



Journal of Research in Weed Science

Journal homepage: www.jrweedsoci.com



Original Research

Ethno-medicinal and AMF diversity conservation aspects of some weeds of Himachal Pradesh, India

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ARTICLE INFORMATION

Received: 17 June 2020

Revised: 25 July 2020

Accepted: 14 September 2020

Available online: 20 September 2020

DOI: [10.26655/JRWEEDSCI.2021.1.4](https://doi.org/10.26655/JRWEEDSCI.2021.1.4)

KEYWORDS

AMF spores

Root colonization

Weed

ABSTRACT

The present investigation was focused on enumeration of medicinal potential of weeds and biodiversity of Arbuscular mycorrhizal fungi (AMF) associated with them. For AMF analysis, fourteen medicinal weeds were selected, roots and their respective rhizospheric soil samples collected from different localities of Hamirpur district, Himachal Pradesh. The results revealed that number of AM spores in the rhizosphere of plant was not correlated to percent of AM root colonization. The highest percentage of root colonization was reported in *Solanum nigrum* (73.54±07.15 %) and minimum in *Ageratum conyzoides* (22.22±00.55 %). AM spore count was recorded maximum in rhizospheric soil sample of *Parthenium hysterophorus* (135.32±06.05 spores per unit 50g soil) and minimum (32.26±04.10 spores per unit 50g soil) in *Fumaria officinalis*. Twenty five AM species belonging to four genera i.e. *Acaulospora*, *Entrophospora*, *Gigaspora* and *Glomus* were isolated during course of study. *Calotropis procera* preserve maximum AM spore richness in their rhizospheric soil followed by *Solanum nigrum* and least in *Amaranthus viridis*. Among variety of spores, *G. geosporum* is most frequently occurred species in studied soil samples. The study confirmed the weeds potential to provide hostile environment for conservation, sporulation and propagation of competent AM spores to ensure their ubiquitous distribution.

Introduction

Utilization of plant and their products are as old as human civilization. Despite of reaching advancement in healthcare system, modern civilizations still depends on plant products. Plants are generally rich sources of herbal products and most of them used for human welfare especially to reduce the human pain and suffering from many diseases. Now-a-days throughout the world

several thousands of weed plants are confirmed for therapeutic potential and few drug plants are cultivated (Dobhal et al., 2006). Weeds are widespread in its distribution and act as major biological constraints that limit crop productivity by exerting competition pressure over crop plant for efficient uptake of both natural and applied fertilizers (Mushtaq et al. 2019). The distribution of weeds, adverse impact on crop production and ethnomedicinal uses has been well documented (Randall, 1996; Hassan and Marwat, 2001) but little information is available on microbes having biofertilizer potential associated with rhizosphere soil of weed plants. Many rhizospheric soil microbes form symbiotic association with plants, among them AM fungi are widespread and associated with 80% of all terrestrial plant species. This symbiotic association is usually concerned with regulating the function and biodiversity of the terrestrial ecosystems by producing underground networks, composed of hyphae and spores; that interconnect a number of unrelated individual plant species (Bonfante and Genre, 2010). Upon infection of host plant roots, the fungi produce hyphae that grow into the intercellular space of root cortex, produce extremely branched structures (arbuscules), which are the site of exchange for nutrients and carbon between interacting components; storage structures i.e. vesicles. These fungi receive photosynthates from colonized plant and in return enhance the plant ability for nutrient acquisition (Smith and Read, 2008). This fungal partner of symbiotic association belonging to glomeromycota that corresponds to eight different genera such as *Acaulospora*, *Archaeospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Paraglomus*, *Sclerocystis* and *Scutellospora* (Morton and Redecker, 2001). These genera form symbiosis with variety of plant varying from bryophytes to angiosperms and even single isolate can colonize variety of plants showing ecological specificity (Allen and Boosalis, 1983). Numerous eco-physiological studies have confirmed importance of AM symbiosis in the soil-plant interface, such as improving plant nutrition by better nutrient and water uptake, production of phytohormones, abiotic and biotic stress tolerance. Moreover, symbiosis is also helpful to improve soil structure by production of glomalin that bind small soil particles to form large aggregates. These beneficial influences of indigenous AM fungi on plant health were closely linked with type of fungi and its allocation in soil. So, exploration of microbial diversity associated with weeds of specific region is primarily important in to utilizing these fungi as bio-fertilizer for cultivation of threatened plants. However, commercial utilization of AM fungi in agriculture is relying on the development of capable plant growth promoting strains of AM, which are superior among all native AM fungi found in soil. Therefore, analysis of soil samples belongs to different regions is mandatory to find out of abundance as well as type of indigenous AM fungi present in rhizosphere of the weed plant. Keeping in view the importance of AM fungi, exploration of AMF biodiversity associated with some weed plants growing in agricultural land and non-agricultural land is therefore, necessary from

conservation point of view, formation of an efficient future inoculum and its applicability for better production of seedling as well as their survival in adverse condition. So, present investigation was planned to enumerate medicinal importance and estimate AM fungi associated with some commonly grown weeds. The present study being the first, reports the incidence and diversity of AM fungi associated with some common weeds of Hamirpur district, Himachal Pradesh.

Materials and Methods

Study area

The Hamirpur district is situated in south-west part of the Himachal Pradesh state and geographically located between 31°25' N and 31°52' N and between 76°18' E and 76°44' E. It occupies a total geographical area of about 1,118 Sq. KMs and this hilly track is covered by shivalik range with 400-1100 meter elevation range. The average maximum and minimum temperature ranges from 40°C to 20°C respectively. It is bounded on the north by Kangra and mandi districts in the east, on the south by Bilaspur and on the west by Una district. The district falls in the category of humid sub-tropical zone.

Sampling procedure

Seasonal field trips were performed from 2017 to 2018, in order to collect soil and fine root samples for assessment of AM diversity associated with some weed plants found in wheat crop field in Hamirpur district of Himachal Pradesh, India. Five each plant species were 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 18-30 cm, and stored in sterilized polythene bags at 4-8°C temperature for further processing in the laboratory.

Isolation, Quantification and Identification of AM spores

Isolation of AM spores was done by using 'Wet sieving and decanting technique' Gerdmann and Nicolson (1963). Sieves of different pore sizes i.e. 150µm, 120µm, 90µm, 60µm and 45µm are used. Firstly, soil samples were subjected to dry at room temperature for 48 hours than 50 g of dried composite soil sample was dissolved in 500 ml water. After stirring, soil solution was allowed to settle down over night. On next day, decanting water on a series of sieves arranged in descending order of pore size from top to bottom on which spores were trapped. The trapped spores were transferred to whatman Filter Paper No.1 by repeated washing with tap water. AM spores were counted by Gridline intersect method' proposed by Gaur and Adholeya (1994) under stereobinocular microscope at 60X magnification. Then spores were picked up by hypodermic needle

under stereo-binocular microscope and mounted in polyvinyl lactic acid alcohol (PVLA). Identification of AM spores was done on the basis of morphotaxonomic criteria using INVAM international collection of vesicular arbuscular mycorrhizal and available identification manual of Walker (1983), Schenck and Perez (1990) and Mukerji (1996).

Assessment of AM fungal root colonization

Mycorrhizal root colonization was done by 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970). The collected roots were cut into 1cm long segments and then 15-30 segments are randomly selected for analysis. 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 (1980).

Percent root colonization was calculated by formula:

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

Assessment of AM species richness, abundance and frequency of occurrence

AM species richness, abundance and frequency of occurrence were estimated from number and type of mycorrhizal species as stated follows:

Species richness (SR) = Number of AMF species in 50g soil sample.

Species abundance (A) = Number of soil samples having particular AMF species.

$$\text{Frequency of occurrence (FO)} = \frac{\text{No of soil samples possessing the spores of particular species}}{\text{Number of soil samples studied}} \times 100$$

Results and Discussion

Survey of different weeds growing in the vicinity of Hamirpur district was done, enumerating their medicinal importance based on the textual survey of different text books and research papers as shown in Table 1. In the present investigation, the survey of weed plants for AM fungi showed occurrence of AMF association and also expressed broad range of unevenness in root colonization, spore density and diversity. Mycelia form is one of major structure that marked occurrence of symbiosis and further intensified by presence of arbuscules and vesicles that arise from mycelium. The Mycelium reported in course of study varies in their shapes and pattern like Y-shaped, H-

shaped, Intrametrical, twisted and parallel forms in the roots of different weed plants. The shape of vesicle varies from round, oval, oblong, pear shaped, rectangular and columnar (Figure 1).

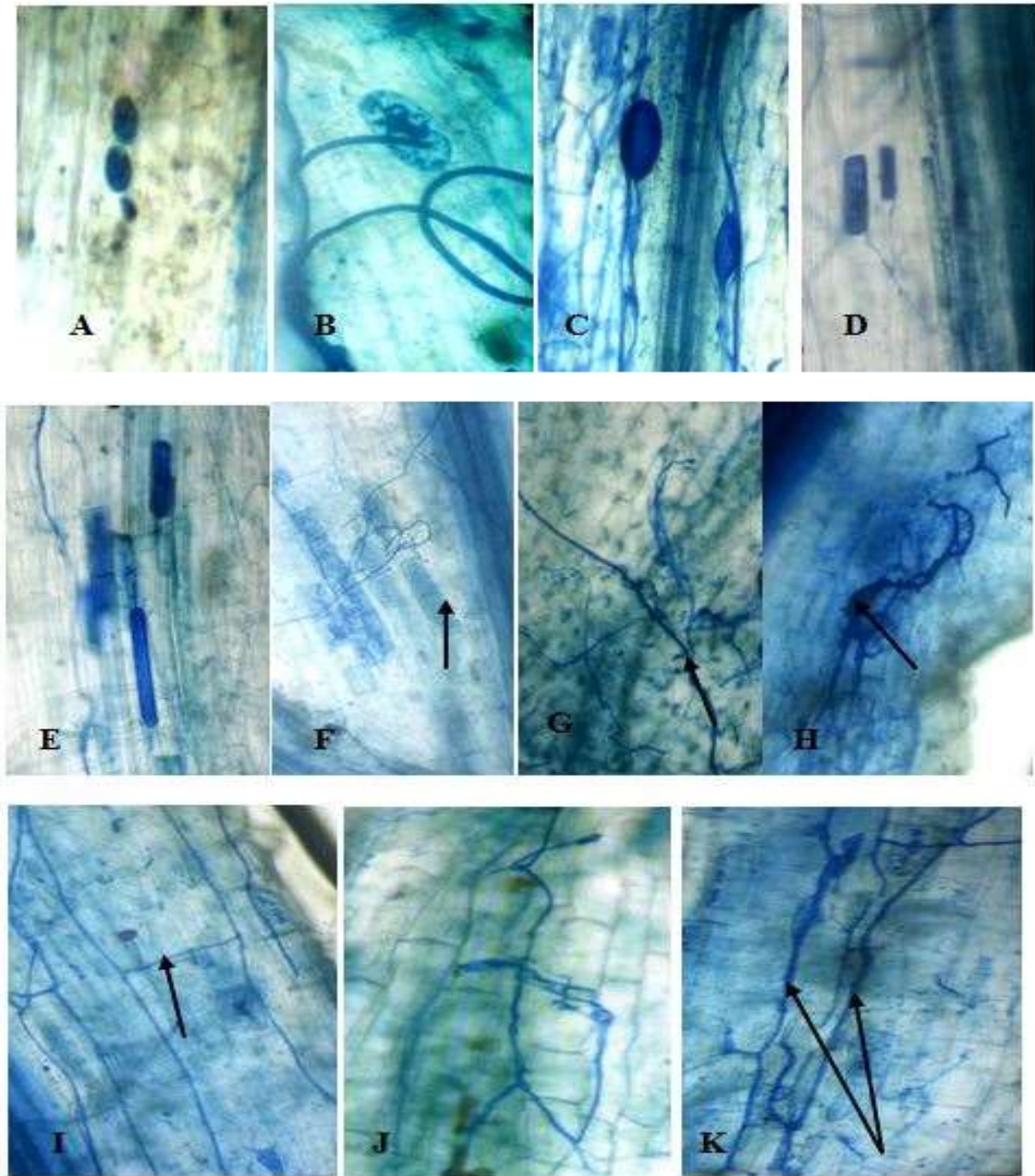


Figure 1. Micrograph showing morphology of mycorrhizal association in weeds. (A) Oval vesicles (B) Oblong vesicle (C) Pear shaped vesicles (D) Rectangular vesicles (E) Columnar vesicles (F) Arbuscules (G) Y-Shaped mycelium (H) Twisted mycelium (I) H- Shaped mycelium (J) Intrametrical mycelium (K) Parallel mycelium at 40x.

Table 1. Medicinal importance of selected weeds for studying AMF association.

Sr. No.	Botanical name	Common name	Plant part used	Medicinal uses
1.	<i>Ageratum conyzoides</i> Linn.	Goatweed, chickweed	Whole plant	Used to cure pneumonia, wound and burn, ulcer, inflammation, spasm, blood infection and bacterial infection.
2.	<i>Amaranthus viridis</i> Linn.	Jungali chaulay	Leaves, root, seeds	Used for treatment of pain, asthma, ulcer and fever. Also known for improving appetite.
3.	<i>Anagalis arvensis</i>	Biliputi	Seeds, shoot	Used for treatment of gout, leprosy, hydrophobia, rheumatism and hepatic and renal troubles.
4.	<i>Argemone maxicana</i>	Mexican poppy	Seeds and roots	Yellow sap of plant is used for treatment of Jaundice, hepatitis, scabies and other liver disorders. The seeds are beneficial to prevent cough and asthma.
5.	<i>Calotropis procera</i> (Ait.) R. Br.	Arka, Ak, Akada	Leaf, flower, root, latex	Flower powder with triphala churan used to cure asthma, latex used against arthritis while bark paste is used against cutaneous infections.
6.	<i>Chenopodium album</i>	Bathuaa	Seeds	Seeds act as stimulant, antispasmodic and diuretic properties.
7.	<i>Cynodon dactylon</i> Pers.	Doob grass	Leaf, root	Used to cure nasal bleeding, fever and dysentery.
8.	<i>Euphorbia hirta</i> Linn.	Doodhli	Stem, Leaves	Stem and leaf extracts are used in jaundice, diuresis, dysentery, bronchitis, asthma, worm infestations in children, pimples, gonorrhoea, digestive problems, and tumors.
9.	<i>Fumaria parpviflora</i> Linn.	Pitpapra	Whole plant	Utilized against indigestion and fever. It also possesses hepato-protective, anti-diabetic, anti-inflammatory, antipyretic, analgesic, dermatological, antimicrobial properties
10.	<i>Parthenium hysterophorus</i> Linn.	Chambara, Gajarghas	Leaves, stem	Decoction of plant is used to treat dysentery and fever.
11.	<i>Rumex hastatus</i> Linn.	Khatmith	Whole plant	Fresh juice of the plant is used to cure dysentery while tubers are chewed to relieve aches in the throat.
12.	<i>Stellaria media</i>	Common chickweed	Leaf, flower	Anti-rheumatic and anti-inflammatory in action.
13.	<i>Solanum nigrum</i> Linn.	Makoi	Whole plant	Freshly prepared extract of the plant is effective in the treatment of cirrhosis of liver. Ripen fruits are utilised as liver tonic and to treat cardiovascular disease.
14.	<i>Taraxacum officinale</i>	Dudhli	Rhizome	Decoction of boiled rhizome is used for the treatment of Jaundice, hepatitis and other liver disorders

Mycelium from is present in all weeds and vesicles were observed in *Ageratum conyzoides*, *Calotropis procera*, *Chenopodium album*, *Euphorbia hirta*, *Parthenium hysterophorus*, *Rumex hastatus*, *Solanum nigrum* and *Taraxacum officinale*. Weeds like *Amaranthus viridis*, *Cynodon dactylon*, *Fumaria officinalis* and *Stellaria media* are observed to have arbuscular type of infection among 14 medicinal weeds. Only three plants i.e. *Calotropis procera*, *Parthenium hysterophorus* and *Taraxacum officinale* were infected with all kind of mycorrhizal infection in root regimes. Minimum root colonization was observed in *Ageratum conyzoides* (22.22±00.55 %) and maximum recorded in *Solanum nigrum* (73.54±07.15 %). A variation in establishment and development of AM Fungi in roots of different medicinal weed plant species of Asteraceae family has been observed. *Rumex hastatus* and *Taraxacum officinale* were infected with mycelium, arbuscules and vesicles while, *Ageratum conyzoides* possesses only vesicles and mycelium in their roots. The extent of root

colonization was depends over compatibility of host plant with particular AM spores, availability and diversity of mycorrhizal spore, positively related with environmental factors like soil pH, nutrient content and temperature (Rajkumar et al. 2012; Kumar et al. 2019). Moreover seasonal variations are also responsible to alter spore population in rhizospheric soil and bring changes in physiology of symbiosis (Vogeti et al. 2008). The high level of AM root colonization is corresponds to exudation of easily oxidisable metabolites which attract particular AM species to accelerate level of colonization (Steinkellner et al. 2007). Our results correspond to the findings of Gunwal et al. (2014) who also observed low root colonization due to edaphic properties which are not appropriate for mycorrhizal infection. 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 variety of plants of Haryana (Kumar et al. 2013; Chauhan et al. 2013)

Arbuscules with fine branches are commonly observed in young cortical cells reported in pre-reproductive stage of host plant and are more susceptible to AM colonization as well as more potent to cope up with high nutrient requirement. Moreover, hyphal coils are also reported in host that can perform the potential role of arbuscules in later stages as suggested by Jahan (2005). Variations reported in AMF establishment and development in our study are relevant with earlier investigation results obtained by Rahman et al. (2003) and Carrenho et al. (2007) who observed that AM variations are positively relying on differential preference of AM fungi to their host, competence of AM species, difference in quality and quantity of released root exudates of the plant in the soil. Carrenho et al. (2002) studied influences of root exudates on root colonization and reasoned that qualitative and quantitative difference in root exudates control variations in mycorrhization. The variability in nutritional requirements of host plants may have direct effect on percent of host root colonization, spore count and frequency of occurrence of particular AM species. The nutrient deficit soil more specifically phosphorous and spore degradation by other competent rhizospheric micro-organism are also responsible for differences reported in AM infection among members of same family. Moreover, poorly developed root architecture and scarcity of fine root hairs to AM fungus for colonization might be a reason for insufficient fungal mass development.

The results of the rhizospheric soil sample analysis for spore density have been presented in Table 2. Arbuscular mycorrhizal spore count varies from (32.26±04.10) in *Fumaria officinalis* to (135.32±06.05) in *Parthenium hysterophorus* per 50 g of soil sample. Among the families, Asteraceae was found to possess highest spore count while Papaveraceae with least spore count. Highest spore count in rhizospheric soil samples were corresponds to type of host species, plant

phenology, root phenology, root production and spore germination rate. Total 25 AM spores were isolated from fourteen medicinal weeds and belongs to four genus i.e. *Acaulospora*, *Entrophospora*, *Gigaspora* and *Glomus*. *Glomus* was the dominant genus and have 12 identified species followed by *Acaulospora* (7), *Gigaspora* (4) and *Entrophospora* (2) (Table 3 and Figure 2).

Table 2. Occurrence and distribution of AMF species among selected weeds of Hamirpur district of Himachal Pradesh.

Sr No	Botanical name	Family	Type of mycorrhization			AM spore density/ 50g soil	AM root Colonization (%)	AM Species richness	AM fungal spores
			M	V	A				
1.	<i>Ageratum conyzoides</i>	Asteraceae	+	+	-	104.66±2.75	22.22±00.55	8	1,4,6,11,14,19,21,25
2.	<i>Amaranthus viridis</i>	Amaranthaceae	+	-	+	84.21±05.24	39.05±07.87	5	3,12,19,21,23
3.	<i>Anagalis arvensis</i>	Primulaceae	+	-	-	56.61±02.12	43.74±01.20	8	7,9,12,15,19,20,22,24
4.	<i>Argemone maxicana</i>	Papaveraceae	+	-	-	131.6±35.21	54.83±06.82	7	5,8,11,13,16,17,23
5.	<i>Calotropis procera</i>	Asclepiadaceae	+	+	+	110.4±13.408	62.57±18.649	14	2,4,6,7,8,10,11,13,15,16,18,19,22,25
6.	<i>Chenopodium album</i>	Chenopodiaceae	+	+	-	78.68±02.01	63.36±01.25	9	1,4,7,9,10,13,16,19,22
7.	<i>Cynodon dactylon</i>	Poaceae	+	-	+	58.31±07.11	27.45±05.13	6	13,15,19,22,23,25
8.	<i>Euphorbia hirta</i>	Euphorbiaceae	+	+	-	87.00±11.937	71.23±13.125	12	1,4,7,8,11,13,16,17,19,21,23,24
9.	<i>Fumaria officinalis</i>	Papaveraceae	+	-	+	32.26±04.10	62.12±07.18	6	15,17,19,21,24,25
10.	<i>Parthenium hysterophorus</i>	Asteraceae	+	+	+	135.32±06.05	45.52±02.21	9	1,2,4,6,7,9,13,17,19
11.	<i>Rumex hastatus</i>	Polygonaceae	+	+	-	110.26±06.03	35.26±06.51	8	6,9,12,14,17,19,20,22
12.	<i>Stellaria media</i>	Caryophyllaceae	+	-	+	52.03±03.32	65.49±09.60	11	3,5,7,9,13,14,16,17,20,22,23
13.	<i>Solanum nigrum</i>	Solanaceae	+	+	-	110.2±20.65	73.54±07.15	13	1,4,6,7,9,12,13,16,17,19,21,23,24
14.	<i>Taraxacum officinale</i>	Asteraceae	+	+	+	65.80±12.814	58.07±08.339	12	2,5,6,9,12,14,15,17,18,20,22,24

Above discussed results are in accordance with the results of Thapa et al. (2015), Misbah et al. (2017) and Deori and Dutta (2019), who also found the dominance of *Glomus* sp. in their course of study. The AM spores richness was observed maximum in *Calotropis procera* (14) followed by *Solanum nigrum* (13) while minimum in *Amaranthus viridis* (5). *Glomus geosporum* is most abundant and frequently occurred species in studied soil samples among all isolated species while *Acaulospora laevis*, *Gigaspora gigantean* and *Glomus formosanum* are least occurred AM spore (Table 3). In present investigation, no positive correlation was observed between AM spore number and percent root colonization, and similar observations were reported by other investigators (Radhika and Rodrigues, 2010; Khakpour and Khara, 2012). The high root colonization and low spore count might be possible due to efficient sporulation of AM fungi during favourable conditions

Shanker et al. (1990) whereas in plants with least infection, the AM spores are unable to compete with other soil microbes or the unfavourable soil characteristics Kumar and Mahadevan (1984). Moreover, low sporulation rate was found to be favoured by poor fungal biomass development and high rate of spore degradation by other soil microbes. Our results shows wide spread distribution of *Glomus* in soil samples which is relevant with the findings of Thapa et al. (2015), who also observed supremacy of *Glomus* species in their course of study. This ubiquitous distribution of *Glomus* species could be attributed by wide range of adaptability to continue to exist in acidic as well as in alkaline soils while, genus *Acaulospora* is next most diverse and it shows preference to form symbiosis with vascular-phyta commonly growing in acidic soil (Rajkumar et al. 2012) Occurrence of high AM spore density in some weeds has been contributed by stumpy nutrient status, high aeration, optimum moisture and the undisturbed conditions of the soils. AM fungal species can infect all potential hosts but some are more susceptible for AM invasion than other, and can invade hosts only under ideal conditions.

Table 3. List of isolated AMF species, abundance and frequency of occurrence in different weeds grown in Hamirpur district of Himachal Pradesh.

Sr. no.	Isolated AMF species	Species abundance	Frequency of occurrence
1.	<i>Acaulospora bireticulata</i> F.M. Rothwell & Trappe	5	20
2.	<i>A. foveata</i> Trappe & Janos	3	12
3.	<i>A. laevis</i> Gerdemann & Trappe	2	9
4.	<i>A. scrobiculata</i> Trappe	6	27
5.	<i>A. splendid</i> Sieverd., Chaverri & I. Rojas	3	12
6.	<i>A. trappii</i> Ames And Linderman	6	27
7.	<i>Acaulospora</i> sp.	7	31
8.	<i>Entrophospora</i> sp.1 (unidentified)	3	12
9.	<i>Entrophospora</i> sp.2 (unidentified)	7	31
10.	<i>Gigaspora gigantea</i> Gerdemann & Trappe	2	9
11.	<i>G. rosea</i>	4	16
12.	<i>G. margarita</i>	5	20
13.	<i>Gigaspora</i> sp.	8	36
14.	<i>Glomus albidum</i> Walker and Rhodes	4	16
15.	<i>G. clarum</i> Nicolson & Schenck	5	20
16.	<i>G. clavisporum</i> (Trappe) R.T Almedia & N.C.schenck	6	27
17.	<i>G. fasciculatum</i> (Thaxtex) Gerd and Trappe emend walker	8	36
18.	<i>G. formosanum</i> Wu and Chen	2	9
19.	<i>G. geosporum</i> (Nicolson & Gerdemann) Walker	11	44
20.	<i>G. hoi</i> Berch and Trappe	4	16
21.	<i>G. lamellosum</i> Dalpe, Koske & Tews	5	20
22.	<i>G. macrocarpum</i> Tul and Tul	7	31
23.	<i>G. mosseae</i> (Nicolson & Gerdemann) Gerdemann & Trappe	6	27
24.	<i>G. pallidum</i> Hall	4	16
25.	<i>G. reticulatum</i> Bhattacharjee & Mukerji	4	16



Figure 2. Micrograph showing morphology of different mycorrhizal spores isolated from rhizospheric soil of weeds.

AMF species richness in the rhizospheric soil of respective host plant might be associated with organic matter that may assist root colonization of specific host plant. In soil, the presence of organic matter which serves as a nutrient sink for the plants could also regulate the intensity of

mycorrhization due to its positive influences on soil fertility, aggregation, water-holding capacity and the degree of compaction (Siddiqui and Pichtel, 2008; Franzluebbers, 2002). Moreover, AM species richness related to diversity of host species and composition of mycorrhizal fungi in the soil indicates about the community structure of plants (Van der Heijden et al. 1998).

Conclusion

It can be concluded from the present study that all studied weed plants have medicinal values and harbour mycorrhizal association however, diversity of arbuscular mycorrhizal fungi differ in different weeds and the degree of AMF infection is controlled by biotic and abiotic components of an environment. The dominance of *Glomus* and *Acaulospora* sp in the soil makes it more favourable AM fungi for the mass multiplication and can be utilized for increasing growth and productivity of threatened plants. Moreover, this type of investigations may also be important while comparing the effect of different anthropogenic activities on AM Fungi associated with selected plant species. Also researchers could focus towards escalating the AMF population which showed better performance in cultivation of threatened plant. 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 agro-climatic conditions.

Acknowledgements

The authors are thankful to Department of Botany, Kurukshetra University Kurukshetra and Forest Pathology Division, Forest Research Institute, Dehradun for their laboratory facilities to carry out this work.

Conflicts of Interest

No conflicts of interest have been declared.

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Cite this article as: Ashish Kumar, Anil Gupta, Ashok Aggarwal, Jitinaksh Pratap Singh, Vipin Parkash. 2021. Ethno-medicinal and AMF diversity conservation aspects of some weeds of Himachal Pradesh, India. *Journal of Research in Weed Science*, 4(1), 43-56. DOI: [10.26655/JRWEEDSCI.2021.1.4](https://doi.org/10.26655/JRWEEDSCI.2021.1.4)