J. Biol. Chem. Chron. **2017**, *3(2)*, 01-04
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco/jbcc/

Arbuscular Mycorrhizal Fungal Association in Some Medicinal Plant Species in the Hills of Himachal Pradesh, India

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(Received 17 July, 2017; Accepted 13 Sept, 2017; Published 26 Sept, 2017)

ABSTRACT: Medicinal and aromatic plants (MAPs) are used in various systems of medicines in different parts of the globe. In addition to conventional cultivation of MAPs, recent emphasis is on exploiting useful and appropriate soil microorganisms present in the rhizosphere of medicinal plants. In the present investigation, attempts were made to screen out arbuscular mycorrhizal fungal (AMF) biodiversity from different medicinal plants. A total of ten plants from six different families were screened for arbuscular mycorrhizal (AM) spore number and root colonization. The mycorrhizal root colonization ranged from 21.35±5.94 to 100.0±0.00 percent. *Cichorium intybus* showed maximum root colonization and *Curculigo orchiodes* showed minimum mycorrhizal root colonization. The spore number ranged from 52.00±4.35 to 178.0 ± 6.2. The highest mycorrhizal spore count was found in *Centratherum anthelminticum* and lowest in *Cichorium intybus*. It was found that number of spores in the rhizosphere of plant was not related to the intensity of AM root colonization. Arbuscular mycorrhizal colonization and spore population varied significantly in different plant species as showed by results.

Keywords: Medicinal plants; Arbuscular mycorrhizal fungi; Biodiversity; Himachal Pradesh.

INTRODUCTION: Arbuscular mycorrhizal (AM) fungi are the group of fungi which is symbiotically associated with the roots of plants. This symbiosis occurs in more than 80% of the plant species.[1] The demand of MAPs has been increasing rapidly with the consumption of herbal drugs. This led to an increase in the cultivation of MAPs in order to maintain a steady supply to support the increasing demand due to decline in their natural population. Knowledge about the presence and diversity of AMF is an essential first step to utilizing these fungi in any application. The distribution and abundance of AM fungi in medicinal plants has been well documented. In a survey on AM association with three different endangered species of *Leptadenia reticulata, Mitragyna parvifolia* and *Withania coagulans*, high diversity of AMF was observed.[2] About 50 species of medicinal plants from 19 families have been studied in the association with AM fungi.[3] Likewise, a total of twelve plants from six different families were screened for arbuscular mycorrhizal (AM) spore number and root colonization.[4] However, not enough has been focused on the mycorrhizal association with medicinal plant species. Hence, the present study is attempted to investigate the diversity of AM fungi associated with medicinal plant species in the hills of Himachal Pradesh.

MATERIAL AND METHODS:

Study Site: The study was undertaken at different sites located in the hills of Himachal Pradesh. Wide differences in geo-physical features accounts for considerable variation in climate and rainfall in different sub-regions of the state. Physio-geographically, the state is a part of Himalayan system.

Field Sampling: The plant grew under natural environmental conditions. The three healthy individuals for each medicinal plant species were randomly selected for collection of rhizospheric soil and root samples during course of investigation. A total ten plant species and thirty soil samples were collected. The samples of each plant were collected for further processing for the isolation of AM spores and studying mycorrhizal root colonization.

Estimates of AM root colonization: The mixed soil and roots samples of each plant species were packed

in polyethylene bags, labeled and brought to the laboratory. The soil samples were air-dried at room temperature. Roots were washed to remove soil particles and processed by 'Rapid Clearing and Staining Method'.[5] Assessment of root colonization was done by estimating total percentage of root colonization by root slide technique[6]. The percentage of mycorrhizal root colonization was determined by the following equation:

Percentage AM root colonization =

$$\frac{\text{Number of arbuscular mycorrhiza positive segments}}{\text{Total number of segments examined}} \times 100$$

Extraction, Quantification and Identification of AM fungal Spores: Rhizospheric soil samples were wet sieved for AM spores using the technique of Gerdemann and Nicolson (1963)[7]. The quantification of AM spores was done by Grid Line Intersect Method[8]. The spores were identified by using keys of Walker (1986), Schenk and Perez (1990), Kumar *et al.* (2009a)[9, 10 & 11].

RESULTS AND DISCUSSION: AM fungi are ecologically important root symbionts of most terrestrial plants. Root samples of all the plant species showed a wide range of variation in terms of AM root colonization. The mycorrhizal structures present in the roots included mycelium, vesicles and arbuscules. Mycelia of various type like Y-shaped, H-shaped, coiled and parallel mycelia were reported in the roots of different plants. Vesicles of different shapes were observed. In some roots, vesicles were present in chain, in groups of two and more. Colonization rate of AM fungi in the rhizosphere of 10 medicinal plants species are presented in Table 1. The percentage of root colonization ranged from 21.35±5.94 to 100.0±0.00. The highest colonization was observed in *Cichorium intybus* and lowest in *Curculigo orchiodes*. The AM spore number ranged from 52.00±4.35 to 178.0 ± 6.2.

Table 1: Arbuscular mycorrhizal studies of some medicinal plants in the hills of Himachal Pradesh.

Sr. No.	Botanical Name	Common name	Family	Type of Infection			AM spore count / 50gm. of soil	% AM Root Colonization
				#M	#V	#A		
1.	*Bacopa monnieri*	Brahmi	Scrophulariaceae	+	+	-	74.00±6.24	29.62±6.41
2.	*Berberis aristata*	Daru- haldi, Kashmal	Berberidaceae	+	-	+	106.6±5.50	31.34±5.62
3.	*Bergenia ligulata*	Pashan- bhed	Saxifrugaceae	+	+	+	118.66±2.51	58.33±8.33
4.	*Centratherum anthelminticum*	Kalijiri	Asteraceae	+	+	+	178.0 ± 6.2	59.01 ± 8.70
5.	*Chrysanthemum indicum*	Guldaudi	Asteraceae	+	-	+	95.66±3.21	76.74±9.41
6.	*Cichorium intybus*	Chikori	Asteraceae	+	+	+	52.00±4.35	100.0±0.00
7.	*Cirsium falconeri*	Falconer's thistle, Leeh	Asteraceae	+	+	-	86.00±5.00	35.57±4.19
8.	*Citrus jambhiri*	Ghamirdi	Rutaceae	+	-	+	87.66±1.52	39.09±11.39
9.	*Citrus medica*	Kagji nimbu	Rutaceae	+	-	+	143.0 ± 1.4	43.74 ± 1.20
10.	*Curculigo orchiodes*	Kali musli	Amaryllidaceae	+	+	-	149.33±2.08	21.35±5.94

** Mean of three replicates; # M-Mycelium, V-Vesicles, A-Arbuscules; + : Present; - : Absent; ± : Standard deviation*

The highest mycorrhizal spore count was found in *Centratherum anthelminticum* and lowest in *Cichorium intybus*. It was found that number of spores in the rhizosphere of plant was not related to the intensity of AM root colonization. All 10 medicinal plants were infected by AM fungi, but the degree of colonization and the spore number varied among plant species. This may due to differences in the ability of

AM species to sporulate.[12] The variation in root colonization may be due to the exudation of toxic metabolites resulting in substances in proximity to the roots which attracts the AM fungi such as production of easily oxidisable compounds resulting in increased colonization and physiological difference between species.[13,14] In this study, high levels of AMF colonization and low sporulation were found in some plant species. This may have been due to inadequate fungal biomass development and poor root development or high rates of spore degradation or predation by other soil organisms.[15 & 16] Similarly, a high range of AM sporulation and root colonization was also reported in some plants species of Himachal Pradesh.[4 &17] 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 supported the conclusion of Wang *et al*. (2004)[18], that the host species apparently had direct effects on spore density and colonization of AM fungi.

CONCLUSION: The AM colonization and diversity in the medicinal plants species in the hills of Himachal Pradesh were investigated in the present study. From the research, it could be concluded that the biodiversity of AM fungi was abundant. The degree of colonization and the spore number varied markedly among plant species. As mycorrhizal symbiosis is a highly evolved mutualistic relationship for plant establishment, it seems that more attention should be paid to the predominant AM fungi during the establishment of seedlings, process of cultivation in order to increase the productivity and reduce the fertilizer application required for obtaining economic production of medicinal plants under field conditions.

ACKNOWLEDGEMENTS: The authors (AK & CM) are grateful to Kurukshetra University, Kurukshetra to provide financial assistance to carry out the work.

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