

Diversity of Arbuscular Mycorrhizal Fungi Associated With the Medicinal Plants from Gwalior-Chambal Region of Madhya Pradesh-India

K.K. Koul, Shuchi Agarwal and Rafiq Lone

School of Studies in Botany, Jiwaji University Gwalior (M.P), 474011, India

Abstract: Gwalior-Chambal region with an area of more than 45,000 km². is famous for its unique physiognomy-ravines (loc. Beehad), lying north of the central Indian state of Madhya Pradesh (M.P) classified as arid with very hot summers and mild cold winters. Rainfall is mainly during monsoon months July to September and averages around 80-90 cm. Extensive cultivation of oil crop mustard (*Brassica sps.*) is done with low yields. The area under study has now been declared a preferential area for the cultivation of medicinal and aromatic plant species, adapted to ravines, so as to improve economic potential of nearly 20 million rural populace of this area. Study of Arbuscular Mycorrhizal Fungi (AMF) and their association with the plants in the area is not available.. The plants were collected with their roots and their respective rhizospheric soil. The fresh roots were subjected to AMF analysis. AMF spore density was done assessing spore number per 100gm rhizospheric soil. INVAM and other updated literature was employed to characterize AMF species on the basis of spore morphology. 110 plant species collected randomly from their habitats spread over 54 families show dominantly polysporal association with AMF. On the higher side 6 species of AMF are symbionts of *Aloe vera*. Per 100gm of rhizospheric soil the mean spore density varies with highest around 166.22 in *Aloe vera* to the lowest of 5.33 in *Abutilon indicum*. 23 species seem as first time being reported as mycorrhizic.

Key words: *Mycorrhiza* · *Medicinal plants* · *AMF Taxonomy* · *Gwalior-Chambal area*

INTRODUCTION

Mycorrhiza is a symbiotic association between a soil borne fungus and the roots of a plant [1]. Mycorrhizae form a mutualistic relationship with the roots of nearly eighty percent of plant species examined [2]. Being one of the largest producers of pharma preparations based on herbs, India is now an established partner in the global medicinal herb trade. [3]. There are number of reports of medicinal plants with secondary metabolites harbouring AMF associations in their roots [4-9]. An enhanced P uptake and also elevated physiological and metabolic efficiency due to efficient P utilization resulted by mycorrhizal symbiosis are established [10]. Besides P, Fe, Zn and Cu uptakes are also enhanced by such association. [10, 11]. AMF fungi associated with the medicinal plants has not only enhanced the growth of medicinal plants but also improved the active principle content [6, 12].

The collection area under present study is an area of unique physiognomy famously known as Gwalior-

Chambal ravines world over and 'behaad' locally. It covers a large area of 2.387 million hectares of ravines spread over 4 states of India; the major part of more than 45000 km² being Gwalior-Chambal region. The Government of Madhya Pradesh state has declared the area under present study as a preferential area for the cultivation of medicinal and aromatic plants. The study is also important, since literature perusal did not show any authentic study regarding AMF status of this unique geomorphological area-ravines which account for 3.67 million hectares in India. Once inventorised, the same can be used for improvement of cultivated herbs and their active principles in all the four states.

MATERIALS AND METHODS

Materials: Collection of medicinal plants with their roots and soil was done randomly from different sites in 8 districts which include various habitats. After bringing these plants to Lab the roots were separated and analyzed when fresh. The rhizosphere soil mixtures of the host

species were collected, air-dried and then stored in plastic bags till processing. Identification of host plants was confirmed by comparisons with the holotypes deposited in the herbarium of the School of Studies in Botany, Jiwaji University, Gwalior and also with the assistance provided by the Institute of Ethnobiology of the School of Studies in Botany, Jiwaji University. The area was surveyed on bimonthly basis to cover all seasons. Only those species are reported here which are already described as medicinal or used as a spice or a condiment of any sort. Despite 300 plants collected only 110 were randomly selected; irrespective of being cultivated, ornamental and/or escapes in wild.

Methods

Root Clearing and Staining Technique: The method described by Phillips and Hayman [13] and modified by Kormanik [14] was employed for root clearing and staining with trypan blue.

Analysis for Root Colonization: AMF colonization and its extent was visualized in the root tissue of each plant species by using frequency distribution method proposed by Biermann and Lindermann [15] and same was assessed by random selection of 10 equal length roots. Each root was divided into ten 1cm long segments, which were then cleared, stained and arranged on slides. Slides were observed under compound microscope to score for any structures associated with mycorrhizal fungi *viz.*, hyphae, vesicles or arbuscules in each segment.

Percent root colonization for each plant was calculated using following relation:

$$\text{Percent colonization} = \frac{\text{Total number of colonized root segments}}{\text{Total number of root segments examined}} \times 100$$

Spore Separation and Quantification: Separation of AMF spores from the rhizospheric soil from each plant was done by using wet sieving and decanting method proposed by Gerdemann and Nicolson [16] from 100 gm sample of the soil. The rhizospheric soil collected from various spots of collection for a specific plant species was pooled, mixed and then the sample taken. The samples were replicated four times and data presented as the mean spore density per 100gm rhizospheric soil with statistical standard deviation.

Spore density was calculated as the total number of spores recorded by sieving and collected into petri plates [17]. Quantification was carried out as per Lugo and Cabello [18] in 10 cm diameter petri dishes with a gridline

of 1 cm square under a stereoscopic microscope at 50x. Ten divisions were counted and related to the total number of spores by using the method as modified by Mc Kenney and Lindsey [19]. Spores of different types were collected with the help of micropipette and transferred on the glass slide containing polyvinyl lactoglycerol (PVLG) as suggested by Koske and Tessier [20] and Omar [21] and was further modified with or without Melzers reagent, [22]

Identification of AMF Spores: The AMF fungi were identified using manual provided by Schencz and Perez [23] and compared with original species descriptions and reference isolates described by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi. Intact spores were used for AMF characterization, on the basis of their spore morphology and wall characters which included colour, shape, size and hyphae respectively [23-26]. Spore colour range extended from colourless to brown or dark brown and as such colour of spore wall and germinal wall layers was observed both in intact and crushed spore in either water or PVLG [27]. The spore shape and size was based on at least 50 intact spores mounted in a drop of lactic acid. The dimensions were determined by micrometry.

RESULTS

The area surveyed in the present study is recognized into two administrative divisions namely Gwalior and Chambal, both having Head Quarters in Gwalior (M.P) India. The unique feature of this area is the special topography presented by the ravine formations; very commonly known as Chambal ravines. Lying on the northeast and north west directions of Gwalior area is defined by Bhind district in the earlier direction and the areas of Amba, Porsa, Shoopur Kalan and Morena in the later direction respectively. On the south east of Gwalior lie Dabra and Datia and on the south-west Shivpuri and Guna. The area is classified as arid with very hot summers and mild cold winters. Rainfall is mainly during monsoon months and averages around 80-90 cm annually.

Plant species along with their AMF characterizations are presented in the Table 1. It also presents the individual and combined range of the plant species being colonized by the AM fungi. Except for three species *Amaranthus viridis* of the family Amaranthaceae, *Chenopodium ambrosioides* of the family Chenopodiaceae and *Raphanus sativus* of the family Brassicaceae, all other 107 of 110 species were mycorrhizal. AMF show a large

Table 1: The percentage frequency of root colonization, mean AMF spore density, the number of AMF spore species of AMF with each individual host species and also the type of colonization associated with the species of angiospermic plants studied from the Gwalior-Chambal area

Host species	AMF structures Present in root	Average percent root colonization	Sporal status	No. of AMF spore species in rhizosphere	Mean spore density 100 gm ⁻¹ of rhizospheric soil.	AMF species associated (for species see no's in the legend)
<i>Achyranthes aspera</i>	HAV	55	Polysporal	2	17.66 ± 2.51	21,23
<i>Adhatoda vasica</i>	HV	43	Polysporal	4	15.33 ± 0.57	13,23,18,39
<i>Aegle marmelos</i>	HAV	80	Polysporal	3	17.66± 2.08	13,23,18
<i>Allium cepa</i>	HAV	70	Polysporal	2	17.66 ± 1.15	15,23
<i>Allium sativum</i>	HAV	62	Polysporal	2	12.33± 1.51	13,18
<i>Ailanthus excelsa</i>	HAV	45	Monosporal	1	6.33 ± 1.52	41
<i>Aloe vera</i>	HAV	90	Polysporal	6	162.66 ± 2.081	18,23,14,28,41,6
<i>Alternanthera sessilis</i>	HAV	86	Polysporal	2	47.66 ± 3.37	24,37
<i>Andrographis paniculata</i>	HAV	65	Monosporal	1	22.33 ± 2.48	24
<i>Anagallis arvensis</i>	HAV	57	Monosporal	1	14.33 ± 2.30	41
<i>Amaranthus viridis</i>	-	-	-	-	-	--
<i>Argemone mexicana</i>	HAV	45	Polysporal	2	9.66± 0.53	21,23
<i>Artemisia nilagrica</i>	HAV	28	Polysporal	5	37.33 ± 2.64	41,21,23,26,32
<i>Asparagus racemosus</i>	HAV	75	Polysporal	3	10.66 ± 0.51	22,31,25
<i>Azadirachta indica</i>	HAV	80	Polysporal	3	42.33 ± 4.93	6, 24, 15
<i>Bacopa monnieri</i>	HA	35	Polysporal	3	25.33 ± 0.57	28, 21, 23
<i>Barleria prionitis</i>	HAV	53	Polysporal	2	13.33 ± 1.51	41, 23
<i>Begonia roxburghii</i>	HAV	75	Polysporal	2	23.33 ± 1.52	4, 11
<i>Berberis aristata</i>	HAV	35	Polysporal	4	141.66 ± 1 0.57	29, 28, 24, 15
<i>Bauhinia variegata</i>	HAV	75	Polysporal	2	8.33 ± 1.15	15, 13
<i>Boerhaavia diffusa</i>	HAV	42	Polysporal	4	86.33 ± 2.51	12, 17, 13, 28
<i>Calotropis procera</i>	HAV	80	Polysporal	2	124.66 ± 12.30	41, 07
<i>Cyanodon dactylon</i>	HAV	72	Polysporal	2	109.33 ± 11.15	41, 28
<i>Cassia angustifolia</i>	HAV	42	Monosporal	1	41.66± 0.57	27
<i>Carica papaya</i>	HAV	60	Polysporal	2	57.33 ± 1.52	28, 5
<i>Cassia fistula</i>	HAV	75	Polysporal	2	45.00 ± 2.64	15, 28
<i>Catharanthus roseus</i>	HAV	60	Polysporal	3	26.66 ± 1.154	15, 24, 41
<i>Centella asiatica</i>	HAV	35	Polysporal	2	34.66± 2.15	21, 23
<i>Chenopodium ambrosioides</i>	-	-	-	-	-	--
<i>Chlorophytum tuberosum</i>	HAV	70	Monosporal	1	117.66± 10.57	28
<i>Citrus aurantium</i>	HAV	65	Polysporal	3	155.3333 ± 10.53	10, 23, 37
<i>Clitoria ternatea</i>	HAV	62	Polysporal	3	37.66± 1.52	23, 15, 28
<i>Cocculus hirsutus</i>	HAV	65	Polysporal	2	25.33 ± 0.57	15, 20
<i>Coleus barbatus</i>	HAV	65	Polysporal	3	66.66± 1.52	24, 23
<i>Convolvulus pluricaulis</i>	HAV	70	Polysporal	2	12.66667±1.527525	28, 12
<i>Commiphora wightii</i>	HAV	60	Polysporal	2	76 .00± 11.23	14, 19
<i>Coriandrum sativum</i>	HAV	38	Monosporal	1	7.66± 0.67	28
<i>Costus speciosus</i>	HAV	46	Monosporal	1	3.66± 0.57	04
<i>Crinum latifolium</i>	HAV	90	Polysporal	4	11.66± 0.43	6, 15,23, 28
<i>Curculigo orchiooides.</i>	HAV	37	Polysporal	3	123.66± 12.30	28, 23, 24
<i>Curcuma longa</i>	HAV , Spores	89	Polysporal	4	174.66± 11.57	31, 28, 24, 13
<i>Cymbopogon citratus</i>	HAV	58	Polysporal	2	122.66± 10.77	41, 16
<i>Cyperus rotundus</i>	HAV	66	Polysporal	2	21.66± 5.77	31, 04
<i>Dalbergia sisso</i>	HAV	60	Polysporal	2	153.66 ± 12.32	37, 2
<i>Datura stramonium</i>	HAV	60	Polysporal	2	95.66± 11.15	16, 28
<i>Euphorbia hirta</i>	HAV	71	Polysporal	2	85.33 ± 1.54	15, 23
<i>Eclipta alba</i>	HAV	60	Polysporal	2	27.00± 1.30	26, 24
<i>Ficus benghalensis</i>	HAV	42	Polysporal	3	42.00 ±4.60	28, 37, 41
<i>Ficus racemosa</i>	HAV	75	Polysporal	3	74.33 ± 6.52	22, 28, 26
<i>Ficus virens</i>	HAV	77	Polysporal	2	136.33± 11.52	12, 14
<i>Foeniculum vulgare</i>	HAV	64	Polysporal	2	155.66± 12.88	10, 36
<i>Glycyrrhiza glabra</i>	HAV	43	Polysporal	3	53.66± 3.57	14, 34, 19
<i>Gymnema sylvestre</i>	HAV	36	Polysporal	3	21.33 ±2.33	28, 24, 15
<i>Hibiscus rosa-sinensis</i>	HAV	55	Polysporal	3	33.33 ± 4.15	22, 24, 16
<i>Ipomoea carneaefistulosa</i>	HAV	42	Monosporal	1	12.33 ± 1.15	23

Table 1: Continued

Host species	AMF structures Present in root	Average percent root colonization	Sporal status	No. of AMF spore species in rhizosphere	Mean spore density 100 gm ⁻¹ of rhizospheric soil.	AMF species associated (for species see no's in the legend)
<i>Jatropha curcas</i>	HAV	60	Monosporal	1	6.33 ± 1.52	22
<i>Lantana camara</i>	HAV	83	Polysporal	2	11.33 ± 0.57	28, 26
<i>Lawsonia inermis</i>	HAV	40	Polysporal	2	17.66± 1.63	32,18
<i>Leucas aspera</i>	HAV	54	Polysporal	2	10 .00± 1.73	28, 6
<i>Musa paradisiaca</i>	HAV	50	Polysporal	2	14.66± 1.73	10, 42
<i>Mentha longifolia</i>	HAV	70	Polysporal	2	46.66± 1.52	23,26
<i>Mimosa pudica</i>	HAV	60	Polysporal	2	23.33± 2.51	20,28
<i>Momordica charantia</i>	HAV	60	Polysporal	3	16.66± 2.51	22,32,18
<i>Moringa oleifera</i>	HAV	65	Polysporal	3	38.66± 5.57	23,4,5
<i>Murraya koenigii</i>	HAV	72	Polysporal	3	14.33 ± 5.57	33,37,23
<i>Nardostachys jatamansi</i>	HAV	45	Polysporal	2	12.66 ± 1.33	28,15
<i>Nelumbo nucifera</i>	HAV	42	-	-	-	--
<i>Nicotiana plumbaginifolia</i>	HAV	53	Polysporal	2	13.66± 2.08	30,22
<i>Ocimum sanctum.</i>	HAV	76	Polysporal	2	43.66± 2.30	39,3
<i>Origanum majorana</i>	HAV	56	Polysporal	2	6 .00± 1.13	1,34
<i>Oxalis corniculata</i>	HAV	84	Polysporal	2	164.66± 15.17	28,15
<i>Papaver somniferum</i>	HAV	30	Polysporal	2	31.33± 0.57	20,32
<i>Parthenium hysterophorus</i>	HAV	35	Monosporal	2	42.66± 2.08	24,26
<i>Phyllanthus amarus</i>	HAV	69	Polysporal	3	122 .06± 12.33	37,38,23
<i>Phyllanthus emblica</i>	HAV	79	Polysporal	3	135.00 ± 13.66	12,19,40
<i>Picorrhiza kurroa</i>	HAV	51	Polysporal	2	13.00± 1.00	24, 28
<i>Piper betel</i>	HAV	65	Polysporal	2	63.66± 1.52	31, 5
<i>Piper longum</i>	HAV	53	Polysporal	3	21..36±3.21	5, 26, 11
<i>Pongamia pinnata</i>	HAV	36	Polysporal	4	14 .00± 1.73	6, 28,24, 23
<i>Punica granatum</i>	HAV	65	Polysporal	2	82 ± 1	15, 40
<i>Raphanus sativus</i>	-	-	-	-	-	--
<i>Rauwolfia serpentina</i>	HAV	35	Monosporal	1	15.33± 1.52	37
<i>Ricinus communis</i>	HAV	62	Monosporal	1	17.43±2.33	--
<i>Rosa damascena</i>	HAV	56	Monosporal	1	08.00±1.5	35
<i>Salmalia malabarica</i>	HAV	60	Monosporal	1	8.33 ± 2.081	15
<i>Saraca asoca</i>	HAV	53	Polysporal	2	32.33 ± 1.15	8, 39
<i>Sida cordata</i>	HAV	19	Polysporal	2	17.00± 2.15	28, 24
<i>Solanum nigrum</i>	HAV	61	Polysporal	2	65.33 ± 2.51	23, 12
<i>Sapindus mukorossi</i>	HAV	41	Monosporal	1	8.66± 0. 87	36
<i>Spilanthus acmella</i>	HAV	81	Polysporal	3	61.66± 6.57	33, 22, 18
<i>Syzygium cumini</i>	HAV	78	Polysporal	4	18.33 ± 0.57	18, 23, 22, 37
<i>Tagetes erecta</i>	HAV	78	Polysporal	4	15.66± 1.52	24, 28, 23, 40
<i>Tamarindus indica</i>	HAV	35	Monosporal	1	15.00 ± 1.33	24
<i>Terminalia arjuna</i>	HAV	60	Monosporal	1	76.33 ± 5.27	24
<i>Terminalia bellerica</i>	HAV	60	Monosporal	1	53.00 ± 6. 42	18
<i>Terminalia chebula</i>	HAV	62	Monosporal	1	17.33 ± 2.081	23
<i>Tinospora cordifolia</i>	HAV	75	Polysporal	4	67.66± 5.52	37, 6, 15, 24
<i>Thevetia nerifolia</i>	HAV	60	Polysporal	3	61.66± 4.52	2, 15
<i>Thuja orientalis</i>	HAV	60	Monosporal	1	10.66±0.57	28
<i>Trigonella foenum- graecum</i>	HAV	75	Polysporal	3	47.00± 6.66	38, 18, 23
<i>Tridax procumbens</i>	HAV	45	Polysporal	2	34.33 ± 2.08	15, 32
<i>Vitex negundo</i>	HAV	23	Polysporal	2	12.66± 1.52	24, 4
<i>Cissus quadrangularis</i>	HAV	65	Polysporal	2	17.66± 1.52	22, 40
<i>Valeriana wallichii</i>	HAV	53	Monosporal	1	2.33 ± 1.15	28
<i>Withania somnifera</i>	HAV	40	Polysporal	5	116.33 ± 11.52	24, 26, 16, 23, 15
<i>Ziziphus nummularia</i>	HA	21	Polysporal	4	14.00 ± 2.33	9, 37, 31, 23
<i>Zingiber officinale</i>	HAV, Spores	85	Polysporal	5	124.33± 15.27	23, 26, 22,24, 41

1. *Acaulospora denticulate*, 2. *A. lacunose*, 3. *A. colossica*, 4. *A. delicata*, 5. *A. koskei*, 6. *A. laevis*, 7. *A. rehmi*, 9. *Gigaspora decipiens* 11. *G. rosea*, 12. *G. albida*, 13. *G. gigantea*, 14. *Glomus caledonium*, 15. *G. claridum*, 16. *G. clariodium*, 17. *G. clarum*, 18. *G. clavosporum*, 19. *G. convolutum*, 20. *G. coronatum*, 21. *G. diaphanum*, 22. *G. eburneum*, 23. *G. etunicatum*, 24. *G. fistulosum*, 25. *G. geosporum*, 26. *G. intraradices*, 27. *G. lamellosum*, 28. *G. luteum*, 29. *G. manihotis*, 30. *G. mosseae*, 31. *G. pallidum*, 32. *G. spurcum*, 33. *G. versiforme*, 34. *G. viscosum*, 35. *Scutellospora fulgida*, 36. *S. heterogama*, 37. *S. persica*, 38. *S. reticulata*, 39. *S. calaspera*, 40. *S. coralloidea*, 41. *S. gregaria*, 42. *S. pellucid*, 43. *S. verrucosa*, 44. *S. caulospora*, *H* = hyphe, *A* = arbuscule, *V*= visicle

Table 2: Percentage presence of various AMF structures in the roots of plants.

S.No	AMF Structures in the roots	Number of host plants showing AMF structures	Percentages of total plant species
1.	Hyphae (H)	107	97.27
2.	Arbuscules (A)	105	95.45
3.	Vesicles (V)	104	94.45
4.	HAV	104	94.27
5.	HA	105	95.36
6.	HV	104	94.45
7.	Spores (S)	02	1.18
8.	No infection	03	2.72

H= Hyphae, V= Vesicles, A= Arbuscules, S= Spores

Table 3: Number of the AMF rhizospheric species associated with their respective genus in the soils of various plants species

S. No	Name of the AMF Genus	Number of Species of AMF Genus
1.	<i>Glomus</i>	21
2.	<i>Scutellospora</i>	10
3.	<i>Acaulospora</i>	07
4.	<i>Gigaspora</i>	04

variational range of root colonization in the plant species ranging from very low of 19 percent in *Sida cordata* of Malvaceae to the highest of 90 percent in *Aloe vera* and *Crinum latifolium* of Liliaceae and Amaryllidaceae families respectively. Only ten of the 110 plant species have 80-90 percent colonisation (~9%) and 30 show AMF root colonisation between 60-80 percent. Therefore, nearly 60 percent of the plants analysed show either none or less than 60 percent individual colonisation.

All the 107 mycorrhizal species show hyphal presence in roots (Table 2) and more than 95 percent of these plants show presence of either hyphae and arbuscules or hyphae and vesicles or all the three structures individually. Presence of AMF spores in roots was almost negligible, being present in around 2 percent of the plant species. 21 plants show monospory in their rhizospheric soils accounting to 17 percent of the total species analysed and rest of the 85 plant species show polyspory in the soils associated with their roots therefore, accounting to nearly 83 percent (Table 1). *Aloe vera* rhizosphere showed as many as 6 types of AMF spores, followed by 5 in *Artemisia nilagrica*, *Withania somnifera* and *Zingiber officinales*. Highest mean spore density of 174 amongst the rhizosphere of various species was in *Curcuma longa*. Only 4 plant species showed a sporal density of more than 150 and just 8 plants showed a spore density between 100-150. Less than 10 spores in the rhizosphere were shown by about 10 plant species. Four species amongst these being without AMF spores in their rhizosphere viz., *Amaranthus viridis*, *Chenopodium ambrosoides*, *Nelumbo nucifera* and *Raphanus sativus*, however, except for *Nelumbo nucifera* other 3 without any of the AMF structure/s in their roots.

The study showed a total of 42 AMF species associated with the medicinal plants. And are predominantly distributed amongst 4 genera. Genus *Glomus* is represented by 21 species which is around 50 percent of the total AMF species, followed by 10, 07 and 04 species of genus *Scutellospora*, *Acaulospora* and *Gigaspora* respectively (Table 3).

DISCUSSION

AMF are now going to be an integral part of sustainable agricultural practice, reclamation of the land and restoration of denuded habitats for both routine conventional crops as well as newly introduced or to be introduced other crops [2]. Taking M.P. state as whole with an area of 308,252 km² eastern M.P. rhizospheric AMF work is constantly being reported by Singh [4]. However, the mycorrhizal status of the western and central parts of M.P. seems to have hardly been ascertained. Gwalior region has been classified as arid having very hot dry summers and very low precipitation in winters. The monsoon rainfall is low in comparison to the national averages. The variation in soil composition and texture are classified as poor in nutrients like nitrogen, medium in phosphorus and organic carbon and just above average for available potash [28-29]. The area has varied topographical composition of ravines, valleys, many seasonal riverines, hillocks and flat land. This provides for diverse species spectrum [30], which peaks in the high rain season. These edaphic and rugged climatic conditions induce growth of an assorted flora and plantation crops. The present study reveals that 107 of the 110 species investigated are mycorrhizal. Percent root colonization and rhizospheric spore number seem to show no correlation. The findings here are to some extent in agreement with those of Mohan *et al.*, Sudha and Ammani and Gaur and Kaushik [31-33]. It may however, be said after a sizable effort, that nearly 23 species assessed here for mycorrhizal relationship may be first time reports as mycorrhizal and that too for

Gwalior Chambal region. Plants belonging to three families namely Amaranthaceae, Chenopodiaceae and Brassicaceae did neither show any root colonization with AMF nor did their rhizospheric soil show any AMF spores irrespective of the time and site of collection. The failure of plants of these families to form such association has been reported elsewhere also [34-36]. The barrier to such association by the plants of these families has been ascribed to inhibitory substances produced by plants, for example brassinolides by the members of Brassicaceae or certain other substances like alkaloids, tannins or phenolics by other plants. Absence of spore in the soil of *Nelumbo nucifera* seems to be due to deep aquatic habitat of the root.

The limited AMF generic diversity distribution to just four in the Gwalior-Chambal area seems an interesting observation. This though needs further investigation. Only genus *Glomus* seems to show a high adaptative value with the medicinal plants of this area, since 50 percent of the total AMF species enumerated here belong to this genus and rest being represented by other three genera of *Scutellospora*, *Acaulospora* and *Gigaspora*. The hardness and predominance of various *Glomus* species seems to be a general observation reported under certain ecosystems by others also, wherein the study of certain medicinal plants *vis a vis* AMF was done [34, 37-43]. It can be assumed that the majority of species of genus *Glomus* may have developed a stronger adaptive mechanism of symbiosis with different plant hosts as some sort of a co-evolving mechanism [41]. This can be corroborated by a report of *Glomus pyriforme* and *G. albidum* growing in symbiotic association with the earliest diazotrophic cyanobacteria *Nostoc* spp. in poor phosphate soils [44]. Similar reports has very recently been published by Deotare and Wankhede, [45]. One can therefore, conclude that the species of genus *Glomus* seem to have developed adaptive mechanisms for association with hosts even under extreme environmental conditions as are presented by the climatic and edaphic factors in Gwalior-Chambal region. High incidence of polyspory amongst the species spread over large spectrum of families also hints towards specialized adaptation. Since failure of any one species to associate is offset by being responsive to various other species.

CONCLUSION

The present study is the first such report on AMF in the vast unique topographical area of Gwalior-Chambal ravines of central India. Being a study

on the adapted medicinal cum aromatic plants of the region can lay the foundation for further such work for many of the arid regions of the world. The observation here also shows a polyspore adaptation of AMF-plant interaction to ward off inhibitive mechanisms for symbiosis. Nearly 23 plant species seem to be first report for being mycorrhizic.

ACKNOWLEDGEMENTS

Authors thank the Head, School of Studies in Botany and Prof. A.K. Jain, Honorary Director, Institute of Ethnobiology, Jiwaji University for providing facilities.

REFERENCES

1. Kirk, P.M., P.F. Cannon, J.C. David and J.A. Stalfers, 2001. Ainswrth and Bisby's Dictionary of the fungi. 9thed. CAB International, Wallingford, U.K.
2. Wang, B. and Y.L. Qui, 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, 16(5): 299-363.
3. Ahmedullah, M. and M.P. Nayar, 1999. Red Data Book of Indian Plants. Vol. 4, Botanical Survey of India, Calcutta, India
4. Singh, M., P. Singh and D. Vyas, 2011. Mycorrhization in medicinal plants. *Mycorrhizal News*, 23: 14-21.
5. Manjunath, G.T.S. and N. Reddy, 2003. Occurrence of vesicular arbuscular mycorrhizae in medicinal plants of MPC Area, Sandur, Karnataka. *Vigyana Ganga (Sci. Technol.)*, 3: 42-47.
6. Basu, M. and N.K. Srivastava, 1998. Root endophytes in medicinal plants: Their population and effect ICCP 98 G.T.S. Baylis, 1967. Experiments on the ecological significance of phycomycetous mycorrhizas. *New Phytol.*, 66: 231-243.
7. Rao, Y.S.G., C.K. Suresh, N.S. Suresh, R.R. Mallikarjunaiah and O.F. Bagyaraj, 1989. Vesicular arbuscularmycorrhizae in medicinal plants. *Indian Phytopathol.*, 92: 476-478.
8. Abbott, L.K. and A.D. Robson, 1982. The role of vesicular-arbuscular-mycorrhizal fungi in agriculture and the selection of fungi for inoculation. *Australian Journal of Agricultural Research*, 33: 389-408.

9. Taber, R.A. and J.M. Trappe, 1982. Vesicular arbuscular mycorrhiza in rhizomes, scale like leaves, roots and xylem of ginger. *Mycologia*, 74: 156-161.
10. Mohammad, M.J. and H.I. Malkawi, 2004. Root, shoot and nutrient acquisition responses of Mycorrhizal and Nonmycorrhizal wheat to phosphorous application to highly calcareous soils. *Asian Journal of Plant Sciences*, 3(3): 363-364.
11. Achakzai, A.K.K., M.O. Liasu and O.J. Popoola, 2012. Effect of mycorrhizal inoculation on the growth and phytoextraction of heavy metals by maize grown in oil contaminated soil. *Pakistan Journal of Botany*, 44(1): 221-230.
12. Zubek, S. and J. Blaszowski, 2009. Medicinal plants as hosts of arbuscular mycorrhizal fungi and dark septate endophytes. *Phytochem. Rev.*, 8: 571-580.
13. Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, 55: 158-161.
14. Kormanik, P.P., W.C. Bryan and R.C. Schultz, 1980. Procedures and equipment for staining large numbers of plant root samples for endomycorrhizal assay. *Can. J. Microbiol.*, 26: 536-538.
15. Biermann, B. and R.G. Linderman, 1981. Quantifying vesicular-arbuscular mycorrhizae: A proposed method towards standardization. *New Phytol.*, 87: 63-67.
16. Gerdman, J.W. and T.H. Nicolson, 1963. Spores of mycorrhizal *Endogen* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.*, 46: 235-244.
17. Gaur, A. and A. Adholeya, 1994. Estimation of VAM spores in the soil: A modified method. *Mycorrhiza News*, 6: 10-11.
18. Lugo, M.A. and M.N. Cabello, 2002. Native Arbuscular Mycorrhizal Fungi (AMF) from mountain grassland (Cordoba, Argentina) I. Seasonal variation of fungal spore diversity. *Mycologia*, 94: 579-586.
19. McKenney, M.C. and D.L. Lindsey, 1987. Improved methods for quantifying endomycorrhizal fungi spores from soil. *Mycologia*, 79: 779-782.
20. Koske, R.E. and B. Tessier, 1983. A convenient, permanent slide mounting medium. *Mycol. Soc. Am. Newslett.*, 4: 59-64.
21. Omar, M.B., L. Bolland and W.A. Heather, 1979. A permanent mounting medium for fungi. *Bull. Brit. Mycol. Soc.*, 13: 31-32.
22. Morton, J.B., 1988. Taxonomy of VA mycorrhizal fungi: classification, nomenclature and identification. *Mycotaxon*, 32: 267-324.
23. Schencz, M.C. and Y. Perez, 1990. Manual for the identification of VA Mycorrhizal Fungi. INVAM, Gainesville, USA., pp: 280.
24. Walker, C. and J.M. Trappe, 1993. Name and epithets in the Glomales and endogonales. *Mycol. Res.*, 97: 339-344.
25. Wu, C.G., 1993. Glomales of Taiwan. III. A comparative study of spore ontogeny in *Sclerocystis* (Glomaceae, Glomales). *Mycotaxon*, 47: 25-39.
26. Morton, J.B. and G.L. Benny, 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, *Glomineae* and *Gigasporineae* and two new families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of Glomaceae. *Mycotaxon*, 37: 471-491.
27. Kornerup, A. and J.H. Wanscher, 1983. *Methuen Hand book of Colour*. 3rd Edn., E. Methuen and Co. Ltd., London, UK., pp: 252.
28. Tiwari, R.J., K. Dwivedi and S.K. Verma, 1994. Multiple relationship of soil properties with nutrient content of leaves and crop yield on sodic vertisol. *Crop Res.*, 8: 52-56.
29. Sharma, S.C., V.S. Rajput and R.J. Tiwari, 1995. Status of available manganese in Harsi command area soils of Madhya Pradesh. *Crop Res.*, 9: 441-443.
30. Jain, A.K., 1992. Ethnobotanical studies on Sahariya tribals of Madhya Pradesh with special reference to medicinal plants. *Journal of Economic and Taxonomic Botany, Add. Ser.*, 10: 227-232.
31. Mohan, V., M. Bappamal, N. Malathy and P. Monokaran, 2005. Distribution of Am fungi in association with some medicinal plants of Tamil Nadu. *Indian For.*, 131: 784-797.
32. Sudha, K. and K. Ammani, 2010. Arbuscular mycorrhizal fungi in medicinal plants in Thrissun district, Kerala. *Mycorrhiza News*, 21: 13-18.
33. Gaur, S. and P. Kaushik, 2011a. Biodiversity of Vesicular Arbuscular Mycorrhiza Associated with *Catharanthus roseus*, *Ocimum* spp. and *Asparagus racemosus* in Uttarakhand State of Indian Central Himalaya. *International Journal of Botany*.

34. Mohan Kumar, V. and A. Mahadevan, 1984. Do secondary substances inhibit mycorrhizal associations? *Curr. Sci.*, 53: 377-378.
35. Hirsch, A.M. and Y. Kapulnik, 1998. Signal transduction pathways in mycorrhizal associations: Comparisons with the Rhizobium-legume symbiosis. *Fungal Genet. Biol.*, 23: 205-212.
36. Verma, N.K., 1998. Effect of VA mycorrhiza on the growth and P uptake in *Eupatorium adenophorum* Spring. (Asteraceae) grown in soil amended with soluble phosphate. *J. Natl. Bot. Soc.*, 52: 41-45.
37. Ram, U. and S. Bhadauria, 2009. Vesicular-arbuscular mycorrhizal association with some medicinal plants growing on alkaline soils of Manipuri district, Uttar Pradesh. *Mycorrhiza News*, 13: 12-14.
38. Gupta, M.L., A. Khaliq, R. Pandey, R.S. Shukla, H.N. Singh and S. Kumar, 2000. Vesicular-arbuscular mycorrhizal fungi associated with *Ocimum* spp. *J. Herbs Species Med. Plants*, 7: 57-63.
39. Gupta, A.K., S. Chaturvedi and A.K. Sharma, 2009. Arbuscular mycorrhizal fungal diversity in some medicinal plants. *Mycorrhiza News*, 20: 10-13.
40. Panwara, J. and J.C. Tarafdar, 2006. Distribution of the three endangered medicinal plant species and their colonization with arbuscular mycorrhiza. *J. of Arid Environ.*, 65(3): 337-350.
41. Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel *et al.*, 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396: 69-72.
42. Mahmood, I. and R. Rizvi, 2010. Mycorrhiza and Organic Farming. *Asian Journal of Plant Sciences*, 9(5): 241-248.
43. Gaur, S. and P. Kaushik, 2011b. Influence of Edaphic Factors on Distribution of Mycorrhiza Associated with Medicinal Plants in Indian Central Himalayas. *Journal of Biological Sciences*, 11(5): 349-358.
44. Kluge, M., D. Mollenhauer, E. Wolf and A. Schübler, 2002. The *Nostoc-Geosiphon* endocytobiosis. In *Cyanobacteria in Symbiosis*. Edited by A.N. Rai, B. Bergman and U. Rasmussen, Kluwer Academic Publishers, pp: 19-30.
45. Deotare, P.W. and T.B. Wankhede, 2010. Arbuscular mycorrhizal fungal diversity and distribution around natural salt lake of Lonar, Maharashtra, India. *Mycorrhiza News*, 21: 9-12, 7(1): 31-41.