Investigating the Association of Arbuscular Mycorrhizal Fungi with Selected Ornamental Plants Collected from District Charsadda, KPK, Pakistan

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Abstract: This study investigates Arbuscular Mycorrhizal Fungi (AMF) spore population and root colonization in 25 different ornamental families (35 species) collected from District Charsadda, at early winter and summer seasons. The spore density of AMF ranged from 23-80% and root colonization from 0-100%. The lowest spore density was recorded in *Canna indica purpurea*, *Eriodictyon californicum*, *Lagerstroemia indica* and the highest spore density was recorded in *Jasminum sambc*. The AMF root colonization was observed to be the lowest in *Portulaca indica*, *Dracaena* (Dragon tree) and the highest in *Alocasia indica*, *Chlorophytum borivilianum*, *Eriodictyon californicum*, *Cesturnum nocturnum*, *Euphorbia milli*. The present study also reports that roots of a few species of family *Portulacaceae* have not been colonized by AMF. 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).

Key words: Arbuscular mycorrhizal fungi, root infection, spores density, ornamental plants, district charsadda

INTRODUCTION

Ornamental plants are plants that are normally grown for ornamental purpose and not expected to be used for their nutritional value. These plants have enormous contribution towards horticultural industry and play important role in our daily life (Chandler, 2013). The term mycorrhiza is rooted from two Greek words “myco” means fungus and “rhiza” means root. Mycorrhiza is described as a mutual sharing of life; whereby the fungus is the major partner of the plant that supply food, growth hormones and protection to plant roots from pathogens and the plant in return offers high energetic material to the fungus (Alizadeh, 2011). The AM fungi form mutualistic association with majority of land plants and provide several advantages to host plants (Krishnakumar *et al.*, 2013).

Plants have always been important in human history as a source of food, feed, medication and housing. Due to increased use of flowers, ornamental plants are getting more attention worldwide. Several conventional and biotechnological approaches have been practiced to enhance the quality and quantity of ornamental plants (Brundrett, 2002).

The AM fungi are obligate symbiont, which cannot be grown without living plant host. They have been shown to improve plant growth, due to increased uptake of phosphorous (P) and other essential nutrients (Requena *et al.*, 2001). They have also been demonstrated in reduced soil erosion, due to improved water absorption (Dhar and Mridha, 2003), reduced disease occurrence, salt

and drought tolerances (Evelin *et al.*, 2009; Tonin *et al.*, 2001). Among the ornamental plants, *Petunia* (*Petunia hybrida*) is very common and widespread in Riyadh, as it can be grown under reduced irrigation and high temperature. Not much is known whether *petunia* form mutualistic association with AM fungi (Gaur *et al.*, 2000; Shamshiri *et al.*, 2012; Reddy *et al.*, 2009).

The critical factors, involved in the sustainable production of mycorrhizal ornamental plants, are the choices of growth substrate and fertilizing method. Therefore, this study investigated the effects of commercially available arbuscular mycorrhizal inocula and mode of organic mineral fertilization on mycorrhizal colonization and growth of three species of ornamental conifers, under a nursery-container production system (Falkowski and Matysiak, 2010).

MATERIALS AND METHODS

Study 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 m. The climatic conditions in District Charsadda are harsh, as there is little rainfall throughout the year, with an average temperature of 22.5°C. The average annual rainfall is 460 mm.

Collecting soil samples: Soil samples, containing spores and roots from the colonized 35 ornamental and medicinal plants, were retrieved during the study (Table 1). The

Table 1: Detailed information regarding plant species, families, growth stage and collection date

Plant species	Family	Growth stage	Season	Date of collection
<i>Portulaca grandiflora</i>	Portulacaceae	Flowering	Winter	20.11.2014
<i>Calendula officinales</i> (tagetes)	Asteraceae	Flowering	Winter	20.11.2014
<i>Chrysanthemum indicus</i>	Asteraceae	Flowering	Winter	15.02.2015
<i>Coreopsis lenceolata</i>	Asteraceae	Flowering	Summer	17.05.2015
<i>Gladiolus dalenii</i>	Iridaceae	Fruiting	Winter	20.11.2014
<i>Aloe vera</i>	Xanthorrhoeaceae	Mature	Summer	15.05.2015
IVY (<i>Hedera</i>)	Araliaceae	Fruiting	Winter	21.11.2014
<i>Tradescantia pallida</i>	Commelinaceae	Flowering	Summer	15.05. 2015
<i>Celosia cristata</i>	Amaranthaceae	Flowering	Winter	21.11.2014
<i>Alternethra purple knight</i>	Amaranthaceae	Flowering	Winter	26.12.2014
<i>Amaryllis</i> (<i>Hippeastrum</i>)	Amaryllidaceae	Flowering	Summer	15.05.2015
<i>Narcissus poeticus</i>	Amaryllidaceae	Flowering	Winter	14.12.2014
<i>Cynodon dactylon</i>	Poaceae	Fruiting	Winter	5.01.2015
<i>Cymbopogon</i>	Poaceae	Vegetative	Winter	22.02.2015
<i>Duranta erecta</i>	Verbenaceae	Fruiting	Summer	15.05.2015
<i>Duranta</i> (Golden duranta)	Verbenaceae	Fruiting	Winter	26.11.2014
<i>Jasminum officinale</i>	Oleaceae	Flowering	Winter	22.02.2015
<i>Jasminum humile</i>	Oleaceae	Flowering	Winter	22.02.2015
<i>Jasminum sambac</i>	Oleaceae	Flowering	Winter	22.11.2014
<i>Epipremnum aureum</i>	Araceae	Vegetative	Winter	16.01.2015
<i>Alocasia indica</i>	Araceae	Fruiting	Winter	28.11.2014
<i>Dypsis Lutescens</i>	Araceae	Vegetative	Winter	28.11.2014
<i>Chlorophytum</i>	Asparagaceae	Flowering	Winter	25.02.2015
<i>Dracaena</i> (Dragon tree)	Asparagaceae	Flowering	Winter	27.11.2014
<i>Canna indica purpurea</i>	Cannaceae	Flowering	Summer	15.05.2015
<i>Eriodictyon californicum</i>	Boraginaceae	Vegetative	Summer	15.03.2015
<i>Lagerstroemia speciosa</i>	Lythraceae	Fruiting	Winter	25.02.2015
<i>Gardenia jasminoides</i>	Rubiaceae	Fruiting	Winter	20.02.2015
<i>Bryophyllum delagoense</i>	Crassulaceae	Flowering	Summer	16.05.2015
<i>Senegalia Senegal</i>	Febaceae	Flowering	Winter	22.11.2014
<i>Pittosporum tobira</i>	Pittosporaceae	Flowering	Summer	15.05.2015
<i>Cesturnum nocturnum</i>	Solanaceae	Fruiting	Winter	17.01.2015
<i>Chamaecyparis lawsoniana</i>	Cupressaceae	Fruiting	Winter	26.11.2014
<i>Araucaria araucana</i>	Araucariaceae	Fruiting	Winter	27.11.2014
<i>Euphorbia milli</i>	Euphorbiaceae	Flowering	Winter	26.12.2014

collected soil and root samples, from each of the plant studied, were used for isolation of AMF spores and studying related features.

AMF spores isolation and their quantification: The spores of AM fungi were isolated by “Wet Sieving and Decanting Technique” demonstrated by Gerdemann and Nicolson (1963). The isolated AMF spores were quantified, using Grid Line Intersect Method (Gaur and Adholeya, 1994). The pictures of the spores being counted were taken, using Nikon Coolpix S6200.

Identification of AMF spores: The spores of AM fungi were identified, using the guidelines given by the earlier studies (Morton and Benny, 1990; Mukerji, 1996; Schenck and Perez, 1990; Morton and Redecker, 2001; Sharma *et al.*, 2009; Kumar *et al.*, 2009). The identification of these fungi was done, following the manual of Schenck and Perez (1990) and was also compared with reference species description demonstrated by INVAM (International culture collection of vesicular arbuscular mycorrhizal fungi). For the characterization of AM fungi, various characteristics of spores, such as, morphology, shape, color and size, were studied. For

examining the spore size and shape up to 50 spores were taken in a drop of lactic acid. The dimensions of spores were determined, using micrometry.

Root clearing and staining technique: For roots cleaning and staining, a method, reported by Phillips and Hayman (1970) and Kormanik *et al.* (1980), was used.

Separation and quantification of spores: The AMF spores from the 100 gm roots zone soil were isolated using wet sieving and decanting method (Gerdemann and Nicolson, 1963). 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/100 gm soil with standard deviation. The total number of spores obtained by wet sieving was referred to as Spore density. Quantification of spores was performed employing the procedure reported by Lugo and Cabello, (2002) in petri plate of 10 cm diameter with gridline of 1cm square taking help of stereoscopic microscope at 100x. In this study, we counted 10 divisions and relate it to total number of spores employing the procedure, given by McKenney and Lindsey (1987).

Root colonization analysis: The colonization of AM fungi and its extent was demonstrated from the randomly chosen root tissue of each plant studied using frequency distribution method (Biermann and Lindermann, 1981). For this purpose, roots were cut in 1cm segments, cleared and stained. 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.

RESULTS AND DISCUSSION

This study shows that 33 out of 35 species examined were mycorrhizal fungi. The present work reports that the 35AMF species linked with the ornamental plants are broadly disseminated among 3 genera, i.e., Genus *Glomus*, *acaulospora* and *Sclerocystis*, characterized by all species, respectively. The limited AMF generic diversity shown for District Charsadda is encouraging and needs to be further explored.

In our study, only genus *Sclerocystis* has been reported to present high adaptation with ornamental plants. Since the total AMF species enumerated here belong to this genus and the remaining are characterized by the other two genera of *Glomus* and *Acaulospora*. The results indicated that 33 out of 35 Ornamental plants, examined during the study, exhibited the presence of arbuscular mycorrhizal association. The root colonization

range in AMF is assumed to be variable which range from very low of 20% in *Alternethra purple knight* (family *Amaranthaceae*) and *Duranta erecta* (family *Verbenaceae*) to the highest of 100% in *Eriodictyon californicum* (family *Boraginaceae*), *Euphorbia milli* (family *Euphorbiaceae*), *Cesturnum nocturnum* (family *Solanaceae*), *Eriodictyon californicum* (family *Boraginaceae*) and *Chlorophytum borivilianum* (family *Asparagaceae*). Only 4, out of 35 plants' species, have 0-20% root colonization, 13 plant species have 30-60% colonization, 9 species show AMF root colonization between 60-80% and the other 9 have shown 80-100% root colonization.

Spores density: The spore density of AMF ranged from 23-80% in the rhizospheric soil of 25 different ornamental families (35 species). Different morphotypes of AMF corresponding to two orders were recorded from the rhizosphere, i.e., *Acaulospora* species, *Glomus* species (Fig. 2). These results have demonstrated that *Glomus* was the dominant specie. These results are in agreement with the results, obtained by Burni *et al.* (2013), which show that *Glomus* species were dominant, followed by *Acaulospora* and *Sclerocystis* in *Aloe vera* (*Xanthorrhoeaceae*). The results were also comparable with the results of Zandavalli *et al.* (2004), who have reported the presence of *Glomus clarum* in *A. angustifolia*, *Glomus pansihalus* in *Jasminum sambac* (Fig. 1c), *Glomus amphisorum* in *Hedra helix* (Fig. 1d),

Fig. 1(a-f): AMF spores in ornamental plants, (a) *Schlerocystis* spp., (b) *Schlerocystis* spp., (c) *Glomus pansihalus*, (d) *Glomus amphisorum*, (e) *Glomus multisubstensum* and (f) *Acaclospora mellea*

Fig. 2: Spore Density (SD) of each of three AMF species G: *Glomus* species, AC: *Aciculospora* species, Scl: *Sclerocystis* species) associated with ornamental plants species in District Charsadda, Khyber Pakhtunkhwa

Fig. 3: Total spore density of AMF associated with the species of ornamental plants studied from the District Charsadda, KPK

Glomus multisubstentum in *Chrysanthemum indicus* (Fig. 1e), *Sclerocystis* spp., in *Narcissus poeticus* (Fig. 1a-b) and *Aciculospora mellea* in *Tradescantia indica* (Fig. 1f). These results were supported by the results of Younis *et al.* (2013), reporting the number of spores found in the root zones of *Tetralina articulata* (Cupressaceae) and *O. oleaster* (Oleaceae) with spores of 3 different genera of AM fungi (*Glomus*, *Scutellospora* and *Aciculospora*). The lowest spore density was recorded in *Canna indica purpurea*, *Eriodictyon californicum*, *Lagerstroemia indica* and the highest spore density was recorded in *Jasminum sambac* (Fig. 3). These findings also support the findings of Kumar *et al.* (2012), who intimated the presence of *Glomus*, *Aciculospora* and

Sclerocystis in *Aster amellus* (Asteraceae). Zaman *et al.* (2008a) have also demonstrated the AMF spore density in the *compositae*.

Root colonization: The AM fungi are considered to be an essential part of sustainable agriculture, playing a role in the recovery of agriculture land and bared habitats for both classical and new crops (Wang and Qiu, 2006). They have received significant attention in agriculture especially in the field of horticulture. These fungi have been reported to form a key ecological and economical cluster of soil borne fungi which establish mutualistic relationship with most of the plant families.

Table 2: Percentage of frequency occurrence of AM fungi in the rhizosphere soils of ornamental plants of Charsadda

Families	Species	Root infections				Frequency (%)
		E H.	IH.	Arb.	Ves.	
Portulacaceae	<i>Portulaca indica</i>	-	-	-	-	0.0
Asteraceae	<i>Calendula officinales</i>	++	-	-	++	75.0
	<i>Chrysanthemum indicus</i>	+	+	-	+	75.0
	<i>Coreopsis lenceolata</i>	+++	-	+	++	80.0
Iridaceae	<i>Gladiolus dalenii</i>	++	++	-	-	50.0
Xanthorrhoeaceae	<i>Aloe vera</i>	+++	++	-	+	70.0
Araliaceae	(<i>Hedera</i>)	+++	++	-	++	87.5
Commelinaceae	<i>Tradescantia pallida</i>	-	+++	-	+++	80.0
Amaranthaceae	<i>Celosia cristata</i>	+++	-	-	-	66.0
	<i>Alternethra purple knight</i>	++	++	-	-	20.0
Amaryllidaceae	<i>Amaryllis</i>	+	++	-	+	60.0
	<i>Narcissus poeticus</i>	+	+	-	-	50.0
Poaceae	<i>Cynodon dactylon</i>	-	-	-	-	50.0
	<i>Cymbopogon</i>	++	++	-	++	66.0
Verbenaceae	<i>Duranta erecta</i>	+	+	-	++	20.0
	<i>Duranta</i>	+	++	-	+	40.0
Oleaceae	<i>Jasminum officinale</i>	+	++	-	-	50.0
	<i>Jasminum humile</i>	++	+++	-	-	66.6
	<i>Jasminum sambac</i>	++	++	+	-	50.0
Araceae	<i>Epipremnum aureum</i>	+	-	-	+++	33.3
Aracaceae	<i>Alocasia indica</i>	-	++	-	-	100.0
	<i>Dypsis Lutescens</i>	+	++	-	+	50.0
Asparagaceae	<i>Chlorophytum borivilianum</i>	-	-	-	-	100.0
	<i>Dracaena</i>	++	++	+	-	Nil
Cannaceae	<i>Canna indica purpurea</i>	++	-	-	-	33.3
Boraginaceae	<i>Eriodictyon californicum</i>	+	++	-	++	100.0
Lythraceae	<i>Lagerstroemia speciose</i>	++	++	-	-	50.0
Rubiaceae	<i>Gardenia jasminoides</i>	+++	++	-	+	75.0
Crassulaceae	<i>Bryophyllum delagone</i>	+++	++	+	-	90.0
Febaceae	<i>Senegalia Senegal</i>	+++	+++	-	+	80.0
Pittosporaceae	<i>Pittosporum tobira</i>	++	++	-	-	83.3
Solanaceae	<i>Cesturnum nocturnum</i>	++	++	-	++	100.0
Cupressaceae	<i>Chamaecyparis lawsoniana</i>	-	+	+	-	50.0
Araucariaceae	<i>Araucaria araucana</i>	++	++	-	-	50.0
Euphorbiaceae	<i>Euphorbia milli</i>	-	++	++	+++	100.0

Nil: -, Low: +, Medium: ++, High: +++ EH: External Hyphae, IH: Internal hyphae, Arb.: Arbuscules, Ves.: Vesicles

In the studied plants, root colonization was reported from 0-100%. No colonization in the roots of *chlorophytum borivilianum* by AM fungi was reported here (Table 2). Our results matched with the results of Silva and Yapa (2010), who have reported maximum root colonization in *Asparagus racemosus*. The present study has also reported 50% root colonization in *Gladiolus dalenii*, 75% colonization each in *Calendula officinali* and *Chrysanthemum indicus* (Table 2). These results also support the results of Kumar *et al.* (2012), who also examined AMF root colonization and spore density in *Crysanthemum leucanthemum* and *Gladiolus grandifloras*. Root colonization reported in *Aloe vera* in our study was 70% (Table 2), which is closer to 95% reported by Burni *et al.* (2013). These results were also supported by results of Burni and Hussain (2011), in families like *Alocasia indica* (100%), *cynodon dactylon* (50%), *cymbopogon* (50%), *Euphorbia milli* (100%), *Senegalia Senegal* (80%), *Gardenia jasminoides* (75%) and *Cesturnum nocturnum* (100%). Our results of getting

more vesicles, arbuscules and external hyphae (Table 2) were in agreement with results of Gorski (2002) and Zaman *et al.* (2008b), who had reported 66.6% AMF effect in *Kalanchoe pinnata*.

CONCLUSION

It is concluded from this study 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. When spore number were high, the percentage colonization was low but as spore number began to decline the colonization increased. The highest spore numbers were recorded during August to October, while most mycorrhizal colonization recorded during December to March. Fluctuations in spore numbers were 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 was also observed that extra radical hyphae were more helpful in providing nutrients to host plant than do the root hairs.

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