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Biodiversity of Endophytic Mycorrhiza in Some Ornamental Flowering Plants of Solan, Himachal Pradesh

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ABSTRACT: Arbuscular mycorrhizae (AM) are the important mutualistic symbionts of the soil edaphon in most agro-ecosystems. The present experiment was conducted in 2011-2012 to investigate the AM fungal status (AM root colonization, AM spore count and AM diversity) of fifteen ornamental flowering plants. AM root colonization ranged from 14.28 ±2.08 to 100.00 ± 0.0 percent and AM spores in the rhizospheric soil of different ornamental plants also varied significantly. Maximum AM root colonization and spore count was observed in *Senecio cineraria* (100.00 ± 0.0) and *Gladiolus grandiflorus* (172.00 ±4.35) respectively. Twenty three different species of AM fungi belonging to five genera i.e. *Glomus, Acaulospora, Sclerocystis, Gigaspora* and *Entrophospora* were reported. *Glomus* was reported to be dominant genera followed by *Acaulospora.* The study confirmed that biodiversity of arbuscular mycorrhizal fungi differ in different plant species.

Key Words: Arbuscular mycorrhizae, Biodiversity, Ornamental flowering plants, *Glomus*, *Acaulospora*

INTRODUCTION

Since the beginning of life, plants have served humankind as source of fuel, food, clothing, shelter and medicine. Due to large demand of flowers, cultivation of ornamental plants has received an impetus in the recent years. So, the enhancement of growth and flowers production is desirable. Many new modern techniques, methods, strategies and plant associations have been put into use to improve the quantity and quality of plant resources. Microbial populations are key component of soil plant system where they are immense in a network of interactions affecting plant development. Mycorrhizal evolution is hypothesised to have progressed from endophytic to balanced symbiotic associations where both partners are interdependent due to the exchange of limiting resources (Brundrett, 2002). About 80% of all terrestrial plant species are known to be forming this type of symbiosis (Smith and Read, 1997). They play a role in shaping plant community structure by increasing the mineral supply to plants, improving water uptake and retention and thus drought tolerance (Lapointe and Molard, 1997). The efficient utilization of AM fungal diversity is of crucial importance in sustainable plant production systems. To preserve the precious ornamental plants, it is essential to know the community of AM fungi in their rhizosphere soil. Therefore, the present work was aimed at understanding the diversity and species richness of AM fungi associated with ornamental plants of Solan.

MATERIAL AND METHODS

Study site: The study was undertaken at different sites located in Solan district, Himachal Pradesh. Solan is located at 30.92^0 N, 77.12^0 E and have a geographical area is 1936 Sq.Km. The climate of the area is generally sub- temperate, semi- humid characterized by cold winter with mild summer and moderate rainfall.

Collection of soil samples: Soil samples along with secondary and tertiary roots of three individuals for fifteen ornamental plants were collected during course of investigation from 2011-2012. The samples of each plant were collected for further processing for the isolation of AM spores and studying mycorrhizal root colonization.

Isolation and quantification of AM spores: AM spores were isolated by using 'Wet Sieving and Decanting Technique of Gerdemann and Nicolson (1963) and quantified by 'Grid Line Intersect Method' (Adholeya and Gaur, 1994). The photography of the counted spores was taken with the help of Nikon coolpix S6200 with an adapter tube.

Identification of AM fungi: Following criteria were used for the identification of AM spores i.e. colour, size, shape, wall structure, surface ornamentation of spores, size of hyphae, bulbous suspensor, number and arrangements of the spores in the sporocarps.

These AM spores were identified by using the key of Walker (1986), Scheneck and Perez (1990), Morton and Benny (1990), Mukerji (1996), Morton and Redecker (2001), Sharma *et al.* (2008) and Kumar *et al*. (2009).

AM root colonization: Root colonization was studied by "Rapid Clearing and Staining Method" of Philips and Hayman (1970). The percentage of mycorrhizal root colonization was calculated by following formula;

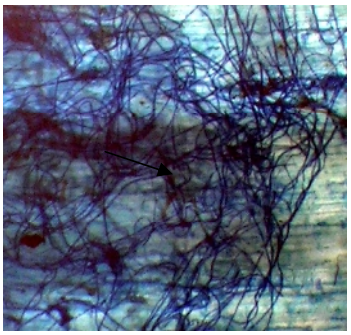
$$\% \text{ age root colonization} = \frac{\text{No. of root segments infected}}{\text{Total no. of root segments studied}} \times 100$$

RESULTS AND DISCUSSION

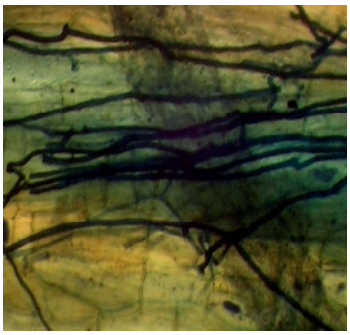
Among the different types of mycorrhizae, arbuscular mycorrhizal fungi has gained much importance in the field of horticulture. Several symbiotic groups, phosphorous solubilizers, plant growth promoters and other such beneficial micro-organisms are reported from different soils. AM fungi form an important ecological and economical group of soil fungi that develop symbiotic associations with the vast majority of plant families.

In the present investigation, biodiversity of AM fungi (AM root colonization, AM spore and AM diversity) associated with fifteen ornamental flowering plants were studied. In case of AM root colonization, root samples of all the fifteen plants showed a wide range of variation as shown in Table 1 Differences among hosts were observed in the amount of hyphae, arbuscules and vesicles produced by the fungi which could be attributed to growth and development characteristics among hosts and AM fungi. The mycorrhizal structure present in roots included mycelium, vesicles and arbuscules. Various types of mycelia like Y-shaped, H- shaped, coiled and parallel mycelia were observed in the roots of different plants. Extensive mycelial growth was also observed in some root samples. Different types of vesicles like rounded, oval, beaked, elliptical and elongated were observed (Plate-I). It is evident from the results that both vesicles and arbuscules type of colonization was reported in six plants species and mycelium was reported in all the plants. The plant species that belonged to the families Iridaceae, Malvaceae, Solanaceae and Asteraceae only showed the presence of vesicles while arbuscules were absent. Likewise, in plants namely *Dahlia variabilis*, *Jacobinea carnea* and *Nerium indicum* only showed arbuscules and vesicles were absent. In *Hydrangea paniculata* and *Rosa indica* it was observed that both vesicles and arbuscules were absent (Table 1). AMF root colonization ranged from 14.28 ± 2.08 to 100.00 ± 0.0 percent. It is envisaged from the result (Table 1) that maximum AMF root colonization was found in *Senecio cineraria* ((100.00 ± 0.0) and the least was observed in *Jacobinea carnea* (14.28 ± 2.08). Similarly, *Rosa indica* (94.44 ± 9.62), *Catharanthus roseus* (91.07±7.78), *Dahlia variabilis* (88.88± 9.62), *Nerium indicum* (79.16± 7.21) and *Hydrangea paniculata* (75.00± 12.5) were found to have high AM root colonization as shown in Table 1. The result also indicated that mycorrhizal infection varied within the same family. In family Asteraceae, *Dahlia variabilis* (88.88± 9.62), *Tagetes patula* (66.68±8.26), *Aster amellus* (60.31±5.49) and *Crysanthemum leucanthemum* (43.45± 6.27) showed varied degree of mycorrhization. Similar was the observation made in families like Apocynaceae where *Catharanthus roseus* (91.07± 7.78) and *Nerium indicum* (79.16 ± 7.21) showed a difference in AM root colonization. AM infection improves plant survival and growth by enhancing the root's ability to absorb moisture, macro and micro-nutrients from the soil. Survey of arbuscular mycorrhizal fungi has been carried out in different parts of world from time to time, vesicular arbuscular mycorrhizal fungi has the potential to influence the ecosystem processes, thereby determine the plant communities and its ability to induce a wide variety of growth responses in coexisting plant species (Klironomos *et al*., 2000).

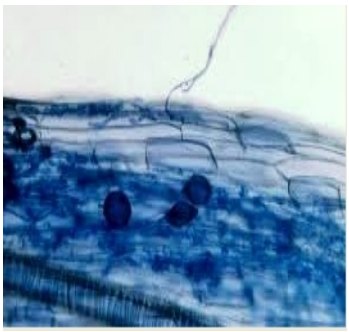
PLATE-I: Types of mycorrhizal mycelia, vesicles and arbuscules (*100X)



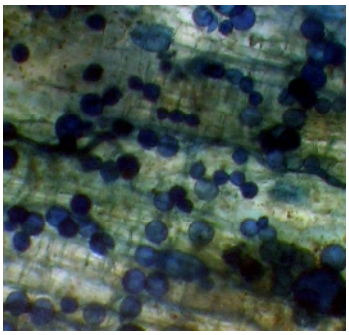
***Intramatrical mycelium**



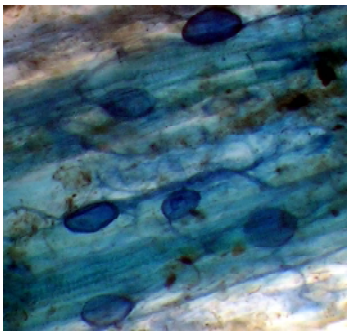
***Parallel mycelium**



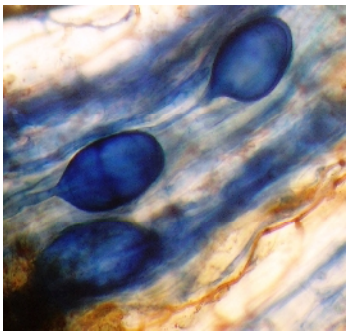
***Mycelium, vesicles & arbuscules**



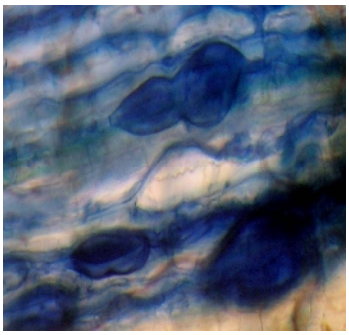
***Rounded Vesicles**



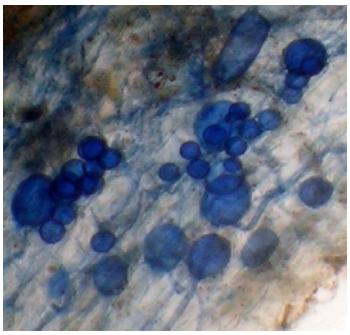
***Oval shaped vesicles**



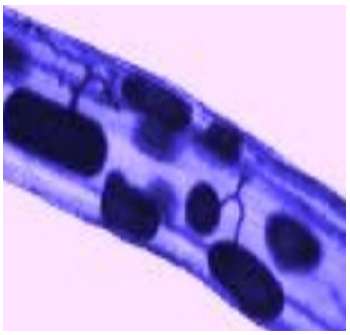
***Elliptical vesicles**



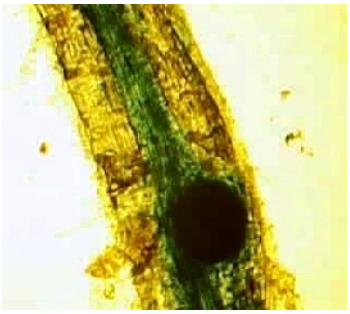
***Beaked**



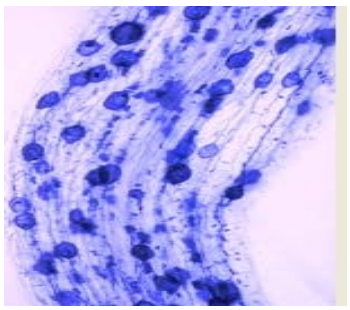
***Different vesicles in a root**



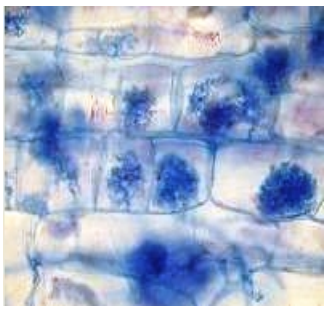
***Elongated vesicles**



***AM spore infecting root**



***Scattered vesicles**



***Root colonization by arbuscules**

Table 1. Endomycorrhizal studies of some ornamental flowering plants of district Solan, (HP).

Sr. No.	Botanical name	Family	Type of infection #M	# V	#A	AM spore count/ 50gm. of soil	% AM root colonization
1.	*Aster amellus*	Asteraceae	+	+	+	102.00±5.00	60.31± 5.49
2.	*Catharanthus roseus*	Apocynaceae	+	+	+	65.00± 3.60	91.07± 7.78
3.	*Crysanthemum leucanthemum*	Asteraceae	+	+	+	124.00±2.64	43.45± 6.27
4.	*Dahlia variablis*	Asteraceae	+	-	+	94.66 ±4.50	88.88± 9.62
5.	*Geranium pelargonium*	Geranaceae	+	+	+	74.66± 4.16	37.89± 4.77
6.	*Gladiolus grandiflorus*	Iridaceae	+	+	-	172.00±4.35	63.36 ± 1.58
7.	*Hibiscus rosa sinensis*	Malvaceae	+	+	-	90.33 ±3.05	37.33± 0.28
8.	*Hydrangea paniculata*	Saxifragaceae	+	-	-	103.00 ±4.00	75.00± 12.5
9.	*Jacobinea carnea*	Acanthaceae	+	-	+	112.66 ±4.50	14.28 ±2.08
10.	*Lilium rubescens*	Liliaceae	+	+	+	57.66 ±3.21	38.09 ± 8.24
11.	*Nerium indicum*	Apocynaceae	+	-	+	122.33± 3.05	79.16± 7.21
12.	*Rosa indica*	Rosaceae	+	-	-	104.00±3.60	94.44 ± 9.62
13.	*Salvia splendens*	Labiatae	+	+	+	121.66 ±6.11	47.61 ± 8.25
14.	*Senecio cineraria*	Solanaceae	+	+	-	71.00±4.00	100.00 ± 0.0
15.	*Tagetes patula*	Asteraceae	+	+	-	76.00 ±2.64	66.68 ± 8.26

Note: #M – Mycelium, V-Vesicles, A- Arbuscules, **+:** Present, **- :** Absent, **±:** Standard deviation.

Table 2: Natural occurence of AM spores with some ornamental flowering plants of district Solan, (HP).

Sr.No	Botanical Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1.	*Aster amellus*	–	–	×	–	–	–	–	×	–	×	–	–	–	–	–	×	–	–	×	–	–	–	×
2.	*Catharanthus roseus*	–	–	×	–	×	–	–	×	–	–	–	–	–	–	×	×	–	–	–	–	–	–	–
3.	*Crysanthemum leucanthemum*	–	–	×	–	–	×	–	–	–	–	–	–	×	–	–	–	×	–	–	–	–	–	–
4.	*Dahlia variablis*	–	–	–	–	×	–	–	×	–	–	–	×	–	–	–	×	–	–	–	–	–	–	–
5.	*Geranium pelargonium*	–	–	×	–	–	–	–	–	–	×	–	–	–	–	–	–	–	–	–	×	×	–	–
6.	*Gladiolus grandiflorus*	×	–	–	–	–	–	×	–	×	–	–	×	–	–	–	×	–	–	–	–	–	–	×
7.	*Hibiscus rosa sinensis*	×	–	×	–	–	–	–	–	–	–	–	×	–	×	–	–	–	–	–	–	×	–	–

8.	*Hydrangea paniculata*	–	×	–	–	–	–	–	–	–	–	×	–	–	–	–	×	–	–	–	–	–	–	–
9.	*Jacobinea carnea*	–	–	–	–	–	–	–	×	–	–	–	×	–	–	–	–	–	–	–	–	×	–	–
10.	*Lilium rubescens*	–	×	×	–	×	–	×	–	–	–	–	×	–	–	–	×	–	×	–	–	×	–	–
11.	*Nerium indicum*	–	–	–	–	–	–	–	×	–	–	–	×	–	×	–	–	–	–	–	–	–	–	–
12.	*Rosa indica*	×	–	×	–	–	–	×	–	–	–	×	–	–	–	×	–	–	–	–	–	×	–	×
13.	*Salvia splendens*	–	–	×	×	–	×	–	×	–	–	–	–	–	–	–	×	–	–	–	–	–	–	–
14.	*Senecio cineraria*	×	–	–	–	–	–	–	×		×	–	×	–	–	–	×	–	–	–	–	–	–	–
15.	*Tagetes patula*	–	×	×	–	–	–	×	–	–	–	–	–	×	–	–	–	–	–	–	–	–	–	–

X = Present - = absent

Type of AM fungi: 1. *Acaulospora bireticulata* **2.** *A.lacunosa* **3.** *A.laevis* **4.** *A.rehmii* **5.** *A.spinosa* **6.** *A.trappei* **7.** *Entrophosphora infrequens* **8.** *Gigaspora gigantea* **9.** *G.nigra* **10.** *Glomus constrictum***11**. *G.deserticola* **12.** *G. fasciculatum* **13**. *G.indica* **14.** *G.aggregatum* **15**. *G.microcarpum* **16.** *G.mosseae* **17**. *G.fuegianum* **18.** *G.pallidum* **19.** *G.pansihalos* **20**. *G.scintillans* **21**. *Sclerocystis ceremoides* **22.** *S.duscii* **23.** *S.sinuosa.*

The data presented in Table 2 showed that the population of AM spore in the rhizosphere soil of different ornamental plants varied significantly. The density of AM spore varied from 57.66 ±3.21 to 172.00 ± 4.35. The maximum number of AM spore population was recorded in the rhizospheric soil of *Gladiolus grandiflorus* (172.00 ±4.35) and it was followed by *Crysanthemum leucanthemum* (124.00±2.64), *Nerium indicum* (122.33± 3.05), *Salvia splendens* (121.66 ± 6.11), *Jacobinea carnea* (112.66 ± 4.50), *Rosa indica* (104.00±3.60), *Hydrangea paniculata* (103.00 ±4.00) and *Aster amellus* (102.00±5.00). The minimum number of AM spore population was recorded in *Lilium rubescens* (57.66±3.21) and *Catharanthus roseus* (65.00± 3.60). Different AM spores isolated from the rhizosphere of studied ornamental flowering plants were identified to find out the species biodiversity and richness. A variety of spores were recovered from the rhizosphere of fifteen ornamental flowering plants (Table 2). It can be envisaged from Table 2 that twenty three different species of AM fungi belonging to five genera i.e. *Glomus*, *Acaulospora*, *Sclerocystis*, *Gigaspora* and *Entrophospora* were screened from rhizospheric soil of different plants. *Glomus* was found to be dominant genera (11 species) followed by *Acaulospora* (6 species), *Sclerocystis* (3 species), *Gigaspora* (2 species) and *Entrophospora* (1species). AM fungi belonging to genus *Glomus* were dominant and *Entrophospora* was rare. Among dfferent species of *Acaulospora*, the most abundant and most frequent species reported in different ornamental flowering plants was *A.laevis* (9) followed by *A. bireticulata* (4). Similarly, among *Glomus* species, *G. mosseae* was reported in eight plants followed by *G. fasciculatum* (7). In case of *Gigaspora*, *Sclerocystis* and *Entrophospora*, the more abundant species recognized in ornamental flowering plants were *Gigaspora gigantea* (7), *Sclerocystis ceremoides* (5) and *Entrophosphora infrequence* (4) respectively. *Glomus* sp. was found in all the plants. *Gladiolus grandiflorus* was the only plant with great abundance of mycorrhizal fungi and have all the five genera of AM fungi. *Lilium rubescens* and *Rosa indica* were found to be associated with four genera of AM fungi. Maximum numbers of species were reported from *Lilium rubescens* (8) and *Rosa indica* (7). Minimum numbers of species were recorded from *Hydrangea paniculata* (3), *Jacobinea carnea* (3) and *Nerium indicum* (3).

From the above results it is clear that biodiversity of arbuscular mycorrhizal fungi differ in different plants. The extramatrical hyphae are more efficient in nutrient uptake than root hairs (Allen, 2007). In several studies, a high degree of AM root colonization shows high localized bioprotective effect whereas intermediate and low levels of AM root colonization showed very less bioprotective effect (Vierheilig, 2008). The variation in root colonization may be due to the exudation of toxic metabolites resulting in substances in proximity to the roots which attract the AM fungi such as production of easily oxidisable compounds resulting in increased colonization physiological difference between species (Albert and Sathianesan, 2009). The smaller diameter and the faster growth of hyphae

than of the roots permit the exploitation of soil particles with smaller pores and the surpassing of the nutritional depletion zone which appears around the rhizosphere (Smith and Read, 1997). Ornamental plants are often grown in soil amended with large quantities of composted organic matter and most of the amendments are known to enhance sporulation and root colonization by AM (Singh, 2000). The composition of the AM community may be strongly influenced by the host species through differential effects on hyphal growth and sporulation (Eom *et al.*, 2000). It has also been shown that more than one fungal species can colonize roots of an individual plant in a natural ecosystem (Ahulu *et al.*, 2007). Differences among hosts were observed in the amount of hyphae, arbuscules and vesicles produced by the fungi which could be attributed to growth and development characteristics among hosts and AM fungi (Kramnik *et al.*, 2007). Recently, it was showed that AMF abundance, species richness and species diversity varied among different sampling sites, as some of the sites were irrigated and some were non irrigated (Kumar *et al*., 2012). Tejavathi *et al.* (2011) reported the positive correlation between percent mycorrhizal colonization and plant growth in *Andrographis paniculata*. Bargali (2011) claimed that the number of plants possessing vesicles are always higher than the plants bearing arbuscules. These results confirmed that the roots of majority of the plants colonized were mature as vesicles are storage organs and generally produced in older region of the infection. However, Zhang *et al.* (2012) also reported a varied degree of AM root colonization in ephemeral plants that range from 7% to 85%.

Similarly, Kubato *et al*. (2005) also described that the morphology of AM type is the result of interaction between both the plant and fungal species. The increased level of mycorrhizal colonization caused by AM inoculum and higher phosphorus content in the leaves of *Physocarpus opulifolius*, *Spiraea japonica* and *Potentilla fruticosa* species did not correspond with higher biomass of plants (Bozena and Grzegorz, 2010). The observed high richness of Glomeromycota in the agricultural ecosystems indicates the need to obtain comparable descriptive soil fungal community data from a more diverse range of agricultural ecosystems. This unique richness of AMF could be speculatively attributed to the agro-climatic conditions and or type of host species (Kumar and Garampalli, 2012).

Arbuscular mycorrhizal fungi of the phylum Glomeromycota live in symbiosis with a majority of land plants. The AM fungi are well known to enhance the nutritional status of several ornamental flowering plants and thereby aid in increased growth and yield. 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. The management of their population in the soil is an essential tool for overall plant health in the present scenario of sustainable crop productivity.

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