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Occurrence and Distribution of Arbuscular Mycorrhiza in Wheat and Maize Crops Grown in Northern Areas of North West Frontier Province, Pakistan

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Abstract: Arbuscular Mycorrhizal (AM) fungi are of considerable interest because of their ability to form symbiotic associations with various crops species. Rhizosphere soil samples were collected both from fertilized and unfertilized soils along with wheat and maize roots during the year 2005. In wheat, maximum numbers of white spores were found in Kanju, Beha, Besham, Bunir, Mingora, Alpuri, brown spores in Pirsabak, Mingora and Beha and black spores in Badwan, Shangla, Bunir, Marghuzar and Besham soil series with fungal infection rates ranged from 32 to 68% in roots of wheat crop. In maize, highest concentrations of white spores were found in Pirsabak, Besham, Kanju, Shangla, Bunir and Marghuzar, brown spores in Marghuzar Shangla Badwan, Beha, Khwazakhela and Alpuri and black spores in Pirsabak Khwazakhela and Besham soil series with 24 to 60% infection rates in roots of maize crop. Results suggest that spores density in soil and roots infection intensity varied from one site to another under different agro-ecological conditions and higher AM infections rates were observed in soil with low organic matter contents.

Key words: AM fungi % Fungal spores % Maize % Nutrient concentration % Roots infection and wheat

INTRODUCTION

The ability to exploit the natural resources constitute a major step towards economic prosperity for developing country like Pakistan as chemical fertilizers are expensive, short and may cause the problems of environmental pollution [1]. Arbuscular Mycorrhizal (AM) fungi are distributed worldwide [2]. Infection of crop roots with AM fungi can improve their uptake of nutrients, particularly of phosphorus and increase crop production [3,4]. Response of tropical and subtropical cultures to endomycorrhizal fungi has been reported by Jaizme and Azcon [5]. The endomycorrhizal fungi are obligate symbiotic fungi, the hyphae of which develop mycelia, arbuscules and in most fungal genera vesicles in roots. These hyphae can explore an area around the root which far exceeds that available to root hairs. It is the ability of these hyphae to absorb relatively immobile or fixed elements (P, Zn, Cu) in acid or alkaline soils, especially in the plants with coarse root systems [6,7].

Association of AM fungi with plant roots can help plants to overcome water stress by stomata regulation in plants [8]. There is a great lack of information on the ranges of specific soil variables under which specific AM fungal species occur and thus on conditions which may be tolerable or optimum for them. Very limited information is available about the incidence of AM in Pakistan [9-11]. No detailed and systematic studies have been conducted on the precise status of mycorrhizal association of plant in different ecological zones of Pakistan and particularly in NWFP. This research project was planned to conduct the comprehensive field survey in both nutrient deficient and fertile soils to evaluate AM fungi in wheat-maize cropping system of Peshawar vale, NWFP as wheat and maize crops rotation is very common in the area with cultivation on 0.742 and 0.506 million ha area having production of 1.03 and 0.87 million tones of wheat and maize, respectively in NWFP [12].

MATERIALS AND METHODS

Field survey was conducted during the year 2005 to determine the status of AM fungal spores concentrations in soil and their colonization in roots of wheat and maize crops in different soil series of Malakand division.

Soil and Roots Sampling: Rhizosphere soil samples attached with plant roots were collected from fertilized soil with better crop growth conditions as well as from unfertilized soil with poor plant growth conditions along

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with plant roots randomly from 10-15 plants to make a composite sample from wheat and maize crops of different soil series of Malakand area. Half of the soil sample was stored at 4°C for the determination of mycorrhizal spores while remaining half of sample was air dried, ground and passed through 2 mm sieves for the analysis of soil physical and chemical characteristics. Crop root samples were also stored at temperature of 4°C for the estimation of mycorrhizal infection rates.

Soil Analysis: Total N concentration of soil samples was determined by the kjeldhal method of Bremner [13]. Soil texture was determined by hydrometer method as described by Koehler *et al*. [14]. AB-DTPA extractable P, K, Cu, Fe, Zn and Mn were analyzed by the method as described by Soltanpour and Schawab [15]. Soil pH and electrical conductivity were determined in soil and water suspension of 1:5 by McClean [16]. Soil organic matter content was determined by using $K_2Cr_2O_7$ as an oxidizing agent as described by Nelson and Sommer [17].

Isolation of AM Fungal Spores from Soil: Spores of AM fungi were isolated from soil by wet-sieving and decanting techniques as described by Brundrett [18]. Soil was suspended in water and then passed through sieves of different sizes. Spores concentrations of different size, shape and color were observed in different numbers under binocular microscope, which were grouped as high (>3333 spores kg^{-1}soil), medium (1400-3333 spores kg^{-1}soil) and low (<1333 spores kg^{-1}soil) according to the criteria as developed by Brundrett [18]. These spores were identified according to their morphological characteristics including shape, size, colour, distinct wall layer, attached hyphae and surface orientation of spores as described by Schenck and Parez [19].

Estimation of AM Fungal Infections: Infection rates by AM fungi in the roots of wheat and maize crops were determined by staining the mycorrhizal chitin with lactic-trypan blue according to the procedure as described by Koske and Gemma [20]. The presence of vesicles, arbuscules or hyphae (minimum or maximum) are measured by the techniques as described by Giovannetti and Mosse [21]. Soil organic matter contents and spores density were correlated with roots infections rates by AM fungi in wheat and maize crops of this area.

RESULTS AND DISCUSSION

Soils of Malakand division ranged from moderate to very good land and is either canal irrigated or moderate dry farm land with mean annual rainfall from 800 to 1500 mm [22].

Physical and Chemical Characteristics of Soils of the Study Area: Data given in Table 1 showed that pH values of the well managed fertile rhizosphere soil of this area ranged from 6.88 to 8.10, electrical conductivity from 0.067 to 0.433 dS m^{-1}, soil organic matter content ranged from 1.82 to 2.53% and lime from 2.9 to 14.1%. In marginal rhizosphere soils, pH values ranged from 6.17 to 8.02, electrical conductivity from 0.044 to 0.287 dS m^{-1}, lime

Table 1: Soil physical and chemical characteristics of Malakand division

Soil series	Location	Soil type	pH (1:5)	E.C. (dS m^{-1})	SOM (%)	Lime (%)	Soil textural class
Badwan	Badwan	S-I	7.85	0.210	2.27	7.1	Sandy loam
Kanju	Landaki	"	8.01	0.169	2.10	13.6	Sandy loam
Bunir	Shahi Nagar	"	8.06	0.229	2.14	14.1	Clay loam
Pirsabak	Kabal	"	7.93	0.173	2.42	6.9	Silt loam
Beha	Beha	"	8.10	0.220	2.14	7.8	Loam
Marghuzar	Manglore	"	7.51	0.433	1.82	13.3	Sandy loam
Mingora	Charbagh	"	7.03	0.145	2.53	2.9	Silt loam
Khwazakhela	Khwazakhela	"	7.75	0.082	2.00	5.8	Silty clay loam
Shangla	Shangla	"	6.88	0.163	1.89	12.4	Silt loam
Alpuri	Alpuri	"	7.40	0.308	2.12	2.9	Silt loam
Besham	Besham	"	7.11	0.067	2.43	3.8	Silt loam
Badwan	Badwan	S-II	7.96	0.189	2.13	7.4	Sandy loam
Kanju	Landaki	"	8.02	0.176	1.86	12.7	Sandy loam
Bunir	Shahi Nagar	"	7.96	0.287	1.59	14.5	Clay loam
Pirsabak	Kabal	"	7.28	0.173	1.92	3.6	Silt loam
Beha	Beha	"	7.88	0.188	1.54	5.3	Loam
Marghuzar	Manglore	"	7.23	0.148	1.10	7.1	Sandy loam
Mingora	Charbagh	"	7.32	0.165	1.30	3.3	Silt loam
Khwazakhela	Khwazakhela	"	7.53	0.189	1.63	6.4	Silty clay loam
Shangla	Shangla	"	6.17	0.059	1.47	10.5	Silt loam
Alpuri	Alpuri	"	6.83	0.158	1.88	2.9	Silt loam
Besham	Besham	"	7.07	0.044	1.98	2.8	Silt loam

S-I= Fertilized soil with better crop growth conditions, S-II= Unfertilized soil with poor plant growth conditions

Table 2: Soil nutrients concentrations of Malakand Division

				P	K	Zn	Fe	Cu	Mn
Soil series	Location	Soil types	N (%)	(mg Kg^{-1})					
Badwan	Badwan	S-I	0.333	10.3	270.5	0.83	15.84	4.8	127.7
Kanju	Landaki	"	0.223	11.8	294.3	0.71	9.64	5.6	135.1
Bunir	Shahi Nagar	"	0.228	9.1	210.3	0.46	18.62	5.3	42.5
Pirsabak	Kabal	"	0.249	9.2	201.7	0.81	18.34	7.4	45.8
Beha	Beha	"	0.350	10.7	235.1	1.05	17.90	9.3	83.5
Marghuzar	Manglore	"	0.306	12.1	192.0	1.44	17.30	6.2	45.5
Mingora	Charbagh	"	0.341	6.8	122.4	1.41	27.10	8.8	55.3
Khwazakhela	Khwazakhela	"	0.298	6.3	148.5	0.64	25.90	9.6	38.7
Shangla	Shangla	"	0.263	10.9	139.3	0.56	41.28	6.3	104.5
Alpuri	Alpuri	"	0.303	10.2	166.7	1.45	25.92	9.1	62.7
Besham	Besham	"	0.298	10.5	115.2	1.14	30.14	5.9	65.5
Badwan	Badwan	S-II	0.298	4.5	117.2	0.71	14.50	3.4	106.1
Kanju	Landaki	"	0.220	10.3	236.6	0.29	8.72	4.9	103.9
Bunir	Shahi Nagar	"	0.215	3.6	139.1	0.17	16.06	4.5	28.6
Pirsabak	Kabal	"	0.211	8.7	202.2	0.73	12.12	5.6	41.2
Beha	Beha	"	0.298	6.1	227.9	0.78	17.38	8.3	52.4
Marghuzar	Manglore	"	0.228	7.1	160.4	0.86	10.36	4.5	27.1
Mingora	Charbagh	"	0.241	5.7	115.3	1.04	21.40	7.9	48.7
Khwazakhela	Khwazakhela	"	0.290	5.5	108.2	0.59	19.26	8.5	36.2
Shangla	Shangla	"	0.260	7.3	119.3	0.31	35.16	6.2	46.8
Alpuri	Alpuri	"	0.248	8.1	116.1	1.03	16.44	8.2	48.8
Besham	Besham	"	0.271	7.4	107.6	0.99	21.04	3.9	42.2

S-I= Fertilized soil with better crop growth conditions, S-II= Unfertilized soil with poor plant growth conditions

contents from 2.8 to 14.5% and soil organic matter contents ranged from 1.10 to 2.13%. Relatively higher soil organic matter contents in fertile and marginal soils were recorded as soils attached with roots contain more organic materials with higher microbial activities [23].

Soil Nutrient Concentrations: Data in Table 2 describe the concentrations of soil total N and AB-DTPA extractable P, K, Zn, Fe, Cu and Mn in soils of Malakand division. Total N ranged from 0.223 to 0.350%, AB-DTPA extractable P from 6.3 to 12.1 mg kg^{-1}, K from 115.2 to 294.3 mg kg^{-1}, Zn from 0.46 to 1.45 mg kg^{-1}, Fe from 9.64 to 41.28 mg kg^{-1}, Cu from 4.8 to 9.6 mg kg^{-1} and Mn ranged from 42.5 to 135.1 mg kg^{-1} in well managed fertile rhizosphere soil of Malakand division. In marginal rhizosphere soil of this area, AB-DTPA extractable P from 3.6 to 10.3 mg kg^{-1}, K from 107.6 to 236.6 mg kg^{-1}, Zn from 0.17 to 1.04 mg kg^{-1}, Fe from 8.72 to 35.16 mg kg^{-1}, Cu from 3.4 to 8.5 mg kg^{-1} and Mn ranged from 27.1 to 106.1 mg kg^{-1} and total N ranged from 0.211 to 0.298%. Relatively higher total soil N contents in fertile and marginal soils were recorded as soils attached with roots contain more organic materials with higher microbial activities [23].

Density of AM Fungal Spores and their Roots Colonization in Wheat Crop: Density of AM fungal spores varied greatly due to different soil types in different locations (Table 3). In fertile soil, high numbers (>3333 spores kg^{-1}soil) of white spores were found in Kanju, Beha and Besham soil series, brown spores in Pirsabak and Mingora soil series and black spores were recorded in Shangla soil series of Malakand division. Medium (1400-3333 spores kg^{-1}soil) or lower (<1333 spores kg^{-1}soil) numbers of white, brown and black spores were recorded in all other soil series of this area. In marginal soil, maximum numbers of white spores (>3333 spores kg^{-1}soil) were found in Kanju, Bunir, Mingora and Alpuri soil series, brown spores in Beha, Mingora and Pirsabak soil series where as maximum black spores were recorded in Badwan, Bunir, Marghuzar, Shangla and Besham soil series. Medium (1400-3333 spores kg^{-1}soil) or

Table 3: AM fungal spores concentrations and their root colonization in wheat crop

Soil series	Location	Soil types	Spores concentrations			Root infection (%)
			White	Brown	Black	
Badwan	Badwan	S-I	Medium	Low	Low	32
Kanju	Landaki	"	High	Low	Low	36
Bunir	Shahi Nagar	"	Low	Medium	Medium	44
Pirsabak	Kabal	"	Medium	High	Low	48
Beha	Beha	"	High	Low	Low	36
Marghuzar	Manglore	"	Medium	Low	Low	44
Mingora	Charbagh	"	Medium	High	Low	56
Khwazakhela	Khwazakhela	"	Medium	Low	Medium	44
Shangla	Shangla	"	Medium	Medium	High	49
Alpuri	Alpuri	"	Medium	Medium	Medium	42
Besham	Besham	"	High	Medium	Medium	40
Badwan	Badwan	S-II	Medium	Low	High	47
Kanju	Landaki	"	High	Medium	Medium	44
Bunir	Shahi Nagar	"	High	Medium	High	52
Pirsabak	Kabal	"	Low	High	Medium	64
Beha	Beha	"	Medium	High	Low	52
Marghuzar	Manglore	"	Medium	Low	High	64
Mingora	Charbagh	"	High	High	Medium	68
Khwazakhela	Khwazakhela	"	Medium	Medium	Medium	56
Shangla	Shangla	"	Medium	Low	High	59
Alpuri	Alpuri	"	High	Medium	Low	52
Besham	Besham	"	Medium	Low	High	52

< 20 spores (Low), 21-50 spores (Medium) and >50 spores (High) in 15 g soil, S-I= Fertilized soil with better crop growth conditions, S-II= Unfertilized soil with poor plant growth conditions

Table 4: AM fungal spores density and their root colonization in maize crop

Soil series	Location	Soil types	Spores concentrations			Root infection (%)
			White	Brown	Black	
Badwan	Badwan	S-I	Low	Medium	Low	29
Kanju	Landaki	"	Low	Medium	Medium	28
Bunir	Shahi Nagar	"	Low	Medium	Low	24
Pirsabak	Kabal	"	High	Medium	High	48
Beha	Beha	"	Low	Medium	Medium	30
Marghuzar	Manglore	"	Medium	High	Low	34
Mingora	Charbagh	"	Low	Medium	Low	32
Khwazakhela	Khwazakhela	"	Low	Medium	High	37
Shangla	Shangla	"	Medium	High	Medium	34
Alpuri	Alpuri	"	Medium	Low	Medium	48
Besham	Besham	"	High	Medium	Medium	42
Badwan	Badwan	S-II	Medium	High	Low	40
Kanju	Landaki	"	High	Medium	Medium	32
Bunir	Shahi Nagar	"	High	Medium	Low	42
Pirsabak	Kabal	"	Low	Medium	High	60
Beha	Beha	"	Medium	High	Low	42
Marghuzar	Manglore	"	High	Medium	Medium	54
Mingora	Charbagh	"	Low	Medium	Low	40
Khwazakhela	Khwazakhela	"	Medium	High	Medium	50
Shangla	Shangla	"	High	Medium	Low	45
Alpuri	Alpuri	"	Low	High	Medium	56
Besham	Besham	"	Low	Medium	High	50

< 20 spores (Low), 21-50 spores (Medium) and >50 spores (High) in 15 g soil, S-I= Fertilized soil with better crop growth conditions, S-II= Unfertilized soil with poor plant growth conditions

lower (<1333 spores kg^{-1}soil) numbers of white, brown and black spores were recorded in all other soil series of Malakand division.

Root infection rates by AM fungi varied in wheat crop from one site to another. In fertile soil of Malakand division, 32 to 56% AM fungal infection rates were noted in roots of the wheat crop where as 44 to 68% infection rates by mycorrhizal fungi were observed in marginal soil of this area (Table 3). In the area of Malakand division, relatively more roots infections rates in wheat crop by AM fungi were observed in unfertilized soil (S-II) as compared with fertilized (S-I) soil (Fig. 1) and more number of AM fungal spores caused to increase the roots infections rates of wheat crop in this area (Fig. 2). The

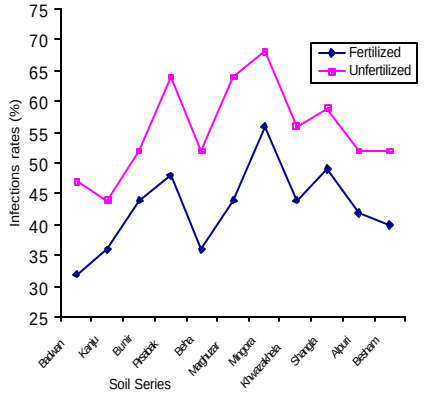

Fig. 1: Comparison of wheat infection rats by AM fungi in fertilized and unfertilized soils

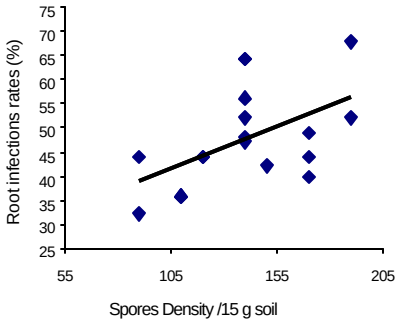

Fig. 2: Relationship between spores density and AM infection rates in wheat roots

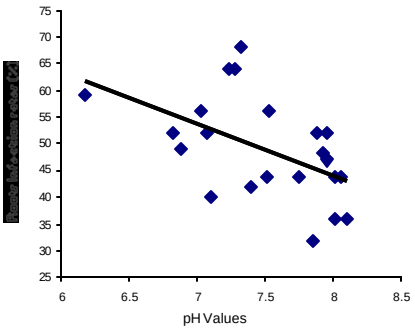

Fig. 3: Relationship between pH values and AM infection in wheat root of Malakand soils

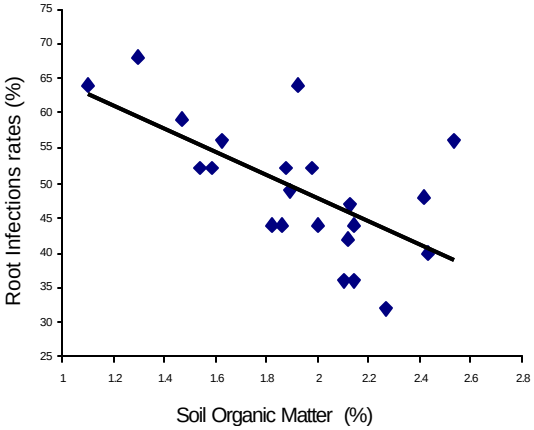

Fig. 4: Relationship between soil organic matter and AM infection rates in wheat roots

relationship between pH values and root colonization indicated that infections rates by AM fungi in wheat crop decreased with increases in soil pH values (Fig. 3) and higher wheat root infections rates by AM fungi were noted in soil with lower organic matter contents (Fig. 4).

Density of AM Fungal Spores and their Roots Colonization in Maize Crop: Data regarding density of AM fungal spores and maize roots infection are given in Table 4.

It can be inferred from the data in Table 4 regarding the AM fungal spores density of different color and their root colonization in maize crop of fertilized and unfertilized soils of Malakand division that high numbers (>3333 spores kg^{-1}soil) of white spores were found in Pirsabak and Besham soil series, brown spores in Badwan, Beha, Khwazakhela and Alpuri soil series and black spores were found in Pirsabak and Besham soil series in fertilized soil of Malakand division. All other soil series of this area gave either medium (1400-3333 spores kg^{-1}soil) or lower (<1333 spores kg^{-1}soil) numbers of white, brown and black spores. In unfertilized soil, maximum numbers of white spores (>3333 spores kg^{-1}soil) were found in Kanju, Bunir, Marghuzar and Shangla soil series, brown spores in Badwan, Beha, Khwazakhela and Alpuri soil series where as maximum black spores were noted in Pirsabak and Besham soil series. Medium (1400-3333 spores kg^{-1}soil) or lower (<1333 spores kg^{-1}soil) numbers of white, brown and black spores were recorded in all other soil series of Malakand division. Data on root infections rates by AM fungi in maize crop ranged from 24 to 48% in fertile soil and that of 32 to 60% in marginal soil of Malakand division (Table 4). Like wheat crop, relatively more roots infections rates in maize crop by AM fungi were observed in marginal soil and more number of AM fungal spores caused increased root infections rates.

Density and their roots infections rates by AM fungi varied in different soil types and locations. The highest number of AM fungal spores and their infections of wheat and maize roots in some soil series of NWFP may be attributed to the root exudates of these crops, which stimulated the germination of mycorrhizal spores and their infection rates under the prevailing conditions of the area. The most plant species in Gramineae family are normally mycorrhizal [24]. Morley and Mosse [25] reported that wheat and maize crops vary in mycorrhizal infections according to cultivars and environmental conditions. Soils, plants, environment and their management mainly affect the mycorrhizal fungi, their development and crop root infections in an ecosystem. The diversity of AM fungal species and their roots infection intensity decline from natural ecosystem to high input agricultural systems. Relatively high numbers of AM fungal spores and their roots colonization in wheat and maize crops were found in marginal soil. Fertilizers applications may counteract super optimum AM fungal populations [26] as the reactions of fertilizers in soils are faster enough and the plants are not or only slightly depend on AM fungi and thus the reproduction of fungi will be low under this conditions, which will result low AM populations and root colonization in such soils generally.

Identification of AM Fungal Spores: Spores of *Glomus fasciculatum* were found in abundance in all soil samples where as spores of *G. intraradices G. mosseae, G. aggregatum*, *Acaulospora melleae, Sclerocystis* and *Sclerocystis pakistanica* [27] were also identified in the soil samples but in lower densities. These identified spores still require further confirmation by crop inoculation, re-isolation and re-identification.

CONCLUSION

It is concluded from the results of this study that relatively higher AM fungal spores and their root colonization in wheat and maize crops were observed in unfertilized soil with varied spores density and infections intensity from one site to another under different agro-ecological conditions. Higher spores density caused increased roots infections intensity in wheat and maize crops and roots infection rates were higher in soil with low organic matter contents. There were almost similar trends of AM fungal density and their roots intensity in wheat and maize crops in area under investigations. Possible interactions of AM fungi with other soil microorganisms, there mass production and management through agronomic practices to further improve their efficiency for economically feasible and sustainable crop production are needed to be focused in future research.

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