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

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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 rhizospheric 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 rhizospheric soil sample of Mimosa pudica (177.4±4.306) and least number of spores in Datura stramonium (47.53±2.76). Forty 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°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 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

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:

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\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 deresis. |
| 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 Conyza Linn. | Gundrya, 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, Gural | 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 flatulence. |
| 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      | Cymbopogon 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 leucorrhoea 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 dysmenorrhoea or period pain. |
| 14      | Indigofera tinctoria Linn. | True indigo, Nil | Fabaceae | Used to cure skin diseases, leucoderma, burns, epilepsy, asthma, blemorrhagia, 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. |
Each value is a mean of five replicates, ±: Standard deviation, A: Arbuscule, V: Vesicle, M: Mycelium, +: present, -: absent.

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

| Sr. No. | Botanical Name            | Type Infection of AM Root (%) | Spore count / 50gm. of soil | AM Colonization (%) | Root AM species richness | AM fungal spores |
|---------|--------------------------|-------------------------------|-------------------------------|----------------------|--------------------------|-----------------|
| 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 conyoides       | + + -                         | 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 variegata       | + - -                         | 91.5±3.384                   | 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 sisso          | - + -                         | 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.
The Journal of Phytopharmacology

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 bivetacalata F.M. Rothwell & Trappe | 23      | Gigaspora sp.5                              |
| 2       | Acaulospora foveate Trappe & Janos           | 24      | Glomus ambispora                            |
| 3       | A. lacunosa Morton                          | 25      | G. clarum Nicolson & Schenck               |
| 4       | A. laevis Gerdemann & Trappe                | 26      | G. claviporum (Trappe) R.T Almedia &N.C.schenck |
| 5       | A. scrobiculata Trappe                      | 27      | G. fasciculata (Thaxtex) Gerd and Trappe emend walker |
| 6       | A. splendid Sieverd., Chaverri & L. Rojas    | 28      | G. formosanum Wu and Chen                  |
| 7       | Acaulospora sp.1                            | 29      | G. geosporum (Nicolson & Gerdemann) Walker |
| 8       | Acaulospora sp.2                            | 30      | G. hoi Berch and Trappe                     |
| 9       | Acaulospora sp.3                            | 31      | G. lamellosam Dalpe, Koske &Tews           |
| 10      | Acaulospora sp.4                            | 32      | G. macrocarpum Tul and Tul                  |
| 11      | Acaulospora sp.5                            | 33      | G. mosseae (Nicolson & Gerdemann) Gerdemann & Trappe |
| 12      | Acaulospora sp.6                            | 34      | G. pallidum Hall                           |
| 13      | Acaulospora sp.7                            | 35      | G. reticulatum Bhattacharjee & Mokerji     |
| 14      | Acaulospora sp.8                            | 36      | Glomus sp.1                                |
| 15      | Entrophospora sp.1                          | 37      | Glomus sp.2                                |
| 16      | Entrophospora sp.2                          | 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      | Sclerotyris 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, Cymbopogon citratus, Dalbergia sissoo, Datura stramonium, Mentha viridis, Mimosa pudica, Pongamia pinnata, Spilanthes acmella, Tinospora cardifolia and Vitek negundo. Out of 22 medicinal plants, Arbuscular type of infections were observed in few plants like Acacia catechu, Adhatoda vasica, Aloe vera, Bryophyllum pinnatum, Cymbopogon 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 Sclerotyris 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 Sclerotyris (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, 28]. Occurrence of high AM spore density might be favoured by the conducive 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

[19-29]
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 variegata* 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 spor density and frequency of mycorrhizal colonization [36, 37]. Phosphorous deficiency and spor 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 colonization 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 spor 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.

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