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Influence of Potassium Humate on Am Contamination and Rhizospheric Mycoflora of Rice (Oryza sativa L)

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Abstract
An experiment was conducted in Institute of Plant Pathology, University of the Punjab, Lahore, to assess the effect of different concentrations of Potassium humate (0, 250, 500, 750 and 1000 mg per Kg) on rhizospheric mycoflora of Basmati Rice (*Oryza sativa* L.) in field experiment. In this regard different parameters of Arbuscular Mycorrhizal infection (%age infection, number of arbuscules, number and size of vesicles, length of mycelium), number of mycorrhizal spores and percentage occurrence of soil borne pathogenic and antagonistic fungi was recorded in the rhizospheric soil of rice. The data calculated for the study of mycoflora did not exhibit any significant different in the AM colonization in roots, number of rhizospheric mycorrhizal spores and percentage occurrence of many fungi. Only *Aspergullus terreus, Rhizopus spp.* and *Aspergillus petrakii* show significant difference in their percentage occurence in different treatments. *Rhizopus spp*. decreases with the increase in the concentration of potassium humate while *Aspergillus petrakii* and *Aspergullus terreus* increased with the increase in potassium humate concentration.
Keywords: *Oryza sativa* L., rice, Potassium humate, Humic acid concentrations, Arbuscular mycorrhiza, fungi.

Introduction

Humic substances (HS) are natural organic poly electrolytes present in the soil humus and stabilized soil organic matter. These molecules have ecological importance, as they intervene in the regulation of a large number of chemical and biological processes that occur in natural ecosystems (Chen *et al*., 2004). Humic acid enhances plant growth, nutrient uptake and improves stress tolerance in plants (Serenella *et al*., 2002) Moreover it acts as disease suppressant by enhancing beneficial soil microbe activity (Manuela *et al*., 1996)**.** Humic acid enhances nutrient uptake like ^{83}Rb,^{137}Cs, ^{54}Mn, ^{65}Zn, ^{88}Y, ^{102}Rh, and ^{75}Se by rice plants and enhances its growth rate (Ozaki T. *et al.,* 2003). Potassium humate is a good source of humic acid. Its stimulation to plant growth is a function of nutrients supply to the plant. A clear significantly positive trend was seen in increasing plant height, stem diameter and root length by increasing the concentration of potassium humate (Sahar *et al.,* 2009).

Arbuscular Mycorrhizae (AM) is mutually beneficial relationship between fungi and plant roots. Colonization of roots by mycorrhizal fungi has been shown to improve growth and productivity of several field crops including legumes, cereals, vegetables and oil crops (Kapoor *et al.,* 2004; Subramanian *et al.,* 2006 & Wang *et al.,* 2006). Mycorrhizal associations increase plant growth and productivity by increasing nutrient element uptake, (Al- Karaki, 2002), and improving resistance to abiotic, (Chen *et al.,* 2006) and biotic, (Arnaud *et al.,* 1994), stress factors. Plants are benefited by the presence of mycorrhizal associates via a variety of mechanisms including improvement of soil structure, mobilization of essential minerals, enhancement of desiccation resistance, and protection from pathogens and herbivores, Smith *et al.,* (1997). It is also reported that AM also increase the crop tolerance against allelopathy and enhance crop growth under this stress (Bajwa *et al.,* 1999). Arbuscular Mycorrhizal root colonization is slightly affected by the humic acid as by increasing the concentration of humic acid in soil, the hyphal growth of *Glomus mosseae* reduces while the production of extraradical mycelium by the mycorrhizal fungus is also increased (Gryndler *et al.,* 2005). Humic acid also increases the growth and dry mass production of ectomycorrhizal basidiomycetes (Hrselova *et al.,* 2007).
This study was designed to check the effect of different concentrations of potassium humate on AM infection in roots and Mycoflora of rhizospheric soil for evaluation of humic acid whether it supports plant associated mycoflora or not.

Materials and Methods

The seeds of Basmati rice were arranged from "Punjab seed corporation", Lahore and were grown in field conditions at 25-38 ± 2°C under different concentrations of potassium humate {0 (control), 250 (T1), 500 (T2), 750 (T3) and 1000 (T4)} for the study of rhizospheric mycoflora in mature plants at the time of inflorescence. Each treatment and control had three replicates with five pots per replicate. Every pot had single plant and size of pots was 8/12" with 2 Kg soil capacity. Pots were filled with clean silt free of pebbles and stones. At the time of harvest three plants from each treatment were carefully uprooted along with rhizospheric soil and taken in laboratory in sampling bags, where rhizospheric soil and roots were separated and processed for the study of different parameters. Percentage mycorrhizal infection, no. of arbuscules and vesicles, size of vesicles and mycelium length was recorded in roots after staining while percentage occurrence of different fungi and

mycorrhizal spores was studied in soil.

The adhering soil from roots was removed with the help of camel hair brush while washing gently under the tap water. The root system of all samples was cut into 1cm^2 pieces and fixed in F.A.A. (Formaline: Acetic Acid : Alcohol in 5:5:90 ratio by volume) in properly labeled McCartney bottles separately. The samples were cleared and stained for analysis of colonization of AM fungi using Phillips and Hayman (1970) procedure. Roots were cleared in 10% KOH solution in an autoclave at 121°C, placed in 0.1N HCl for 2-3 minutes for neutralization and then stained with trypane blue solution (0.05% trypane blue powder in 100ml lactophenol). The sample pieces were mounted on the glass slides in a drop of lactic acid and were observed under low power (40X) of the light microscope. Extent of AM infection was recorded with the help of an already calibrated ocular micrometer. This was done by randomly focusing the plant material under the microscope and by measuring the hyphae. The number of vesicles and other structures were also recorded in the same way. Photography was performed at every step.

Rhizospheric soil was screened for the associated AM spores and soil borne fungi. Spore extraction was done following wet sieving and decanting method of Gerdeman and Nicolson (1967). Density and diversity of spores was recorded. Spores were identified using synoptic key by Morton (1988) and Schenck and Perez (1990). Different fungi were isolated from soil at 2% Malt extract agar medium (MEA) (Gallowey *et al.,* 1952). Different techniques were used for the isolation of the fungi, including sprinkle method {a pinch of soil (definite quantity) was sprinkled over the prepared plate of 9 cm diameter}, (Agnihothrudu, 1962), and serial dilution technique for inoculation (Bishop *et al.,* 2008). Dilution 10^{-2} was inoculated (0.5ml) on the 2% MEA plate. The inoculated plates were incubated at 25±2^{o}C temperature for 5 days and the data for the percentage occurrence and total number of each fungal colony was recorded. Isolated fungi were purified and identified with the help of Fungal Culture Bank of Pakistan, University of the Punjab, Lahore. All the data was statistically analyzed by computing Standard Error, Least Significant Difference; (LSD) and Duncan's New Multiple Range Test.

Results and Discussion

AM infection in roots

The data regarding the number of vesicles and arbuscules with different concentrations of potassium humate is shown in table 1. No significant effect of potassium humate concentrations on (arbuscular mycorrhizal) AM infection in rice was recorded. All the treatments and control showed 100% AM infection. However greater numbers of arbuscules per cm root piece were found in control (340.6) while minimum were recorded in T4 (64.4). A decrease in number of arbuscules was recorded with increase in concentration of potassium humate. While the number of vesicles tend to increase with the increase in the concentration of potassium humate as maximum number of vesicles (6.9) were found in T4 whereas minimum were found in control (0.2). The size of vesicle was found maximum in T2 and least value was found in control. The length of mycelium was observed to decrease with increase in the concentration of potassium humate. Vallini *et al*., (1993) and Gryndler *et al*., (2005) also reported the same results they depicted that Arbuscular Mycorrhizal root colonization is slightly affected by the humic acid as by increasing the concentration of humic acid in soil, the hyphal growth of *Glomus mosseae* reduces while the production of extraradical mycelium by the mycorrhizal fungus is also increased.

Percentage occurrence of spores in soil

Mycorrhizal spores/10g soil

The data regarding number of mycorrhizal spores is shown in Table 2. Number of spores in rice plant does not show any significant difference by introduction of potassium humate. AM spores separated from rice soil by wet sieving method and spores were identified by the synoptic key developed by Gerdman and Trappe. Species of *Glomus, Gigaspora* and *Acaulospora* were found. The difference among treatments means remained statistically nonsignificant however maximum spores of were found in T4 while minimum were recorded in T3. No significant difference was recorded in total number of spores isolated from the rhizospheric soil of the differect treatments and control (Vallini *et al*., 1993 & Gryndler *et al*., 2005).

Soil borne fungal spores

The data obtained from the percentage occurrence of different soil fungi indicated that most of the fungi did not affected by humic acid while few show significant difference (Siddiqui Y. *et al.,* 2009). *Aspergullus niger, Aspergullus flavus, Aspergullus aculeatus, Aspergullus terreus, Alterneria alternata, Rhizopus spp., Aspergillus petrakii, Aspergillus tamarrii* and *Fusarium soldai* were isolated from the soil. *Aspergullus terreus, Rhizopus spp.*and *Aspergillus petrakii* show significant difference in their percentage occurence in different treatments. *Rhizopus spp*. decreases with the increase in the concentration of potassium humate while *Aspergillus petrakii* and *Aspergullus terreus* increased with the increase in potassium humate concentration (Table 2).

Table 1: Effect of Potassium humate on AM infection in root and rhizospheric soil f rice (size taken at 40X)

Treatments	Control	T1	T2	T3	T4
Percentage infection (%).	100	100	100	100	100
Number of arbuscules	340.6a	226.7a	112.8a	90.1a	67.4a
Number of vesicles	0.2a	2.7a	5.3a	6.1a	6.9a
Size of vesicle (µm) [lengthxwidth]	4.55X8.75a	8.23X13.27a	15.77X23.55a	13.97X22a	12.16X20.44a
Length of mycelium (µm)	243.3a	176.05a	108.8a	107.7a	106.6a
Number of spores per gram soil					
Glomus fasiculatum	6.98	8.75	9	7.85	9.03
Acaulospora sp.	-	-	2.56	-	-
Gigaspora spp.	10.65	-	-	-	3.94
Glomus mossease	-	-	-	-	6.84
Total number of mycorrhizal spores	17.63a	8.75a	11.56a	7.85a	16.91a

Table 2: Effect of Potassium humate on percentage occurrence of different soil borne fungi in rhizspheric soil of rice

Treatments	Control	T1	T2	T3	T4
Aspergullus niger	4.18a	1.84a	1.31a	1.169a	0.29a
Aspergullus flavus	1.33a	0.79a	0.66a	1.29a	1.73a
Aspergullus aculeatus	11.28a	6.07a	4.55a	4.9a	5.51a
Aspergullus terrus	63.35b	77.35ab	81.26a	79.51a	77.53a
Alterneria alternata	5.36a	3.15a	1.77a	1.06a	0.29a
Rhizopus spp.	4.96a	1.83ab	0b	0b	0b
Aspergillus petrakii	1.34c	1.83c	2.15c	6.55b	11.01a
Aspergillus tamarrii	2.09a	2.92a	4.41a	2.52a	0.86a
Fusarium solani	6.09a	4.20a	3.87a	3.32a	2.74a

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