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Species Diversity of Arbuscular Mycorrhizal Fungi (AMF) in the Rhizospheric Soil of *Spilanthes acmella* Murr.

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ABSTRACT: Microbes are very important components of soil plant complex where they show a network of interactions affecting plant health, growth and development. In the present investigation, attempts were made to screen out arbuscular mycorrhizal fungal (AMF) species from rhizospheric soil of *Spilanthes acmella*- an important medicinal plant. A total of ten species of arbuscular mycorrhizal (AM) spores were screened out. AM fungi belonging to genus *Glomus* were dominant. Root samples also showed a wide range of variation in terms of AM root colonization. It was found that number of spores in the rhizosphere of plant was not related to the intensity of AM root colonization. Arbuscular mycorrhizal spore population varied significantly in *S.acmella* as shown by results.

Keywords: Medicinal plants; Arbuscular mycorrhizal fungi; *Spilanthes acmella*.

INTRODUCTION: Among all the natural resources, one, that provides its invaluable support to sustain human race, is plantation. Besides sustenance, the plants have been used as therapeutic aid for alleviating various human ailments from very earlier times[1]. The sustainable use of these plant resources through *in-situ* and/ or *ex- situ* conservation becomes imperative. The present wave of awareness around environment and natural products is an opportunity for planned sustainable utilization of our valuable forest and cultivation bioresources in the Indian Himalayan zone.

The mycorrhizal symbiotic association appears to have evolved with plants since the colonization of dry land by plant began as a survival mechanism for fungi and higher plants, thus allowing each to survive in the existing environment of low temperature, low soil fertility, periodic drought, diseases, extreme environments and other stress situations[2].

Wang and Qiu (2006)[3], concluded that the mycorrhizas are present in 92% of plant families (80% of species) and most of these plants are colonized by fungi of the phylum Glomeromycota, which form VAM (Vesicular Arbuscular Mycorrhiza) or AM (Arbuscular Mycorrhiza), the ancestral and predominant form.

Keeping in view all these important aspects, the present investigation was undertaken to find out the abundance and distribution of AM fungi with *S.acmella.*

Description of Selected Medicinal Plants:

Spilanthes acmella

Common Name: Akarkara, Alakada
Family: Asteraceae
Parts used: Leaves, flower head and roots.

S.acmella is an indigenous herb, grown as an annual herb throughout the tropics. Plant is erect or ascending stout herb, more or less pubescent, some time hairy. Stem 30-60 cm, usually decumbent near the base. Leaves are opposite, petiolate, broady ovate, narrow at base, acute or obtuse at apex. Flower heads discoid or radiate, conical, solitary on long stalks. Flowers are white or yellow in colour, mostly bisexual. Achenes flattened, each enclosed in a scale.

A number of constituents have been isolated from *S.acmella* e.g. spilanthol isobutylamide and triterpenoids[4,5]. It has been found that the plant possess vasorelaxant and antioxidant activity as well as antimicrobial activity[6]. Different bioactive metabolites have also been reported from *S.*acmella[7,8].

Medicinal Importance: *S.acmella* is the well known 'toothache plant', also commonly used as a spice. The whole plant is claimed to possess medicinal properties. It has a long history of use as a folklore remedy e.g. for toothache, rheumatism and fever[9]. In pharmaceuticals, the plant has found applications as an antitoothache, formulation for pain relief, swelling and gum infections and mouthwashes. Moreover, plant extract is added to body and beauty care cosmetics as a fast acting muscle relaxant to accelerate repair of functional wrinkles. As a nutritional supplement, small amount of the plant extract have been used for taste improvement as a sweetner.

MATERIAL AND METHODS:

Study Site: The study was undertaken 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 of medicinal plant species were randomly selected for collection of rhizospheric soil and root samples during course of investigation. The samples of each plant were collected for further processing for the isolation of AM spores and studying mycorrhizal root colonization.

Estimation 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'[10]. Assessment of root colonization was done by estimating total percentage of root colonization by root slide technique[11].

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)[12]. The quantification of AM spores was done by Grid Line Intersect Method[13]. The spores were identified by using keys of Walker (1986), Schenk and Perez (1990), Kumar *et al.* (2009)[14,15,16].

RESULTS AND DISCUSSION: Basic knowledge of the fungal diversity associated with medicinal and aromatic plants may be useful for the conservation point of view. AM fungi are ecologically important root symbionts of most terrestrial plants. Root samples of 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 types 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.

The species diversity and natural occurrence of AM spores isolated from the rhizosphere of studied medicinal plants is depicted in Table I. A total of ten AM fungal taxa were identified in the soil samples. AM fungi belonging to genus *Glomus* were dominant followed by *Gigaspora* and *Acaulospora*. A large number of spores were isolated and variation in spore population was observed in current study. A total of seven species of *Glomus*, two species of *Gigaspora* and one species of *Acaulospora* were isolated (Figure 1). The high spore number in the rhizosphere soils of studied medicinal plants supported the conclusion of Wang *et al*. (2004)[17] in that the host species apparently had direct effects on spore density and colonization of AM fungi. The features favoring the higher population may either be the conductive to edaphic conditions for sporulation like low nutrient status, high aeration and optimum moisture or the undisturbed conditions of the soils which allowed sufficient time for the buildup of mycorrhizal spores[18,19]. Spore population is affected by a wide range of soil, climatic, fungal and host factors[20].

The present results are in conformity with the findings of several workers who reported the dominance of the *Glomus* in various natural ecosystems[21]. Manoharachary *et al.* (2008)[22] screened thirty five AM fungi associated with sixteen medicinal and aromatic plants belonging to family Apocyanaceae and reported *Glomus* to be most dominant genera represented by twenty one species followed by *Acaulospora* with seven species. The involvement of intercellular or intracellular mycorrhizal associations or association of more than one mycorrhizal fungus with single host plant species might be attributed to their physiological, ecological and genetical variability[23]. The sporulation pattern of *Glomus* might bring about the dominance of the taxon. Spores of *Glomus* are grown in

cluster and sporulate more frequently than others. Twenty three species of AM fungi, which belong to genus *Glomus* were reported from some medicinal plants of Himachal Pradesh[16]. The soil and plant types were found to be more or less important factors contributing to such an existence of *Glomus*. Wang *et al.* (2008)[24] has reported the species of *Glomus* are the most widespread and abundant, followed by *Acaulospora* in agricultural soils of Sichuan Province of mainland China. More species of *Glomus* than that of *Acaulospora* was recognized by different workers while they worked on various natural ecosystems[25,26].

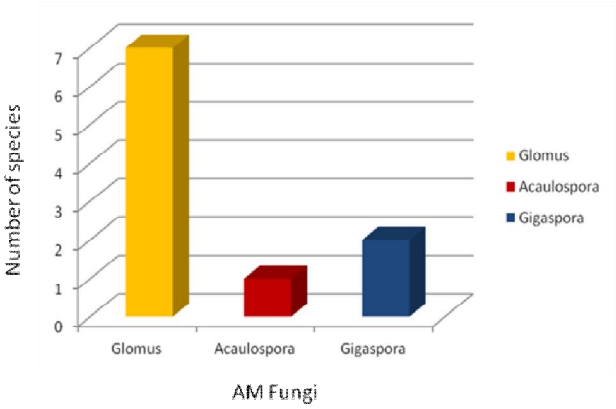

Figure 1: Number of AM species associated with *Spilanthes acmella.*

Table 1: Natural occurrence of AM spores with medicinal plants.

Sr.No.	Name of Plant	AM Fungi
1.	***Spilanthes acmella***	***A. laevis*** Gerdemann & Trappe
		Glomus aggregatum Schenck & Smith emend. Koske
		G. fasciculatum (Thaxter) Gerdemann & Trappe emend. Walker & Koske
		G. geosporum (Nicolson & Gerdemann) Walker
		G. minutum Blaszk, Tadych & Madej
		G. mosseae (Nicolson & Gerdemann) Gerdemann & Trappe
		G. indica Mano, Sharat & Adholeya
		G. segmentatum Trappe, Spooner & Ivory
		Gigaspora albida Schenck & Smith
		G. gigantea (Nicol. & Gerd.) Gerd. & Trappe

CONCLUSION: Much of the data presented in our work elucidates patterns of spore distribution and their identification. This type of study could be the beginning of further research pursuits that will utilize such symbiotic fungi to manipulate the host in different ways. More studies are being emphasized to select the suitable indigenous AM fungal strains for the establishment and management of natural ecosystem such as medicinal plants and to make the people conscious about the role of mycorrhiza as a tool to maintain and manage the herbal flora of the state by environmental friendly approach.

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