

Soil inhabiting fungi and nematodes under rice-wheat cropping system

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ABSTRACT

Several pathogenic and non-pathogenic fungi, bacteria and nematodes dwell in the soil and thereby influence the crop growth. Under Rice-Wheat cropping system, use of pesticides in wheat is relatively less as compared to rice. Present studies on soil biological aspects were undertaken under Rice-Wheat rotation. *Trichoderma viride* was isolated from DWR experimental as well as farmers' zero tilled wheat fields. *Bipolaris sorokiniana* was obtained from farmers' fields under rice-wheat system. *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Penicillium* sp, *Mucor* sp, *Bipolaris sorokiniana*, *Alternaria alternata*, and *Fusarium* sp, have been found at various growth stages of the rice and wheat crops. Various treatments applied in rice season have shown to affect mycoflora and nematodes in wheat season. As observed, bioagent population (colony forming units) was very low and negligible at the time of sowing of wheat. The beneficial fungus, *Aspergillus niger* was present at both the growth stages while *T. viride* was available only at ear head emergence stage. Among nematodes, *Tylenchorhynchus* was present in very high numbers in all the three farmer's fields and its highest population was recorded in the month of April 1997. The population of *Helicotylenchus* was more or less at equilibrium in all the treatments. The highest population of *Hoplolaimus* was recorded in October 1997 whereas of *Tylenchorhynchus* and *Helicotylenchus* in April 1998. All the nematodes showed increasing trends from early January/late December 1997 onwards. Soil biota of rhizosphere colonize the root system and act as growth promoters besides being biocontrol agents against plant diseases and nematodes. Colonization of plant root system can lead to reduced pathogen attack directly or indirectly through production of antimicrobial substances and posing competition for space and nutrients as well as through induction of systemic resistance.

Key words: soil inhabiting fungi, Rice-Wheat system, soil nematodes

Soil, being an important component of the agricultural system, is likely to play a vital role in crop production and root health. Several of the pathogenic and non-pathogenic organisms, like fungi and nematodes dwell in the soil and influence the crop growth in the field. The wheat system is relatively free from the use of pesticides, while the other crops in the rotation get a lot of pesticides in the form of weedicides, insecticides and fungicides. All these factors ultimately influence the soil biota and root health (Singh *et al.* 2002; Bochus and Shroyer 1998; Dill-Mackey and Jones 2000). Not much information is available on the status of these soil-inhabiting biota under different cropping systems. Only limited information on rice-wheat system has been published on these aspects during the recent past (Sharma and Nagarajan 2000; Dabur *et al.* 2002; and Singh *et al.* 2002). Keeping these factors in view, studies were conducted on population dynamics of soil-borne fungi and nematodes under rice-wheat rotation.

MATERIAL AND METHODS

The studies were carried out during three crop seasons. In the first phase, preliminary analysis of soil borne fungal species was done from a wheat field under rice-wheat rotation during 1995-96 and 1996-97 crop seasons and variety used was HD 2329. Samples collected from the farmers' fields in the districts of Kaithal and Karnal were also analyzed. In all, 63 soil samples were analyzed at seedling stage of the crop and of these, 12 were from the farmers' fields. In the second phase, rice was planted under eight different planting conditions during 1997-98 and 1998-99

while wheat was sown under conventional system during the following rabi seasons. Soil samples were drawn at various growth stages during wheat crop season and analyzed for fungal species as well as the soil-borne nematodes. During 1996-97, soil samples were drawn from five random spots in a field of 1000m² size. During the 1997-98, the treatments in rice season were as here under:

- T1 Non-basmati rice (var. Pusa-44) direct seeded, weedicide used
- T2 Non-basmati rice (var. Pusa 44), direct seeded, no weedicide
- T3 Basmati rice (Traori Local) direct seeded, weedicide used
- T4 Basmati rice (Traori Local) direct seeded, no weedicide
- T5 Non-basmati rice (Pusa 44) transplanted, weedicide used
- T6 Non-basmati rice (Pusa 44) transplanted, no weedicide
- T7 Basmati rice (Traori Local) transplanted, weedicide used
- T8 Basmati rice (Traori Local) transplanted, no weedicide

During wheat season of 1997-98 and 1998-99, the wheat variety UP 2338 was used and the treatments T-1 to T-8 had similar position with regard to weedicide. The weedicide used during rice season was Machete (Butachlor

@ 1.5 kg per ha) while during wheat season, stomp 30EC (pendimethalin was applied @ 3.3 l/ha (1kg a.i./ha) in 500 litres of water/ha at 0-3 days after sowing. The plot size was 100m² during both the seasons. The soil samples were drawn from five random spots from the top 15 cm soil during both the seasons. The five samples thus drawn were shade dried and mixed together. These were then crushed into fine soil, mixed thoroughly and again a small sample of 100 gm was drawn from it. For each isolation, 1 gm soil was taken from each of these samples and dilutions were made in sterilized distilled water to get dilution of 10² level. One ml of this dilution was spread on sterilized PDA in 90 cm diameter petriplates. For each sample, three replications were kept. These were incubated in the B.O.D. at 25 ± 1°C for 7-10 days and the colonies were observed and identified using stereobinocular as well as light microscope.

For nematodes analysis, the soil samples were collected from three farmers fields in Karnal district during 1996-97 crop season, whereas during 1997-98, the soil samples were collected from the DWR experimental field under rice-wheat system. The samples in this case were collected at three stages, viz., before sowing of wheat, during grain-filling stage and after harvest wheat. The nematode frequency was worked out in 250 g of soil in each case.

RESULTS AND DISCUSSION

SOIL MYCOFLORA

During 1995-96 crop seasons, 51 samples from the experimental field, yielded the following fungi: *Alternaria alternata*, *Curvularia lunata*, *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *Cladosporium* sp., *Trichoderma*

viride and some *Mycelia sterilia*. From farmers' fields, 12 samples were analyzed which yielded the following fungi: *Alternaria alternata*, *Cladosporium* sp., *Curvularia lunata*, *Aspergillus flavus*, *A. niger*, *Penicillium*, *Bipolaris sorokiniana* (= *Helminthosporium sativum*), *Trichoderma viride*, some *Mycelia sterilia* and two species of unidentified fungi.

It was interesting to isolate from the root zone of wheat seedlings, the bioagent *Trichoderma viride* from both the experimental as well as farmers' fields. This was isolated from a zero till plot of wheat in Distt. Kaithal. Another interesting feature is the isolation of *B. sorokiniana* from farmers' fields under rice-wheat rotation in Kaithal district (Haryana).

Fifteen samples were analyzed after harvest of wheat crop (before transplanting of rice) from the same field from where samples were analyzed when wheat crop was at seedling stage. These yielded *A. flavus*, *A. niger*, *C. lunata*, *Penicillium* sp., *Mucor* sp., *B. sorokiniana*, *A. alternata*, *Fusarium* sp. and two unidentified species.

During 1996-97 crop season, the samples were drawn from experimental field of var. UP 2338 under basmati rice-wheat rotations. In all, 10 species of fungi including *Trichoderma viride* were isolated at boot leaf stage and at harvest (Table 1). The frequency of this beneficial fungal species increased from 3.0 x 10² to 14.5 x 10² cfu/gm soil during the course of the crop cycle, viz., at booting stage and at harvest, respectively. This fungal species, however, was not isolated before sowing of wheat.

In all 13 fungal species were recovered from various treatments during wheat crop season. The most dominant species

Table 1 Fungal species isolated from the field of wheat var. UP 2338 under rice-wheat rotation during 1996-97 at different periods of time

Name of fungus	Frequencies (no. of colonies/gm of soil x 10 ²)		
	Before sowing of wheat	Booting stage	After harvesting of wheat
<i>Aspergillus flavus</i>	1.75	2.80	4.05
<i>Aspergillus niger</i>	0.20	1.20	11.25
<i>Cladosporium</i> sp.	3.00	8.82	7.28
<i>Fusarium</i> sp.	6.50	7.19	6.00
<i>Mucor</i> sp.	3.60	2.20	-
<i>Trichoderma viride</i>	-	3.00	14.30
<i>Rhizopus</i> sp.	-	-	2.25
<i>Penicillium</i> sp.	-	1.53	2.56
<i>Alternaria alternata</i>	-	-	2.00

seems to be *F. oxysporum* in some of the treatments during all the four dates of sampling. *B. sorokiniana* was recorded from T3 and T4 (direct seeded Basmati plots, with and without weedicide during both rice and wheat crop). Bioagent fungus, *T. viride* was available only in T1 and T8 at the time of sowing. It was recorded from T2, T5 and T8 at booting stage, from T2, T6 and T8 at maturity and from T2, T3, T7 and T8 after harvest. This indicates that the bioagent population (colony forming units, cfu) is very low or negligible at the time of sowing due to which

reason, it is not detected in most of the treatments (Table 2-5). During rice season also, the bioagent was not detected in any of the treatments except T2, where no weedicide was used in any of the crops and the rice was direct seeded non basmati type. Population of *F. oxysporum* was high in direct seeded rice plots (T1-T4), whereas during wheat season, it was only in plots with transplanted basmati rice at the time of sowing.

During 1998-99, the samples were again analyzed from the same fields where wheat var. UP 2338 was grown

Table 2 Fungi isolated from soil samples obtained from the field before sowing of wheat (Nov 1997)

Name of fungi	Frequencies (no. of colonies/gm of soil x 10 ²)							
	T1	T2	T3	T4	T5	T6	T7	T8
<i>Alternaria alternata</i>	0.0	0.0	2.2	0.0	0.0	0.0	2.0	0.0
<i>Aspergillus flavus</i>	0.2	0.8	2.4	0.8	3.2	0.8	1.2	0.0
<i>Aspergillus niger</i>	0.0	0.6	1.6	1.4	0.0	0.0	0.0	0.0
<i>Aspergillus sp.</i> (Golden colour)	0.0	0.0	0.0	0.0	3.0	3.0	3.0	0.0
<i>Fusarium moniliforme</i>	8.8	11.4	13.4	3.2	0.0	0.0	0.8	1.2
<i>Mucor sp.</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Trichoderma sp.</i>	1.0	0.0	0.0	0.0	0.0	0.0	0.0	39.4
<i>Penicillium sp.</i>	0.0	0.0	0.0	2.0	10.0	0.0	0.0	0.0
<i>Penicillium oxalicum</i>	3.8	21.2	20.0	23.6	1.2	22.6	0.0	192
Greenish white colony growth	0.0	0.0	0.0	0.0	0.0	10.0	10.2	10.6
Brown to black colour colony	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dark brown colour colony	0.4	0.0	0.0	0.0	1.4	0.0	2.2	0.0

Table 3 Fungi isolated from soil samples obtained from wheat field at booting stage (February 1998)

Name of fungi	Frequencies (no. of colonies/gm of soil x 10 ²)							
	T1	T2	T3	T4	T5	T6	T7	T8
<i>Alternaria alternata</i>	0.0	0.0	0.0	0.8	14	2.4	0.0	1.0
<i>Aspergillus flavus</i>	1.2	0.02	0.8	1.4	0.0	0.8	1.8	0.0
<i>Aspergillus niger</i>	0.0	0.04	0.8	0.0	6.0	1.6	0.0	2.6
<i>Aspergillus sp.</i> (Golden colour colony)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Fusarium moniliforme</i>	6.6	19.2	6.8	2.0	0.0	0.0	1.0	2.8
<i>Mucor sp.</i>	1.8	0.4	1.4	0.0	0.0	0.0	0.0	0.0
<i>Trichoderma sp.</i>	0.0	15.6	0.0	0.0	22.8	0.0	0.0	26.6
<i>Penicillium sp.</i>	11.8	9.0	1.4	17.0	25.8	33.8	39.8	0.0
Greenish white colony	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brown to black colour colony	0.0	0.0	0.0	1.8	0.0	3.0	0.0	0.0
Dark brown colour colony	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 4 Fungi isolated from soil samples obtained from wheat field at maturity (April, 1998)

Name of fungi	Frequencies (no. of colonies/gm of soil x 10 ²)							
	T1	T2	T3	T4	T5	T6	T7	T8
<i>Alternaria alternata</i>	2.0	0.0	0.0	1.5	1.5	2.3	8.7	2.3
<i>Aspergillus flavus</i>	8.0	1.8	0.0	0.8	0.8	0.7	0.0	1.7
<i>Aspergillus niger</i>	0.8	0.0	1.5	1.0	1.5	0.0	0.0	5.0
<i>Aspergillus sp.</i> (Golden coloured)	0.0	0.0	7.2	0.0	0.0	0.0	0.0	0.0
<i>Fusarium moniliforme</i>	0.0	11.5	16.0	2.7	0.0	0.0	4.0	0.0
<i>Helminthosporium sativum</i>	0.0	0.0	1.5	2.5	0.0	0.0	0.0	0.0
<i>Mucor sp.</i>	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0
<i>Trichoderma sp.</i>	0.0	14.2	0.0	0.0	0.0	18.0	0.0	7.8
<i>Penicillium sp.</i>	16.0	17.0	0.0	0.0	17.5	20.2	0.0	0.0
Brown to black colour colony	22.5	0.0	11.3	0.0	3.0	0.0	0.0	0.0

Table 5 Fungi isolated from soil samples obtained from wheat field after harvest (before preparation of field for rice)

Name of fungi	Frequencies (no. of colonies/gm of soil x 10 ²)							
	T1	T2	T3	T4	T5	T6	T7	T8
<i>Alternaria alternata</i>	0.0	0.0	0.0	1.4	1.0	0.0	2.0	0.0
<i>Aspergillus flavus</i>	1.10	0.0	1.5	1.0	11.8	9.8	0.0	2.2
<i>Aspergillus niger</i>	0.0	0.0	1.5	1.0	0.0	0.0	0.0	0.8
<i>Fusarium moniliforme</i>	3.3	9.4	10.0	11.0	4.4	4.2	16.0	0.0
<i>Trichoderma sp.</i>	0.0	11.2	18.8	0.0	0.0	0.0	3.8	8.4
<i>Penicillium sp.</i>	0.0	11.2	18.8	0.0	6.0	0.0	11.4	1.6
White cottony growth	1.2	11.4	2.5	0.0	0.0	0.0	0.0	0.0
Brown to black coloured colony	0.5	0.3	0.0	2.4	0.0	0.0	0.0	0.4
Whitish yellow growth	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.8
	0.0	0.2	1.8	0.6	0.4	1.0	0.0	0.8

after the rice crop (same treatments as during 1997-98). The following fungal species were isolated:

- T1 *Aspergillus flavus*, *Penicillium sp.*, *Fusarium moniliforme* and brown coloured sterile mycelium.
- T2 *Fusarium moniliforme*, *Penicillium sp.*, *Trichoderma viride* and Cottony white sterile mycelium.
- T3 *Fusarium moniliforme*, *Aspergillus flavus*, *Penicillium sp. T. viride* and *A. niger*
- T4 *Alternaria alternata*, *F. moniliforme*, *A. flavus*, *A. niger*, white colony with yellow centres.
- T5 *F. moniliforme*, *Penicillium sp.*, *Aspergillus sp.* and white sterile colony.
- T6 *F. moniliforme*, *Penicillium sp.* *Aspergillus sp.* and white sterile mycelium
- T7 *Alternaria alternata*, *Aspergillus flavus*, *F. moniliforme* and *T. viride*.
- T8 *A. flavus*, *A. niger*, *T. viride sp.*, *Penicillium sp.* and white sterile mycelium

The occurrence of fungi like *Aspergillus* and *Penicillium*, may not be of importance as far as their pathogenicity is concerned, but they are likely to influence the mycoflora in the rhizosphere.

The beneficial fungus *Aspergillus niger* was present at both the growth stages while *T. viride* was available only at earhead emergence. The population of *A. niger* remained almost constant. Total frequency of the fungal species (cfu of soil) was higher at earhead emergence stage than at tillering.

Trichoderma viride was not isolated in initial stages but built up at later stages of growth. This may be due to the reason that the fields remain under water during rice season, hence, the fungus seems to be unable to survive under submerged conditions and starts building up during wheat season. Moreover, the use of organic manure in the form of FYM is on the decline in this region, hence, the population of beneficial fungi like *T. viride* is likely to decline.

SOIL-BORNE NEMATODES

During wheat crop season 1996-97, soil samples from three farmers' fields yielded *Tylenchorhynchus*, *Hirschmanniella*

and *Pratylenchus*. Of these, *Tylenchorhynchus* was present in very high numbers in all the three fields (population per 250 gm soil 176.30, 403.3, 26.2), while the other two were very low in all these fields. *Hirschmanniella* and *Pratylenchus* were present (population 6.6/250 gm soil sample) in only one field while these were absent in the other two fields (Table 6).

Table 6 Nematode population in soil samples from farmers' fields under R-W system (Karnal district) during 1996-97

Nematode species	Farmers' fields in village Beejna, Distt. Karnal (No. per 250 g of soil)		
	Field no. 1	Field no.2	Field no.3
<i>Tylenchorhynchus</i>	176.30	403.3	26.60
<i>Hirshmaniella</i>	0.0	6.6	0.0
<i>Pratylenchus</i>	6.6	0.0	0.0
Total no. of nematodes (Population/250 g soil)	182.90	409.9	26.6

During 1997-98 nematode population was monitored in the Rice-Wheat rotation at the experimental field. *Tylenchorhynchus* was found to be the most important nematode since its population was low (30 per 250 g soil) before sowing, increased during mid season and again showed signs of decrease after harvest. Same trend was observed in case of the other nematode species, *Hoplolaimus* and *Helicotylenchus* (Table 7).

Nematode population was again monitored during 1998-99 wheat crop season in the eight treatments from where samples were studied for soil mycoflora. The population of *Tylenchorhynchus* remained more or less

Table 7 Nematode population in R-W system (frequency per 250 g of soil) during 1997-98

Nematode species	Before sowing	Mid-season	After harvest
<i>Tylenchorhynchus</i>	30	116	100
<i>Hoplolaimus</i>	12	40	28
<i>Helicotylenchus</i>	15	30	20

constant in different treatments in the months of October 1997, January 1998 and April 1998. In treatment No 6, the population of this nematode went as high as 325 in the month of April 1998. The population of *Helicotylenchus* was more or less at equilibrium in all the 7 treatments in all the three months but unusually went high in treatment no. 8 during April 1998. The highest population of *Hoplolaimus* was recorded in the month of October 1998 in treatment no 4. The April 1998 population of *Tylenchorhynchus* and *Helicotylenchus* have shown the highest levels in treatment no. 8.

In general all the nematodes showed increasing trends from early January/late December onwards. It shows that in spite of good crop, the temperature in the soil plays important role. The effect of weedicide was not significant on plant parasitic nematodes.

Soil biota including fungi and bacteria (Rhizobacteria) live in the plant rhizosphere and sometimes colonize the root system and act as growth promoters and/or biocontrol agents against plant diseases (Kloepper and Beauchamp 1992; Liu *et al.* 1995). Colonization of plant root system can lead to reduced pathogen attack directly or indirectly through production of antimicrobial substances or competition for space and nutrients, and also through induction of systemic resistance (Kloepper and Beauchamp 1992). Hence, the population dynamics of fungi and nematode species in the soil under rice-wheat system, carry significance. Thus, studies are further needed to monitor the role of the soil-biota which can lead to making of suppressive soils in relation to certain soil-borne diseases and nematodes.

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