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Fungi Associated With Rice-Wheat Cropping System in Relation to Zero and Conventional Tillage Technologies

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Abstract: Soil mycoflora play an important role in agricultural economy of a country. Soil borne pathogen produces serious losses to the yield of a crop. In rice-wheat cropping system due to continuous cropping and reduced rotation, soil borne pathogens have become increasingly important with the yield decline. The current study was made to have the knowledge about soil borne fungi associated with rice and wheat crop in rice-wheat cropping system under zero and conventional tillage technologies. One hundred and seven fungal species belonging to 54 genera were isolated from foliar parts, roots and soil on general and specific media. Forty three genera including 59 species were isolated for the first time from rice, wheat plants and soil of rice-wheat cropping system. The fungi were categorized into four groups (pathogenic, saprophytic, toxin producing and beneficial). ***Aspergillus flavus*** was identified as only toxin producing fungus and isolated from both rice and wheat crops. ***Trichoderma*** spp were isolated from both crops and ***Paecilomyces*** spp were isolated only from wheat fields and are known as beneficial fungi. Among the pathogenic fungi ***Bipolaris sorokiniana***, ***Fusarium oxysporum*** and ***Rhizoctonia solani*** were isolated as pathogenic fungi both from rice and wheat soils. ***Alternaria trtiticina***, ***Fusarium equiseti***, *F. **grameniarum***, *F. **poae***, *F. **solani*** and ***Pythium*** sp. were isolated only from wheat fields whereas ***Nigrospora oryzae***, ***Rhizopus oryzae*** and ***Sclerotium oryzae*** were isolated only form rice fields. Total fungal colony counts isolated from soil on five specific media show some effect of zero tillage on soil mycoflora.

Key words: Fungi, rice-wheat cropping

Introduction

Wheat and rice are the main crops grown in Pakistan. The area is reported at 8463 thousand hectares for wheat and 2515.4 thousand hectares for rice for the year 1999-2000 with the production of 21078.6 thousand tones and 5155.6 thousand tones respectively (Anonymous, 1999). The main growing areas of rice and wheat are concentrated in Punjab where farmers are practicing their conventional methods in rice-wheat cropping pattern. But in current scenario zero tillage is considered as starting point in the evolution of sustainable agriculture and this technology has been introduced on experimental basis to save the time and resources. Soil borne fungi are considered major yield constraints in many agro-ecosystems, yields of cereals

are affected by reductions in emergence, plant vigour and tillering. Among these fungi, number of soil borne pathogens seriously affect the crop and cause great loss to agriculture economy (Marther and Cunfer, 1993) as ***Rhizoctonia solani*** sheath blight is now the most important fungal pathogen of rice in Bangladesh (BRRI survey data). Number of soil borne fungi like ***Cochliobolus sativus***, ***Sclerotium rolfsii***, ***Rhizoctonia solani***, ***Fusarium*** spp. ***and Gaeumannomyces gramins*** causes root rot, crown rot and sheath blight of rice and wheat but they are not reported from Pakistan (Ali ***et al.***, 1992). ***Drechslera sorokiniana*** and ***Fusarium*** spp are reported as causative agents of foot and root rot in Barani areas of Pakistan (Iftikhar and Aslam, 1987). Recently ***Fusarium*** spp. and ***Helminthosporium*** spp. have been isolated as pathogens of root rot and ***Alternaria*** sp and ***Helminthosporium*** sp as pathogens of foliar spots in main rice- wheat cropping areas of Punjab (Iram ***et al.***, 1999).

Three years study has been conducted to gain the knowledge of fungi associated with Rice-Wheat cropping system including pathogens and associated competitors/antagonists under resource conservation technologies.

Materials and Methods

Sampling

Two sites were selected in main rice-wheat cropping areas at Muridke and Bhalwal in Punjab with two farmers at each site and each farmer having one field with conventional tillage and one with zero/minimum tillage. Sampling was done at maturity stage of rice crop in September/October and wheat crop in March.

Sampling was done in selected fields at 10 points along a diagonal transit as in IRRI protocols (Bridge ***et al.***, 1999). All points have 5 paces apart, starting 10 paces into the field. From each sampling site plant include tillers, roots and soil were dug down to a depth of 20 cm by using the narrow bladed sampling tool. Soil adhere to the roots was left intact and plants were placed in polythene bag (one plant/ bag) and brought to the laboratory for further analysis.

Isolation of fungi from plant parts

Pieces of leaves with spots and lesion on root were cut and surface sterilized in 1% Clorox (commercial NaOCl) solution for 1 min then rinsed in sterilized distilled water. Pieces were plated on potato dextrose agar medium (PDA) after drying on sterilized filter paper. Five pieces were placed in 9 cm size Petri plates and incubated for 7 days at 22°C. Fungal cultures isolated from root and foliar portions were transferred on PDA slants for further study. Slides were prepared in lactophenol and cotton blue stain and examined under stereo and light microscope.

Isolation of fungi from soil

A portion of adhered soil (approx. 100 cm^3) was manually removed from roots and all ten soil samples were mixed to form one composite sample. One gram of soil from composite sample was used for preparation of suspension (= 10^{-1} dilution) of each sample by placing in 9 ml sterilized distilled water in a sterile universal tube. The tube was caped tightly and shook for 30 min. Similarly 2nd dilution (=10^{-2} dilution) was prepared by taking 1 ml from first 10^{-1} dilution and

added it to a fresh 9 ml of diluent and shook well and in similar fashion the Third dilution (10^{-3}) was prepared.

The 10^{-2} and 10^{-3} dilution were used for inoculation on five different media including MEA (Malt extract agar), MEA+ Benomyl, MEA+ Rose Bengal, TW+ actidione-Keratin bait and TW+ hemp seed bait. Inoculation was done by adding 0.1 ml of suspension and spread immediately using a sterile L - shaped spreader. Plates were sealed with tape and incubated at 22 ± 2°C. Observations were made daily and slides were prepared from mature fungal colonies and identified.

Results and Discussion

Total one hundred and seven fungal species belonging to 54 genera were isolated from foliar parts, roots of plants and soil of rice and wheat cropping system in zero and conventional tillage. These fungi are grouped as pathogenic, beneficial, toxin producing and saprophytic (Fig. 1). Out of which 12 pathogenic genera including 16 species were isolated from plant parts and soil of rice-wheat cropping system during year March, 2000 to October, 2002. Six pathogenic genera having 7 species were isolated from wheat under zero tillage and 8 genera including 11 species under conventional tillage, whereas, 5 pathogenic genera were isolated from rice crop under zero tillage and 8 genera in conventional tillage. Four pathogenic genera (***Bipolaris sorokiniana***, ***Fusarium oxysporum***, ***Phytophthora megasperma*** and ***Rhizoctonia solani***) were common in wheat and rice. Blight causing fungus ***Alternaria triticina*** was isolated from stem of wheat in conventional tillage field. Pathogen is seed and soil borne and survives on host residue in soil. *A. **padwickii*** was isolated from plant part of rice. Fungus is seed borne and with other fungal genera causes discolouration of grains, seed rotting, lesions on roots, seedling blighting in water seeded rice. ***Bipolaris sorokiniana*** is major pathogen of cereal crops all over the world (Hill ***et al.***, 1983; Diehl, 1979; Fedle-Moen and Harrison, 1987) also causes leaf spot in favourable conditions. Pathogen was mainly isolated from foliar parts and roots of wheat and also from rice plants under both technologies (Table 1). It was isolated from soil during wheat season where conventional method was practiced. ***Bipolaris*** infects wheat root, leaves and seedlings. The frequent isolation from plant parts is due to its seed and soil borne nature. ***Fusarium*** spp. are responsible for head scab and root rot in cereals while ***Fusarium*** spp including *F. **graminearum***, *F. **oxysporum***, *F. **solani*** with *Drechslera. **sorokiniana*** are important in foot and root rot syndrome of wheat (Diehl, 1979; Iftikhar ***et al.***, 1991). ***Fusarium gramenearum*** was isolated from plant parts under both technologies in rice crop and from wheat roots under conventional till. Other Fusarium species including *F. **oxysporum and F. poae*** were isolated from plant parts in rice and wheat crop (Table 1). ***Fusarium poae*** was also identified among fungal pathogens isolated from soil under zero tillage practice in wheat season. ***Fusarium solani*** was isolated from soil during wheat season where conventional tillage was practiced. ***Cephalosporium gramineum*** is soil borne only true vascular pathogen in wheat which causes Cephalosporium stripe to wheat. It was isolated from foliar parts of wheat irrespective of technologies. ***Nigrospora*** species are mainly saprophytes but attack when plants become weak by disease, insect, pests or poor nutrition. ***Nigrospora oryzae***, which is a weak pathogen of rice and infects the leaves and leaf sheaths, was also isolated from rice leaves in both zero and conventional tillage and from soil in zero tillage

Table 1: Groups of fungi isolated from plant parts and soil of rice wheat cropping system in relation to conventional and zero tillage technology during March 2000 to March 2002

		Crop							
		Rice				Wheat			
		C-T		00-T		C-T		0-T	
Group	Name of fungi	Plant parts	Soil	Plant parts	Soil	Plant parts	Soil	Plant parts	Soil
Pathogenic	*Alternaria triticina*	-	-	-	-	+	-	-	-
	A. padwicki	+	-	-	-	-	-	-	-
	Bipolaris sorokiniana	+	-	+	-	+	+	+	-
	*Cephalosparium grameneum**	-	-	-	-	+	-	+	-
	Cochliobolus miybeanus	+	-	+	-	-	-	-	-
	Fusarium gramenearum	-	-	-	-	+	-	-	-
	F.oxysparum	+	-	-	-	+	-	+	+
	*F. poae**	-	-	-	-	-	+	-	-
	F. solani	-	-	-	-	+	+	+	-
	Nigrospora oryzae	+	-	+	+	-	-	-	-
	*Pythium sp**	-	-	-	-	+	-	-	-
	*Phytophthora megasperma**	-	+	+	-	+	+	+	+
	Rhizoctonia solani	+	+	-	-	+	-	+	-
	Rhizopus oryzae	-	-	-	+	-	-	-	-
	Scleratium oryzae	+	-	-	-	-	-	-	-
	*Verticillium sp**	-	-	-	-	+	-	+	-
Beneficial	*Paecilomyces inflatus**	-	-	-	-	-	+	-	+
	Paecilomyces sp	-	-	-	-	+	+	+	+
	*Trichoderma viride**	-	-	-	-	-	+	-	-
	Trichoderma sp	-	-	-	-	+	+	-	+
Toxin producing									
	Aspergillus flavus	+	+	+	-	+	+	+	+
Saprophytic	*Acremanium fusidioides**	+	+	+	+	+	+	+	+
	*Acremanium sp**	+	+	+	+	+	+	+	
	*Acrophialophora fusispara**	-	-	-	-	-	+	-	+
	Acrophialophora sp*	-	-	-	-	-	+	-	+
	Alternaria alternate	+	-	-	-	-	-	-	-
	*A. dianthicola**	-	-	-	-	-	-	+	-
	A. longipes	-	-	-	-	+	-	-	-
	Alternaria sp	+	-	+	-	+	+	+	+
	A. tenussima	-	-	-	-	-	+	-	-
	*Amylosporium sp**	-	-	-	-	-	+	-	-
	*Arthrobotrys arthrobotryoides**	+	-	-	-	-	-	-	-
	Aspergillus condidus	+	+	+	+	+	+	+	+
	A. fumigatus	-	-	-	-	-	+	-	+
	A. niger	+	+	+	+	+	+	+	+
	Aspergillus sp	+	+	+	+	+	+	+	+
	A. terreus	-	-	-	-	-	+	+	+
	Aureabasidium sp*	-	-	-	-	-	+	-	-

Table 1: Continue

		Crop							
		Rice				Wheat			
		C-T		00-T		C-T		0-T	
Group	Name of fungi	Plant parts	Soil	Plant parts	Soil	Plant parts	Soil	Plant parts	Soil
	Basipetospora sp*	-	-	-	-	-	+	-	-
	Bipolaris sp*	-	-	-	-	-	-	+	-
	Botrytis sp*	-	-	-	-	-	+	-	+
	Cephalosporium sp	-	-	-	-	-	-	-	+
	Chaetomium globosum	+	+	+	+	+	+	+	+
	Chaetomium.sp	+	+	+	+	+	+	+	+
	Chrysosporium sp*	-	-	-	-	-	+	-	-
	*Cladosporium chlorocephalum**	+	+	+	+	+	+	+	+
	C . cladosporioides	+	+	-	+	+	+	+	+
	*C. gallicola**	-	-	+	-	-	-	-	-
	C. herbarum	-	-	-	-	-	-	-	-
	Cladosporium sp	+	+	+	+	+	+	+	+
	*Cochliobolus lunatus**	-	-	-	-	-	-	-	+
	Coniothyrium sp*	+	+	+	-	+	+	-	+
	*Corynascus sepedonium**	-	-	-	-	-	-	-	+
	Curvularia lunata	+	+	+	+	+	+	+	+
	C. penniseti	-	-	-	-	-	-	-	+
	Curvularia sp	+	+	+	+	+	+	+	+
	C. tuberculata	+	-	+	-	-	-	-	-
	*Cylindrocarpon sp**	-	-	-	-	-	+	-	-
	*Doratomyces stemonitis**	-	-	-	-	-	-	-	+
	Drechslero halodes	+	-	+	-	+	+	+	+
	*D. pedicellata**	-	-	-	-	+	-	+	+
	Drechslera sp	-	-	-	-	-	+	-	-
	D. spicefer	-	-	-	-	-	-	-	+
	*Emericella rugulosa**	-	-	-	-	-	-	-	+
	Emericella sp	-	-	-	-	-	-	-	+
	*Epicoccum nigrum**	-	-	-	-	+	-	+	+
	*Fusarium sacchari**	+	+	+	+	+	+	+	+
	Fusarium sp	+	+	+	+	+	+	+	+
	*F.udum**	-	-	-	-	-	+	-	-
	*Gabarnaudia betae**	-	-	-	-	-	-	-	+
	*Geotrichum candidum**	-	+	-	+	-	-	-	-
	Gliocladium sp	-	+	-	-	-		-	-
	Helminthosporium sp	+	+	+	+	+	+	+	+
	*H. tetramera**	-	-	-	-	+	-	+	-
	Humicola sp*	+	+	+	+	+	+	+	-
	*Hymenopsis trochiloides**	-	-	-	-	-	-	-	+
	*Macrophamina phaseolina**	-	-	+	-	+	+	-	+
	Micromucor sp*	-	-	-	-	-	+	-	+

Table 1: Continue

		Crop							
		Rice				Wheat			
		C-T		00-T		C-T		0-T	
Group	Name of fungi	Plant parts	Soil	Plant parts	Soil	Plant parts	Soil	Plant parts	Soil
	Monilia sp*	+	+	-	+	+	+	+	+
	Monocillium sp*	+	+	+	+	+	+	+	+
	Monosporascus sp*	-	-	-	-	-	-	+	-
	Mortierella sp*	-	-	-	-	+	+	+	+
	*Mucor genevensis**	+	+	+	+	+	+	+	+
	*M.hiemalis**	-	-	-	-	-	-	-	+
	Mucor sp	+	+	+	+	+	+	+	+
	Nigrospora sp	-	-	-	+	+	+	+	+
	*N. sphaeria**	-	-	-	-	-	+	-	-
	*Penicillium aurantiogriseum**	-	+	-	+	+	+	+	+
	*P. chrysogenum**	-	-	-	-	-	-	+	-
	P. oxalicum	-	-	-	-	-	-	-	+
	*P.pinophilum**	-	-	-	-	-	+	-	+
	*P. raquefortii**	-	-	-	-	-	+	-	-
	Penicillium sp	-	+	-	+	+	+	+	+
	Periconia sp*	+	-	-	-	-	-	-	-
	*Phoma nebuloso**	-	-	-	-	+	-	-	+
	*P. pereupyrena**	-	-	-	-	+	-	-	-
	Phoma sp	-	-	-	-	-	-	-	+
	*P. sorghina**	-	-	-	-	-	-	-	+
	*Phytophthora infestans**	+	-	-	+	+	+	-	+
	Rhizopus sp	-	+	-	+	+	+	+	+
	Scopulariopsis sp*	+	-	+	-	-	+	+	-
	*Setosphoeria rastrata**	+	-	+	-	-	-	-	-
	*Stachybotrys bisbyi**	+	+	-	+	+	+	-	+
	*S. elegans**	-	-	-	-	-	+	-	+
	Stachybotrys sp*	+	+	-	+	+	+	-	+
	Stemphyllium sp	-	-	-	-	+	-	+	-
	Ulocladium sp	-	-	-	-	+	-	-	-

* New record

application. It was also isolated from soil in rice season. Brown spot of rice is caused by ***Cochliobolus miybeanus*** (anamorph ***Bipolaris oryzae***) and was isolated from rice foliar parts. Among zoosporic fungi, ***Pythium*** sp was isolated from roots of wheat in conventional fields. This seed borne fungus helps in seedling blight and mainly cause root rot of rice. Another water loving fungus, ***Phytophthora megasperma*** was isolated from plant parts and soil of rice-wheat cropping system (Table 1). The species of this fungus causes water mold to rice crop. Being a pathogen to both rice and wheat, needs to have pathogenicity test. ***Macrophomina phaseaolina*** is soil borne fungi and pathogenic to most of the crops but not reported as a pathogen of rice and wheat crop, it was isolated in low

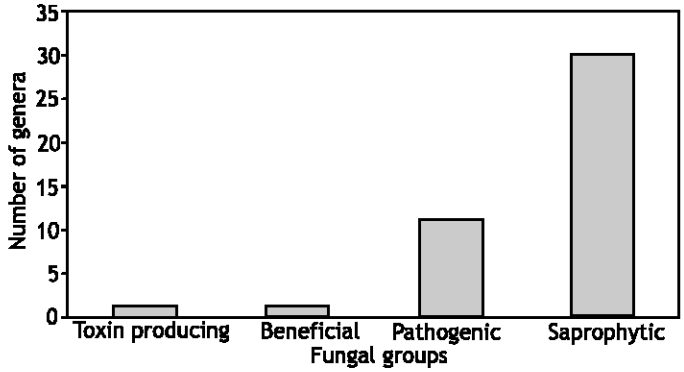

Fig. 1: Different Groups of Fungi associated with Rice wheat cropping system

frequency from roots of rice plants only under zero tillage. ***Rhizopus*** spp. is common saprophyte worldwide but in some regions in association with other fungi causes the discoloration of seeds in rice.

A soil borne fungus ***Rhizoctonia solani***, the cause of sheath blight and sheath spot in rice and root rot in wheat, was isolated from both rice and wheat plant parts and from soil during rice season. ***Sclerotium oryzae***, which causes stem rot in rice, was isolated from roots of rice under conventional tillage practice. ***Verticillium*** sp was isolated from stem of wheat plants in both practicing technologies. Its isolation from wheat leaves showing a new finding. Beneficial fungus ***Paecilomyces*** spp (***Paecilomyces inflatus*** and ***Paecilomyces*** sp) and ***Trichoderma*** spp (***Trichoderma viride*** and ***Trichoderma*** sp) were isolated from plant parts of wheat in zero as well as in conventional tillage. The isolation of toxin producing fungus ***Aspergillus flavus*** was very common from plant parts and soil in whole system (Table 1).

Forty-eight fungal genera having 86 species were identified as saprophytes which were isolated from plant parts and soil of the rice-wheat cropping system under conventional and zero tillage practices. During this study 43 genera of 59 species were recorded for the first time from rice-wheat cropping system in relation to zero and conventional tillage (Table 1).

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