ARBUSCULAR MYCORRHIZAL SPORE DENSITY AND ROOT INFECTION IN SOME WEEDS OF ASTERACEAE

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ABSTRACT

The rhizospheric soil containing roots samples of two plants of the same family were collected from the three different locations of Tehsil Charsadda to find out the spore density, spore dominancy, relative abundance, evenness of the spore and the root infections. Three types of spores were found during the study i.e. Glomus (43.64%), Sclerocystis (29.55%) and Acaulospora (26.81%) of the total spores in both Parthenium hysterophorus L. and Conyza canadensis L. The spore density ranges from 75 to 93 spores per 100gm^{-1}. The root colonization ranged from 15.12% to 34.48% in both plants. The highest spore density was recorded in P. hysterophorus while lowest in C. canadensis. Glomus formed highest community about 48.24% and 38.98% in both P. hysterophorus and C. canadensis respectively. The values of relative abundance, specie evenness Sampson index of dominancy and spore density revealed that the community is formed by the Glomus spores followed by Sclerocystis and Acaulospora. In case of root colonization, the most frequent infection was internal hyphal infection followed by the vesicular infection and external hyphal infection

Keywords: Glomus, Sclerocystis, Acaulospora, Parthenium hysterophorus L., Conyza canadensis L., weeds, mycorrhization.

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INTRODUCTION

There has been an increasing consciousness amongst the environmentalists that the renovation and reinstatement of delicate and degraded bionetworks should be considered expansively and methodically (Alizadeh, 2011). The ultimate motive is that the renovation should comprise not only the aboveground systems but also the underground microbes which accompany functionally with plants (Wang & Qiu, 2006). Arbuscular mycorrhizal fungi (AMF) have been believed to be indispensable in maintaining plant-soil systems due to their mutual association which results in formation of arbuscules and other multifunction (Leifheit et al., 2014; Symanczik et al., 2015). It is well established that AMF can affect the plant capability, community structure, biodiversity, and bionetwork unpredictability (Li et al., 2007; Daei et al., 2009; Knegt et al., 2014). Arbuscular mycorrhizas are relationships among plant roots and glomalean fungi and are abundant in usual bionetworks (Baum et al., 2015; Bona et al., 2016). It is assessed that all grass species form 84% AM associations (Boyer et al., 2014; Tobisa & Uchida, 2017). The association is important because it promotes the nutrient uptake of plant host and provides carbon compounds to AMF (Mueller & Bohannan, 2015). In addition to improved nutrient uptake of plant host, other possible advantages include enhanced water dealings, improved growth, and confrontation to pathogens and additional environment friendly anxieties (De Souza et al., 2013; Vasconcellos et al., 2013; Rouphael et al., 2015). Mount up confirmation point towards the mycorrhizal relations can be significant contributing factor of diversity in bio-networks and can change the building and functioning of plant communities in intricate ways (Shahzad et al., 2015; Hart et al., 2014). Due to responses of AM fungal symbiosis, the co-occurring plant species differ significantly in their growth (Bagyaraj et al., 2015; Liang et al., 2015). A large number of research work has been carried out to which includes the efforts of (Li et al., 2017; Moradi et al., 2017) who's reported the work on spore density of the rhizospheric soil and root infection of some plants. Therefore, the study was conducted to report the density of spores and root infection and taxonomic identification of the fungal species and the response of that fungal species to the selected plant of some areas of District Charsadda.

MATERIALS AND METHODS

Soil Sampling

Rhizospheric soil samples were obtained at a depth of 3-10 cm along with roots section taking 3 replicates of each plant. In advance random sampling of soil, the above ground layer was worn out to eliminate the alien constituent part and litter. The soil samples were standardized replication wise before passing through a sieve (<2mm mesh size) to take out unwanted materials. The sampling sites locations were selected about 6km away and the replicates was taken 1km apart from each other during the collection.

Spores extraction

From each plant replicates, 100g rhizospheric soil was taken. For the extraction of spores from the soil, the method of Gerdemann and Nicolson (1963) wet sieving and decanting was used. The entire spore quantity of AM fungi in the processed soil sample was assessed using methods of Gaur and Adholeya (1994). The spores were studied with the help of compound microscope and picked up using micropipette for making slides. Taxonomic identification of spore was done taking help from Schenck and Perez (1990).

Formulas used to measure the AMF communities

- Spore density (S.D) = Spores Numbers in 100g soil
- Relative Abundance (R. A) =

$$\frac{number\ of\ spore\ of\ a\ genus}{total numbers\ of\ identified\ spore\ in\ a\ soil\ samples} X\ 100$$

- Percentage AM root colonization = $\frac{total\ number\ of\ root\ segment\ colonized}{total\ number\ of\ root\ segment\ examined} X\ 100$
- $\text{Evenness} = {}^{H'}/_{\text{H max}}$
- Evenness = H'/H_{max}
- Dominancy of Simpson's index = D = $\sum \left(\frac{Xi}{Xo}\right)^2$

Here maximal H' is denoted by H_{max} which is calculated by H=1n S

Here the total number of identified species per sampling site represented by S. X_i is the population density for an individual specie where, as X_0, the total population densities of the Replicate (An et al., 1993).

Root colonization

For the colonization, the collected root samples were washed with distilled water and stained. The root section was cut into fragments of 1cm length and boiled for about ten minutes (depending upon the toughness of the root section) in 10% KOH solution. It was then captured by fine sieve and washed with distilled H_2O. Post clearance was done with 0.5% H_2O_2 v/v and 0.5% NH_4OH in distilled H_2O. Then the roots were washed using distilled H_2O and treated with 1% HCl. It was stained with methyl blue 0.05% w/v in lattice acid glycerol. Root colonization from each sample was calculated using glass slide technique in which nominated root section was determined microscopically. The presence of hyphae, arbuscules and vesicles on a section was considered infection. All the data were collected in replicates and means were taken for comparison.

RESULTS AND DISCUSSION

During the current study, two plant species (Parthenium hysterophorus L. and Conyza canadensis L.) belonging to same families from three different locations of District Charsadda were assessed for mycorrhizal association. The rhizospheric soil samples along with the host plant's roots were collected from three locations and studied for the spore density and root colonization.

Spore Density

The plants showed from least possible to maximum colonization. Three types of AMF spores (Glomus, Sclerocystis and Acaulospora) were isolated from the rhizospheric soil of both the plants studied (Fig-1). The results of getting high number of Glomus and Acaulospora spores during the study is in agreement with other reports (Sawilska et al. 2010). From both the plants studied, the highest number of spores was demonstrated by Glomus (43.64%), followed by Sclerocystis (29.55%) and Acaulospora genera (26.81%) (Fig-2). These results are in line with other studies conducted (Tao et al., 2004; Dandan & Zhiwei, 2007; Li et al., 2007; Burni et al., 2009; Snoeck et al., 2010; Osborne et al., 2018; Sepp et al., 2018; Koffi et al., 2018) who have reported Glomus the most frequent specie among all types of spores studied. Glomus showed highest adaptive value with both the plants (Table-1). The maximum collective percentage of all types spores was recorded as 36.18% and 35.43% respectively from the rhizospheric soil of both C. canadensis and P. hysterophorus at location 2 which was Umarzai. About 38.98% and 48.24% of Glomus spores were isolated from the rhizosphere of C. canadensis and P. hysterophorus respectively which evidenced that the Glomus was the most frequent among all the collected spores. (Table-1). The highest relative abundance of Glomus (49.33%) was recorded at location/site 3 (Utmanzai) from P. hysterophorus (Fig. 3) rhizosphere. Similar results of highest relative abundance (41.38%) of Glomus was reported from location 1 (i.e. Turangzai) while studying the rhizosphere of C. For both plant species, the highest percentage of spores (i.e. 38.98%, 48.24%) was observed to be for Glomus species (Table-1, [Fig-4]. Our results of getting Glomus in abundance are in

agreement with Mafaziya and Madawala (2015) and Kowalczyk and Blaszkowski (2011) who have demonstrated Glomus the most abundant genera across the studied sites. Our results are also in concordance to results of Mosbah et al. (2018) who have reported Glomus the dominant genus in the rhizospheric zone of R. raetam. The results of present study is in contrast to results of Sarah and Ibrar (2016) where the dominant genus reported was Acaulospora. In our study, the highest species evenness (1.10 & 1.33) and Sampson index dominancy (2.35 & 2.90) was shown by Glomus in both plant species i.e. C. canadensis and P. hysterophorus (Table-2). Our results are in line with previous studies (Zhang et al., 2004; Panwar & Tarafdar, 2005; Su & Guo, 2007; Kamalvanshi et al., 2012; Kavitha, Nelson, 2013).

Root colonization

The high roots infection for both plant species was recorded for internal hyphae, vesicular and external hyphae (35%, 28% and 24% respectively, Fig-5). The minimum infection was recorded for arbuscules i.e. 13.17% (Fig-6). Our results are in line with results of Kumar et al. (2013) who also observed similar findings from different plant species including Asteraceae. Similarly, in case of P. hysterophorus, the maximum value was recorded for the internal hyphal infection i.e. 34.63% followed by the vesicular and internal hyphal infections viz., 25.61% and 22.44%, respectively (Fig-7). These results are in line with results of Koske and Gemma (2001) who has investigated internal and external hyphal infections. Our results are also in line with results of Hemavani and Thippeswamy (2013) who demonstrated maximum root infection from P. hysterophorus. The overall results of root colonization revealed that the highest infection was recorded for C. canadensis followed by P. hysterophorus. These results are in concordance with previous results (Conrad & Segraves, 2012; Rodríguez- Rodríguez et al., 2013; Rozpadek et al., 2014) who have reported the root colonization and spore density from some species of family Asteraceae. In this study, the lowest value was recorded for arbuscules (13%) which is in contrast to findings of Macek et al., (2012) who has reported relatively higher values for arbuscules in the infected roots.

Table-1. Spore density of two wild plants of Tehsil and District Charsadda, Khyber Pakhtunkhwa Province Pakistan

S. NO	Plant specie	Location	Mean number of Gl.	Mean number of Scl.	Mean number of Ac.	Spore Density	Total spores Percent (%)
1	C. canadensis	L1	4.00±0.69	3.22±0.46	2.44±0.29	87	34.25
		L2	3.67±0.71	2.89±0.42	3.44±0.47	90	35.43
		L3	3.33±0.55	3.00±0.71	2.22±0.22	77	30.31
Total Means ± Standard Error			3.67±0.37	3.04±0.30	2.71±0.22	---	---
Total numbers of spores			99	82	73	254	---
% of spore's specie wise			38.98	32.28	28.74	---	---
2	P. hysterophorus	L1	4.67±0.94	2.89±0.54	2.33±0.41	89	34.63
		L2	5.00±1.18	2.22±0.40	3.11±0.54	93	36.18
		L3	4.11±0.65	2.56±0.65	1.67±0.41	75	29.18
Total Means ± Standard Error			4.6±0.53	2.56±0.30	2.38±0.28	---	---
Total numbers of spores			124	69	64	257	---
% of spore's specie wise			48.24	26.84	24.9	---	---

Key; L1: location 1 (Turangzai), L2: Location 2 (Umarzai), L3: location 3 (Utmanzai), Gl: Glomus spore, Scl: Sclerocystis spores, Ac: Acaulospora spores. Each value of L1, L2 and L3 of Gl, Scl and Ac is the grand mean ± Standard error of nine replicates of sieve, having three replicate from each type of sieve.

Table - 2. Species Evenness and Simpson's index of dominance (D) of spores.

Plant Name	Locations	Glomus		Sclerocystis		Acaulospora	
		E	S (I.D)	E	S (I.D)	E	S (I.D)
C. Canadensis	L1	0.40	0.83	0.32	0.67	0.24	0.51
	L2	0.37	0.73	0.29	0.58	0.34	0.69
	L3	0.33	0.79	0.30	0.70	0.22	0.52
Total sum of E. and S (I.D)		1.10	2.35	0.91	1.95	0.8	1.72
P. hysterophorus	L1	0.45	0.94	0.28	0.58	0.23	0.47
	L2	0.48	0.97	0.22	0.43	0.30	0.60
	L3	0.40	0.99	0.25	0.61	0.16	0.40
Total sum of E. and S (I.D)		1.33	2.90	0.75	1.61	0.69	1.47

Key; L1: location 1 (Turangzai), L2: Location 2 (Umarzai), L3: location 3 (Utmanzai), E: Evenness and S (I.D): Simpson's index of dominance.

Table -3. Root colonization of two wild plants of Tehsil Charsadda.

S. NO	Plant specie	Location	Ves.	Arb.	E.H.	I.H.
1	C. candensis	L1	+++	+	++	++++
		L2	+++	+	++	++++
		L3	++	+	+++	++++
2	P. hysterophorus	L1	+++	+	++	++++
		L2	++	+	+++	++++
		L3	+++	++	++	++++

Key; L1: location 1 (Turangzai), L2: Location 2 (Umarzai), L3: location 3 (Utmanzai). Highest: (++++), High: (+++), Medium: (++), Low: (+), Absent: (-), Ves: (Vesicles), Arb: (Arbuscules), E.H: (External Hyphae), I.H: (Internal Hyphae). Each value of L1, L2 and L3 of vesicles, arbuscules and external hyphae is the grand mean of five replicates

Fig. 1. The figure shows the major three types of isolated spores mycorrhiza i.e. Glomus, Sclerocystis and Acaulospora from the rhizospheric soil of the selected plants species.

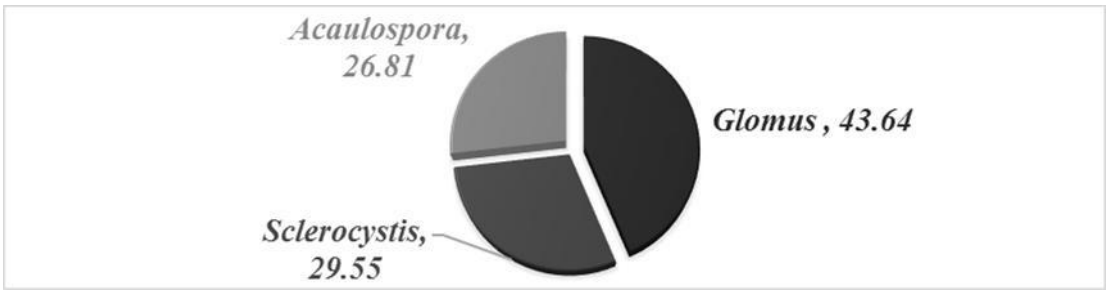

Fig. 2. Combined spore's percent in the rhizospheric soil of both C. canadensis and P. hysterophorus.

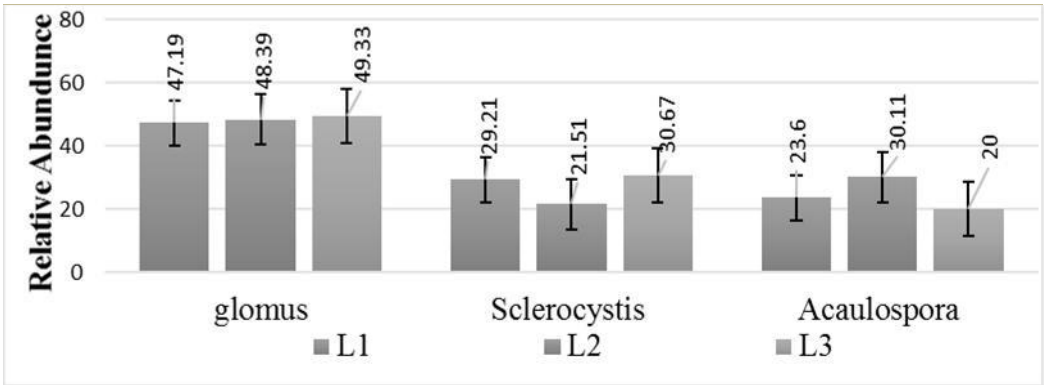

Fig. 3. Relative Abundance of spores in rhizospheric soil of P. hysterophorus.

L1= location 1 (Turangzai), L2= Location 2 (Umarzai), L3= location 3 (Utmanzai)

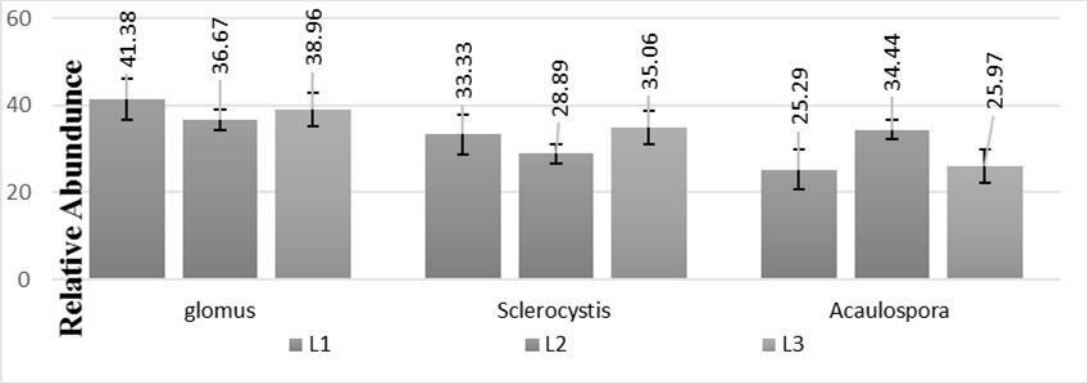

Fig. 4. Relative Abundance of spores in rhizospheric soil of C. canadensis.

L1= location 1 (Turangzai), L2= Location 2 (Umarzai), L3= location 3 (Utmanzai)

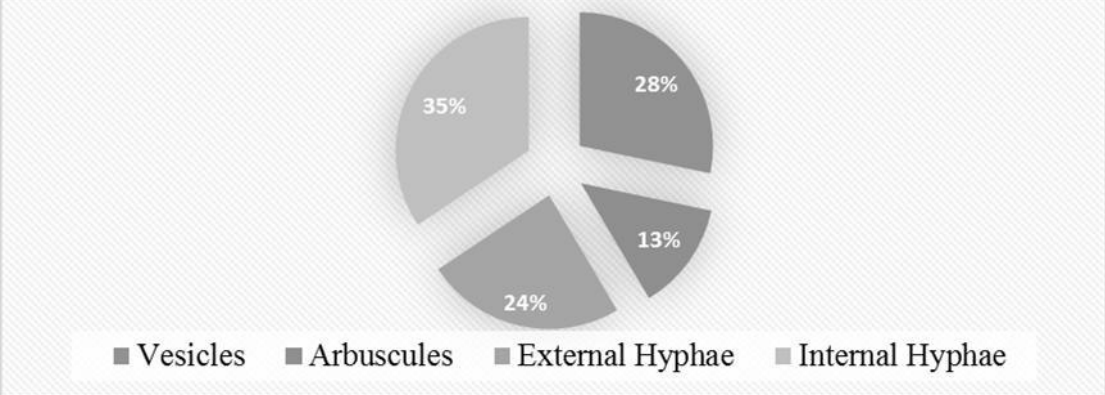

Fig. 5. Combined roots colonization infection of both C. canadensis and P. hysterophorus

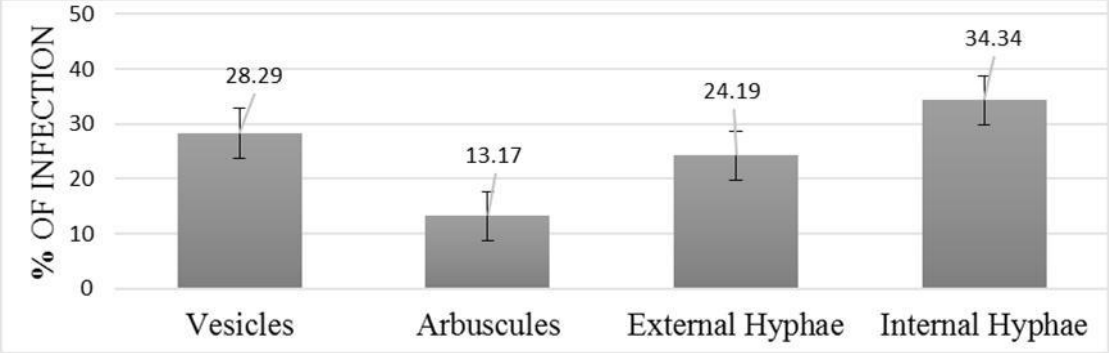

Fig. 6. Percent of root colonization of C. Canadensis by different mycorrhizal infection types.

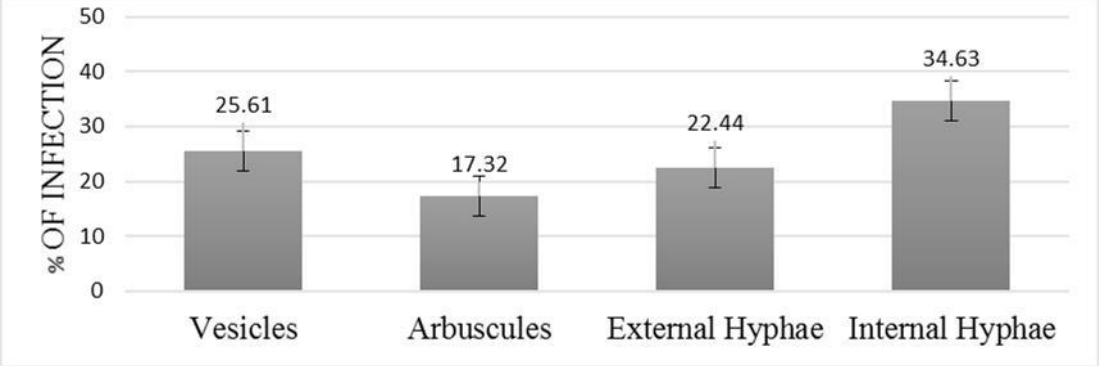

Fig. 7. Percent of root colonization of P. hysterophorus by different mycorrhizal infection types.

CONCLUSIONS

From the present study it was concluded that the Glomus formed the highest community followed by Sclerocystis and Acaulospora. The highest spore density was recorded from the rhizospheric soil of P. hysterophorus at Umarzai site. The most frequent infection was internal hyphal infection followed by the vesicular infection and external hyphal infection. It was also observed that the relation of the mycorrhiza is very important and can help in the nutrients absorption.

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