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EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION IN COMBINATION WITH DIFFERENT ORGANIC FERTILIZERS ON MAIZE CROP IN ERODED SOILS

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Abstract

A pot experiment was conducted to study the effect of inoculating maize (*Zea mays* L. Azam) with Arbuscular mycorrhizal (AM) fungi in 2 different series of North West Pakistan during the year 2007. Data showed significant increase in shoots and roots yield of maize with the inoculation of AM fungi alone and in combination with farm yard manure (FYM), poultry manure (PM) and humic acid (HA) over control and N-P-K treatments. Accumulation of N by maize shoots increased significantly by the addition of HA, PM and FYM plus N-P-K with or without inoculation of AM fungi over the treatments of N-P-K and control. Plants P accumulation increased significantly over control and N-P-K treatments with the inoculation of AM fungi alone and in combination with FYM, PM and HA in missa soil series. In missa gullied soil series, significantly increased plants P accumulation was noted by the treatments of AM inoculation with PM followed by HA. Accumulation of Mn by maize shoots increased significantly with AM inoculation with HA and PM over all other treatments, Fe increased with PM, HA and FYM. Plants Cu accumulation in missa series increased significantly over control and N-P-K treatments by AM alone and in combination with PM, FYM and HA and by AM fungi with PM, FYM and HA in missa gullied series. Maximum Mycorrhizal root infection rate of 51 % was recorded in the treatment of AM fungal inoculation with HA followed by the treatment inoculated with AM fungi with FYM. In missa gullied soil series, Maximum (59 %) and significantly increased roots infection rates over all treatments were observed in the treatment of AM fungal inoculation with HA followed by PM. Spores concentrations of AM fungi increased significantly with AM inoculation alone and with FYM, PM and HA. Maximum spores numbers of 50 in 20 g soil were recorded by the inoculation of AM fungi alone and with HA.

Introduction

Nutrients availability to plants is increased as a results of microorganisms activities in soil (Alexander, 1978). Arbuscular Mycorrhizal fungi (AMF) form association between plants and fungi to colonize roots during the period of active plants growth and are distributed worldwide in the root system of most crops (Gerdeman, 1968). The AMF may improve crops yield by the increased nutrients uptake, particularly P (Tinker, 1975; Menge *et al*., 1987). Similar results have also been reported by Mamatha *et al.*, (2002), Atimanav and Adholeya, 2002 and Joner (2000). These fungi may substantially increase P availability to plants in soils fertilized with rock phosphate (Khan, 1975; Omer, 1998). Associations of AMF with plant roots can help to overcome water stress by stomata regulation (Levy & Krikun, 1980; Robert, 2001: Kevinand Peterson, 1996). Research work so far conducted in this field is often not met by indisputable and consistent results (Allen, 1995; Miller & Jastrow, 1992; Johnson & Pfleger, 1992). Information available about the incidence of AMF status in Pakistan is not sufficient till todate (Shuja *et al*., 1971; Saif, 1975; Saif & Iffat, 1976 and Saif & Parveen, 1977). Few investigators reported the association of AMF in plants (Hussain & Burni, 1993; Burni & Jabeen, 1997).

Maize crop was grown in Messa and Messa gullied soil series of eroded soil. The crop was applied with AMF and farm yard manure, poultry manure and humic acid. Wheat and maize crops rotation is very common in the area with cultivation on 8.40 and 1.04 million ha area in Pakistan and 0.72 and 1.12 million ha area in Khyber Pakhtunkhwa (KP) having total production of 21.30 and 3.11 million tones in Pakistan and 1.10 and 0.78 million tones in KP of wheat and maize, respectively (Anon., 2005-2006). Production of crops in eroded soil is poor due to removal of top fertile soil, losing organic matter and plant nutrients with consequent exposure of the sub-soil with poor fertility status. Out of the total eroded land area in Pakistan, which is about 15.22 m ha, 0.95 m ha area is situated in KPK (Chaudhry & Shafiq, 1986).

There is a lake of detailed and systematic studies in this field of study. Keeping in views the above mentioned facts, this experiment was designed to grow maize crop in eroded soils to investigate the impact of AMF alone and with different organic fertilizers on maize crop in these soils.

Materials and Methods

Pots experiment was carried out to study the scope of inoculating maize crop with AM fungi alone and in combinations with different organic fertilizers in eroded soils of KPK during the year 2007. Maize crop was inoculated with AM fungal spores isolated from fresh wheat soils of research farm of the University. Intensity of roots AM infection, spores density of soil, effects on maize yield with plant nutrients accumulations was determined. The experiment was conducted as two factors randomized complete design. The soils under investigation were sandy loam and loam in texture with pH value of 7.70, electrical conductivity as 0.35 dSm^{-1}, lime 6.10%, organic matter contents 1.16%, total nitrogen 0.123% and AB-DTPA-extractable P as 4.25 mg kg^{-1} in average.

Fertilizers at the rate of 120-90-60 kg N, P and K ha^{-1}, respectively was added to all treatments as urea, single super phosphate and potassium sulphate. Farm yard manure was used at the rate of 20 t ha^{-1} in this experiment contains 0.80% N, 0.21% P and 0.68% K, poultry manure as 20 t ha^{-1} have 3.20% N, 1.83% P and 0.83% K, where as HA as 1 kg ha^{-1} contains 4% N and 0.2% extractable P. Surface soil samples were collected at 0-15 cm depth from moderately (Missa series) and severely (missa gullied series) eroded soils on Chakdara-Kabal road and village Garru Swat area, respectively. Soil samples of 10 kg weight were filled in plastic pots and placed in CR design in three replications under natural conditions (open air). Four maize plants were grown in each pot, which were irrigated at field capacity. All cultural practices were strictly followed throughout the crop growing period. Crop was harvested after 70 days of growth. Plant samples were dried at 65-70^{o}C till constant weight and dry matter yields of maize was recorded.

Soil analysis: Physical and chemical characteristics of the composite soil sample were determined by established standard analytical procedures. Soil texture was determined by Koehler *et al*., (1984) method. pH by McClean, (1982), soil organic matter (SOM) with the method of Nelson and Sommer, (1982), total N (Bhargna & Raghupathi, 1993) and P, Cu, Zn, Fe and Mn by AB-DTPA extractable method of Soltanpour & Schawab (1977).

Plants analysis: The plant samples were oven dried at 65-70^{o}C till constant weight and the ground samples were analyzed by Walsh & Beaton, (1977) method.

Spores isolation and identification: Spores of AMF were isolated from soil by wet-sieving and decanting techniques as described by Gerdeman & Nicolson, 1963 and Brundrett *et al*., 1996. Soil was suspended in water and then passed through sieves of different sizes. Spores suspension is then centrifuged with sugar solution and the spore layer was collected in a petri dish from just above the layer of sugar solution through syringe pipe. 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 & Parez (1990).

Estimation of AM fungal infection: Infection rates by AM fungi in the roots of maize crops collected from eroded land were determined by staining the mycorrhizal chitin by Philips & Hyman (1970) and Koske & Gemma (1989) methods. Small pieces of root samples were placed in 10% KOH at 65^{o}C in such a way that roots were completely covered with KOH to clear the roots path. Root samples are then washed, cooked in 2N HCl at 65^{o}C in a water bath and stained with 0.1% trypan blue. Spores and hyphae turned blue due to their chitin content. Root pieces were then observed with magnification of 40x under the microscope. Morphological characteristics were measured by Giovannetti & Mosse, (1980) procedures.

Statistical analysis: Statistical analysis of data regarding various plants parameters, soil nutrients concentration and their accumulation by plants was done by two factorial Completely Randomized Design using MSTATC package. Combined means of the two soil series were evaluated when no significant interactions were found between soil series and treatments. Means of the two soil series were evaluated independently where significant interactions were found between the soil series and treatments. Mean values of the treatments were compared for Least Significant Difference (LSD) test at $p<0.05$ where F ratio was significant.

Results and Discussion

Soil physical and chemical Characteristics, shoots and roots dry matter yield of maize and plants nutrients accumulations are described in following sections. Plant nutrient concentrations were multiplied by dry matter yield to convert into total amount of nutrients accumulated by plants ha^{-1} (Jarrell & Beverly, 1981; Nye & Tinker, 1977; Barber, 1984).

Shoots and roots dry matter yields: These yields data recorded by the application of AM fungi alone and in combinations with different organic fertilizers are presented in Table 1. Combined means of the two different soil series were analyzed for the treatments effects as there were no significant interactions in maize shoots dry matter yield between the two soil series under investigations.

Table 1. Shoot and roots dry matter yield of maize as affected by the inoculation of AM fungi.

Treatment	Shoot dry weight			Root dry weight		
	(kg ha^{-1})					
	S1	S2	Mean	S1	S2	Mean
Control	2770	2867	2819 c*	1260 e*	687 d*	974
N-P-K	4620	3937	4279 b	1920 d	1320 c	1620
N-P-K + FYM	4827	4093	4460 b	2273 cd	1447 c	1860
N-P-K + PM	4800	4467	4634 b	2280 cd	1847 bc	2064
N-P-K + HA	5000	4000	4500 b	2107 cd	1839 bc	1973
N-P-K + AMF	6967	5300	6134 a	2360 c	2400 b	2380
N-P-K + FYM + PM	6803	5467	6135 a	2853 a	2220 b	2537
N-P-K + FYM + AMF	6933	5800	6367 a	2820 ab	2267 b	2544
N-P-K + PM + AMF	6800	5927	6364 a	2780 b	2733 a	2757
N-P-K + HA + AMF	6167	5733	5950 a	2860 a	2743 a	2802

*Means with different letter (s) in columns are significantly different at $p<0.05$

Data revealed that as compared with N-P-K treatment, shoot dry matter yield of maize showed significant increases with AMF in combination with FYM, PM and HA with no significant difference among these treatments. Addition of FYM, PM and HA with NPK without AM fungal inoculation did not increase this yield.

Roots dry matter yield of maize reacts differently with the inoculation of AM fungi in two different soil series of eroded soil. In missa soil series, roots dry matter yield of maize showed significant improvement with AMF inoculated in combination with PM, FYM and HA when compared with control and N-P-K treatments. Combined inoculation of AM fungi with FYM, PM and HA increased roots dry matter yield over the treatment inoculated with AM fungi alone. In missa gullied soil series, inoculation of AM fungi with PM and HA increased roots dry matter yield of maize significantly ($p<0.05$) over all other treatments. Roots dry matter yield of increased significantly ($p<0.05$) with the inoculation of AM fungi alone and with FYM over the treatments of N-P-K and control. Maximum roots dry matter yield of maize in both series of eroded soils was recorded with AMF and HA.

Accumulation of N and P by maize shoots: Accumulation of N and P by maize shoots as affected by the inoculation of AMF and organic fertilizers are shown in Table 2.

Table 2. Accumulation of N and P by maize shoot as affect inoculation of AM fungi.

Treatment	Plants accumulation					
	N			P		
	(kg ha^{-1})					
	S1	S2	Mean	S1	S2	Mean
Control	68.9 e*	59.6 d*	64.2	2.16 e*	2.07 e*	2.12
N-P-K	102.6 d	95.3 c	98.9	5.05 d	4.08 de	4.57
N-P-K + FYM	136.6 c	91.7 c	114.1	5.29 cd	4.50 de	4.90
N-P-K + PM	138.8 c	94.1 b	116.4	5.35 cd	5.10 d	5.23
N-P-K + HA	116.0 c	99.2 c	107.6	5.78 c	4.81 d	5.30
N-P-K + AMF	143.9 a	113.9 b	128.9	8.46 a	7.25 cd	7.86
N-P-K + FYM + PM	121.1 b	94.4 c	107.7	7.82 b	6.65 cd	7.25
N-P-K + FYM + AMF	140.3 a	119.1 a	129.7	8.48 a	8.21 c	8.35
N-P-K + PM + AMF	148.3 a	127.0 a	137.6	8.33 ab	11.37 a	9.85
N-P-K + HA + AMF	144.6 a	121.0 a	132.8	8.93 a	9.20 b	8.07

*Means with different letter (s) in columns are significantly different at $p<0.05$

Plants N accumulation by maize shoots improved by the addition of HA, PM and FYM plus N-P-K with or without inoculation of AM fungi over the treatments of N-P-K and control in missa soil series. There were no significant difference among the treatments of PM, HA and FYM applied with out AM inoculation. Similarly, treatments of AM fungal inoculation showed no significant difference among each others. In missa gullied soil series, plants N accumulations enhanced in the treatments of AM fungal inoculation with FYM, PM and HA over all other treatments. Maximum plants N accumulation was recorded in the treatment of AM fungal inoculation with PM followed by HA in this soil series.

In missa soil series, improvement in plants P accumulation was found with the inoculation of AM fungi alone and in combination with FYM, PM and HA over all other treatments except treatment of FYM + PM. Maximum P accumulation by maize shoots was observed in the treatments of AM inoculation with HA followed by FYM. In missa gullied soil series, maximum and significantly ($p<0.05$) increased plants P accumulation over all other treatments was noted in the treatment of AM inoculation with PM followed by HA.

Accumulation of Cu and Zn by maize shoots: Accumulation of Cu by maize shoots in missa series of eroded soil increased significantly ($p<0.05$) over control and N, P and K treatments by the inoculation of AM fungi alone and in combination with PM, FYM and HA, while in missa gullied soil series, Cu accumulation by maize plants increased by the combined inoculation of AM fungi with PM, FYM and HA (Table 3).

Zn accumulation by maize shoots in missa soil series increased significantly ($p<0.05$) by the inoculation of AM fungi alone and in combination with FYM, PM and HA. Maximum Zn accumulation by maize shoots was observed in the treatment of AM inoculation with PM. In missa gullied soil series, maximum and significantly ($p<0.05$) increased plants Zn accumulation was noted in the treatment of AM inoculation with HA having no significant difference with PM and FYM applied with AM fungi.

Accumulation of Mn and Fe by maize shoots: Table 4 show that Mn accumulation by maize shoots in eroded soils increased by AM fungi with HA and PM over all other treatments. Inoculation of AM fungi alone and in combination with FYM increased plants Mn accumulation when compared with the treatments of N-P-K and addition of HA, PM and FYM with N-P-K.

Table 3. Accumulation of Cu and Zn by maize shoots as affected by AM fungi.

Treatment	Cu			Zn		
	(kg ha^{-1})					
	S1	S2	Mean	S1	S2	Mean
Control	0.20 e*	0.16 d*	0.28	0.15 d*	0.12 d*	0.13
N-P-K	0.46 de	0.35 cd	0.41	0.48 c	0.32 c	0.39
N-P-K + FYM	0.52 d	0.39 cd	0.46	0.52 c	0.39 c	0.46
N-P-K + PM	0.54 d	0.48 c	0.51	0.51 c	0.49 bc	0.50
N-P-K + HA	0.60 c	0.46 c	0.53	0.53 c	0.39 c	0.47
N-P-K + AMF	0.93 b	0.73 b	0.83	0.81 b	0.68 b	0.75
N-P-K + FYM + PM	0.83 b	0.76 b	0.80	0.84 b	0.67 b	0.76
N-P-K + FYM + AMF	1.13 a	0.97 a	1.05	1.01 a	0.75 ab	0.88
N-P-K + PM + AMF	1.17 a	1.04 a	1.11	1.07 a	0.79 ab	0.93
N-P-K + HA + AMF	1.08 a	0.95 a	1.02	0.93 a	0.88 a	0.91

*Means with different letter (s) in columns are significantly different at $p<0.05$

Table 4. Accumulation of Mn and Fe by maize shoots as affected by AM fungi.

Treatment	Mn			Fe		
	(kg ha^{-1})					
	S1	S2	Mean	S1	S2	Mean
Control	1.97	0.48	1.23 e*	0.26	0.22	0.24 e*
N-P-K	3.34	3.20	3.27 d	1.53	1.34	1.44 d
N-P-K + FYM	3.79	4.05	3.92 d	2.25	1.69	5.63 bc
N-P-K + PM	7.07	7.70	7.39 c	3.34	2.84	3.09 cd
N-P-K + HA	8.30	6.25	8.28 c	4.43	2.60	3.53 cd
N-P-K + AMF	16.11	12.82	14.47 b	7.67	6.55	7.11 b
N-P-K + FYM + PM	9.99	8.68	9.34 c	3.76	5.07	4.42 c
N-P-K + FYM + AMF	13.82	13.01	13.42 b	6.84	6.33	6.59 b
N-P-K + PM + AMF	18.16	15.83	17.00 a	9.35	9.04	9.20 a
N-P-K + HA + AMF	19.11	16.87	17.99 a	6.07	9.55	7.81 ab

*Means with different letter (s) in columns are significantly different at $p<0.05$

Accumulation of Fe by maize shoots enhanced by AM fungi with PM, HA and FYM. Maximum and significantly ($p<0.05$) increased plants Fe accumulation was recorded in the treatment of AM fungal inoculation with PM, followed by the treatment of AM fungal inoculation with HA.

Roots infection intensity and spores concentration of AM fungi in maize crop: Data on maize roots infections intensity and soil spores concentration as affected by the inoculation of AM fungi alone and in combinations with different organic fertilizers are summarized in Table 5.

Table 5. AM fungal root infection intensity and spores concentration in maize as affected by the inoculation of AM fungi.

Treatment	AM infection rate			AM spores concentration		
	(kg ha^{-1})					
	S1	S2	Mean	S1	S2	Mean
	(%)			(/20 g soil)		
Control	1.97	0.48	1.23 e*	0.26	0.22	0.24 e*
N-P-K	3.34	3.20	3.27 d	1.53	1.34	1.44 d
N-P-K + FYM	3.79	4.05	3.92 d	2.25	1.69	5.63 bc
N-P-K + PM	7.07	7.70	7.39 c	3.34	2.84	3.09 cd
N-P-K + HA	8.30	6.25	8.28 c	4.43	2.60	3.53 cd
N-P-K + AMF	16.11	12.82	14.47 b	7.67	6.55	7.11 b
N-P-K + FYM + PM	9.99	8.68	9.34 c	3.76	5.07	4.42 c
N-P-K + FYM + AMF	13.82	13.01	13.42 b	6.84	6.33	6.59 b
N-P-K + PM + AMF	18.16	15.83	17.00 a	9.35	9.04	9.20 a
N-P-K + HA + AMF	19.11	16.87	17.99 a	6.07	9.55	7.81 ab

Means with different letter (s) in columns are significantly different at $p<0.05$
Arbuscular Mycorrhizal fungi (AMF)= 80 spores pot^{-1}

It is evident from the data that infections rate of maize roots by AM fungi improved by the AMF with FYM, PM and HA over all other treatments in missa soil series. Addition of HA with N-P-K and FYM + PM with N-P-K increased roots infection rates significantly ($p<0.05$) over the treatments of control and N-P-K in this soil series. Maximum root infection rate of 51% was recorded in the treatment of AM fungal inoculation with HA followed by the treatment inoculated with AM fungi with FYM. In missa gullied soil series, Maximum (59%) and significantly ($p<0.05$) increased roots infection rates by AM fungi in maize crop over all treatments were observed in the treatment of AM fungal inoculated with HA followed by PM. Inoculation of maize plants with AM fungi alone and with FYM increased roots infection rates over the treatments of N-P-K and control. Spores concentrations of AM fungi increased with AMF and with FYM, PM and HA. Spores concentration was not increased with the addition of HA, FYM and PM without AM fungal inoculation. Maximum spores numbers of 50 in 20 g soil were recorded by AM fungi alone and with HA.

The *Glomus fasciculatum* spores were maximum in all soil samples, while *G. intraradices G. mosseae, G. Aggregatum, Acaulospora mellea* and *Sclerocystis* spores were found in lower concentration. Differences in the means of AM spores concentration in the two different soil series were neglected due to no significant interactions between the two soil series.

Results indicated that inoculation of maize crop with AM fungi alone and in combination with different organic fertilizers improved shoots and roots dry matters yield, and plants nutrients accumulation. Roots infection and spores concentrations increased with the inoculation of AM fungi with different organic fertilizers. Hamel (1996) concluded that AM fungal symbiosis is recognized for its multiple positive effects on plant growth and for its contribution toward improving and maintenance of soil quality.

The enhancement in soil organic matter (SOM) content might be associated with the increased roots dry weight in these treatments. Addition of organic materials along with chemical fertilizers improve the SOM status and decreases pH values slightly (Subramanian & Kumaraswamy 1989). Jadhav & Patil (1996) reported that inoculation of plants with AM fungi has potential to increase shoot dry weight and crops grains yield along with improved N, P and K accumulation by plants. It was further concluded that AM fungi can be established in previously undisturbed and un-sterilized soil to the benefits of crops. Arihara & Karasawa (2000) found that inoculation of wheat, maize, potato and rape with AM fungi alone and in combination with different organic fertilizers in the previous season promoted roots AM infection of succeeding crops, which in turn enhanced the P uptake, crops growth and grains yield. Millner (1990) investigated that inoculation of AM fungi play a major role in plants P accumulation of most agronomically important crops. Wang *et al*., (1997) investigated that the infection of host plant with AM fungi markedly increased plants accumulation of P, Cu, Zn and Mn. Ammani *et al*., (1994) examined roots of 21 grass species and concluded that these species formed multiple mycorrhizal associations with 30 to 70% root infection rates. The dominantly found spore's density of *Glomus fasciculatum* requires further confirmation. Normally 5-15 species may be found in an agro-ecosystems (Sieverding, 1989).

Conclusion

It can be concluded from the results of this experiment that AM fungal inoculation of maize plant in combination with various organic fertilizers have the capability to increase shoots and roots dry matter yield of maize crop in eroded soils under investigations due to the improved mycorrhizal roots infection rates, soil spores density and plants accumulation of N, P, Fe, Cu, Zn and Mn. These fungi may play role as biological measure to reduce the effect of erosion on soil fertility status and increase plants P accumulation in eroded soils by improving the efficiency of P fertilizer for plant growth.

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