Research Article

Arbuscular Mycorrhizal Fungi spores diversity and AMF infection in some medicinal plants of District Charsadda KPK

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Abstract

An investigation has been made about the Arbuscular Mycorrhizal Fungi (AMF) spore population and root colonization in 20 medicinal plants selected and collected from district Charsadda at early winter and summer seasons during session 2014-2015. The spore density of AMF ranged from 80 spores per 100gm⁻¹, and root colonization from 20-80%. The lowest spore density was recorded in *Cestrum nocturnum*, *Ficus carica*, *Aquilaria agallocha* and the highest spore density was recorded in *Sedum dendroideum*, *Cannabis sativa*, *Triticum aestivum*, and *Mentha longifolia*, *Withania somnifera* while absent in *Brassica campestris*, *Amaranthus viridis*. The spore density of AMF and root colonization by these fungi varied from species to species, season to season, and also affected by host plant growth stages (vegetative-fruiting). AM fungi association was more observed at fruiting stage and low at vegetative and flowering phases of host. The most dominant species of AMF were *Glomus* followed by *Sclerocystis* and *Acaulospora* species.

Keywords: Arbuscular mycorrhizal fungi; Root infection; Spores density; Medicinal plants

Introduction

Mycorrhiza is a symbiotic relationship between a fungus and the roots of higher plants [1]. "Mycorrhiza, is a mutualistic association between fungi and higher plants, has been documented extensively, but much less is known about the development of Arbuscular Mycorrhizal (AM) fungi" [2].

AM Fungi is a diverse group of associations which are formed by the co-evolution between fungi and plants and benefits to both partners [3]. The term of mycorrhiza describes symbiotic associations between plants and fungi, these associations are assumed to play an important role in the land colonization by plants due to the ability

of the symbiotic organisms in acquiring nutrients unavailable to non-mycorrhizal individuals [4]. The AMF inoculated plants will improve plant health by enhancing nutrient status, yield and also by promoting the host plants against the pathogens. It is therefore, essential to know the specificity of AMF host pathogen combination for beneficial purpose [5]. ‘‘Mycorrhizal fungi are mostly widespread since they can be found in the roots of most plant species, these characteristics make mycorrhizal fungi a potential powerful tool to be used in actions towards a sustainable management of the environment’’ [6, 7]. Medicinal plants are known as source of ‘phytochemicals, or active compounds’ that are worldwide used for their natural properties, As many medicinal plants selected for this study has pharmaceutical and industrial importance for example, basil is a vital source of essential oil and used for a perfumery, cosmetic, food and pharmaceutical industry [8]. The medicinal herbs in the area grow and present naturally and propagated vegetative (underground rhizomes, stems and bulbs or corms), Medicinal plants play pivotal role for existence of life on the earth Out of approximately 2, 50,000 higher plant species on earth, more than 80,000 are medicinal [9]. Although AMF impact on the performance and accumulation of therapeutic compounds of several medicinal plant species has been well documented [10-14].

Materials and methods

Site

The present study was performed in various parts of Charsadda, Khyber Pakhtunkhwa situated 34°8'43N - 71°43'51E with an elevation of 276 meters. The rainfall throughout the year with an average temperature of 22.5°C. The average annual rainfall is 460 mm.

Survey work

Twenty Roots and Soil samples were collected from different area of district Charsadda during the session of 2014-2015. The areas include Umarzai, Tangi, Mandani, Kuladand, Shakh No: 6 and some plants from Thakht Bhai hills. Plant samples belong to different families, in which family *Labiatae*, *Crassulaceae*, *Poaceae*, *Apocynaceae*, *Lomariopsidaceae*, *Urticaceae*, *Cycadaceae*, *Cupressaceae*, *Amaranthaceae*, *Moraceae*, *Dioscoreaceae*, *Thymelaeaceae*, *Caesalpiniaceae*, *Cannabaceae*, and *Brassicaceae* were represented by their respective species.

AMF spores isolation and its quantification

The spores of AM fungi were isolated by ‘‘Wet Sieving and Decanting Technique’’ demonstrated by [15].

Identification of AMF spores

The identification of these fungi was done by following the manual of [16] and was also compared with reference species description demonstrated by INVAM (International culture collection of vesicular arbuscular mycorrhizal fungi, 2014). For the characterization of AM fungi, various characteristics of spores such as spore morphology, shape, colour and size were studied.

Root clearing and staining technique

For roots cleaning and staining, a method reported by [17] was used. The stained roots samples were visualized using compound microscope for monitoring fungal structures like vesicles, hyphae or arbuscule etc. Percent root colonization for each plant was calculated using following relation:

$$\% \text{ Root colonization} = \frac{\text{Total no. of colonized root segments}}{\text{Total no. of root segments investigated}} \times 100$$

Isolation and quantification of spores

AMF spores from the 100gm roots zone soil were isolated using wet sieving and decanting method [15]. The rhizospheric soil for a particular plant was obtained from different spots, pooled and then mixed to

take representative soil sample. Each sample was taken in triplicate and data represented as mean spore density/100gm soil. The total number of spores obtained by wet sieving was referred to as Spore density.

Results and discussion

Spore density

Selected plants belong to different families at various host growth stages are shown in Table (1). It show the presence of AM fungi ranges from low to maximum colonization Table (2). Except for three species which have low percentage (20%) of spores; *Ficus carica* (Moraceae) *Cestrum nocturnum* (Solanaceae) 18 of the of 20 species were mycorrhizal. The study showed total of 18 AMF species investigated in the selected medicinal plants. *Glomus* is represented about (40-80 %) the total AMF species, followed by (14%) species of genus *Sclerocystis*, *Acaulospora* respectively (Figure 2-4). Our results have demonstrated that *Glomus* was the dominant specie. These results are in agreement with results obtained by [18], which shows that *Glomus* species were dominant followed by *Acaulospora* and *Sclerocystis* in *Aloe vera*

(*Xanthorrhoeaceae*). Eighteen plants show AMF in their rhizospheric soils associated with their roots and nearly 80 percent are colonized. A rhizospheric soil of *Sedum dendroideum* (yellow) show three types of spores. Highest mean spore density is present in *Sedum dendroideum* our results agree with [19]. Who explained the morphology of AM fungi that it is a type of symbiotic association between the plant and fungal species. Only genus *Glomus* seems to show a high adaptive value with the medicinal plants since 50-80 percent of the total AMF species enumerated here belong to this genus and rest being represented by other two genera of *Sclerocystis* and *Acaulospora*. The hardness and predominance of various *Glomus* species seems to be a general observation, that statements are followed [20-21]. Correlate with a report of *Glomus* and *Sclerocystis* growing in symbiotic association with the earliest *Diazotrophic cyanobacteria*, *Nostoc* species in medicinal plants [22]. A similar report has very recently been published by [23].

Table 1. Selected plants belong to different families at various host growth stages

S. No.	Plants	Family	Host stages	Habit
1	<i>Ocimum basilicum</i>	Labiatae	Fruiting	Herb
2	<i>Mentha longifolia</i>	Labiatae	Vegetative	Herb
3	<i>Thuja orientalis</i>	Cupressaceae	Vegetative	Tree
4	<i>Ficus carica</i>	Moraceae	Fruiting	Tree
5	<i>Minta pepita</i>	Labiatae	Vegetative	Herb
6	<i>Amaranthus viridis</i>	Amaranthaceae	Fruiting	Herb
7	<i>Aspidium exaltata</i>	Lomariopsidaceae	Vegetative	Shrub
8	<i>Canabus sativa</i>	Canabiniaceae	Fruiting	Shrub
9	<i>Bohenia variegata</i>	Caesalpenianaceae	Fruiting	Tree
10	<i>Auilaria agallocha</i>	Thymelaeaceae	Vegetative	Shrub
11	<i>Cestrum nocturnum</i>	Solanaceae	Fruiting	Tree
12	<i>Cataranthus roseus</i>	Apocynaceae	Flowering	Shrub
13	<i>Pheonix dactalifora</i>	Cycadaceae	Vegetative	Tree
14	<i>Withania somnifera</i> (L.)	Solanaceae	Vegetative	Shrub
15	<i>Dioscorea rotundata</i>	Dioscoraceae	Vegetative	Herb
16	<i>Cymbopogon</i>	Poaceae	Vegetative	Shrub
17	<i>Sedum spurium-fuldaglu</i>	Crassulaceae	Vegetative	Shrub
18	<i>Sedum dendroideum</i>	Crassulaceae	Vegetative	Shrub
19	<i>Triticum aestivum</i>	Poaceae	Vegetative	Shrub
20	<i>Brassica compestris</i>	Brassicaceae	Vegetative	Shrub

Table 2. AM fungal root colonization in some selected medicinal plants of Charsadda

S. No	Plants	Family	AMF morphs			
			E H. %	IH. %	Arb. %	Ves. %
1	<i>Ocimum basilicum</i>	Labiatae	+++	++	+++	++
2	<i>Mentha longifolia</i>	Labiatae	+++	++	+	+++
3	<i>Thuja orientalis</i>	Cupressaceae	++	++	++	-
4	<i>Ficus carica</i>	Moraceae	+	+	+	+
5	<i>Menta piperita</i>	Labiatae	+	+++	++	++
6	<i>Amaranthus viridis</i>	Amaranthaceae	-	-	-	-
7	<i>Aspidium exaltata</i>	Lomariopsidaceae	++	++	+	++
8	<i>Cannabis sativa</i>	Cannabaceae	+++	+++	+++	+++
9	<i>Bauhinia variegata</i>	Caesalpiniaceae	++	++	+	-
10	<i>Aquilaria agallocha</i>	Thymelaeaceae	+	+	+	+
11	<i>Cestrum nocturnum</i>	Solanaceae	+++	++	+	+++
12	<i>Catharanthus roseus</i>	Apocynaceae	++	++	-	-
13	<i>Phoenix dactylifera</i>	Cycadaceae	++	+	+	++
14	<i>Withania somnifera</i>	Solanaceae	+++	++	+	++
15	<i>Dioscorea rotundata</i>	Dioscoreaceae	+++	+	+	+
16	<i>Cymbopogon</i>	Poaceae	+	++	+	++
17	<i>Sedum spurium-fuldaglut</i>	Crassulaceae	++	++	++	++
18	<i>Sedum dendroideum</i>	Crassulaceae	+++	+++	++	+++
19	<i>Triticum aestivum</i>	Poaceae	++	+++	+	++
20	<i>Brassica campestris</i>	Brassicaceae	-	-	-	-

Absent: [-], Low: [+], Medium: [++], High: [+++], EH (External Hyphae), IH [Internal Hyphae] Arb. [Arbuscules], Ves. [Vesicles]

Figure 1. Spore density (SD) of each of three AMF species (G =*Glomus* species; AC = *Acaulospora* species; Scl = *Sclerocystis* species) associated with medicinal plants (P1-P20) species in district Charsadda Khyber Pakhtunkhwa

Figure 2. *Glomus* species

Figure 3. *Sclerocystis* species

Figure 4. *Acaulospora* spp.

Root colonization

All the 18 mycorrhizal species show hyphal presence in roots (Table 1) and more than 80 percent of these plants show presence of either hyphae and Arbuscules or hyphae and vesicles or all the three structures individually. Twenty two samples of roots and soil were collected from various parts of district Charsadda, Which is belongs to 16 different families. Our results agree with those of [24], who also reported the occurrence of Mycorrhiza in some members of the family Labiatae species ranged from 55-70% Colonized. Nineteen of the studied

plant species formed AMF associations 40-80 percent. AMF mycelium was less 20-30 percent found in the root systems of *Ficus carica* which is belong to family Moraceae, *Aquilaria agallocha* belongs to family Thymelaeaceae, *Dioscorea rotundata* belongs to family Dioscoreaceae, *Cymbopogon* (lemon grass) belongs to family Poaceae. That is generally accepted as conventionally AMF less host *Brassica campestris* belongs to family Brassicaceae, which have no any mycorrhizal infection. These results also agree with the results of [25].

Figure 5. Root colonization (vesicles, arbuscules, external hyphae and internal hyphae) percentage in medicinal plants of district Charsadda

Conclusion

It is concluded from our results that biodiversity of AMF, spore density and root colonization by these fungi varied from species to species, season to season, and also affected by host plant growth stages (vegetative-fruiting). Spore density when studied in summer and winter indicated that the dominant AMF endophytes and plant species in each season were different following great variation. Fluctuations in spore numbers have been attributed to seasonal variations. A variety of AM fungi were found in both seasons where *Glomus* species were found more frequently. There is a specificity of AMF, natural diversity of AM fungi in the soil differ from species to species and differently influence root colonization and spore density. The highest intensity of AM colonization and spore population in the flowering stage also explains the successful interaction of the plants with AM fungi. It has been believed that extraradical hyphae are more helpful in providing nutrients to host plant.

Authors' contributions

Conceived and designed the experiments: T Yaseen & Y Khan, Performed the experiments: Y Khan, Analyzed the data: T Yaseen & Y Khan, Contributed reagents/materials/ analysis tools: T Yaseen & MS hahkeel, Wrote the paper: T Yaseen & Y Khan.

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