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Ashish Kumar
Department of Botany, Kurukshetra University Kurukshetra, Haryana, India

Vipin Parkash
Scientist- E, forest pathology division, Forest Research Institute, Dehradun, India

Anil Gupta
Botany Faculty, Institute of Integrated and Honors studies, Kurukshetra University, Kurukshetra Haryana, India

Ashok Aggarwal
Department of Botany, Kurukshetra University Kurukshetra, Haryana, India

Correspondence:
Ashish Kumar
Department of Botany, Kurukshetra University Kurukshetra, Haryana, India
Email: ashishbotany990[at]gmail.com

Biodiversity of arbuscular mycorrhizal fungi associated with selected medicinal plants of Hamirpur district of Himachal Pradesh, India

Ashish Kumar*, Vipin Parkash, Anil Gupta, Ashok Aggarwal

ABSTRACT

The present investigation was focused on exploration of biodiversity of Arbuscular mycorrhizal fungi (AMF) associated with different medicinal plants. Twenty-two medicinal plants belonging to 14 families were analyzed for AMF colonization. The plant roots and their respective rhizosphereic soil samples were collected from different localities of hamirpur district, himachal pardesh for AMF analysis and spore assessment per 50gm of soil sample of soil. The results revealed that number of AM spores in the rhizosphere of plant was not related to percent of AM root colonization. Highest per cent of root colonization was reported in *Ricinus communis* (86.5±4.68 %) and *Achyranthes aspera* lacks colonization. Highest number of AM spore was found in rhizosperic soil sample of *Mimosa pudica* (177.4±4.306) and least number of spores in *Datura stramonium* (47.53±2.76). Fourty three AM species belonging to five genera i.e. *Glomus*, *Acaulospora*, *Gigaspora*, *Entrophospora* and *Sclerocystis* were isolated during investigation. Maximum AM spore diversity was observed in *Mentha viridis* followed by *Catharanthus roseus* and least diversity related to *Datura stramonium*. The study confirmed that diversity of AM fungi varies with plant to plant.

Keywords: AMF spore, root colonization, medicinal plant.

INTRODUCTION

Despite for reaching advancement of modern civilization, man still depend largely on plants and their products. Medicinal and aromatic plants (MAP) are used in different traditional systems of medicines in different parts of globe. The cultivation of MAP has been increased to sustain increased demand of MAP as a result of excessive consumption of herbal drugs. Therefore, researchers are focused on to increase production of medicinal plant with the help of useful and appropriate soil microbes present in rhizosphere of medicinal plants. Many soil microbes form symbiotic association with plants, among them AM fungi are stand out because of their better effects on plant growth and are associated with 80% of all terrestrial plant species. This fungal partner of symbiotic association belonging to glomeromycota that corresponds to five different genera such as *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* [1] It has been well established that AM fungi improves plant growth in terms of better nutrient uptake, water relations, stress tolerance, production of growth promoting substances and protection from root pathogen [2-5]. So, exploration of microbial diversity is primarily important in to utilizing these fungi as bio-fertilizer for cultivation of valuable medicinal plants. The beneficial influences of indigenous AM fungi on plant health were closely linked with type of fungi and its distribution in soil. However, utilization of AM fungi on a wide scale in agriculture is relying on the development of effective plant -growth-promoting strains of AM, which are superior among native soil population of AM fungi [6]. Therefore, analysis of soil samples belongs to different regions is mandatory for estimation of abundance as well as type of indigenous AM fungi present in rhizosphere of the plant.

Keeping in view the above facts, the study of AMF biodiversity associated with some medicinal plants is therefore, necessary from efficient utilization and conservation point of view. Considering the important status of medicinal plants, the present investigation was concerned to isolate, identify and classify the indigenous AM fungi associated with some commonly grown medicinal plants in Hamirpur district, Himachal Pradesh. The exploration of predominant AM fungi also helpful for formation of future inoculums as well as its application for production of better seedlings and their survival in adverse conditions.

MATERIAL AND METHOD

Sampling

Seasonal field trips were performed from 2016 to 2018, in order to collect soil and fine root samples for assessment of AM diversity associated with some medicinal plants found in Hamirpur district of Himachal Pradesh, India. The plants were randomly selected for sampling from different areas. Soil samples and fine roots from the rhizospheric soil were collected by digging out small amount of the soil close to the plant roots up to the depth of 15-30 cm, and stored in sterilized polythene bags at 4-10^{0}C for further processing in the laboratory.

Isolation, Quantification and Identification of AM spores

Isolation of AM spores were done by using 'Wet sieving and decanting technique' of Gerdman and Nicolson [7]. Sieves of different sizes i.e. 150µm, 120µm, 90µm, 60µm and 45µm are used. 50 gm of composite soil sample was dissolved in water. After stirring, soil solution was allowed to settle down over night. Decanting water on a series of sieves in following order 150µm, 120µm, 90µm, 60µm and 45µm from top to bottom on which spores were trapped. The trapped spores were transferred to whatman filter paper No.1 by repeated washing with water. Then spores were picked up by hypodermic needle under stereo-binocular microscope and mounted in polyvinyl lactic acid alcohol (PVLA). AM spores were counted by Gridline intersect method' proposed by Gaur and Adholeya [8] under stereo-binocular microscope at 60X magnification. Identification of AM spores was done using identification manual [9-11].

AM root colonization

Mycorrhizal root colonization was done by 'Rapid Clearing and Staining Method' of Phillips and Hayman [12]. The collected roots were cut into 1cm segments and then 15 – 30 segments are selected randomly. These roots segments were cleaned in 10% KOH (24 hours), acidified with 1% HCl (20 minutes) and stained with trypan blue stain for 24 hours. After this root segments were destained with lactophenol for a day to remove excess of stain. Now roots were mounted in lactic acid: Glycerol (1:1) solution and examined for AM colonization. Evaluation of root colonization was done by root slide technique of Giovannetti and Mosse [13].

Percent root colonization was calculated by formula:

$$\text{Percentage of AM root colonization} = \frac{\text{No of root segments with infection}}{\text{Total no. of root segments studied.}} \times 100$$

Table 1: Medicinal importance of the plants selected for studying AMF association

Sr. No.	Botanical Name	Common Name	Family	Medicinal Importance
1	*Acacia catechu* (L.f.) Willd.	Khair	Mimosaceae	The bark of the plant is used as an antipyretic as well as anti inflammatory substance. It is also used to relieve psoriasis, anemia, gum problems, leprosy, constipation and skin disorders.
2	*Achyranthes aspera* Linn.	Puthkanda	Amaranthaceae	The seeds are given in cutaneous diseases, hydrophobia, snake bite and to stimulate dieresis.
3	*Adhatoda vasica* Nees.	Basuti	Acanthaceae	Basuti is used as bronchodilator and respiratory antispasmodic. It is utilized to cure cold, asthma, whooping cough and tuberculosis.
4	*Ageratum Conyzoides* Linn.	Gumdrya, Ujadu	Asteraceae	It is used to cure pneumonia, wound and burn, ulcers, inflammations, spasm, blood infection and bacterial infections.
5	*Aloe vera* (Linn.) Burm. f.	Kumar patha	Liliaceae	Leaves are used to cure constipation, skin wound, vaginal infections, diabetes, acne and high cholesterol.
6	*Bauhinia variegata* Linn.	Kachnar, Gurial	Fabaceae	The bark is alterative, astringent and tonic. Used against diarrhoea, ulcer and leprosy. The dried buds are beneficial to treat piles and dysentery. The root decoction is taken to cure dyspepsia and flatulency.
7	*Bryophyllum pinnatum* Kurz.	Air plant, miracle leaf	Crassulaceae	It is used for treatment of ear ache, burns, ulcers, insect bites, diarrhoea, rheumatism and inflammations.
8	*Butea monosperma* (Lamk.) Taub.	Palas Dhak	Fabaceae	Used to treat pyorrhoea, toothache, joint pain. The bark is externally applied on cut and wounds, and orally used to cure intestinal worms.
9	*Cassia fistula* Linn.	Amaltaas, Indian laburnum	Fabaceae	Anti-inflammatory, antioxidant, constipation, antibacterial, insect bites, urinary trouble and blood dysentery.
10	*Catharanthus roseus* (L.) G. Don	Madagascar periwinkle, Sadabhar	Apocynaceae	Plant used for treatment of blood cancer, diabetes, malaria, Hodgkin's disease and malignant lymphomas.
11	*Cymbogopon citratus* (DC.) Stapf.	lemon grass	Poaceae	The plant is used for bronchitis, epilepsy, skin disease, fever and gastric irritations.
12	*Dalbergia sissoo* Roxb.	Sisham, sissoo	Fabaceae	Powder of dried leaves is taken with sugar for the treatment of leucorrhorea and menorrhagia.
13	*Datura stramonium* Linn	Chitta Dhatura, jimson weed, thorn apple	Solanaceae	The leaves and seeds are antiasthmatic, hallucinogenic, epileptic, anaesthetic. Extract of whole plant material is useful in case of dysmenorrhea or period pain.
14	*Indigofera tinctoria* Linn.	True indigo, Nil	Fabaceae	Used to cure skin diseases, leucoderma, burns, epilepsy, asthma, blennorrhagia, hepatitis. Root and stem have laxative, anticephalalgic, antitumour, anthelmintic, promote growth of hair.
15	*Lantana camara* Roxb.	Phool lakdi	Verbenaceae	The leaves and roots are used to cure malaria, respiratory infections, bacterial infection, scabies, skin rashes, inflammation.

16	*Mentha viridis* Linn	Pudina	Lamiaceae	The leaves are given in bronchitis and fever, and a decoction is used as lotion in aphthae.
17	*Mimosa pudica* Linn.	Lajwanti, Chui-mui	Mimosaceae	The roots are use to cure kapha, leprosy, Vaginal and uterine complaints, asthma and Leucoderma. Externally used for oedema, rheumatism and tumour of uterus
18	*Pongamia pinnata* Pierre	Pongam, Indian beech	Fabaceae	Used for treatment of abdominal tumors, rheumatism, diarrhea, dyspepsia, gonorrhea herpes, bronchitis, whooping cough, diabetes, piles, diabetes, leprosy, scabies, and ulcers.
19	*Ricinus communis* Linn.	Arand, Castor	Euphobiaceae	Castor oil is used for facilitating easy birth of child and used as purgative for pregnant women during menses. Leaves are employed externally by nursing mother to increase flow of milk. Ricin acts as blood coagulant and Ricin A is a lectin that possesses antitumor activity. Castor plant is used against diarrhoea, inflammatory disease of intestine, rectum and urinogenital tract and jaundice.
20	*Spilanthes acmella* Murr.	Akarkara	Asteraceae	Root decoction is used as purgative and leaf decoction as diuretic. Flower head chewed to treat toothache and other mouth related troubles. Whole plant is utilized to cure dysentery
21	*Tinospora cardifolia* (wild.)Mier.	Giloe	Menispermaceae	Fresh stem decoction is considered good for the treatment of jaundice and seminal weakness. Starch of stem is mixed with wheat flour and then roasted in butter is recommended for the treatment of Leucorrhorea and menorrhagia.
22	*Vitex negundo* Linn.	Banae, Suraei	Lamiaceae	Amenorrhea, dysmenorrhea, rheumatism, anthelminthic, disease of scalp, chronic ulcer, skin disease. The fruits used in the treatment of reddened, painful, and inflated eyes, headache and arthritic joints.

Table 2: Occurrence and distribution of AMF species among selected medicinal plants of Hamirpur district of Himachal Pradesh.

Sr. No.	Botanical Name	Type of Infection			AM spore count / 50gm. of soil	AM Root Colonization (%)	AM Spore species richness	AM fungal spores
		M	V	A				
1.	*Acacia catechu*	+	+	+	168.1±2.53	65.04±4.64	5	4,10,25,6,30
2.	*Achyranthes aspera*	-	-	-	61.16±2.03	Nil	8	6,11,19,22,29,31,34,43
3.	*Adhatoda vasica*	+	-	+	148.73± 3.34	81.25± 6.30	5	10,13,24,35,37
4.	*Ageratum conyzoides*	+	+	-	104.66±2.75	22.22±0.55	9	3,7,11,19,28,31,35,40,43
5.	*Aloe vera*	+	-	+	60.16±3.105	64.66±8.40	8	1,9,12,18,22,26,37,39
6.	*Bauhinia variegate*	+	-	-	91.5±8.384	14.60±6.16	7	2,5,11,13,17,29,34
7.	*Bryophyllum pinnatum*	+	-	+	71.55±4.105	71.56±2.75	8	7,9,14,27,29,30,37,40
8.	*Butea monosperma*	+	+	-	117.73±5.145	83.2±3.76	6	2,4,13,19,27,39
9.	*Cassia fistula*	+	+	-	98.15±3.21	34.15±0.47	8	5,7,11,14,21,28,33
10.	*Catharanthus roseus*	+	-	-	78±4.44	14.56±2.12	14	5,9,11,13,22,25,31,33,35,36,38,40,42,43
11.	*Cymbogopon citratus*	+	+	+	86.81±10.93	72.55±1.47	12	3,6,7,16,21,27,29,31,33,36,39,41
12.	*Dalbergia sissoo*	-	+	-	119.8±11.54	37.33±5.61	8	2,5,8,12,15,23,31, 35
13.	*Datura stramonium*	+	+	-	47.53±2.76	18.74±1.83	5	1,6,8,25,37
14.	*Indigofera tinctoria*	+	-	-	105.2±9.13	31.04±4.84	13	3,7,9,11,16,18,19,21,24,27,30,33,37
15.	*Lantana camara*	+	-	+	175±2.34	61.00±6.35	8	1,3,5,9,14,21,34,42
16.	*Mentha viridis*	+	+	+	167.76±3.83	28.05±1.33	16	1,6,8,10,12,16,19,21,23,25,28,31,34, 36,39,43
17.	*Mimosa pudica*	+	+	-	177.4±4.306	30.2±4.10	11	5,7,8,12,14,19,23,25,37,40,41
18.	*Pongamia pinnata*	+	+	-	54.95±2.138	26.00±3.02	5	27,32,34,38,42
19.	*Ricinus communis*	+	-	+	120.5±1.8	86.5±4.68	9	7,9,11,15,19,22,26,31,35
20.	*Spilanthes acmella*	+	+	-	116.37±4.56	57.91±2.78	9	5,18,20,23,26,28,33,36,38
21.	*Tinospora cardifolia*	+	+	-	96.85±3.59	74.78±6.32	12	1,6,12,17,19,20,22,26,27,32,34,38
22.	*Vitex negundo*	+	+	-	176.3±7.27	69.77±3.28	8	24,27,31,33,36,37,40,42

Each value is a mean of five replicates, ±: Standard deviation, A: Arbuscule, V: Vesicle, M: Mycelium, +: present, - : absent

Table 3: List of AMF species isolated from different medicinal plants of Hamirpur district of Himachal Pradesh.

Sr. no.	Isolated AMF species	Sr. no.	Isolated AMF species
1	*Acaulospora bireticulata* F.M. Rothwell & Trappe	23	*Gigaspora* sp.5
2	*Acaulospora foveata* Trappe & Janos	*24*	*Glomus ambispora*
3	*A. lacunosa* Morton	*25*	*G. clarum* Nicolson & Schenck
4	*A. laevis* Gerdemann & Trappe	*26*	*G. clavisporum* (Trappe) R.T Almedia &N.C.schenck
5	*A. scrobiculata* Trappe	*27*	*G. fasciculatum* (Thaxtex) Gerd and Trappe emend walker
6	*A. splendid* Sieverd., Chaverri & I. Rojas	*28*	*G. formosanum* Wu and Chen
7	*Acaulospora* sp.1 (unidentified)	*29*	*G. geosporum* (Nicolson & Gerdemann) Walker
8	*Acaulospora* sp.2 (unidentified)	*30*	*G. hoi* Berch and Trappe
9	*Acaulospora* sp.3 (unidentified)	*31*	*G. lamellosum* Dalpe, Koske &Tews
10	*Acaulospora* sp.4 (unidentified)	*32*	*G. macrocarpum* Tul and Tul
11	*Acaulospora* sp.5 (unidentified)	*33*	*G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe
12	*Acaulospora* sp.6 (unidentified)	*34*	*G. pallidum* Hall
13	*Acaulospora* sp.7 (unidentified)	*35*	*G. reticulatum* Bhattacharjee & Mukerji
14	*Acaulospora* sp.8 (unidentified)	*36*	*Glomus* sp.1
15	*Entrophospora* sp.1 (unidentified)	*37*	*Glomus* sp.2
16	*Entrophospora* sp.2 (unidentified)	*38*	*Glomus* sp.3
17	*Gigaspora gigantea* (Nicolson & Gerdemann) Gerdemann & Trappe	*39*	*Glomus* sp.4
18	*G. rosea*	*40*	*Glomus* sp.5
19	*Gigaspora* sp.1	*41*	*Glomus* sp.6
20	*Gigaspora* sp.2	*42*	*Glomus* sp.7
21	*Gigaspora* sp.3	*43*	*Sclerocystis* sp.1
22	*Gigaspora* sp.4		

RESULT AND DISCUSSION

In the present investigation, the survey of medicinal plants for AM fungi showed wide range of variability in terms of root colonization and spore density. Except *Achyranthes aspera*, all medicinal plants selected for study exhibited the presence of AM fungal association. The root colonization was observed in Arbuscules, vesicles and mycelium forms. Different types of Mycelia like Y-shaped, H-shaped, coiled, beaded and parallel mycelia were reported in the roots of different plants. In some cases extensive mycelial growth was also observed. The shape of vesicle varies from elliptical, round, globose, oval, beaked and elongated. Mycelium from is absent in *Achyranthes aspera* and *Dalbergia sissoo,* and vesicles were found in *Acacia catechu, Ageratum conyzoides, Butea monosperma, Cassia fistula, Cymbogopon citratus, Dalbergia sissoo, Datura stramonium, Mentha viridis, Mimosa pudica, Pongamia pinnata, Spilanthes acmella, Tinospora cardifolia* and *Vitex negundo. Out of 22 medicinal plants,* Arbuscular type of infections were observed in few plants like *Acacia catechu, Adhatoda vasica, Aloe vera, Bryophyllum pinnatum, Cymbogopon citratus, Lantana camara, Mentha viridis* and *Ricinus communis.* AMF root colonization ranged from (0.0+ 0.0 %) in *Achyranthes aspera* to (86.5±4.68 %) in *Ricinus communis. Butea monosperma* was observed as second most colonized host plant with 83.2± 3.76 % of AM infection. The high level of AM root colonization is a sign of better fungal- root contact and that increased benefits from AM fungal symbiosis [14]. The extent of root colonization may vary with host plant, growing season, edaphic factors and environmental factors [15-17]. The mycorrhizal root colonization has been reported to be affected by seasonal spore production, seasonal alterations and nutrient accessibility in the soil [18]. The soil temperature and pH have positive influence on AM association, brings changes in physiology of association. The present studies revealed that the percent root colonization of surveyed plants could not be related to spores numbers and its diversity. Similar observation was also made earlier while studying AM fungal diversity associated with some medicinal plant of Haryana [19].

AM spore count varies from *(*47.53±2.76) in *Datura stramonium* to (177.4±4.306) in *Mimosa pudica* per 50 gm of soil sample. Among the families, Mimosaceae followed by Lamiaceae and verbenaceae were found to possess higher spore population while Solanaceae was observed with least spore count. A wide range of variation in spore population was observed in current study. The high spore number in the rhizosphere soils of studied medicinal plants host species, Patterns of spore production, spore quantity etc. are closely related to the plant phenology, root phenology and root production [20]. Total 5 genera i.e. *Glomus, Acaulospora, Gigaspora, Entrophospora* and *Sclerocystis* with 43 different AM species were isolated. *Glomus* was the dominant genus and have 19 species followed by *Acaulospora* (14), *Gigaspora* (7) *Entrophospora* (2) and *Sclerocystis* **(1)**. The AM spores diversity was observed maximum in *Mentha viridis* (16) followed by *Catharanthus roseus (*14) while minimum spore diversities were recorded in more than one plant i.e. *Datura stramonium, Acacia catechu, Adhatoda vasica, Pongamia pinnata*. Our results corroborate well with the findings of other investigators, who reported dominance of *Glomus* sp. while, studying the biodiversity of AM fungi [21-25]. The dominance of *Glomus* species could be due to the fact that they are widely adaptable to the varied soil conditions and survive in both, acidic as well as in alkaline soils [26]. *Acaulospora* sp is second dominant genus and found to be associated with medicinal plant commonly growing in acidic soil [27, 17] Occurrence of high AM spore density might be favoured by the condusive edaphic conditions for sporulation like low nutrient status [28], optimum moisture, high aeration, and the undisturbed conditions of the soils. AM fungal species can infect all potential hosts and some AM species are more preferable to compete for one host than another, even then they may be able to infect the host only under ideal conditions [29]. High species richness in

the rhizosphere of host plant might be associated with organic matter that may assist root colonization of specific host plant.

A variation in development of AMF in roots of different medicinal plant species of fabaceae family has been observed. *Butea monosperma*, *Cassia fistula and Pongamia pinnata* were infected with mycelium and vesicles. Both *Bauhinia variegate* and *Indigofera tinctoria* were infected with mycelium where as *Dalbergia sissoo* was infected only with vesicles. Such variation in mode of infections are also found in Mimosaceae family members like *Mimosa pudica* plant have mycelial and vesicular infection while, *Acacia catechu* credited with all kind of infections i.e. mycelia, vesicular and arbuscular. In our studies, fourteen different medicinal plant species were lacks arbuscules in their root regime. Arbuscules are usually observed in vegetative growth stage of host plant due to availability of new cortical cell for infection and to cope up with high nutrient requirement [30]. So, hyphal coil perform the potential role of arbuscules as suggested [31]. Variations in AMF development are in accordance [32] attributed by differential preference of AM fungi to host plant, difference in quality and quantity of root exudates of the plant in the soil [33-35]. The differential nutrient requirements of host plants may have direct effect on spore density and frequency of mycorrhizal colonization [36, 37]. Phosphorous deficiency and spore degradation by other soil organism are also responsible for variation in AM infection among members of same family. Moreover, availability of root with poor architecture to AM fungus for coloninization might be a reason for inadequate fungal mass development.

CONCLUSIONS

It can be concluded from the present study that all medicinal plants harbour mycorrhizal association however, diversity of arbuscular mycorrhizal fungi species differ in different medicinal plant and the extent of AMF infection is controlled by the host plant as well as environmental factors. AM spore density was found to be maximum in wildly grown medicinal plant as compared to cultivated plant species. These observations could be attributed by Seasonality, edaphic factors, age of host plants, the sporulation abilities of AMF and host dependence. The abundance of *Glomus and Acaulospora* sp in the soil makes it more favoured AM fungi for the mass multiplication and can be utilized for increasing growth and productivity of medicinal plant. Moreover, this type of investigations may also be important while studying the effect of different anthropogenic activities on the AMF. From practical point of view, the use of a species with widespread distribution implies that mycorrhizal inoculum produced with one or many species can potentially be used under different soil and climatic conditions.

Conflict of interest: The authors declare no conflict of interest.

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