

## Research Article



## Arbuscular Mycorrhizal Fungal Spore Density and Root Colonization in Weeds of Carrot field at Charsadda, Pakistan

Tabassum Yaseen<sup>1\*</sup>, Shehzad Ahmad<sup>1</sup>, Khushnood Ur Rehman<sup>2</sup>, Fayaz Asad<sup>1</sup>, Abdul Waheed<sup>1</sup>, Rani Gul<sup>3</sup>, Hussain Gulab<sup>4</sup> and Naveed Akhtar<sup>2</sup>

<sup>1</sup>Department of Botany, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan; <sup>2</sup>Department of Botany, Islamia University, Peshawar, Khyber Pakhtunkhwa, Pakistan; <sup>3</sup>Department of Agriculture Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan; <sup>4</sup>Department of Chemistry, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan.

**Abstract** | Arbuscular mycorrhizal fungi that forming symbiotic associations with plants through roots. It forms the multi colonization systems with the roots and provide benefits to the host plants. The present study was conducted to evaluate the occurrence and distributions of Arbuscular mycorrhizal fungi in the rhizospheric soil of major weeds of District Charsadda. In the present study roots and rhizospheric soil of 15 weeds species belonging to 12 families were collected from Carrot field of District Charsadda and was investigated for the sporulation and root infections types. From the recorded results the highest spore density was found in *Melilotus indica* having spore number 276 which is followed by *Malva neglecta* and *Sonchus asper* having spore number 244, 214 respectively. The lowest spore density was recorded in *Poa annua* having spore number 35. The maximum *Glomus* density was found in *Melilotus indica* having mean value (83.67±1.65) whereas the maximum spore density was recorded for *Acaulospora* and *Sclerocystis* in the rhizospheric soil of *Sonchus asper* and *Fumaria indica* respectively. The root infection was also investigated which showed 20-85% root infection. The highest root colonization was recorded in *Parthenium hysterophorus* (39.67±11.02) whereas the lowest was found in *Fumaria indica* and *Taraxacum officinale* (6.33±5.51), (8.00±4.00) respectively. It is not necessary that the plants having high spore density will have high root colonization the host growth stage of plant influences the diversity and population of Arbuscular mycorrhizal fungi (AMF). Our study revealed that the AMF spore density and root infection is closely related to the soil physicochemical characteristics.

Received | July 23, 2019; Accepted | April 30, 2020; Published | October 17, 2020

\*Correspondence | Tabassum Yaseen, Department of Botany, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan; Email: tabassumyaseen@bkuc.edu.pk

Citation | Yaseen, T., S. Ahmad, K.U. Rehman, F. Asad, A. Waheed, R. Gul, H. Gulab and N. Akhtar. 2020. Arbuscular mycorrhizal fungal spore density and root colonization in weeds of carrot field at Charsadda, Pakistan. *Pakistan Journal of Agricultural Research*, 33(4): 841-848.

DOI | <http://dx.doi.org/10.17582/journal.pjar/2020/33.4.841.848>

Keywords | AMF, Weeds, Carrot field, *Glomus*, *Acaulospora*, *Sclerocystis*

### Introduction

The widespread symbiotic associations of arbuscular mycorrhiza is described as a results of co-progressions actions between plants and fungi where both associates benefit from the exchange of nutrients (Sharma, 2003; Bonfant and Genre, 2008). It is reported that this type of association is most common and about 80% colonization was recorded

from the species of higher plants (Koltai, 2010). In natural ecosystem Arbuscular mycorrhizal fungi (AMF) are regular components of the rhizospheric microflora which is necessary for maintainable plant soil system by establishing symbiotic associations with land plants and form mycorrhiza (Sharma *et al.*, 2009). The fungus improves the capability of plants to absorb nutrients from the soil as well as to protect plants from diseases while the plant provides the

carbon source to the fungus in soluble form (Auge, 2001; Entry *et al.*, 2002; Wardle and Van der Putten, 2002; Gosling *et al.*, 2006; Smith and Read, 2008; Sikes *et al.*, 2009). The new secondary metabolites may also produce due the mycorrhizal associations of fungi with the plants (Venkateswarlu *et al.*, 2008). It may also play a key role in vegetation restoration because of symbiosis with roots of plants; they can improve and stabilize structure of soil which can facilitate mineral absorption by the host plants and affect the structure of population and preserve the diversity of species (Bothe *et al.*, 2010). AMF also recognized to benefit the plants to tolerate heavy metals present in the rhizospheric soil of the plants (Jamal *et al.*, 2002; Turnau *et al.*, 2005). It was reported that the root to shoot transport via phyto extractions and uptake of heavy metals were better in the mycorrhizal colonized plants (Dodd, 2000). In addition, the AMF also reduce the heavy metals in the soil through phyto stabilizations (Khan, 2005). It may also provide other benefits to the host plants i.e. improve salinity and drought tolerance and disease resistance (Auge, 2001, 2004; Ruiz-Lozano, 2003). It is believed that AMF are ecologically important to numerous vascular plants and have the potential to alter the weed species ecology and can affect their growth without disturbing the growth of another major crop (Jordan *et al.*, 2000). Whereas the AMF are not only beneficial but it may also cause negative effects on the growth of the host plants (Van der Heijden, 2002; Klironomos, 2003).

The phylum Glomeromycota of AM fungi are characterized by the production of intracellular absorptive structures Arbuscules and Vesicles are the utmost extensive of soil fungi tracked by ectomycorrhizal fungi (Koide and Mosse, 2004). In the natural ecosystem these are the regular components of rhizospheric microflora which form associations with plants and necessary for sustainable plant soil system. in the multispecies communities and natural plant populations the role of mycorrhiza is poorly understood (Sharma *et al.*, 2009). Several work has been carried out in KP which include the work of Burni and Ilahi (2004), Zainab and Burni (2005), Sharief *et al.* (2005) and Nasrullah *et al.* (2010) about the distribution and occurrence of arbuscular mycorrhizal fungi but there is less information available therefore the present study was conducted to evaluate the occurrence and distributions of AMF in the rhizospheric soil of major weeds of District Charsadda.

## Materials and Methods

Roots of fifteen weeds plants and their respective soil samples were collected from four different areas of Tehsil Charsadda during the session 2017-2018. Plant samples belong to different families, in which *Asteraceae*, *Brassicaceae*, *Cannabaceae*, *Convolvulaceae*, *Euphorbiaceae*, *Fabaceae*, *Fumariaceae*, *Malvaceae*, *Plantaginaceae*, *Poaceae*, *Ranunculaceae*, and *Zygophyllaceae* species. The plants were carefully dug up along with there rhizospheric soil in triplicate and transported to laboratory in polythene bags. The plants roots were gently washed under tap water to remove soil particles, and the rhizospheric soil were shade dried. The roots were fixed in formaline acetic acid.

### Extraction of spores

Rhizospheric soil samples of maize crop roots was collected at different stages. 100 gm of the fine soil was taken and remove the debris and other larger particles and dissolved in the water and kept for about 24 hours' duration when the soil was completely settled down in the bottom of the beaker the water was passed through 2mm sieve to remove the remaining residues. The cleaned water then passed from the three different sizes of sieves i.e. 140, 170 and 400  $\mu\text{m}$  one by one. The remaining residues above the sieves were collected by rubbing the filter paper on the sieves and studied under the compound microscope for the fungal species diversity and density. The spores were collected through needle and kept on the drop of canda balsam and the slides were made then the picture was capture for identification. For the identifications the manual of Hall and Fish (1979), Trappe (1982) were and density of spores were calculated.

### Roots infection observations

For the root infection observations, the standard protocol of Giovannetti and Mosse (1980) and Kormanik (1982) was followed. The roots of the plants were washed with tap water to remove the formaline acetic acid molecules and then cut into fragments and heated for 10-15 minutes in 10% KOH solution. For the bleaching of the pigmented portions of the roots were then kept in alkaline  $\text{H}_2\text{O}_2$  for about 10-15 minutes. The bleached roots segments were then washed with tap water to remove the  $\text{H}_2\text{O}_2$ . After that the segments were treated with 1% HCL for about 1-2 minutes to keep the acidic effect for proper staining. In 0.025% acidic fuchsin the roots segments

were kept and heated for 2-3 minutes. Ten segments of about 1cm length were randomly collected and kept under microscope for the morphological studies of amfa entophyte and microphotographs were taken. The percentage of infections was calculated by means of the mentioned formula.

$$\% \text{ age mycorrhizal infection} = \frac{\text{No.of infected segments}}{\text{Total No.of segments}} \times 100$$

### Physiochemical analysis of soil

The physicochemical parameters include the pH, electric conductivity, soil texture, organic matter i.e. phosphorous, and potassium and nitrogen investigations. The pH and electric conductivity of the soil samples was recorded by the pH meter and EC bridge following the standard protocol of (Jackson, 1967; Black, 1965) respectively. The organic matter was determined by following the procedure of Nelson and Sommer (1982) through below mentioned formula

$$\% \text{ O. M} = \frac{(\text{meq. of K}_2\text{Cr}_2\text{O}_7 \text{ meq of FeSO}_4) \times 0.05 \times 100}{\text{Weight of soil}}$$

## Results and Discussion

### AMF spore density

Roots along with rhizospheric soil of fifteen weed species collected from Carrot field of District Charsadda. The soil was investigated for the occurrence and distribution of mycorrhizal sporulation. Three Genus of spores have been isolated i.e. *Glomus*, *Acaulospora* and *Sclerocystis*. The highest spore density was found in the rhizospheric soil of the species of family Fabaceae with spore number 276, among all the other families. However, the lowest spore density was found for family Poaceae with spore number 35 (Figure 1 and Table 1). The statements were supported by Khakpour and Khara (2012) and (Surtiningsih et al., 2017) in this aspects that who also reported same results of sporulation from both the families respectively. Among all the spore types the *Glomus* genus was found to be dominant followed by *Acaulospora* and *Sclerocystis* (Figure 2). These findings are in agreement with those of (Chen et al., 2001; Pande and Tarafdar, 2004). The genus *Glomus* is predominantly distributed in the soil all over the world, the statement supported by (Minal and Anil, 2012). The genus *Glomus* and *Acaulospora* take short period of time for the production of small spores as compared to other genera which produce

large spores. e.g. *Gigaspora* and *Scutellospora* (Sarkar et al., 2014). The widespread occurrence of *Glomus* may be attributed to their reproduction (Sporocarp formation), lower host preference and wide range of pH tolerance. It is known that soil factors such as pH restrict the distribution of some taxa (Abbott and Robson, 1991). In our study the soil pH is 6.1 (Figure 2) which is favourable for the growth of *Glomus* the statement is supported by Costa et al. (2013) who's reported the highest spore number of *Glomus decipiens* at pH 06.50.

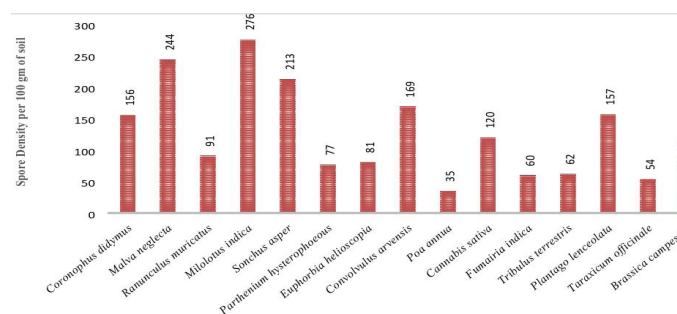


Figure 1: Total AMF spore density in selected weeds of carrot field.



Figure 2: Different types of spores isolated from weeds of carrot field.

The maximum *Glomus* density was recorded in *M. indica* (Fabaceae) with an average spore number of (83.67±1.65), followed by *M. neglecta* and *S. asper* (Malvaceae and Asteraceae) with spore numbers (59.33±1.62) and (53.33±1.50) respectively. Our result agrees with the findings of (Dobo et al., 2016; Mergulhao et al., 2010) who's also studied genus *Glomus* in the above stated families. The minimum *Glomus* density was recorded in *P. annua* (Poaceae) with an average spore number of (9.0±6.00), followed by *T. officinale* and *B. campestris* (Asteraceae) had spore number (15.0±5.00) and (18.33±2.18) respectively. The statement was in agreement to those of Hemavani and Thippeswamy, 2013 and Harikumar et al., 2014. In case of *Acaulospora* the maximum number of spores was recorded in *M. neglecta* and *S. asper* with average spore number (21.67±1.66), and (17.67±1.86). Our results agree with finding of (Bhale et al., 2011) who also reported same results of *Acaulospora* from the species of family Malvaceae and Asteraceae. The minimum number of *Acaulospora* was recorded in *C. sativa* and *F. indica* (Cannabaceae and Fumariaceae)

**Table 1:** AMF spore density in selected weeds of carrot field of Tehsil Charsadda.

Plant Name	Family	AMF Spore Density		
		Mean value of spores of <i>Glomus spp.</i>	Mean value of spores of <i>Acaulospora spp.</i>	Mean value of spores of <i>Sclerocystis spp.</i>
<i>Malva neglecta</i> Wallr.	Malvaceae	59.33±1.62	21.67±1.66	0.33±0.58
<i>Ranunculus muricatus</i> L.	Ranunculaceae	21.33±1.50	09.00±3.00	00.00±0.00
<i>Melilotus indica</i> L.	Fabaceae	83.67±1.65	07.00±1.36	01.33±0.58
<i>Sonchus asper</i> L.	Asteraceae	53.33±1.50	17.67±1.86	00.00±0.00
<i>Parthenium hysterophorus</i> L.	Asteraceae	22.67±2.37	02.67±2.08	00.33±0.58
<i>Euphorbia helioscopia</i> L.	Euphorbiaceae	23.00±2.14	03.33±3.21	00.67±0.58
<i>Convolvulus arvensis</i> L.	Convolvulaceae	49.67±3.84	04.67±2.08	02.00±2.65
<i>Poa annua</i> L.	Poaceae	09.00±1.00	02.33±2.08	00.33±0.58
<i>Cannabis sativa</i> L.	Cannabaceae	35.67±2.29	01.67±1.53	02.67±4.62
<i>Fumaria indica</i> L.	Fumariaceae	15.00±1.11	01.67±1.53	03.33±4.16
<i>Tribulus terrestris</i> L.	Zygophyllaceae	17.67±7.51	02.00±1.00	01.00±1.00
<i>Plantago lanceolata</i> L.	Plantaginaceae	45.67±4.61	03.67±4.73	03.00±3.61
<i>Taraxacum officinale</i> Wigg.	Asteraceae	15.00±5.00	02.00±1.73	01.00±1.00

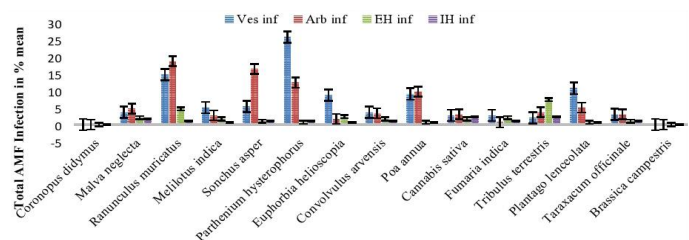
Each value is the grand mean ± Standard deviation of five replicates.

with average spore number of 1.67±1.53 respectively. The statement was supported by Agwa and Al-Sodany, 2003 and Birhane et al., 2017. Among all the spores the *Sclerocystis* was found to be formed small community, the highest number of *Sclerocystis* was recorded in *F. indica*, *P. lanceolata* and *C. sativa* with average spore number of 3.33±4.16, 3.00±3.61 and 2.67±4.62 respectively, whereas in the rhizosperic soil of *R. muricatus* and *S. asper* no *Sclerocystis* was found (Datta and Kulkarni, 2012).

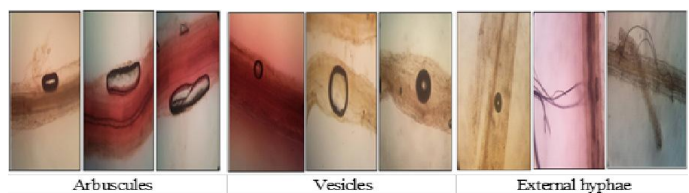
**Root colonization**

All the weed species were also investigated for the root infection (Figure 4). All the species showed Mycorrhizal infection in their roots except (*Brassicaceae*) but the degree of colonization varied among plant species. Typical fungal structures were found, i.e. Arbuscules, Vesicles, External Hyphae and Internal Hyphae. The root infection was recorded from the minimum 20% to the maximum 85%. The highest root colonization was recorded in *P. hysterophorus* with mean percentage 39.67±11.02 which is followed by *R. muricatus* 34.00±15.39. Our result agrees with that of Hemavani and Thippeswamy, 2013; Rodriguez-Rodriguez et al., 2013; Wang and Jiang, 2015 who also reported root infection from same families. The moderate colonization was found in *S. asper* (23.67±10.60) and *P. annua* (20.00±16.37) (Figure 3). The statement supported by Khakpour and Khara, 2012 in this aspects that who's recorded 62.7% colonization in Poaceae. The lowest percentage

of colonization was recorded in *F. indica* (6.33±5.51) followed by *T. officinale* (8.00±4.00). Our results agree with the findings of Shi et al., 2006 who recorded 17% colonization in Asteraceae. The highest vesicular infection was recorded in *P. hysterophorus* whereas the highest arbuscular infection was recorded in *R. muricatus* and *S. asper* the present findings was supported by the results of Birhane et al., 2017 who's reported highest vesicular infection in family Rosaceae and Milaceae and highest arbuscular infection in Boraginaceae (Figures 3 and 4). In the present study the two species *C. didymus* and *B. campestris* of family Brassicaceae showed no AMF colonization, the statement is supported by (Harikumar et al., 2014).



**Figure 3:** Mean percentage of highest root colonization in selected weeds of carrot field. Ves (vesicles), Arb (arbuscules), EH (external hyphae), IH (internal hyphae).



**Figure 4:** Different types of mycorrhizal roots infection.

The soil was also used to evaluate its physicochemical parameters including the pH, electric conductivity, soil texture, organic matter i.e. phosphorous, potassium and nitrogen investigations. The results of the physicochemical parameters revealed that the soil was loamy in nature. The pH and EC was recorded as  $6.14 \pm 0.65$  and  $0.14 \pm 0.12$ . The organic matter for the soil was found to be  $1.27 \pm 0.20$ . The study also reported that the soil contained mostly the element potassium which was  $101.73 \pm 22.12$  followed phosphorous ( $3.01 \pm 2.10$ ) (Table 2). The edaphic characteristic were in agreement to those of Lekberg *et al.* (2007) and Oehl *et al.* (2004) in this aspects that who's also reported the type of soil, soil depth, soil pH and soil fertility.

**Table 2:** Shows mean values of different chemical constituents of soil.

Parameters	Values
pH	$06.14 \pm 0.65$
EC ds/m	$00.14 \pm 0.12$
OM %	$01.27 \pm 0.20$
N%	$00.17 \pm 0.06$
P mg/kg	$03.01 \pm 2.10$
K mg/kg	$101.73 \pm 22.12$
Textural class	Loamy

P: available Phosphorous; K: available Potassium; N: available Nitrogen; OM: organic matter; EC: electrical conductivity; means of fifteen replicates  $\pm$  standard deviation.

## Conclusions and Recommendations

The results of the study concluded that there is a high incidence of AMF associations in the weed plants of studied area. All the weeds of selected locations were colonized except (Brassicaceae) by AMF. This study reports three genus of *Glomus*, *Sclerocystis* and *Acaulospora* from the studied sites on the basis of spore's identification. The abundance of *Glomus* genus is due to Phosphorous (P) deficiency in the area, also the area has normal pH whereas the genus *Acaulospora* prefers to slightly acidic conditions. The vesicles, Arbuscules, External hyphae and Internal hyphae was also reported. All the findings provide the way to the agriculture sector that where is less microflora present their will be deficiency of some minerals as well as efficiency may also be produce due mycorrhizal associations. Whereas the effect on plant communities either in positive direction or it may be in negative.

## Novelty Statement

The study is novel for carrot growers as it guides about weed control in carrot fields. The finding states that *Arbuscular mycorrhizal* fungi (AMF) controls weeds and enhances growth of carrot.

## Author's Contribution

Tabassum Yaseen and Shehzad Ahmad designed experiment, performed and wrote paper. Fayaz Asad paper revised. Khushnood ur Rahman, Abdul Waheed and Rani Gul in data analysis. Hussain Gulab and Naveed Akhtar technical guidance

## Conflict of interest

The authors have declared no conflict of interest.

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