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Variation of Soil Mycoflora in Decomposition of Rice Stubble from Rice-wheat Cropping System

Vibha[1]* and Asha Sinha[2]

[1]*Department of Plant Pathology, Rajendra Agricultural University, Pusa, Samastipur-848521, India*
[2]*Department of Mycology & Plant Pathology, Institute of Agricultural Sciences, B. H.U., Varanasi, India*
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The colonization pattern and extent of decay produced in paddy stubble by soil inhabiting mycoflora were done by using nylon net bag technique. Among the three methods used for isolation of fungi, dilution plate technique recorded the highest number of fungi followed by damp chamber and direct observation method. Nutrient availability and climatic conditions (temperature, humidity and rainfall) influenced the occurrence and colonization pattern of fungi. Maximum fungal population was recorded in October (48.99×10^4/g dry litter) and minimum in May (11.41×10^4/g dry litter). Distribution of Deuteromycetous fungi was more in comparison to Zygomycetes, oomycetes and ascomycetes. In the early stage of decomposition *Mucor racemosus*, *Rhizopus nigricans*, *Chaetomium globosum* and *Gliocladium* species were found primarly whereas at later stages of decomposition preponderance of *Aspergillus candidus*, *Torula graminis*, *Cladosporiun cladosporioides* and *Aspergillus luchuensis* was recorded.

KEYWORDS: Climatic factors, Decomposition, Rice stubble

Rice is the principal food of nearly half of the world's population. After making use of economic part, the remaining portion is mostly wasted except for few crops. Rice stubble constitutes as a part of the root system and just above the ground portion, which is highly rich in cellulose and lignin. It plays an important role in meeting the nutrients demand of rice and succeeding crops, if incorporated into the soil and allowed to decompose. Crop residue decomposition is essential for maintaining health of the soil, increase water holding capacity and improving soil-water-air continuum. Among the microbes involved in decomposition, fungi come under the important group. They grow well under semi-solid fermentation condition and colonize it quickly by virtue of their ability to ramify through solid substrate (Hudson, 1971). The process of decomposition is governed by the succession of fungi at various stage of decomposition (Beare *et al.*, 1993; Valenzuela *et al.*, 2001; Rai *et al.*, 2001; Santro *et al.*, 2002,), nutrient level of soil, crop residue and prevailing environmental conditions (Nikhra, 1981; Coockson *et al.*, 1998; Cruz *et al.*, 2002; Simoes *et al.*, 2002; Mc Tiernan *et al.*, 2003). Information on rice residue decomposition *per* se is scanty in literature. Keeping this in view the present investigation was undertaken to study the decomposition of rice stubble left in the field after harvesting by soil fungi commonly present in the soil under prevailing environmental conditions.

*Corresponding author <E-mail: vibha00@rediffmail.com>

Materials and Methods

In order to study the decomposition of rice stubble by soil mycoflora, the material was collected after the harvest of rice from the experimental site after a week of harvest of rice crop. Decomposition was studied by nylon net bag technique (House and Stinner, 1987). All stubble samples were mixed and cut into small pieces (2~3 cm). Fifty grams of air-dried rice stubble was filled in each nylon net bag (30 cm × 25 cm) with mesh size of 1~2 mm^2. A trench with an area of 4 × 4 × 1 m was dug in field and it was filled with soil where rice wheat cropping system was practiced. All nylon net bags were kept in the trench at a depth of 10 cm. Sampling programme was run from October, 2001 to August, 2002 at monthly intervals. Different media like Czapek's dox agar and Potato dextrose agar medium were prepared for isolation and sub-culturing of fungi respectively. The following three methods were used for the study of rice-stubble inhabiting mycoflora as discussed under.

Direct observation method. The fungi on the decomposing rice stubble were observed under binocular microscope.

Damp chamber incubation method. This method was described by Boeding, 1956. The stubble were cut into 1~2 cm pieces and placed on sterilized blotting paper in Petri dishes. The plates were incubated at 25 ± 2°C for 15 days.

Table 1. Meteorological data standard (month wise) of Varanasi during 2001~2002

Months	Rainfall	Temperature (°C)		Relative humidity (%)	
		Max.	Min.	Max.	Min.
2001					
October	28.4	32.2	21.7	85.0	61.4
November	0.0	29.1	15.0	83.5	40.0
December	0.0	18.9	10.3	88.2	56.7
2002					
January	2.3	22.6	9.8	87.8	47.8
February	9.5	26.3	13.9	87.7	49.7
March	2.3	32.5	16.6	75.5	30.0
April	0.0	37.8	23.0	67.2	26.4
May	10.4	38.4	26.2	65.2	39.0
June	15.3	37.3	27.4	74.5	49.7
July	31.9	36.0	27.8	74.4	55.0
August	76.3	32.7	26.1	87.7	69.5

Dilution plate technique. Warcup (1960) proposed this method for isolation and determination of fungal population. Stubble samples were powdered and one gram of it was suspended into 10 *ml*. Sterilized distilled water further dilution series ($1:10^3$, $1:10^4$, $1:10^5$) were prepared from it. Five replicas with 1 *ml* of each dilution were incubated on Czapek's dox agar with 100 ppm streptomycin at 25 ± 2°C for a week and fungi were recorded at monthly interval. Total number of fungi/g of oven dried stubble was calculated.

The fungal species were identified with the help of literature available (Thom and Raper, 1945; Ellis, 1971; Barnet and Hunter, 1972). Moisture content was determined by drying the sample at 60°C for 24 h and subtracting this value from initial weight of the respective value.

Table 3. pH, moisture content and average number of fungi per g oven dry decomposing rice stubble under experimental conditions

Months	pH	Moisture content (%)	Average number of fungi/g oven dry litter × 10^4
2001			
October	5.9	15.82	48.99
November	6.2	13.33	46.73
December	5.8	11.35	33.92
2002			
January	5.8	12.00	33.24
February	7.00	13.42	31.25
March	7.0	10.22	29.20
April	6.9	8.71	19.08
May	6.8	5.12	11.41
June	6.2	4.54	16.05
July	6.7	2.81	27.55
August	7.0	2.02	22.86

The pH of rice stubble was determined with help of Elico-Electric pH meter and weight loss by means of Litter weight technique (Bocock and Gilbert, 1957). Meteorological data (Table 1) showing maximum and minimum temperature, relative humidity and rainfall were obtained from meteorological observatory.

Results

The monthly and progressive weight loss of rice stubble during decomposition is given in Table 2. The loss in weight of substrate was recorded throughout the decomposition period but was maximum (20.70%) in February. Higher weight loss (11.46%) was also recorded in April. Fluctuating moisture content, pH and average number of

Table 2. Weight loss of rice stubbles during decomposition

Months	Dry wt. of litter (g)	Loss of weight (g)		Per cent loss in weight	
		Monthly	Progressive**	Monthly	Progressive**
2001					
October	7.07(50.00)	–	–	–	–
November	6.87(47.23)	2.77	2.77	2.45(5.54)	2.53(5.94)
December	6.20(38.55)	8.68	11.45	2.29(18.38)	4.78(22.90)
2002					
January	5.57(30.92)	7.63	19.08	4.44(19.79)	6.17(38.16)
February	4.55(20.76)	10.16	29.24	5.73(32.86)	7.64(58.48)
March	4.04(16.34)	4.42	33.66	4.61(21.29)	8.20(67.32)
April	3.38(11.46)	4.88	38.54	5.46(29.87)	8.77(77.08)
May	3.20(9.92)	1.54	40.08	3.67(13.44)	8.95(80.16)
June	3.06(8.94)	0.98	41.06	3.22(9.88)	9.05(82.12)
July	2.77(8.06)	0.88	41.04	3.21(9.84)	9.55(83.88)
August	2.77(7.21)	0.85	42.79	3.24(10.55)	9.25(85.58)
SEm±	0.027			0.014	0.010
CD (P=0.05)	0.079			0.042	0.029

Data in parentheses are original and these data were transformed and statistically analysed.
**Additive values of subsequent months.

fungi/g oven dry litter was presented in Table 3. The maximum number (48.99×10^4 of fungi/g oven dry litter) of fungi was recorded in October and minimum (11.41×10^4 of fungi/g oven dry litter) in May. The pH varied from 5.9 to 7.0 with no definite trend. Moisture content decrease throughout the decomposition process. There was increased moisture content in February in comparison to preceding months, thereafter, it decreased again. The summer months remained almost dry and led to the gradual decrease in moisture content of substrate from April to June. There

Table 4. Fungi recorded on decomposition rice stubble by various methods

Fungi	Methods		
	D.O.	D.C.	D.P.
Mastigomycotina:			
Pythium aphanidermatum (Edson) Fitzpatrick	−	+	+
Zygomycotina:			
Rhizopus stolonifer (Ehrenberg ex. Fr.) Lind	−	−	+
Mucor racemosus Fresenius	−	+	+
Mortierella subtilissima Oudemans	−	+	−
Ascomycotina:			
Chaetomium globosum Kunze	−	+	+
Deuteromycotina:			
Coelomycetes:			
Sphaerosidales:			
Phoma hibernica Grimes, Oconnor and Cummins	−	+	+
Melancoliales:			
Pestalotia mangiferae Pat.	−	−	+
Colletotrichum falcatum Went.	+	−	−
Hyphomycetes:			
Moniliales:			
Moniliacea			
Aspergillus flavus Link.	−	+	+
Aspergillus niger Van Tieghem	+	+	+
Aspergillus luchuensis Inui	−	−	+
Aspergillus candidus Link	−	−	+
Aspergillus sydowi Bainier & Sastary	−	−	+
Aspergillus sulphurieus	−	−	+
Aspergillus terreus Tho	−	−	+
Penicillium rubrum Stoll	−	−	+
Penicillium citrinum Thom	+	+	+
Penicillium chrysogenum Stoll	−	−	+
Trichoderma harzianum Rifai aggr	+	+	+
Gliocladium roseum (Link.) Thom	−	−	+
Dematiaceae:			
Alternaria alternata (Fr.) Keissler	+	+	+
Alternaria solani Sorauer	−	−	+
Alternaria claymydospora	−	−	+
Curvularia lunata (Walker) Boedij	+	+	+
Curvularia pallescens Boeidijn	−	−	+
Drechslera avanecea (Centis ex. Cooke) Shoemaker	+	−	+
Humicola grisea Traaen	+	−	+
Nigrospora sphaerica (Sacc.) Manson	+	+	+
Torula graminis Diem	+	+	+
Epicoccum purpurascens Ehren & Schlecnt	+	+	−
Cladosporium cladosporioides (Fresen) devries	+	+	+
Helminthosporium oryzae (Sacc.)	+	−	+
Bipolaris spp. (Sacc.)	+	−	+
Tuberculariaceae:			
Fusarium semitectum Berkeley & Revenel	+	+	+
Fusarium oxysporum Schlechtendahl	−	+	+
Myrothecium roridum Tode	+	−	−
Mycelia Sterilia			
Dark sterile mycelium	+	+	+

Table 5. Classwise occurrence of fungi and per cent occurrence of various classes colonizing the decomposing rice stubbles under experimental conditions

Classes of fungi	Number of species isolated	Occurrence (%)
Mastigomycotina	**1**	**2.32**
Oomycetes	1	
Zygomycotina	**3**	**6.97**
Zygomycetes	3	
Ascomycotina	**1**	**2.32**
Deuteromycotina	**34**	**79.06**
Coelomycetes	**3**	**6.98**
Sphaeropsidales	1	
Melanconiales	2	
Hyphomycetes	**31**	**72.90**
Moniliales	31	
Moniliaceae	14	
Dematiaceae	13	
Tubercularíaceae	4	
Mycelia sterilia	**3**	**6.98**
Unidentified	**1**	**2.32**
Total number of fungi isolated	**43**	

was gradual decrease in population of fungi from October to March and sharp decrease in April and May. Thereafter, there was marginal increase in population in June but substantial increase in the month of July. In the month of August population of fungi was slightly less than that of July.

Data contained in Tables 4 and 5 revealed the number of fungal species isolated and their classwise distribution, respectively. A total of 40 fungal species were isolated by dilution plate technique, 20 fungal species by damp chamber method and 16 by direct observation method. Dominant fungal species were *Aspergillus flavus*, *A. niger*, *A. candidus*, *Penicillum citrinum*, *Trichoderma harzianum*, *Fusarium semitectum* and dark sterile mycelium.

The common fungi were *Cladosporium cladosporioides*, *Fusarium* species, *Penicillium rubrum*, *Aspergillus luchuensis*, *Curvularia pallescence*. Frequent fungal group constituted *Aspergillus terreus*, *A. sydowi*, *Nigrospora sphaerica* and *Bipolaris* species. Rare occurring fungi were *Epicoccum purpurescens*, *Torula graminis*, *Mortierella subtilissima* etc. Some decomposing mycoflora found throughout the decomposition period were *Aspergillus niger*, *A. flavus*, *Penicillum citrinum*, *Trichoderma harzianum*, *T. viride*, *Alternaria alternata*, *Curvularia lunata* and *Fusarium semitectum*. The duteromycetous fungi constituted 79.06 percent of total fungal population followed by zygomycetes, oomycetes and ascomycetes.

Discussion

Maximum weight loss was recorded in February, 2001. It may be attributed to increased microbial activity due to favourable atmospheric temperature (Table 1), optimum soil moisture condition owing to rainfall towards end of January and increase in soil pH (Table 3). The prevailing temperature during February might also have helped in increased microbial activities which resulted in remarkable loss in weight. The significant correlation with weight loss and rate of decomposition owing to environmental factors were reported earlier by Coockson *et al.*, 1998 and Beare *et al.*, 2002. Salamanca *et al.* (2003) observed that decrease in weight due to leaching effect of rainfall and synergistic action of microbes and soil fauna.

Optimum soil moisture content affects the marked increase in soil fauna, its distribution and colonization on substrate was reported by Vijay and Naidu, 1995 and Beare *et al.*, 2002. Whereas, pH increases in soil on the incorporation of higher biomass into the soil to increase the bioactivity thereby, resulting in weight loss (Zimmermann and Frey, 2002).

The maximum fungal population was recorded in October. It may be attributed to senescent stubble provide enough moribund tissues and the surface area for the activities of initial colonizer to allow the succession which are unable to appear on fresh decaying tissues and narrow C : N ratio. Rate of decomposition of root residues in the soil after incorporation remained higher in the first and second week, which gradually slowed down and finally become steady owing to bacterial and fungal population. Berkenkamp *et al.* (2002) observed the decomposition of added organic starts just after its incorporation. They opined that senescent stubble provide enough morbid tissue and surface area for the activity of mycoflora. Sariyildiz and Anderson (2003) reported that the decomposition of organic amendment were initially rapid and then plateued. Due to abundance of rice residue and favourable atmospheric condition, increase in microbial population due to increase in amount of applied root residue in soil was observed by Sameni and Pour (2001). The significance of increasing temperature and decreasing relative humidity of air, resulting in decline of fungal population during summer months has also been earlier reported by Khanna (1964) and Cruz *et al.* (2002). While Mc Tiernan *et al.* (2003) observed that wet and warm climatic conditions had more recalcitrant effect on litter decomposition. In the last stage of decomposition, fungal colonization is mainly governed by nutritional level rather than environmental conditions (Ambush and Jensen, 1997; Coockson *et al.*, 1998).

The distribution of higher percentage of Duteromyceteous fungi suggests that the fungi belonging to this class are strong colonizer of the decaying substrate with better adaptability, high competitive ability and their higher percentage of distribution whereas, those of Phycomycetes and Ascomycetes were weak colonizers was reported by (Pathak and Sinha, 1995; Sinha 1992; Rai *et al.*, 2001;

Santro *et al.*, 2002). The order of fungal succession upon a natural substrate reflects the sequential release of different organic and inorganic nutrients along with interaction between each individual and substratum besides the competition between individual fungi (Macauley and Throwes, 1966; Aneja, 1988; Hobbies *et al.*, 2003).

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