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AM Fungal Diversity in Selected Medicinal Plants of Haryana, India

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Abstract: This study deals about bio-diversity of arbuscular mycorrhizal fungi (AMF) associated with medicinal plants of Haryana. Medicinal plants play an important role in supporting healthcare system in India. According to the World Health Organisation (WHO), 80 % of the rural population in developing countries utilizes locally available medicinal plants for their primary healthcare needs. Twenty one AM fungal taxa belonging to five genera based on spores were isolated from fourteen medicinal plants collected from Yamunanagar and Panchukula district of Haryana. The dominant genus was *Acaulospora* (13 sp.) followed by *Glomus* (4 sp.), *Gigaspora* (2 sp.), *Scutellospora* and *Sclerocystis* (1 sp.) each. AM root colonization ranged from (22.0±2.74) in *Amaryllis belladonna* to (81.1±1.82) in *Shorea robusta*. Isolated number of AM spores varied in count from (25.8±2.28) in *Prosopis cineraria* to (178.6±4.39) in *Jasminum sambac*. The data shows that there was no positive correlation between percentage mycorrhizal root colonization and AM spore density.

Key words: Medicinal plants · AM fungi · Root colonization · Spore count

INTRODUCTION

A large number of microorganisms are found to be associated with the roots of plant in their natural habitat. Among them the most important and highly evolved association is that formed by arbuscular mycorrhizal fungi (AMF). The arbuscular mycorrhizal (AM) fungi take an important position amongst various microorganisms colonizing the rhizosphere of plants. The occurrence of endomycorrhizal fungi (AM) in the soil, their association with both forestation and agricultural crops are well documented [1, 2]. AM fungi have emerged as potential biofertilizers, a cheap environmentally friendly alternative to expensive chemical fertilizers [3]. The main advantage of mycorrhiza is its greater soil exploration and increasing uptake of P, N, K, Zn, Cu, S, Fe, Mg, Ca and Mn and the supply of these nutrients to the host roots [4, 5]. AM fungal colonization of plant roots has also been suggested to increase plant tolerance to pathogens thereby acting as a biocontrol agent [6].

The establishment of symbiotic association of AM fungi with plant roots has much similarty to nodule formation in legumes by non- pathogenic rhizobia [7]. The AM fungi are worldwide in distributation. Agricultural management practices, environmental conditions might affect AM fungal communities both qualitatively and quantitatively [8]. Identification of endophytes within roots is important for the development of AM fungal inoculants of the most prevalent AM fungus identified. Another method commonly used to identify the AM fungus is based on the spore morphology, after retrieving the spores from the soil [9].

It is an established fact that medicinal plants play a great role in our life. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant sources [10]. Medicinal plants represent a rich source of antimicrobial agents. In India the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times [11]. Therefore many workers have conducted studies on natural association of mycorrhizal fungi to find out their diversity of different type of vegetation. In a survey, forty two AM fungal species of five genera namely, *Glomus, Acaulospora, Scutellospora*, *Gigaspora* and *Ambispora* were discovered from the rhizosphere soil, associated with thirty selected medicinal plants species, among the AM fungal colony, *Glomus* was found to be the most dominant genus and the most dominant AM fungal species is *Glomus fasiculatum* [12]. In a survey on AM association in three different species of *Cassia* viz.

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Cassia alata, *Cassia sophera* and *Cassia occidentalis* by [13], it was found that all of the three species possess AM association under natural conditions. Similarly, [14] found the AM association with some important medicinal plants in Suburban area of Mumbai. [15], observed mycorrhizal root colonization in twenty species of tree legumes in the Eastern Ghats and found altogether 21 species of AMF belonging to six genera viz. *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora*. [16] studied on 46 medicinal plant species of herbs and shrubs in the western ghats of Karnataka region and identified 36 AMF taxa in which *Glomus* sps. were found to be dominant followed by *Acaulospora* sps, *Gigaspora* sps, *Scutellospora* sps, *Paraglomus* sps and *Pacispora* sps. They are found in most of the ecosystems like woodlands, dense rain forests, grasslands, arid and semi arid deserts [17]. There are many reports of mycorrhizal occurrence status and diversity in medicinally important plants of India [18, 19]. Hence, the present study is aimed to determine the AMF spore population in rhizosphere soils and its colonization in 14 different medicinal plants of Haryana districts of India (Table 1).

Table 1: Important medicinal uses of the plants selected for studying mycorrhizal association.

Sr.No.	Botanical name	Part used	Medicinal importance
1.	*Aloe barbadensis* Mill	Leaves	The gel or mucilage is a wound- heating agent and shows antibacterial, antifungal and anti-inflammatory properties. Aloe gel preparations are used for burns, scalds, sunburns and wounds.
2.	*Amaryllis belladonna* L.	Whole plant	Help to treat chicken pox, colds, fever, flu, measles, mumps, pancreatic diseases and whooping cough.It is used to treat all types of Gastro-intestinal disorders, menopause, urinary tract problems and skin problems.
3.	*Bina orellana* L.	Root, bark, seeds, leaves	It is useful in gonorrhoea, jaundice, antioxidant, antitumor activity. Protects the skin against the ultraviolet rays of the sun. It is also used as a general skin tonic and to heal skin.
4	*Cissampelos parieta* L.	Root	It is a blood purifier and has anti- inflammatory property. Used in cough and dyspnoea, skin disorders. Helpful in indigestion, abdominal pain, diarrhoea and dysentery.
5	*Ficus racemosa* L.	Root, bark, leaves, fruits, latex	Used for treating intestinal worms, leucorrhea, fatigue, bowel complaints, blood impurity, bronchitis, epitasis, skin diseases, diarrhea, constipation and urinary system diseases.
6.	*Gmelina arborea* Roxb.	Root, leaves, bark	Stem-wood bark was found to be hypoglycaemic, stem-bark antiviral, leaf demulcent, bark anticephalagic, root galactogogue, demulcent, tonic, stomachic and laxative. The leaf removes foetid discharges from ulcers. A paste of leaves is applied to the head for the relief of headache in fevers.
7.	*Hemidesmus indicus* L.	Roots	Dried roots constitute the drug useful in fever, skin diseases, loss of appetite, syphilis, leucorrhoea and urinary complaints.
8.	*Jasminum sambac* (L.) Aiton	Roots, leaves, Flowers	Helpful in treatment of number of ailments, including disease of eyes, itching, headache, ulcer, wound, insanity and epilepsy.
9.	*Mesua ferrea* L.	Stamens of flowers	Phenolic constituents of the seed oil revealed a potent antiasthmatic effect. Essential oil from fruits showed antifungal and antibacterial activity. Stamens of flowers prescribed in haemoptysis, blood poisoning, chronic dysentery, diarrhoea with blood and skin eruptions.
10.	*Operculina terpethum*	Root, stem, bark	The main part of plant that is used as medicine is its root. The plant has laxative property and thus effective in curing constipation, relieving flatulence and colic condition. It is also useful in periodic fevers as it helps in reducing body temperature. This herb is used for the treatment of many other diseases like arthritis, dropsy, gout, jaundice.
11.	*Phyla nodiflora* (L.) Greene	Leaves	Plant is diuretic, stomachic, astringent to the bowels and good for the ulcers, wounds, asthma, bronchitis, pain in knee- joints. Leaves and fruits are eaten to relieve the initation of internal piles.
12.	*Prosopis cineraria* (L.) Druce	Leaves, flowers, bark	Flower is pounded mixed with sugar and used during pregnancy as safeguard against miscarriage. Water soluble extract of the residue from methanol extract of the stem bark exhibits anti- inflammatory properties. The smoke of the leaves is good for eye- troubles. The bark is prescribed for scorpion sting.
13.	*Rosa indica* L	Fruit and flower	Fruits are said to be applied in china to wounds, sprains and ulcers, stomach problems. The root is bitter and astringent, used as a tonic. The leaves are considered a good vulnerary.
14.	*Shorea robusta* Gaertn. f.	Heartwood, flowers, gum-resin	Resin exhibits stomachic, astringent, anti-dysentric, antiseptic and antigonorrhoeic properties. Powdered flowers or heartwood used for emaciation and anaemia due to loss of blood and goitere. Fumes of gum-resin were inhaled during asthamatic attacks and bouts of cough.

MATERIALS AND METHODS

Collection of Root and Soil Samples: Soil samples were collected from different areas of Haryana. The samples were collected from the rhizosphere of each plant at a depth of 15-30 cm and were kept in polythene bags at 5-10 ^{0}C for isolation of mycorrhizal spores and root colonization. Five sets of roots and rhizospheric soil samples were thoroughly mixed and a composite sample was taken for analysis.

Isolation of Dominant AM Spores from Soil Samples: Isolation of dominant AM spores was done by 'Wet sieving and decanting technique' by [20]. With this technique, 50g of soil were soaked in 250 ml water for 24 h. The supernatant was then passed through a gradient of sieves with pore sizes ranging from 150 µm to 45 µm arranged one above the other in an ascending order. Each sieve was then washed in water which was filtered through Whatman No.1 filter paper. This filter paper was then observed under a stereobinocular microscope to observe various kinds of spores. The spores were mounted on Polyvinyl lactic acid (PVLA) for further studies.

Quantification of AM Spores and Identification of AM Fungi: This was done by the Grid line intersect method [21]. Spores were counted under a stereobionocular microscope with a tally counter. The AM spores were identified with the identification manuals by [22- 24].

Assessment of AM Fungal Colonization: Roots were collected from the soil blotted dry to determine fresh and dry root weight, P content and mycorrhizal root colonization. Mycorrhizal root colonization was done by the rapid clearing and staining method [25]; The percentage of AM root colonization was determined as under:-

$$\text{Percent root colonization} = \frac{\text{Number of root segments colonized}}{\text{Number of root segments}} \times 100$$

RESULTS AND DISCUSSION

All typical AM features such as arbuscules, vesicles, intracellular hyphal coils, extra and intraradical hyphae were observed in the root samples of all the fourteen medicinal plants. Mycelia of various types coiled, beaded, Y- shaped, H- shaped and parallel were present in roots of different plants. In the present study, arbuscules were seen only in four plants i.e. *Jasminum sambac*, *Mesua ferrea*, *Phyla nodiflora*, *Shorea robusta*. According to [26] arbuscules are normally found during active vegetative growth due to availability of new cortical cells and high nutrient requirement by the host. At later stages, arbuscule formation decreases and vesicle formation increases. Structurally, arbuscules are haustoria like where phosphates and fixed carbon molecules are exchanged. Vesicles of different shapes like oval, elongated, beaded, globose, round etc were found in all the medicinal plants studied. Vesicles act as storage organs for food.

Root Colonization of AM Fungi: The medicinal plants were studied for mycorrhizal colonization. AMF root colonization ranged from (22.0±2.74) in *Amaryllis belladonna* to (81.1±1.82) in *Shorea robusta* (Table 2). [27] revealed that percent root colonization depends on soil quality where the low nutrient status of the soil enhances the rate of infection. Variation occurs in root colonization due to different nutrient requirements of the host plant [28] The level of colonization indicates its deep susceptibility towards AM fungi. Variation in the percentage of AM colonization indicated that the pattern of colonization might be regulated at the species level. [29] recorded varying degree of infection among different plants within a family. Similarly in the present investigation, *Gmelina arborea* of the family Verbenaceae supported the formation of both vesicles and mycelium, while *Phyla nodiflora* of the same family supported vesicles, arbuscules and mycelium. Similarly, variation in the infection levels may also be due to the reason that host response can differ with fungal species and seasonal development [30]. Moreover, environmental factors have strong and sometimes unpredictable effects on infection as well as functioning of the AM endophytes.

Spore Count of AM Fungi: The AM spore population varies from (25.8±2.28) in *Prosopis cineraria* to (178.6±4.39) in *Jasminum sambac* per 50 g of soil (Table 2). Both the species richness and spore density of AM fungi depend upon the area, season and yearly variation in precipitation and temperature [31]. The study also indicates variation in spore number and diversity in the rhizosphere of the fourteen plants surveyed may be because of soil texture, climate and other soil factors. These findings were in accordance with the study conducted by other researcher [32]. In present investigation, the root infection rate could not be correlated with the spore population. Similar observation was also made by [33], under field conditions.

Table 2: Occurrence of arbuscular mycorrhizal fungi with some medicinal plants of Haryana.

S. No.	Name	Common name	Family Name	Presence of M	V	A	Spore Count	% root colonization
1	*Aloe barbadensis* Mill.	Kumari	Liliaceae	+	+	-	*71.8± 3.03	*40.5 ± 1.25
2	*Amaryllis belladonna* (L.)	Lily	Amaryllidaceae	+	+	-	44.6 ± 2.07	22.0 ± 2.74
3	*Bixa orellana* (L.)	Lipstick tree	Bixaceae	+	+	-	56.0 ± 1.58	62.5 ± 2.76
4	*Cissampelos parietal*(L.)	Patha	Menispermaceae	+	+	-	44.0 ± 3.39	42.0 ± 2.05
5	*Ficus racemosa* (L).	Indian fig	Moraceae	+	+	-	40.8± 2.68	52.5 ± 2.92
6	*Gmelina arborea* Roxb.	Gmelina	Verbenaceae	+	+	-	46.2 ± 3.42	33.0 ± 4.11
7	*Hemidesmus indicus* (L). R. Br.	Anantamool	Asclepiadaceae	+	+	-	53.8 ± 2.59	41.4 ± 2.05
8	*Jasminum sambac* (L.) Aiton	Jasmine	Oleaceae	+	+	+	178.6 ± 4.39	74.6 ± 4.22
9	*Mesua ferrea* (L.)	Iron wood tree	Clusiaceae	+	+	+	42.8± 1.92	71.9± 2.86
10	*Operculina terpethum*	Indian jalap	Asteraceae	+	+	-	45.6 ± 1.95	63.1 ± 3.32
11	*Phyla nodiflora* (L.) Greene	Frog fruit	Verbenaceae	+	+	+	37.6 ± 2.07	42.3 ± 2.54
12	*Prosopis Cineraria* (L.) Druce	Jand	Fabaceae	+	+	-	25.8 ± 2.28	39.5 ± 1.19
13	*Rosa indica* (L.)	Rose	Rosaceae	+	+	-	53.6 ± 2.70	61.9 ± 2.72
14	*Shorea robusta* Gaertn. f.	Sal	Dipterocarpaceae	+	+	+	74.6 ± 4.82	81.1 ± 1.82

* = Mean of five readings, M = Mycelium, V = Vesicle, A = Arbuscule, + = Present, - = Absent, ± = Standard deviation.

Table 3: AM spore diversity associated with rhizosperic soil of medicinal plants of Haryana.

S.No.	AM fungi	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P 13	P 14
1	*Acaulospora elegans*	+	_	_	_	+	_	_	_	_	+	_	_	_	_
2	*Acaulospora foveata*	_	_	_	_	_	_	+	+	_	_	_	_	_	_
3	*Acaulospora gerdemanii*	_		_	_	_	_		_	_	_	_	_	_	_
4	*Acaulospora lacunosa*	+	+	_	_	_	_	+	_	_	+	_	_	+	+
5	*Acaulospora laevis*	+	_	_	_	+	_	_	_	_	_	_	_	_	_
6	*Acaulospora margarita*.	_	_	_	_	_	_	_	+	_	_	_	_	_	_
7	*Acaulospora mellea*	_	+	_	_	_	+	_	_	_	_	_	_	_	_
8	*Acaulospora nicolsonii*	_	_	_	_	_	_	_	_	_	_	_	_	_	+
9	*Acaulospora rehmii*	_	_	_	_	_	_	_	_	_	_	_	_	_	_
10	*Acaulospora scrobiculata*	_	_	_	_	+	+	_	_	+	+	_	_	_	
11	*Acaulospora spinosa*	_	_	_	_	_	_	_	_	_	_	_	_	_	+
12	*Acaulospora trappii*	_	_	_	_	+	_	_	_	_	_	_	_	_	_
13	*Acaulospora* sp.	_	_	_	_	_	+	_	+	_	_	_	_	_	_
14	*Gigaspora margarita*	_	_	+	_	_	_	_	_	_	_	_	_	_	_
15	*Gigaspora* sp.	_	_	_	+	+	_	_	_	_	_	_	_	_	_
16	*Glomus constrictum*	_	_	_	_	+	_	_	_	_	_	_	_	_	_
17	*Glomus fasciculatum*	_	_	+	+	+	_	_	_	_	_	_	+	_	_
18	*Glomus intraradices*	_	_	_	_	_	_	_	_	_	_	_	_	_	_
19	*Glomus* sp.	_	_	_	_	+	_	_	+	_	_	_	_	_	_
20	*Sclerocystis* sp.	+	_	_	_	_	_	_	_	_	_	_	_	_	_
21	*Scutellospore* sp.	+	+	+	_	_	_	_	+	_	_	+	_	_	_

P1 –*Aloe barbadensis*, P2 – *Amaryllis belladonna* , P3 – *Bixa orellana*, P4– *Cissampelos parieta*, P5 –*Ficus racemosa* , P6-*Gmelina arborea*, P7 –*Hemidesmus indicus*, P8 –*Jasminum sambac* , P9 - *Mesua ferrea*, P10-*Operculina terpethum*, P11- *Phyla nodiflora,* P12- *Prosopis cineraria,* P13- *Rosa indica,* P14- *Shorea robusta* + shows present ; _ shows absent

According to [34], these differences might be due to the presence of diverse type of VAM fungi in the rhizosphere soil of individual plant species or might be a manifestation of greater host susceptibility to AM fungi.

Diversity of AM Fungi: AM spores isolated from the rhizosphere of different medicinal plants are depicted in Table 3. Total five genera with 21 different AM species were isolated. The dominant genus was *Acaulospora* (13 sp.) followed by *Glomus* (4 sp.), *Gigaspora* (2 sp.), *Scutellospora* and *Sclerocystis* (1 sp.) each. Maximum diversity of AM species was reported from *Ficus racemosa* (8 sp.) minimum from *Rosa indica*, *Prosopis Cineraria, Phyla nodiflora, Mesua ferrea* (one sp.) each. The dominance of *Acaulospra* was also obtained by [35]. [36] observed *Acaulospora* and *Glomus* as the dominant mycospecies in the roots of medicinal plants. The possible reasons for the predominance of *Acaulospora* species are often associated with acidic soils [37] and *Glomus* species with different temperature and pH preferences for germination [38].

CONCLUSIONS

It can be concluded from the present study that the widespread presence of *Acaulospora* and *Glomus* in the soil makes it more favoured fungi for the mass multiplication as well as seedling inoculation for better growth of the medicinally important plants. All the plant species harbour mycorrhizal association however, these plants must possess adequate amount of mycorrhizal colonization at the planting stage in order to survive and perform better in adverse plantation sites. Mycorrhizal spore density was found to be higher in wild medicinal plant species as compared to cultivated species. These observations could be attributed to the undisturbed nature of the ecosystem. There are many factors that could disturb spore proliferation in a given host rhizosphere. Seasonality, edaphic factors, age of host plants, the sporulation abilities of AMF, host dependence have been previously reported to be amongst these factors [39]. The percent root colonization, spore density and its richness has been correlated with climatic and edaphic factors of the study area and also with the host dependence of AMF species.

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