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Effect of tillage practices in rice-wheat cropping system on diversity of soil inhabiting mycoflora of calcareous soil of Bihar

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ABSTRACT

Fungal diversity of soil under contrast tillage practice with and without residue incorporation in rice-wheat cropping system of calcareous soil was studied. Soil samples were collected from rice-wheat cropping system using dilution plate technique and compared with the distribution of fungal propagules in different treatments. out of twenty-two principal genera isolated and identified, Aspergillus and Penicillium were the most frequent followed by Fusarium, Cladosporium, Alternaria, Trichoderma, Curvularia and Epicoccum. Community structure indicates conventional tillage system without brown manure as supportive to fungal diversity, richness, evenness etc. Data indicates that different agricultural practices impact soil inocula of beneficial, alkalophilic, thermophilic and saprophytic fungi in their production area. Conservation tillage with brown manure was augmentive towards selected group of fungi and support more number fungal population to conventional till system.

Key words: *Fungal diversity, community structure, conventional and conservation tillage system.*

Shrinking culturable land, nutrient sucking cropping pattern, intoxication of rice-wheat cropping system with nitrogenous fertilizer coupled with decreasing crop productivity are the concomitant problems of Indian subcontinent. Tillage induced shift in agro-ecosystem is gaining importance to influence the soil mycoflora, who regulates the natural ecosystem by recycling the soil organic matter. The primary effect of tillage is to disturb the soil profile that resulted in differential reaction by mycoflora inhabiting to it. The soil microbial community has not been well characterized in agro-ecosystem (Wardle, 1995), particularly in conventional tillage (CT) and no tillage (NT) system which are the prominent management practices (Frey *et al,* 1999), in different parts of the world. Fungi play an important role in soil ecosystem as major decomposer of plant residue, releasing nutrient that sustain and stimulate plant growth in the process. Saprophytic fungi appeared to play a major role in regulating the decomposition of residue in no-till system; fungi also appear to contribute more to the formation and stabilization of aggregates in no-till compared to tilled soil (Beare *et al,* 1992). Interactions among different fungal (thermotolarent, alkalophilic, toxin producing *etc.*) groups can have either beneficial or detrimental effect on crop. Greater understanding of pattern of fungal distribution could lead to more precise management of these mycoflora for beneficial purposes in agriculture. The present paper deals with the effect of conservation and conventional tillage practices in rice-wheat cropping system with and without brown manure on soil mycofloral distribution and diversity in calcareous soil of Bihar. Apart from measuring the similarity in fungal occurrence in different system, they are also grouped according to their qualitative traits. This investigation will certainly enrich the knowledge of the researcher to plan their operational practices towards enhancing the beneficial organism in rice-wheat cropping system.

MATERIALS AND METHODS

The study area is situated on the flood prone zone of southern-western bank of river Buri Gandak (25^{o}-59'N; 84^{o}-48'E) with calciorthent soil (free $CaCO_3$,- 28.2 percent); soil contains organic carbon (0.38percent), P(4.1ppm), K(46ppm), pH(8.3), EC($0.73 dSm^{-1}$), Zn(0.64 mg kg^{-1}), Cu(1.38 mg kg^{-1}), Fe(6.34 mg kg^{-1}), and Mn(3.68 mg kg^{-1}). The experiment was conducted at research farm of Rajendra Agricultural University, Pusa, Bihar in north-eastern part of India, under conservation and conventional tillage with and without brown manure in randomised block design during 2007-08. Brown manuring was done with *Sesbania* which was grown in half of each plot and killed after 30 days of sowing by 2, 4D. The sampling was done after the harvest of rice and wheat crop in October and March (2007-2008) respectively. Sampling was done in selected fields at ten points along a diagonal transit as in IRRI protocols (Bridge *et al,* 1999).

Soil sample collected from different points were mixed to form one composite sample of respective tillage group. One gram of soil from composite sample was used for preparation of ($=10^{-1}$ dilution) of each sample by placing 9 ml sterilised distilled water in a sterile conical flask. The mixture was then shaken and diluted to final concentration of 10^2, 10^3, 10^4. From fourth dilution, 0.1 ml was used for inoculating on malt extract agar (MEA), rose bengal agar (RBA) and sterile water containing hemp seed wait at $25\pm2^{o}C$ for 15 days. The colonies were transferred to test tubes with PDA (potato dextrose agar). Each isolates were identified to genera and if possible to species level with literature available. Thermo-tolerant fungi were grown on MEA at $40\pm2^{o}C$ for 15 days, beneficial and toxin producing fungi were characterized on the basis of character cited in different literature after growing them individually on MEA, PDA and RBA media. Alkalophilic fungi were isolated on alkaline corn meal agar (ACMA) media by dilution plate technique (Nagai *et al.,* 1995). ACMA was prepared with solution A (17g of CMA powder, 900ml of distilled water) and solution B (3g of Na_2CO_3, 3g of $NaH_2PO_4.2H_2O$, 100ml of distilled water). After sterilization; 900ml of solution A and 100ml of solution B were mixed. The final pH of the mixture was about 9.7. The fungus was isolated after 1-3 wk of incubation at $25\pm2^{o}C$.

The following indices were used to measure the diversity which is an important tool for biologist to quantify the mycoflora.

The isolation frequency (Fr) of genera was calculated according to Gonzalez *et al.* (1995)

Frequency (%) =

$$\frac{\text{number of sample with species or genus}}{\text{Total number of sample}} \times 100$$

Species richness (S) = The total number of species in the community.

Simpson's dominance index (D) = $1-\sum_{i=1}^{S}(p_i)^2$

This was calculated for each treatment based on relative proportion of species to total number of isolated fungal isolates.

Shannon's diversity index (H') = $-\sum_{i=1}^{S} p_i . \log_2(p_i)$

Where p_i is the proportion of species i that contribute to total diversity.

Sorensen's similarity index (SI) = 2j / (a+b)

Where j is the number of species common to both seasons/ sample type and a is the number of species in tillage type I/ sample type A, b being the number of species in tillage type II/ sample type B (this index is equal to 1 in case of complete similarity and 0 if they have no species in common).

RESULTS AND DISCUSSION

This study compared fungal population at genera level in conservation and conventional tillage with and without residue incorporation in high pH soil using community structure analysis. The results indicate that the mycofloral population was different under tillage practices in Rice-Wheat cropping system. CTW (+B) and C_OTR (-B) had the highest (129) and lowest (95) numbers of filamentous fungi, respectively. Higher fungal population in conservation than conventional tillage system, suggest that fungal population responded to the management practice and organic matter incorporation. It is well established that agricultural management practices can affect soil chemical properties and the number and activity of numerous soil micro-organism (Lowlor *et al*., 2000; Balota *et al*., 2004). The decrease in the efficiency of the use of carbon sources by soil micro-organisms under CN, as evident by greater carbon dioxide evolution per unit microbial biomass under CN compared with NT system (Hayness, 1999). The presence of 22 principal genera including *Absidia, Alternaria, Aspergillus, Cladosporium, Curvularia, Epicoccum, Fusarium, Geotricum, Drechslera, Humicola, Mortierella, Monilia, Nigrospora, Penicillum, Pestolatia, Rhizoctonia, Mucor, Trichoderma, Chaetomium, Paecilomyces, Pythium, Torula* were recorded, considering the frequency of genera defined as percentage of sample in which each genera was present (Table 1).

Aspergillus and *Penicillium* were the most frequent under rice-wheat cropping system, being the greatest in conservation tillage rice and wheat with brown manure while fall was recorded in conventional tillage rice and wheat without brown manure. *Fusarium* and *Cladosporium* were more frequent in all the treatments followed by *Alternaria, Trichoderma, Curvularia* and *Epicoccum*. *Paecilomyces* with lower frequency was recorded in all the treatments. *Geotrichum* and *Mortieriella* were found to be associated only with C_OTR (-B) with higher frequencies with both the systems. *Pestolatia, Torula* and *Rhizoctonia* were nearly uniformly distributed in all the tillage practices. Genera of *Pythium* and *Nigrospora* were recorded only with Rice ecosystem. Occurrence of *Dreschslera* and *Humicola* were almost identical with minor exception to *Humicola,* which was not recorded in case of C_OTW (-B). Increase in fungal abundance in conventional tillage rice to conventional tillage wheat, reflects influence of weather parameter on fungal colonization. Fungal abundance in soil at site along the precipitation gradient increased from the driest to wettest (Frey *et al.*, 1999).

Table 1. Frequency percent of Genera recovered from different tillage operations.

Genera	CTR(+B)	C_OTR(-B)	CTW(+B)	C_OTW(-B)
Absidia	0	2.10	0	1.88
Alternaria	2.97	4.21	3.10	4.58
Aspergillus	26.73	16.84	27.90	19.26
Cladosporium	6.93	5.26	3.87	2.75
Curvularia	3.96	2.10	3.10	1.83
Epicoccum	2.97	2.10	1.55	1.83
Fusarium	6.93	9.47	6.97	10.09
Geotricum	0	2.10	0	2.75
Drechslera	1.98	2.10	0.77	0.91
Humicola	1.98	1.05	0.77	0
Mortierella	0	3.15	0	2.75
Monilia	0	2.10	0.77	3.66
Nigrospora	1.98	1.05	0	0
Penicillium	22.77	16.85	17.05	9.17
Pestolatia	0.99	1.05	1.55	0.91
Rhizoctonia	0.99	1.05	0	0.91
Mucor	0	2.10	1.55	1.83
Trichoderma	4.95	4.21	1.55	2.75
Chaetomium	1.98	4.21	0	1.83
Paecilomyces	1.98	1.05	1.55	0.91
Pythium	0	1.05	0	0
Torula	0.99	1.05	1.55	0.91
N	101	95	129	109

N= total no. of isolated fungi; CTR(+B)= conservation tillage rice(with brown manure); C_OTR(-B)= convention tillage rice(without brown manure); CTW(+B)= conservation tillage wheat(with brown manure); C_OTW(-B)= convention tillage wheat(without brown manure).

Table 2 represents the highest (3.45) diversity index in C_OTR (-B) followed by CTR (+B), C_OTW (-B) and CTW (+B). Highest (0.92) Dominance index was recorded with C_OTR (-B) while least (0.85) in CTR (+B). Evenness index of conventional tillage rice and wheat without brown manure were similar (0.15) alongwith higher values of richness and dominance index to conservational tillage rice-wheat with brown manure. On the basis of species composition, Sorensen's similarity index showed higher values in conventional tillage rice-wheat without brown manure (0.67), followed by conservation and conventional tillage rice with and without brown manure (0.66), conservation and conventional tillage wheat with and without brown manure (0.65). The most different were conventional rice and conservation wheat (0.57) and conservation rice and conventional wheat (0.50). The higher (r =0.99)

Table 2. Community structure of soil mycoflora under different tillage practices.

Cropping pattern	Diversity index	Dominance index	Evenness index	Richness index
CTR(+B)	2.98	0.85	0.18	16
C_OTR(-B)	3.45	0.92	0.15	22
CTW(+B)	2.43	0.88	0.16	15
C_OTW(-B)	2.98	0.93	0.15	19

N= total no. of isolated fungi; CTR(+B)= conservation tillage rice(with brown manure); C_OTR(-B)= convention tillage rice(without brown manure); CTW(+B)= conservation tillage wheat(with brown manure); C_OTW(-B)= convention tillage wheat(without brown manure).

values of correlation coefficient indicates that there was similar trend of fungal distribution in conventional tillage rice-wheat without brown manure. The strong (r=0.84) correlation also existed between the richness and dominance index, calculated to know about the relation between the two indices used for studying the fungal population dynamics in rhizosphere of pigeonpea crop. Diversity, dominance, evenness and richness were higher in conventional tillage to conservational. This probable due to redistribution of older C and N, applied fertilizer and fungal resting structure *vis-à-vis* micro-climate of the soil through ploughing. Consequently, conventional tillage enhanced diverse fungal groups whereas; conservation tillage was prone towards selective groups of fungi. Change in microbial biomass may be indicative of future change in amount of SOM since the microbial community respond rapidly to the disturbance or altered residue input (Gupta *et al.*, 1994).

Except, cellulose decomposing *Chaetomium globosum* and *Monilia* sp. CTW(+B)was devoid of all the other beneficial fungi in conventional and conservation tillage system with and without brown manure in rice-wheat cropping system (Table 3). Toxigenic fungi, *Aspergillus niger* and *A ochraceous*

Table 3. Different group of fungi isolated and identified from rice-wheat system.

Group	Fungal species	CTR (+B)	C_OTR (-B)	CTW (+B)	C_OTW(-B)
Beneficialfungi	*Trichoderma viride*	+	+	-	+
	T. harzianum	+	+	-	+
	Trichoderma sp.	+	+	-	+
	Paecilomyces sp.	+	+	-	+
	Chaetomium globosum	+	+	+	+
	Monilia sp.	-	+	+	+
Toxigenic Fungi	*Aspergillus flavus*	+	+	+	+
	A. ochraeceous	+	+	+	+
	A. parasiticus	+	-	+	-
Thermophilic fungi	*A. niger*	+	+	+	+
	A. tamarii	+	-	+	-
	A. fumigatus	+	+	-	-
	Humicola gresea	+	+	+	-
	Mucor sp.	-	+	+	+
Alkalophilic fungi	*Alternaria* sp.	+	+	+	+
	Penicillium sp.	+	+	+	+
	Fusarium oxysporum	+	+	+	+
	F. solani	+	+	+	+
	Aspergillus sp.	+	+	+	+
Saprophytic fungi	*Pythium* sp	-	+	-	-
	Pestolatia sp.	+	+	-	+
	Torula graminis	+	+	+	+
	Drechslera oryzae	+	+	+	+
	Epicoccum nigrum	+	+	+	+
	Alternaria alternata	+	+	+	+
	Curvularia lunata	+	+	+	+
	Cladosporium cladosporioides	+	+	+	+
	Geotrichum candidum	-	+	-	+
	Mortierella alliacea	-	+	-	+
	M. vinacea	-	+	-	-
	Nigrospora sphaerica	+	+	+	-
	Aspergillus sydowii	+	+	+	+
	A. flavipes	+	+	+	+
	A. terreus	-	+	+	-
	A. luchuensis	-	+	+	-

N= total no. of isolated fungi; CTR(+B)= conservation tillage rice(with brown manure); C_OTR(-B)= convention tillage rice(without brown manure); CTW(+B)= conservation tillage wheat(with brown manure); C_OTW(-B)= convention tillage wheat(without brown manure).

were isolated from both the system whereas *A parasiticus* was recorded only once from conservation system. Distribution of thermophilic fungi was erratic in studied ecosystem except *Aspergillus niger*. Alkalophilic fungi showed homogeneous distribution in rice-wheat cropping system irrespective of cropping system and residue incorporation. Dematiaceous fungi of saprophytic group were rather more uniformly distributed in rice-wheat cropping system. List of isolated saprophytic fungi was much higher to beneficial, toxigenic and thermophilic fungi. Saprophytic fungi appeared to contribute more to the formation and stabilization of aggregates in soil (Beare *et al.,* 1992).

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