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Field Evaluation of Arbuscular Mycorrhizal Fungi in Wheat-Maize Cropping System in Hazara Division of North West Frontier Province

M. Sharif, M.S. Sarir and Nasrullah
Department of Soil and Environmental Sciences, NWFP Agriculture University Peshawar, Pakistan

Abstract: Arbuscular Mycorrhizal (AM) fungi are of considerable interest because of their ability to form symbiotic associations with 85% plants and their potential to use as a biofertilizer. Rhizosphere soil and roots samples of wheat and maize were collected from marginal and fertile soils of Hazara division from soil series extensively use for the production of wheat and maize crops. Physicochemical characteristics of the soil under investigations were determined. AM fungal spores were isolated from soil, identified and their infections rates in the roots were determined. Data indicated that soil pH values of Hazara division ranged from 6.38 to 7.66, soil organic matter content ranged from 1.20 to 1.97% and lime from 1.5 to 11.5%. Maximum numbers of AM fungal white spores were found in Battagram and Abbottabad, brown spores in Chamba and black spores in Abbottabad and Chamba soil series in fertile soil whereas in marginal soil, high numbers of white spores were found in Dedal, Battagram and Abbottabad, brown spores in Dedal and Mansehra and black spores in Chamba, Jaba and Haripur soil series of this area. In fertile soil, 28 to 43% AM fungal infection rates were noted in roots of wheat crop where as 44 to 56% infection rates were observed in marginal soil of this area. Results suggest that comparatively higher AM fungal spores and their root colonization in wheat and maize crops were observed in marginal soil than fertile soil with varied spores density and infections intensity from one site to another. More AM fungal spores density caused higher roots infection intensity in wheat and maize crops of the area and higher AM infections rates were observed in soil of around neutral pH values with low organic matter contents.

Key words: AM fungi, soil spores density, plants roots infection, identification, soil types

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 (Freney and Simpson, 1983). Microorganisms cause a series of dynamic biological and biochemical reactions such as organic matter decomposition, new material synthesis, rock weathering, element transformation in the soil and thus affect the nutrients availability for plants (Alexander, 1978).

Root system is a unique micro site for the association of symbiotic and non-symbiotic free-living microorganisms. Arbuscular Mycorrhizal (AM) fungi are distributed worldwide (Gerdeman, 1968). Non-mycorrhizal plants occur in habitats where the soils are very dry, saline, waterlogged, severely disturbed and where soil fertility is extremely high or extremely low. Mamatha *et al.* (2002), Gaur and Adholeya (2002) and Joner (2000) reported improvement in crops yield and nutrient accumulation with mycorrhizal inoculation. 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 (Allen, 1995; Mosse, 1973). There are indications to show that AM fungal associations with plant roots can help plants to overcome water stress by stomata regulation in plants (Kevin and Peterson, 1996; Robert, 2001).

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 (Saif and Parveen, 1977; Burni *et al.*, 1995; Burni and Jabeen, 1997). No detailed and systematic studies have been conducted on the precise status of mycorrhizal

Corresponding Author: M. Sharif, Department of Soil and Environmental Sciences, NWFP Agriculture University Peshawar, Pakistan

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 Hazara division, 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 (Agricultural Statistics of Pakistan, 2004).

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 potential soil series for these crops in Hazara division identified by the Peshawar regional office of the Directorate of Soil Survey of Pakistan.

Soil and roots sampling: Rhizosphere soil samples were collected both from marginal as well as fertile soil along with plant roots randomly from 10-15 plants for a composite sample from wheat and maize crops of different soil series in Hazara division of NWFP. Half of the collected soil sample was stored at temperature of 4°C for the determination of mycorrhizal spores while remaining half of soil 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 (1996). Soil texture was determined by hydrometer method as described by Koehler *et al.* (1984). AB-DTPA extractable P, K, Cu, Fe, Zn and Mn were analyzed by the method as described by Soltanpour and Schawab (1977). Soil pH and electrical conductivity were determined in soil and water suspension of 1:5 by McLean (1982). Soil organic matter content was determined by using $K_2Cr_2O_7$ as an oxidizing agent as described by Nelson and Sommer (1982).

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 *et al.* (1996). 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 *et al.* (1996). 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 (1990).

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 (1989). The presence of vesicles, arbuscules or hyphae (minimum or maximum) are measured by the techniques as described by Giovannetti and Mosse (1980). 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

Soil of Hazara division generally ranged from poor dry rainfed farmed land to very good canal irrigated land. This area is moderately deep, excessively drained to well-drained land with great potential for the production of wheat and maize crops. The area is level to nearly level with mean annual rainfall from 1000 to 1600 mm.

Soil physicochemical characteristics of hazara area: Data Presented in Table 1 showed that soil of Hazara division is clay loam, silty clay loam, loam and silt loam with pH values of the well managed fertile rhizosphere soil ranged from 6.58 to 7.91, electrical conductivity from 0.041 to 0.400 dS m^{-1}, soil organic matter content ranged from 1.77 to 1.97% and lime from 2.3 to 10.3%. In marginal rhizosphere soils, pH values ranged from 6.38 to 7.66, electrical conductivity from 0.033 to 0.387 dS m^{-1}, soil organic matter content from 1.20 to 1.69% and lime ranged from 1.5 to 11.5%. Maximum wheat root infections rates by AM fungi in area of Hazara division were observed in soil with low organic matter contents as compared with high contents of soil organic matter in this area (Fig. 3).

Density of AM fungal spores and their roots colonization in wheat crop of hazara area: Data collected indicated that the density of AM fungal spores varied in different soil types and locations (Table 2). In fertile soil of Hazara division, maximum numbers (>3333 spores kg^{-1}soil) of white spores were found in Battagram and Abbottabad 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

Table 1: Soil physicochemical characteristics of Hazara division

Soil series	Location	Soil type	pH (1:5)	E.C. (dS m^{-1})	SOM (%)	Lime (%)	Soil textural class
Battagram	Battagram	Fertile	6.58	0.041	1.77	3.9	Silt loam
Mansehra	Mera	″	7.05	0.075	1.95	3.1	Silty clay loam
Jaba	Arbora	″	7.47	0.065	1.97	2.5	Loam
Dedal	Dedal	″	7.26	0.067	1.88	2.3	Loam
Abbottabad	Mirpur	″	7.68	0.273	1.85	5.1	Silty clay loam
Chamba	Goharabad	″	7.73	0.380	1.79	3.7	Clay loam
Haripur	Haripur	″	7.91	0.400	1.77	6.7	Silty clay loam
Darwesh	Darwesh	″	7.59	0.217	1.83	10.3	Silty clay loam
Battagram	Battagram	Marginal	6.64	0.065	1.69	3.3	Silt loam
Mansehra	Mera	″	7.51	0.083	1.45	8.6	Silty clay loam
Jaba	Arbora	″	7.21	0.076	1.20	2.3	Loam
Dedal	Dedal	″	6.38	0.033	1.45	1.5	Loam
Abbottabad	Mirpur	″	7.30	0.222	1.55	2.1	Silty clay loam
Chamba	Goharabad	″	7.15	0.172	1.47	2.8	Clay loam
Haripur	Haripur	″	7.66	0.208	1.33	6.9	Silty clay loam
Darwesh	Darwesh	″	7.51	0.387	1.41	11.5	Silty clay loam

Table 2: AM fungal spores concentrations and their root colonization in wheat crop of Hazara division

			Spores concentrations			
Soil series	Location	Soil types	White	Brown	Black	Root infection (%)
Battagram	Battagram	Fertile	High	Low	Medium	40
Mansehra	Mera	″	Medium	Low	Low	28
Jaba	Arbora	″	Medium	Low	Low	34
Dedal	Dedal	″	Medium	Low	Medium	36
Abbottabad	Mirpur	″	High	Medium	Low	32
Chamba	Goharabad	″	Medium	Medium	Medium	43
Haripur	Haripur	″	Medium	Low	Medium	32
Darwesh	Darwesh	″	Medium	Low	Low	28
Battagram	Battagram	Marginal	High	Low	Medium	52
Mansehra	Mera	″	Medium	High	Low	49
Jaba	Arbora	″	Low	Low	High	44
Dedal	Dedal	″	High	Medium	Low	46
Abbottabad	Mirpur	″	High	Low	Medium	52
Chamba	Goharabad	″	Low	Low	High	56
Haripur	Haripur	″	Medium	Medium	High	50
Darwesh	Darwesh	″	Medium	Low	Medium	48

<20 spores (Low), 21-50 spores (Medium) and >50 spores (High) in 15 g soil

Table 3: AM fungal spores concentrations and their root colonization in maize crop of Hazara division

			Spores concentrations			
Soil series	Location	Soil types	White	Brown	Black	Root infection (%)
Battagram	Battagram	Fertile	Medium	High	Medium	42
Mansehra	Mera	″	Low	Medium	Low	32
Jaba	Arbora	″	Medium	Medium	High	42
Dedal	Dedal	″	High	Medium	Low	32
Abbottabad	Mirpur	″	Medium	High	Low	42
Chamba	Goharabad	″	Medium	Medium	Medium	36
Haripur	Haripur	″	High	Low	Medium	32
Darwesh	Darwesh	″	Medium	Low	Medium	32
Battagram	Battagram	Marginal	Medium	High	Medium	50
Mansehra	Mera	″	Medium	Medium	Low	40
Jaba	Arbora	″	High	High	Medium	59
Dedal	Dedal	″	Medium	High	Low	44
Abbottabad	Mirpur	″	Medium	Medium	Low	49
Chamba	Goharabad	″	High	Medium	High	56
Haripur	Haripur	″	Low	Medium	High	45
Darwesh	Darwesh	″	High	Low	Medium	44

<20 spores (Low), 21-50 spores (Medium) and >50 spores (High) in 15 g soil

this area. In marginal soil, high numbers of white spores (>3333 spores kg^{-1}soil) were found in Dedal, Battagram and Abbottabad soil series, brown spores in Mansehra soil series and black spores were recorded in Chamba, Jaba and Haripur soil series of Hazara division (Table 2). Root infection rates by AM fungi were also varied in wheat crop from one site to another. In fertile soil of Hazara division, 28 to 43% AM fungal infection rates

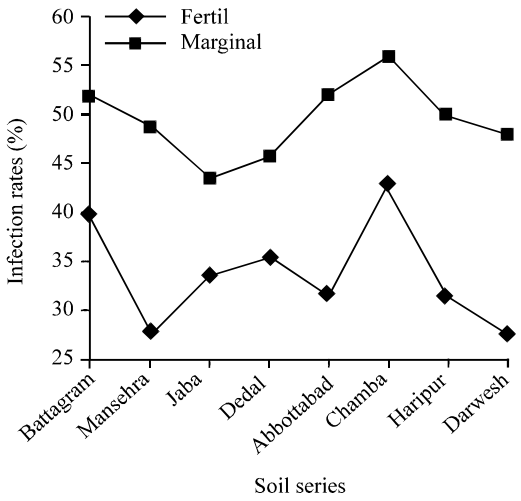

Fig. 1: Comparison of wheat infection rates by AM fungi in fertile and marginal soils of Hazara area

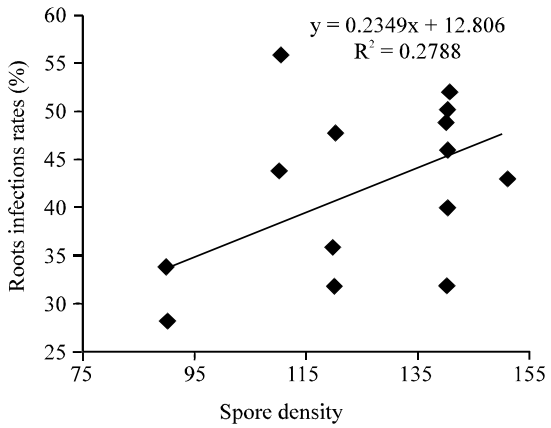

Fig. 2: Relationship between spores density and AM infection rates in wheat roots of Hazara area

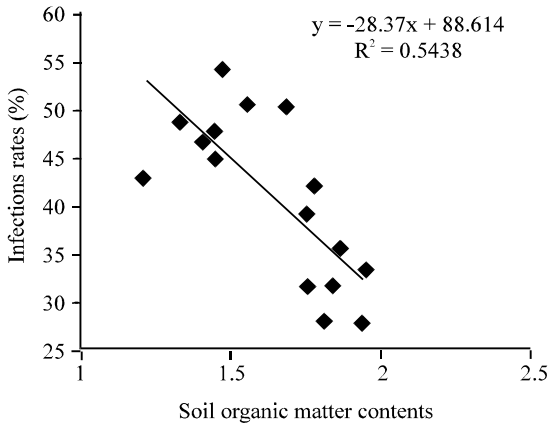

Fig. 3: Relationship between soil organic matter and AM infection rates in wheat roots of Hazara soil

were noted in roots of the wheat crop where as 44 to 56% infection rates by mycorrhizal fungi were observed in marginal soil of this area (Table 2).

Higher roots infections rates in wheat crop by AM fungi were observed in marginal soil of Hazara divisions when compared with fertile soil of this area (Fig. 1). Rates of AM fungal infections in roots of wheat crop were increased with the increase in spore density in the area of Hazara division (Fig. 2) and higher AM fungal infections rates were noted in soil with low soil organic matter contents (Fig. 3).

Density of AM fungal spores and their roots colonization in maize crop of hazara area: In fertile soil of Hazara division, maximum numbers (>3333 spores kg^{-1} soil) of white spores were found in Dedal and Haripur, brown spores in Battagram and Abbottabad and black spores in Jaba 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 this area. In marginal soil, high numbers of white spores (>3333 spores kg^{-1} soil) were found in Jaba, Chamba and Darwesh soil series, brown spores in Battagram, Jaba and Dedal soil series and black spores were recorded in Chamba and Haripur soil series of Hazara division (Table 3). Root infections rates by AM fungi were also varied in maize crop from one site to another. In fertile soil of Hazara division, 32 to 42% AM fungal infection rates were noted in roots of maize crop where as 40 to 59% infection rates by mycorrhizal fungi were observed in marginal soil of this area (Table 3). Like wheat crop, higher spores numbers were found in maize roots of marginal soil, which caused increased maize roots infection rates by AM fungi in this soil. Higher AM fungal infections rates were found in soil of around neutral pH values with low soil organic matter contents.

Density of AM fungi and their roots infection rates varied in different soil types and locations. Soils, plants, root exudates, environment and their management mainly affect the mycorrhizal fungi, their development and crop root infections in an ecosystem. The most plant species in Gramineae family are normally mycorrhizal. Wheat and maize crops vary in mycorrhizal infections according to cultivars and environmental conditions (Morley and Mosse, 1976). 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, which may be resulted more sustainable and less risky agricultural production if optimum possible and favorable conditions are provided to benefit the crops by these fungi under the different potential stress conditions. Fertilizers applications may counteract super optimum AM fungal populations (Sieverding, 1990) 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 (Fig. 4 and 5).

Identification of AM fungal spores: Spores of *Glomus fasciculatum* (Fig. 7) were found in abundance in all soil samples where as spores of *Glomus intraradices*, *Glomus mosseae*, *Glomus aggregatum*, (Fig. 6) *Acaulospora melleae, Sclerocystis* and *Sclerocystis pakistanica* (Iqbal and Bushra, 1980) were also identified in the soil samples but in lower densities. Further confirmation of these spores is still required by crop inoculation, re-isolation and re-identification. Indigenous AM fungal communities generally contain several fungal species. Normally 5-15 species may be found in agro-ecosystems. The spatial distribution of AM fungal species can vary and even when the number is

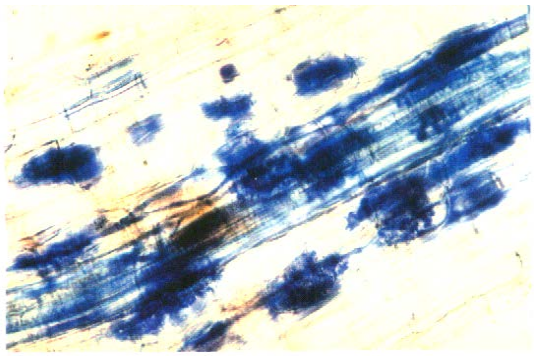

Fig. 4: Arbuscles in root cortex of wheat formed by AM fungi

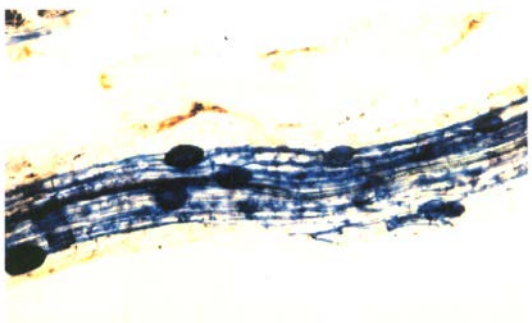

Fig. 5: Vesicles in root cortex of wheat formed by AM fungi

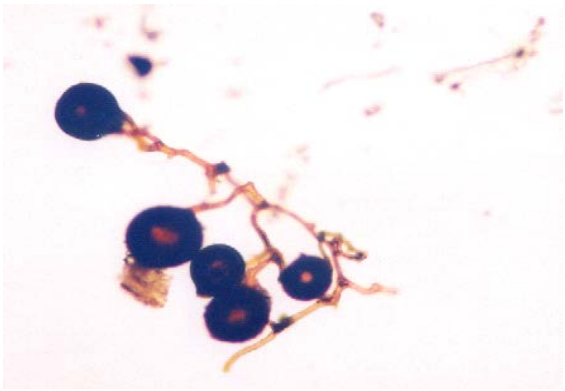

Fig. 6: *Glomus aggregatum*

Fig. 7: *Glomus fasciculatum*

the same at two different soil sites, the species composition of the fungal population can be completely different (Sieverding, 1990). The spores of AM fungi are very distinctive. They range in diameter from 10 μm for *Glomus tenue* to more than 1000 μm for some *Scutellospora* species. The spores can vary in color from hyaline (clear) to black and in surface texture from smooth to highly ornamented.

It can be concluded from these investigations that comparatively higher AM fungal spores density and their roots infection rates in wheat and maize crops were observed in marginal soil than fertile soil with varied spores density and infections intensity from one site to another under different agro-ecological conditions. Data indicated that higher AM fungal spores density caused increased roots infections intensity in wheat and maize crops. AM fungal roots infections rates were higher in

soil of around neutral pH values with low soil organic matter contents and there were almost similar trends of AM fungal density and their roots intensity in wheat and maize crops in areas under investigations.

The problems of mass production of AM fungal inoculum for field crops, their possible interactions with other soil microorganisms and management through agronomic practices to further improve their efficiency for economically feasible and sustainable crop production under different agro-ecological zones of Pakistan are needed to be focused in future research of this area.

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