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ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ASSOCIATED WITH THE RHIZOSPHERE OF *MENTHA ARVENSIS* L., AND *M.LONGIFOLIA* HUDS

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Abstract

Present investigations were carried out to identify, quantify and find out the AMF relationship with chemical characteristics of rhizospheric soils of *Mentha arvensis* and M *longifolia*, growing in some cultivated and natural sites of KPK (Khyber PakhtunKhawa) respectively. Seven AMF species with *M.arvensis* and 11 with *M.longifolia* were recorded. The dominant Genus was *Glomus* (11 species) followed by *Acaulospora and Gigaspora* (2 species each). The AMF spore densities in studied sites were ranged from 30-245 spores/10 gr. soil. AM colonization ranged from 26.87 – 100 %. Coefficient of correlation (r) was estimated statistically to find out the relationship between AMF spore densities and chemical characteristics of soils.

Introduction

Arbuscular mycorrhizal fungi are regular component of rhizosphere microflora in natural ecosystem and are necessary for sustainable plant soil systems by establishing symbiotic associations with most land plants and form mycorrhizae (Sharma *et al*., 2009). AM fungi inhabit a variety of ecosystems including agriculture lands, forest, grasslands and many stressed environments. They colonize roots of most plants including bryophyte, pteridophyte, gymnosperms and angiosperms (Wang & Zao, 2008).The role of mycorrhizae in natural plant population and multispecies communities remains poorly understood (Sharma *et al.,* 2009). VAM fungi are ubiquitous in occurrence and some workers have reported their presence from various locations in Pakistan (Saif & Khan, 1975; Jalal-ud-din & Anwar, 1991). However, there is less information about their occurrence and distribution in the soils of KPK (Burni & Ilahi, 2004; Zainab & Burni, 2005; Sharief *et al.,* 2005; Nasruallah *et al*., 2010) Khyber Pakhtun Khawa of Pakistan is very rich in medicinal and aromatic flora but only few reports are available regarding their mycotrophic status and associated AMF flora (Burni *et al*., 2007, 2011). Knowledge about the presence and diversity of AMF in a specific area is an essential step for utilizing these fungi in any application (Wang *et al*., 2008). Keeping in view the importance of these environment friendly micro-organisms the present work was undertaken in two selected species of *Mentha* growing in KPK.

Materials and Methods

A survey was done for the quantitative and qualitative studies of AM fungi associated with the rhizosphere of soils of two selected *Mentha* species. The soil samples were collected from various sites of KPK (Peshawar, Haripur, Havelian and Manshera). Soil samples were collected from various study sites following the method of (Jalaluddin & Anwar, 1991). For the extraction of AM spores decanting technique was followed (Gerdemann & Nicolson, 1963). For the quantitative estimation of AM spores the plate method was used (Kormanik *et al*., 1980). Density of spore per 100 gm of soil was estimated by the standard method (Stahl & Christensen, 1982). The data on the quantitative distribution of AM spores were statistically analyzed. Relationship between AM spores and soil characteristics were found by computing coefficient of correlation (r). AM colonization levels in the roots of studied plants were assessed by following the procedures of Phillips & Hayman, (1970) and Mohammad *et al.,* (1995). Soil pH was determined by McClean, (1982) AB-DTPA extractable P, K and micronutrients were determined by the method as described by Soltanpour & Schwab, (1977). Total Nitrogen concentration was done by Kjeldhal method (Bremer & Mulvaney, 1982) Lime content (Black, 1965), SOM (Nelson & Sommers, 1982).

Results

Identification: According to latest taxonomy, AM fungi belong to the phylum Glomeromycota, including 4 families and 12 genera. (Schubler *et al.,* 2001; Redecker & Phillip, 2006). Various characteristics such as spore morphology, color, substending hyphae, internal cell contents, wall characteristics were taken for the identification of mycorrhizal endophytes. Standadard monographs i.e., Schenck & Parez, (1990), Morton & Redecker, (2001) were followed for the identification of spores. Eleven species were recovered from *M. longifolia* rhizosphere and 7 species from *M. arvensis* . Based on morphological characteristics of spore they were placed under 3 genera viz., *Acaculospora* ,*Gigaspora* and *Glomus.*

Following species were identified in rhizospheric soil samples. *Acaulospora leavis, Acaulospora mellae, Glomus fasiculatum, Glomus etunicatum, Glomus claroides, Glomus microcarpum, Glomus australe, Glomus intraradicus, Glomus aggregatum, Glomus inveranum, Glomus mosseae,Gigaspora albida* and *Gi. gigantea*, (Tables 1a & 2a Figs 7-12).

Taxonomic distribution of AMF spp.: The results of the present study (Tables 1a & 1b) revealed the maximum number of *Glomus* species in the soils of studied sites i.e., *Glomus fasiculatum, Glomus aggregatum, Glomus microcarpum* were seen in all the soils samples of Manshera,Haripur,Havelian and Peshawar. Only one specie of *A. mellae* was seen in the soils of Havelian and Peshawar while species of *Gigaspora* were prevalent only in the soil of Havelian. Similarly maximum number of *Glomus* species were seen in the soil of Manshera and Havelian, and one specie of *A. leavis* were seen in the soils of Manshera and Peshawar.

Table 1a. Taxonomic distribution of AMF spp., in various Districts of KPK in the rhizospheric soils of *Mentha longifolia*.

S. No.	AM fungi	Manshera	Haripur	Havelian	Peshawar
1.	*A. melleae* Spain & Schenck	-	-	+	+
2.	*G. fasiculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske	+	+	+	+
3.	*G. etunicatum* Becker and Gerdermann	+	+	-	-
4.	*G. claroides* Schenck & Smith	+	+	-	-
5.	*G. microcarpum* Tul & Tul	+	+	+	+
6.	*G.australe(* Berkeley*)*Berch	+	-	+	-
7.	*G. intaradicus* Shenck &Smith	+	+	+	-
8.	*G. aggregatum* Schenck & Smith	+	+	+	+
9.	*G. constrictum*Trappe.	+	+	+	-
10.	G.*mosseae*.Gerdeman&Trappe.	+	+	+	+
11.	*Gigaspora.gigantea* Gerdemann and Trappe	-	-	+	-

+ = Present, - = Absent

Table 1b. Taxonomic distribution of AMF Spp. in various Districts of KPK in the rhizospheric soils of *Mentha arvensis*.

S. No.	AM fungi	Manshera	Haripur	Havelian	Peshawar
1.	*A. leavis* Gerdeman & Trappe	+	-	-	+
2.	*G. microcarpum* Tul & Tul	+	+	+	+
3.	*G. fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske	+	+	+	+
4.	*Glomus inveranum Hall.*	+	+	+	+
5.	*G. mosseae* Gerdemann and Trappe.	+	+	+	+
6.	*G. aggregatum* Schenck & Smith	+	+	+	+
7.	*Gigaspora.albidaSchenck*& Smith	-	-	+	-

+ = Present, - = Absent

Root colonization and and spore density of AM fungi:

Roots of all studied plants exhibited AM association. (Tables 2a & 2b). Root colonization was characterized by the presence of external hyphae,internal hyphae,Arbuscules and vesicles. Arbuscular infections were common in M.*longifolia* where as vesicular infections were more prevalent in *Mentha arvensis* (Table 2a & 2b, Figs 1-8). The results of spore densities means given in (Tables 2a & 2b) revealed that the 4 studied areas of KPK were different with respect to the total number of densities of AMF spores. Among the 4 districts of KPK, maximum being recorded in Manshera (245±2.06) and minimum in Havelian (50±0.26).

Relationship between AMF spores and chemical characteristic of soil of studied sites: The relationship between AMF spores densities and chemical characteristics of soils for both *Mentha* species was determined by computing the co efficient of correlation (r) statistically. The soil samples were analyzed for chemical characteristics. The results are given in Tables 3a & 3b.

Table 2a. Total densities of AM spores from *Mentha longifolia* rhizosphere of various districts of KPK/10gm soil.

Districts	AM colonization %	Spore density means
Manshera	100 ± 2.02	245 ± 2.06
Haripur	80.68 ± 1.45	134 ± 1.29
Havelian	70.25 ± 1.36	150 ± 0.9
Peshawar	40.50 ± 2.25	190 ± 1.89

± Standard deviation

Table 2b. Total densities of AM spores from *Mentha arvensis* rhizosphere of various districts of KPK.

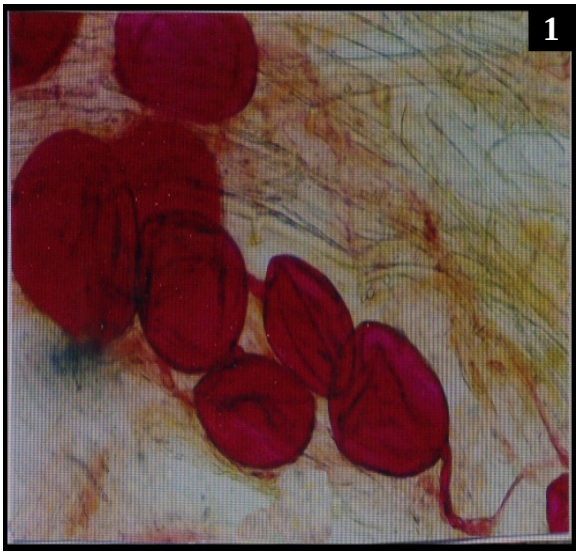
Districts	AM colonization %	Means
Manshera	90.78 ± 1.12	152 ± 3.08
Haripur	66.90 ± 1.88	187 ± 1.07
Havelian	30.34 ± 1.12	50 ± 0.56
Peshawar	26.87 ± 0.99	138 ± o.88

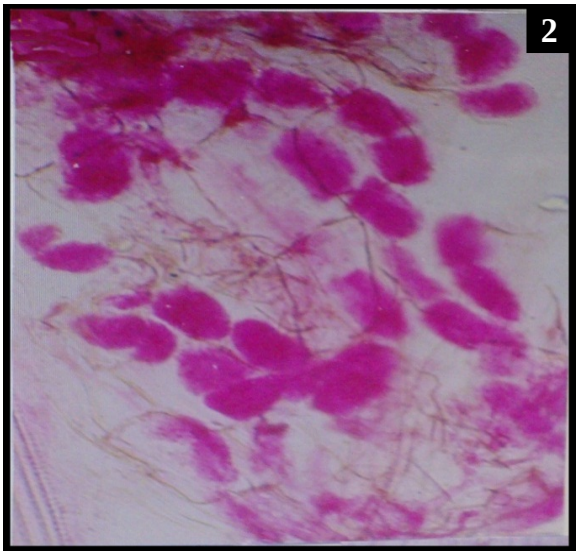
± Standard error

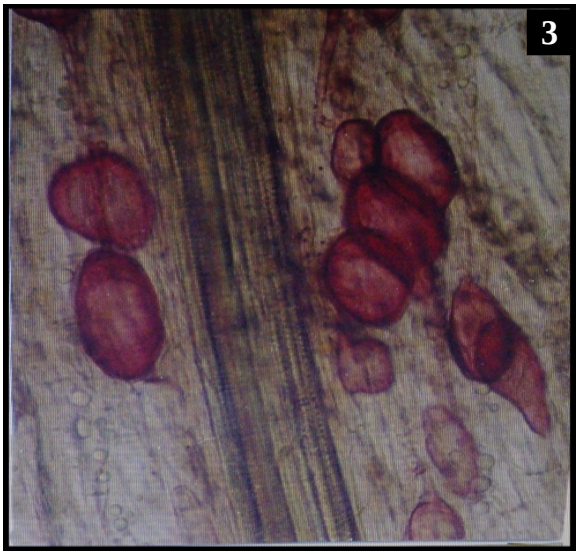
Discussion

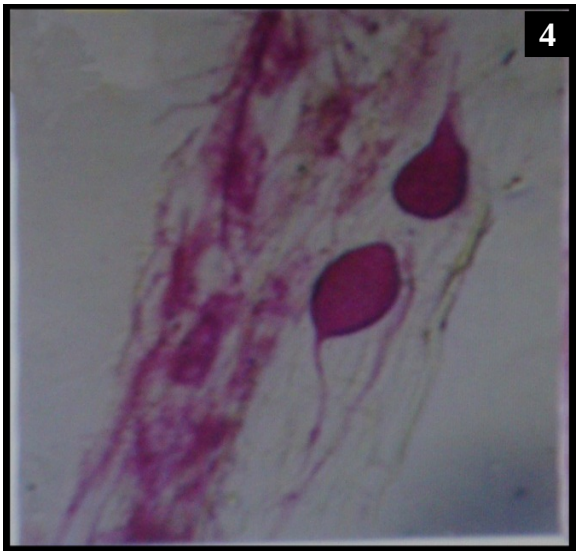
Mycorrhizal fungi are essential component of the rhizospheric microflora in natural ecosystems and important for sustainable plant-soil-systems due to their symbiotic efficiency (Sharma *et al.*, 2009). AMF reproduce by forming spores and sporocarps in the soil. These spores are at the same time a means of identification of these fungi. The results of the present investigation confirmed the ubiquitous nature of AMF spores. Fifteen AMF species in studied soil samples were identified. *Glomus* species was the most common AM fungi in the soils of study sites. Our findings corroborate with the finding of Morton (1988) that the genus *Glomus* is a predominantly distributed genus in the soil all over the world. *Glomus* species were common and made up for more than 75% of total isolates followed by *Acaulospora* and *Gigaspora*. Dominancy of *Glomus* in the present study is in the agreement with the findings of (Panawar & Tarafdar, 2006; Pande & Tarafdar, 2004; Burni & Illahi, 2004; Mridha & Dhar, 2007; Sharma *et al*., 2009; Burni *et al*., 2009). Further Wang & Shi, (2008) found a total of 122 AM species in the rhizosphere of different plants in various environment of China.The most common and widely distributed genus was *Glomus* (64)species followed by *Acaulospora* (26) species.Zang *et al*. (2003) and Tao *et al*., (2004) also found the dominancy of *Glomus* The predominance of *Glomus* species under varying soil conditions might be due to the fact that they are widely adaptable to the varied soil conditions and survive in acidic as well as in alkaline soils (Pande & Tarafdar 2004).

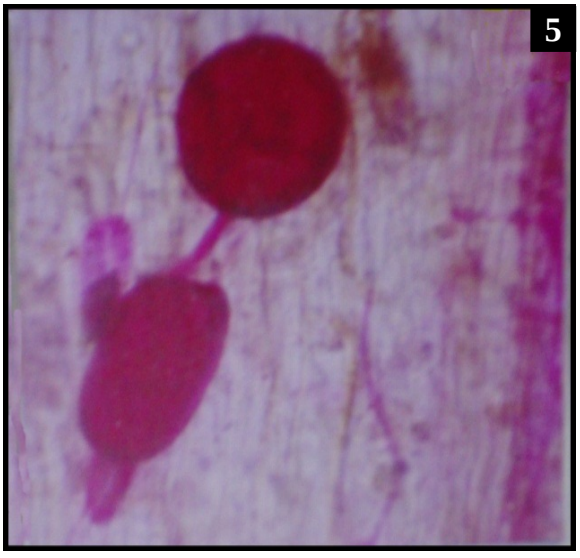
Among the identified species of *Glomus*, *G.fasciculatum* was the most common in all study sites. Our result correlate with the findings of Sharief *et al*., (2005) who found *G.fasciculatum* to be the most predominant in the soils of KPK. Moreover from Pakistani soils Nasim *et al*., (1999) also found the spores of *Glomus fasciculatum* associated with grasses. Jalaluddin & Anwar, (1991) also reported the occurrence of *G. fasciculatum* in wheat fields of Sindh and Punjab. In the present study *Glomus aggregatum* was also common species. Our results are in accordance with Jalaluddin & Anwar, (1991) found this species in the wheat fields of Sindh.

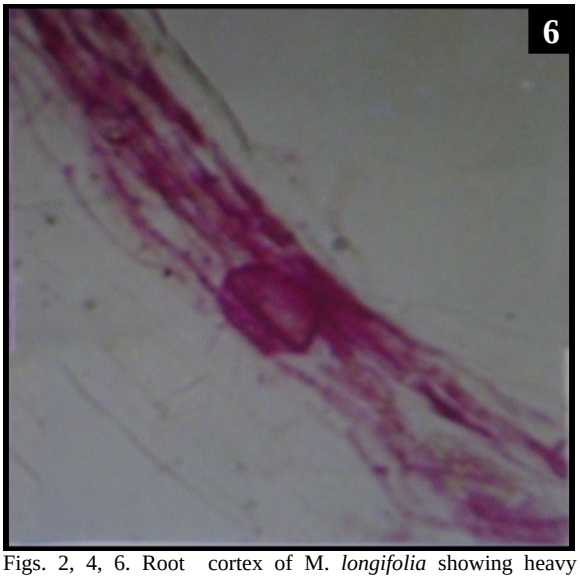






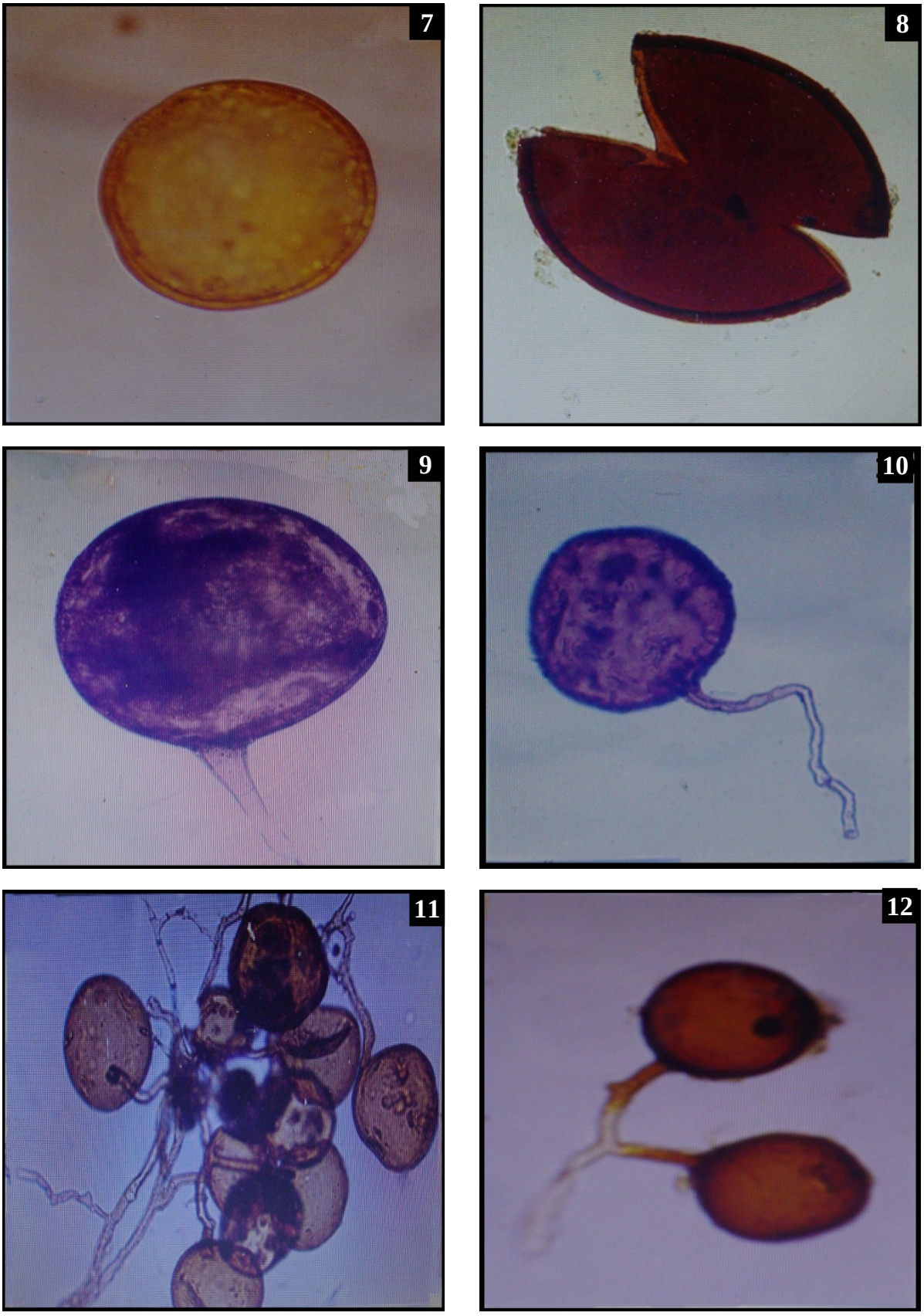






Figs. 1,3,5. Root cortex of *Mentha arvensis* showing heavy vesicular infections.

Figs. 2, 4, 6. Root cortex of M. *longifolia* showing heavy arbuscular infections.



Figs. 7, 8, 9, 10, 11, 12 showing the spores of *Acaulospora melleae,G. etunicatum, G. claroide, G. fasiculatum, G. aggregarum, G. australe* respectively.

Table 3a. Chemical analysis of rhizospheric soil samples of *M. longifolia* from various studied sites of KPK and co-efficient of correlation (r) of soils chemical characteristics and AM spore densities.

Soil properties	Units	Manshera	Haripur	Havelian	Peshawar	r-Values
PH		8.o8 ± 0.01	7.51 ± 0.02	7.85 ± 0.03	8.00 ± 0.01	+0.9
OM	%	1.77 ± 0.03	1.03 ± 0.04	0.78 ± 0.01	0.95 ± 0.05	+0.9
Lime	%	1.50 ± 0.01	0.70 ± 0.01	11.4 ± 0.21	10 ± 0.02	-0.6
N	ppm	0.350 ± 0.01	0.270 ± 0.02	0.341 ± 0.07	0.411 ± 0.02	+0.8
P	ppm	2.60 ± 0-02	2.25 ± 0.02	5.89 ± 0.03	3.99 ± 0.02	-0.8
K	ppm	186.9 ± 0.05	109.2 ± 0.04	156.2 ± 0.01	124 ± 0.05	+0.07
Zn	ppm	0.524 ± 0.06	0.99 ± 0.01	0.148 ± 0.02	0.614 ± 0.07	-0.13
Fe	ppm	16.84 ± 0.01	12.89 ± 0.01	13.00 ± 0.01	15.07 ± 0.08	-0.14
Cu	ppm	1.65 ± 0.03	2.90 ± 0.01	2.60 ± 0.01	1..40 ± 0.01	-0.1
Mn	ppm	19..25 ± 0.01	9.45 ± 0.02	10.40 ± 0.01	13.60 ± 0.01	-0.9

± Standard error

Table 3b. Chemical analysis of *M. arvensis* soil samples from various studied sites of KPK and co-efficient of correlation (r) of chemical soils characteristics and AM spores densities.

Soil properties	Units	Manshera	Haripur	Havelian	Peshawar	r-Values
PH		7.80 ± 0.02	7.05 ± 1.10	7.00 ± 0.8	7.25 ± 0.02	+0.7
OM	%	3.04 ± 0.01	4.52 ± 0.07	3.90 ± 0.03	2.50 ± 0.02	+0.1
Lime	%	2.50 ± 0.04	11.7 ± 0.03	8.75 ± 0.01	11.3 ± 0.05	+0.2
N	ppm	0.254 ± 0.01	0.238 ± 0.03	0.38 ± 0.04	0.254 ± 0.02	+0.9
P	ppm	3.17 ± 0.05	5.99 ± 0.07	6.1 ± 20.01	2.76 ± 0.01	-0.5
K	ppm	312.8 ± 0.06	223.8 ± 0.04	180 ± 0.01	300.2 ± 0.02	+0.2
Zn	ppm	0.23 ± 0.01	0.19 ± 0.04	0.88 ± 0.02	0.142 ± 0.06	-0.8
Fe	ppm	17.82 ± 0.01	14.46 ± 0.01	11.16 ± 0.02	16.06 ± 0.03	-0.9
Cu	ppm	1.30 ± 0.07	2.65 ± 0.01	2.00 ± 0.03	2.70 ± 0.04	-0.3
Mn	ppm	17.45 ± 0.01	10.75 ± 0.03	12.20 ± 0.04	16.80 ± 0.02	-0.3

Arbuscular mycorrhizal fungi have a broad ecological range and play an important role in ecosystem diversity.The population of AMF varies and their distribution is affected by various abiotic and biotic factors (Mohammad *et al*., 2003). Change in total spore population in different ecosystems have been reported by various workers e.g. in dunes (Louis & Lim, 1987), Savana (Saif, 1986) and Tropical rain forest (Mridha & Dhar, 2007).

In the present study, total AMF spore densities in different soils of *Mentha* species of KPK ranged from 50-245 spores/10g of soil. Read *et al*., (1976) reported that grassland soils yielded 800 to 8000 or more spores/100g soil. Similarly several scientists from different countries including Australia, New Zealand (Mosse & Bowen, 1984) Scotland (Daft & Nicolson, 1974), Pakistan (Sharief *et al*., 2005; Nasrullah et.al., 2010) have confirmed that AMF spore vary considerably from place to place according to physical and chemical nature of the soil. Saif & Khan, (1975) found 750 AMF spores/100 grams of soil in wheat fields of northern areas. Jalaluddin & Anwar, (1991) identified AMF flora in different ecological zone of Punjab and Sindh. Kumar *et al.,* 2010 studied the arbuscular fungal dynamics in the rhizospheric soils of five medicinal plants and found AMF spore density ranged from 19.33 to 68.66/10gm soil of *Mentha spicata*. Radhika & Rodrigues, (2010) noticed spore density of 85spores/100gm in the rhizospheric soil of *Mentha* sp. The present results with respect to quantitative distribution of AMF spore of various studied sites showed a great deal of variations. Our results favoured by the results mentioned by Anwar & Jalaluddin, (1993). They indicated that variations in the quantitative distribution may be due to the different soil physico-chemical condition such as pH, texture and mineral nutrients.

Therefore in the present investigations an attempt was made to find out the possible relationship between AM fungi and the chemical characteristics of the soil. The relationship was worked out by computing the co-efficient of correlation (r) statistically. As evident from the results, the soil pH of studied sites ranged between 7.00-8.08. As reported by Kamal & Prasad, (1995) that alkaline pH (8.6) is suitable for AMF spore population. A positive correlation between soil pH and AMF spores was found. These results are in line with the finding of Panawer & Tarafder (2006) which examined that the pH and the number of propagules are positively correlated. Phosphorus concentration in the studied soils ranged from 2.25-5.99ppm. A negative correlation was found between soil phosphorus and AMF spores. Janaki & Manoharachary (1994); Korthari & Singh (1996); Panawer & Tarafdar (2006) also found negative correlation between AMF spores and soil phosphorus. Generally the population of AMF spores and phosphorus are inversely related to each other (Hao *et al*., 1991). But results negate the finding of (Chandrasekara *et al*., 2005) who did not found any significant relationship with available phosphate.

Beside this a positive correlation were found between soil nitrogen (N) and AMF spores. Our results matched with the findings of Panawer & Tarafdar (2006); Joshi & Singh (1995) and Aquilaria *et al*., (1998) who found that number of spores was positively correlated with nitrogen in the soil. There was a positive correlation between K and AMF spores. Our results are also supported by Joshi & Singh, (1995) who reported that positive correlation occurred between AMF spores and potassium (K). Moreover a positive correlation between AMF spores and organic matter was also found. Our results are supported by Anwar & Jalaluddin, (1993) and Burni & Ilahi, (2004)

who reported that soil of Sindh and Khyber Pakhtunkhwa fields have high organic matter contents were also rich in AMF spores number. This may be attributed to the fact that organic matter increases the water holding capacity of soil which may enhance the sporulation of AMF.

In the present investigations the extent of root colonization varied in the studied *Mentha* species and these variations may be attributed to the differential preference of the AM fungi to various species (Khade & Rodrigues, 2009).These findings are also favoured by various workers who reported that root colonization of AMF is genetically controlled (Raju *et al*., 1990; Karangiannidis *et at*., 1997). Karangiannidis *et al*., (1997) reported significant differences between 4 grapevine rootstocks regarding their abilities to form AM association. Recently Burni *et al*., (2011) also reported variations in AM root colonization in various members of Lamiaceae. Different edapho-climatic factors like soil type, nutritional status of soil, soil pH, organic matter, soil moisture, rain fall, temperature etc. may be responsible for variations in root colonization and spore population (Sharma *et al*., 1986).

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