Labiatae	Bhimber	Fr.	Herb	Sandy loam	95	95	0	32.51	17.25
Oxalis corniculata L.	Oxalidaceae	Punjeri	F1.	Herb	Sandy	92	92	20	60.50	17.33
Peganum harmala L.	Rutaceae	Muzaffarabad	F1.	Herb	Clayey	10	10	0	28.19	13.32
Phaseolus mungo L.	Papilionaceae	Bhimber	Veg.	Herb	Loamy clay	40	40	20	21.23	28.17
P. Aconitifolius Jacq.	Papilionaceae	Bhimber	Veg.	Herb	Loamy sand	90	50	90	27.42	11.05
			Fr.	Herb	Loamy sand	60	60	10	47.55	0
Physalis minima L.	Solanaceae	Kalery	Veg.	Herb	Sandy	60	60	0	17.28	0
Plantago ovata L.	Plantaginaceae	Chikar	Fr.	Herb	Clay	50	30	30	60	21.04
P. Lanceolata L.	Plantaginaceae	Sudhen galli	Fr.	Herb	Clay	70	70	0	23.09	16.37
Polygonum sp.	Polygonaceae	Chinari	Veg.	Herb	Clayey	60	60	0	14.24	56.44
Punica grantum L.	Punicaceae	Nikyal	F1.	Tree	Clayey	60	60	0	55.48	27.04
Pupalia lappaceum L. Juss.	Amaranthaceae	Chella Bandi	F1.	Herb	Sandy loam	95	95	30	12.78	83.15
Ricinus communis L.	Euphorbiaceae	Muzaffarabad	Veg.	Shrub	Clay	89	89	0	30.82	19.35
Salvia moorcroftiana Wall.	Labiatae	Muzaffarabad	F1.	Herb	Sandy loam	30	0	0	24.13	0
Sauromatum guttatum Schoot.	Araceae	Muzaffarabad	Veg.	Herb	Clayey	30	10	30	66.08	0
Saussurea heteromalla	Asteraceae	Leepa	F1.	Herb	Clayey	30	0	30	20.55	12.02
Saxifraga sp.	Saxifragaceae	Leepa	Veg.	Herb	Clayey	90	75	0	98.02	0
Sesamum indicum L.	Pedaliaceae	Chapper	Veg.	Herb	Sandy loam	50	50	50	37.08	0
Solanum nigrum L.	Solanaceae	Mirpur	Veg.	Herb	Loamy	30	0	30	25.18	0
S. Surattense Burm.	Solanaceae	Bhimber	Veg.	Herb	Loamysilt	90	90	50	95.17	0
Trianthema pentandra L.	Ficoidaceae	Chapper	F1.	Herb	Sandy	70	70	20	37.18	21.91
Tribulus terrestris L.	Zygophyllaceae	Bhimber	F1.	Herb	Sandy loam	40	40	20	78.08	37.50

Table 1: Continued

Plant species	Family	Locality	Age	Habit	Soil type	Vesicular arbuscular mycorrhizal infection				
									Extent of hype 100 cm^{-1}	
						General VAM infection %	Vesicular infection%	Arbuscular infection%	Aseptate hyphae	septate hyphae
Trigonella foenum-graecum L.	Papilioonaceae	Muzaffarabad	Veg.	Herb	Siltyclay	40	40	0	58.42	12.35
Triticum aestivaum L.	Gramineae	Muzaffarabad	Fr.	Grass	Clayey	60	60	30	11.56	22.42
Valeriana wallchii DC.	Valerianaceae	Hevali	Veg.	Herb	Clayey	50	40	10	37.05	0
Verbascum thapsus L.	Scorphulariaceae	Muzaffarabad	F1.	Herb	Silty loam	30	0	0	28.55	0
Viola canescens Wall ex Roxb.	Violaceae	Pattika	F1.	Herb	Silty clay	30	30	30	67.12	20.02
V. odorata L.	Violaceae	Dheer Kot	F1.	Herb	Clayey	70	70	10	40.00	0
V. tricolor L.	Violaceae	Muzaffarabad	F1.	Herb	Silty clay	40	40	0	31.17	0
Withania somnifera (L.) Dunal	Solaceae	Muzaffarabad	F1.	Shrub	Sandy loam	70	20	70	44.11	0
Xanthium strumarium L.	Asteraceae	Bhimber	Veg.	Herb	Sandy loam	50	40	50	21.00	7.056

Veg, vegetative stage of the plant species, Fl, flowering stage of the plant species
Fr, fruiting stage of the plant species, VAM, Vesicular- Arbuscular Mycorrhizae

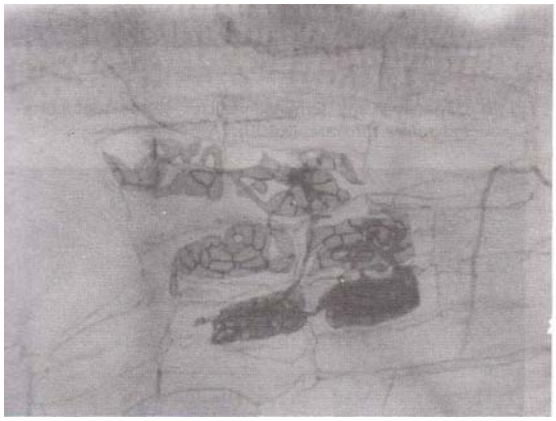

Fig. 1: Septate hyphae forming coils within root cortical cells of *Atropa belladona*. (X400)

Fig. 3: A root segment showing dichotomously branched appressorium with entry point in *Ajuga bracteosa*. (X400)

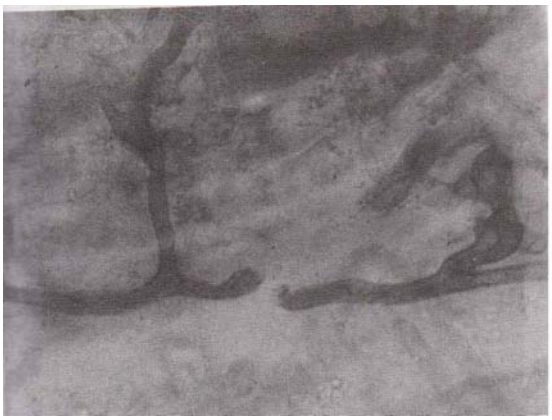

Fig. 2: Septate hyphae forming clamp-connections in the root cortex of *Solanum surattense*.(X400)

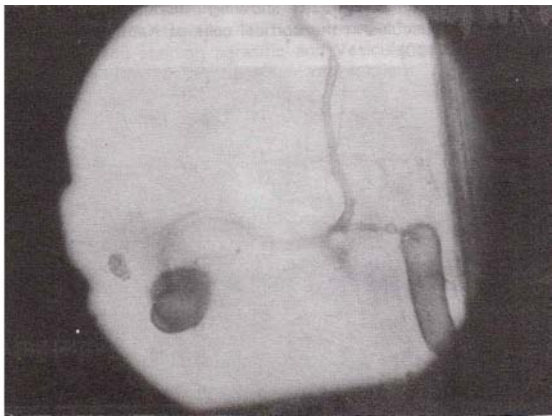

Fig. 4: Elongated and oval vesicles on the same mycelium in the root cortex of *Ocimum basilicum*. (X400)

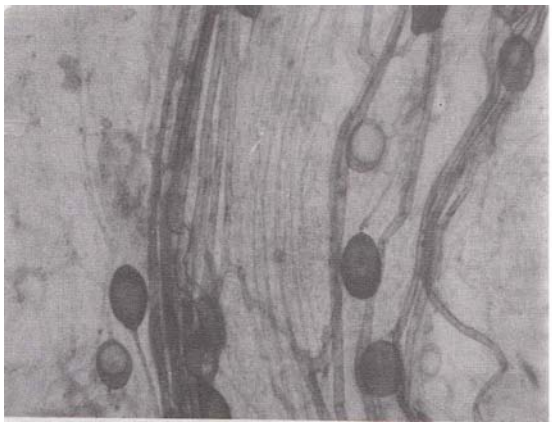

Fig. 5: A portion of root showing hyphae with terminal vesicles in *Eucalyptus* sp. (X400)

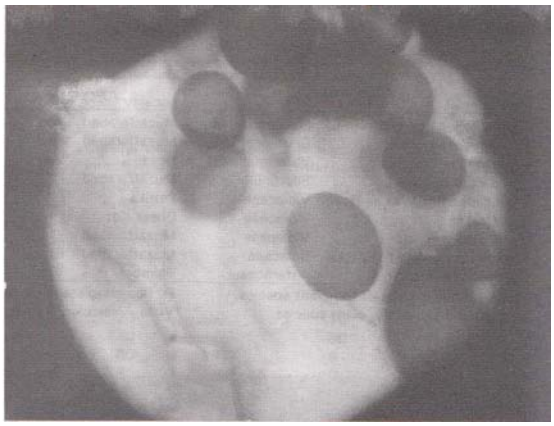

Fig. 8: Large oval and terminal vesicles in the root cortex of *Indigofera trifoliata*. (X400)

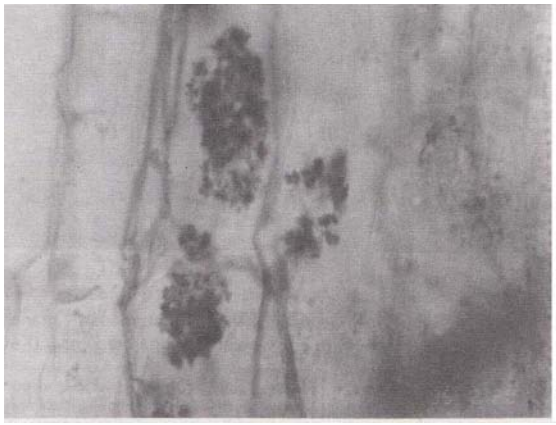

Fig. 6: A root segment showing dichotomously branched arbuscules in the cortical cells of *Xanthium strumarium*. (X400)

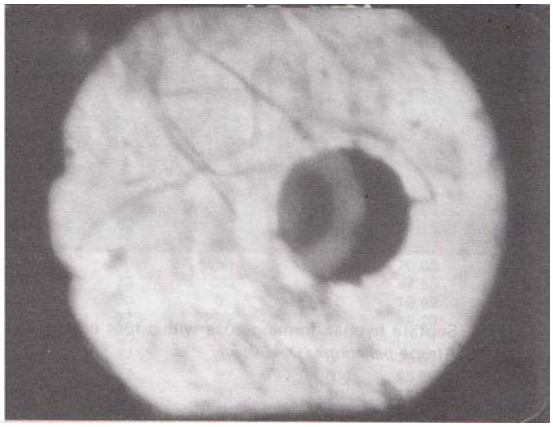

Fig. 9: A sporocarp with several hyphal connections in the cortex of a root segment of *Trigonella foenum– raecum*. (X400)

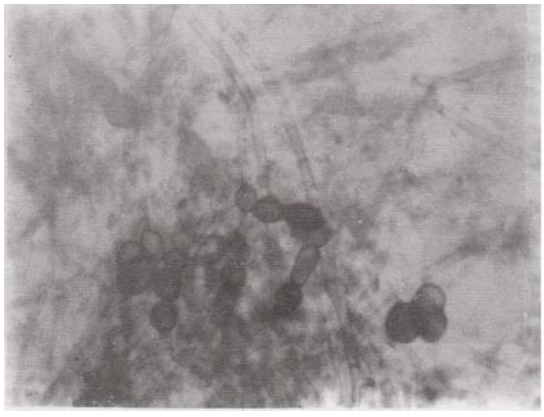

Fig. 7: Septate mycelium with thick – walled, rounded and elongated spores in the root cortex of *Coriandrum sativum*. (X400)

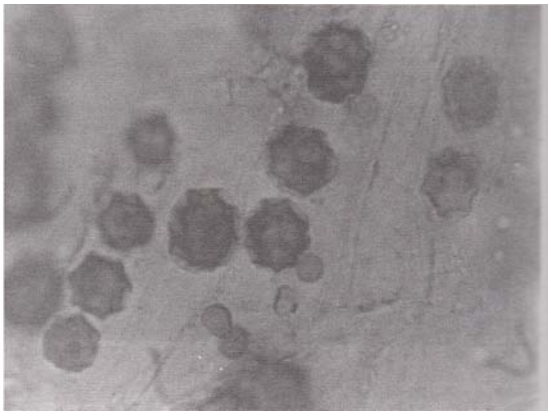

Fig. 10: Star – shaped VAM spores within root cortical cells of *Anagallis arvensis* . (X400)

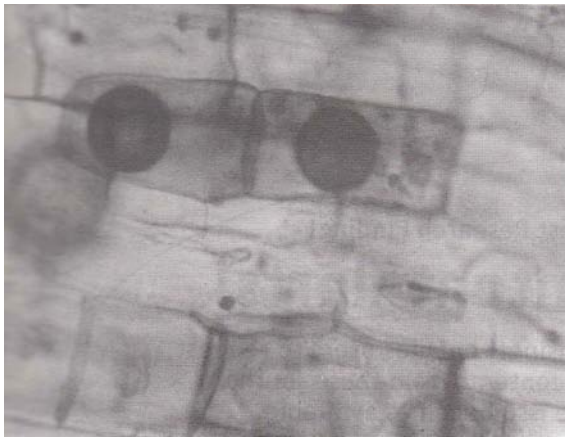

Fig. 11: *Datura alba* , thick – walled spherical vesicles within the root cortical cells. (X400)

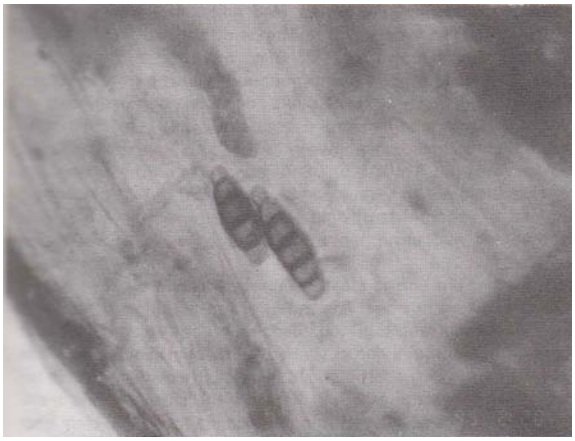

Fig. 12: *Xanthium strumarium*, pathogenic fungus forming conidia within root cortex. (X400)

Spores of various sizes and shapes; *Coriandrum sativa* (Fig. 7) were noticed individually and/ or in the form of root-attached sporocarpsl; *Trigonella foenum-graecum* (Fig. 9) mostly in mature plants of which rounded oblong, echinulated and star-shaped; *Anagallis arvensis* (Fig. 10) double wall spores were most pronounced. The diversity of vesicles and spores indicates the multiple VA infections. The invasion of roots by fungi other than VA endophytes; *Xanthium strumarium* (Fig. 12) was also observed. Variation in the VAM infection percentage may be attributed to differences in physico-chemical and biological characteristics of the soil including their VAM inoculum. The incidence of mycorrhizal colonization may very with season, soil moisture availability (Staffeldt and Vogt, 1975), habitat (Miller, 1979) and composition of a plant community (Hirrel *et al.*, 1978 and Read *et al.*, 1976).

These findings confirms the statement that mycorrhize are of universal occurrence (Micolson, 1967), beneficial for plant (Mosse, 1973 and Tinker, 1975) and have a significant role in ecosystem functioning (Baylis, 1976).

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