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Variation in Soil Mycobiota Associated with Decomposition of *Sesbania aculeata* L.

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ABSTRACT

The aim of the present study was to investigate the variation in soil mycobiota involved in the decomposition of *Sesbania aculeata* L. in soil. Decomposition of *Sesbania aculeata* (Dhaincha) was studied by nylon net bag technique under experimental conditions. The colonization pattern by soil inhabiting mycobiota was studied by standard methods. Among the three methods used for isolation and enumeration of fungi, dilution plate technique recorded the highest number of fungi followed by damp chamber and direct observation method. Nutrient availability and climatic conditions influenced occurrence and colonization pattern of mycobiota. Maximum fungal population was recorded in July ($48.95 \pm 0.20 \times 10^4$ of fungi/g oven dry litter) and minimum in June ($19.78 \pm 0.20 \times 10^4$ of fungi/g oven dry litter). The distribution of Deuteromycetous fungi was much more (74.47%) than Zygomycetes, Oomycetes and Ascomycetes. In the early stage of decomposition *Mucor racemosus*, *Rhizopus stolonifer*, *Chaetomium globosum* and *Gliocladium roseum* were found where as at the later stages of decomposition preponderance of *Aspergillus candidus*, *Cladosporium cladosporioides*, *Curvularia lunata* and *Aspergillus luchuensis* was recorded.

Key words: Climatic factors, decomposition, fungal communities, green manure, mycobiota, *Sesbania aculeata* L.

INTRODUCTION

Green manures have become a vital concern particularly in the context of sustainable agriculture. Use of *Sesbania* as a green manure crop is a common practice in South Asia. Like most of the green manure crops, *Sesbania* belongs to the family Leguminosae and its subfamily is Papilionoideae. Species of the genus *Sesbania* are known for exceptionally fast growth rates as well as a very high affinity for association with several nitrogen-fixing Rhizobia in the soil that cause formation of numerous and large nodules in the plant roots. Besides this it is shown to be a rich source of nutrients from organic to inorganic through various activities of microorganism (Palaniappan, 1994; Hundal *et al.*, 1992; Brar and Sidhu, 1995; Singh *et al.*, 2007). Effects of the age on the decomposition period of *Sesbania aculeata* (Bhardwaj, 1982) and other green manure crop (Atkinson and Cairns, 2001; Wardle *et al.*, 2009) and colonization pattern of culturable decomposers during decomposition of green manures (Akpor *et al.*, 2006) have been studied. Crop residue decomposition is essential for maintaining health of the soil, increasing water holding capacity and improving soil-water-air conditions. Among the microbes involved in the

decomposition, fungi come under the important group. They grow well under semi-solid fermentation conditions 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 its various stages (Valenzuela *et al.*, 2001; Rai *et al.*, 2001; De Santo *et al.*, 2002; Osono, 2005; Kodsueb *et al.*, 2008; Gusewell and Gessner, 2009), nutrient level of soil, crop residue and prevailing environmental conditions (Cookson *et al.*, 1998; Cruz *et al.*, 2002; Simoes *et al.*, 2002; Mc Tiernan *et al.*, 2003; Xingbing *et al.*, 2009; Wood *et al.*, 2009). The various groups of soil mycobiota involved in the decomposition of *Sesbania aculeata* L. contribute differentially and have stage specific occurrence in the decomposition process. Therefore the primary aim of present study is to investigate various soil mycobiota involved in the decomposition of *Sesbania aculeata* L.

MATERIALS AND METHODS

For the study the decomposition of *Sesbania aculeata* L. in experimental conditions by soil mycobiota, the material was collected after harvesting of the *Sesbania* crop from the experimental site (Agriculture farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005). Decomposition was studied by nylon net bag technique (House and Stinner, 1987). The *Sesbania* (Dhaincha) plants were cut into small pieces (2-3 cm). Fifty gram of air-dried green manure pieces was filled in each nylon net bag of 30×25 cm size with mess size of 1-2 mm². A trench with an area of 4×4×0.1 m was dug in the field. All nylon net bags were kept in trench at depth of 10 cm and trench was filled with soil. Sampling programme was run from July, 2008 to June, 2009 at monthly intervals. Four samples (nylon net bags) were used for the experiment to determine weight loss, moisture content and pH and for the isolation of fungi for each month. Different media viz., Czapek's dox agar, carboxymethylcellulose agar and Potato dextrose agar medium were used for screening, isolation and sub-culturing of mycobiota associated with decomposition. The following three methods were used for the study of inhabiting mycobiota of *Sesbania aculeata* as discussed under.

Direct observation method: The fungi on the decomposing *Sesbania aculeata* were observed under binocular microscope (Garrett, 1981).

Damp chamber incubation method: This method was described by Boeding (1956). The samples were cut into 1-2 cm pieces and placed on sterilized blotting paper in Petri dishes. These Petri dishes were incubated at 25±2°C for 15 days.

Dilution plate technique: Warcup (1960) proposed this method for isolation and determination of fungal population. Samples were powdered and 1 g of it was suspended into 10 mL sterilized distilled water. Further dilution series (1:10³, 1:10⁴, 1:10⁵) were prepared from it. Five replicates with 1 mL of each dilution were incubated on Czapek's dox agar medium, potato dextrose agar medium and cellulose methyl agar medium with 100 ppm streptomycin at 25±2°C for 6-7 days and fungi were recorded. This method was repeated at monthly interval to observe the monthly occurrence of decomposing mycobiota. Total number of fungi/g of oven dried sample was calculated.

Statistical analysis: The data was analyzed using CRD design and result was expressed in terms of LSD (least significant difference).

Table 1: Meteorological data standard (month wise) of Varanasi during 2008-2009

Months	Rainfall (mm)	Temperature (°C)		Relative humidity (%)	
		Max.	Min.	Max.	Min.
2008					
July.	358.40	31.76	26.42	87.00	73.20
August	328.00	32.90	26.08	87.75	72.00
September	56.00	33.05	25.53	82.25	65.25
October	Nil	33.02	20.08	75.40	48.00
November	Nil	28.63	14.75	79.75	40.75
December	Nil	24.40	12.23	86.25	52.50
2009					
January	1.00	23.62	11.12	87.60	49.40
February	Nil	28.00	12.45	76.00	33.50
March	Nil	33.72	16.80	53.75	25.50
April	Nil	39.38	22.44	37.20	13.00
May	17.40	38.50	26.45	63.50	36.00
June	20.80	40.85	28.20	56.50	32.00

The fungal species were identified with the help of literature available (Thom and Raper, 1945; Raper and Thom, 1949; Ellis, 1971; Barnett and Hunter, 1972; Gilman, 1975). Moisture content was determined by drying the samples at 60°C for 24 h and subtracting this value from initial weight of the respective value. The pH of samples was determined with the help of Elico-Electric pH meter and weight loss by means of litter weight technique (Bocock, 1964). Meteorological data (Table 1) showing maximum and minimum temperature, relative humidity and rainfall were obtained from meteorological observatory, Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

RESULTS

The monthly and progressive weight loss of *Sesbania aculeata* samples during decomposition is given in Table 2, the loss in weight of substrate was recorded throughout the decomposition period but was maximum (30.60%) in January. Higher weight loss (28.61%) was also recorded in November. Fluctuating moisture content, pH and average number of fungi/g oven dry litter is presented in Table 3. The maximum number ($48.95 \pm 0.20 \times 10^4$ of fungi/g oven dry litter) of fungi was recorded in July and minimum ($19.78 \pm 0.20 \times 10^4$ of fungi/g oven dry litter) in June. The pH varied from 5.69 ± 0.01 to 7.21 ± 0.02 with no definite trend. Moisture content showed climatic factors associated variation throughout the decomposition period. The summer months remained almost dry and led to the gradual decrease in moisture content of substrate from January to April. There was gradual decrease in population of fungi from July to December and marginal increase in population in January. Thereafter, there was sharp decrease from February to June.

The number of fungal species isolated from decomposing *Sesbania aculeata* L. is given in Table 4, a total of 42 fungal species were isolated by dilution plate technique, 23 fungal species by damp chamber method and 16 by direct observation method. Dominant fungal species were *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium rubrum*, *Trichoderma harzianum*, *Fusarium semitectum* and dark sterile mycelium.

The common fungi observed during the study were *Cladosporium cladosporioides*, *Fusarium* species, *Penicillium citrinum*, *Aspergillus luchuensis*, *Curvularia lunata*, *Aspergillus terreus*,

Table 2: Weight loss of *Sesbania aculeata* L. samples during decomposition period

Months	Dry weight of litter (g)	Loss of weight (g)		Percent loss in weight	
		Monthly	Progressive	Monthly	Progressive**
2008					
July	50.00	-	-	-	-
August	46.78	3.22	3.22	6.44	6.44
September	37.99	8.79	12.01	18.79	24.02
October	29.78	8.21	20.22	21.61	40.44
November	21.26	8.52	28.74	28.61	57.48
December	16.40	4.86	33.60	22.86	67.20
2009					
January	11.38	5.02	38.62	30.60	77.24
February	10.08	1.30	39.92	11.42	79.84
March	8.82	1.26	41.18	12.50	82.36
April	8.15	0.67	41.85	7.59	83.70
May	7.40	0.75	42.60	9.20	85.20
June	6.41	0.99	43.59	13.37	87.18
LSD (p = 0.05)	0.42			0.02	0.03

Values are mean (n = 3). **Additive value of subsequent months

Table 3: pH, moisture content and average number of fungi per g oven dry decomposing green manure (*Sesbania aculeata* L.) under experimental conditions

Months	pH	Moisture content (%)	Average No. of fungi per gram of oven dry green manure X10 ⁴
2008			
July	7.21±0.02	44.74±0.30	48.95±0.20
August	6.82±0.10	36.76±0.80	48.28±0.98
September	6.59±0.09	28.82±0.21	41.46±0.13
October	6.50±0.20	24.60±0.91	36.83±0.56
November	6.88±0.06	23.80±0.26	35.46±0.15
December	6.85±0.09	24.20±0.20	34.06±0.10
2009			
January	5.83±0.07	26.58±0.17	36.54±0.02
February	5.69±0.01	22.76±0.98	33.86±0.26
March	5.71±0.13	18.85±0.64	24.64±0.80
April	6.15±0.11	16.26±0.56	22.54±0.19
May	6.35±0.05	17.54±0.33	21.85±0.48
June	6.46±0.15	23.80±0.46	19.78±0.20

Values are mean (n = 3) ±SD

A. sydowi, *Nigrospora sphaerica* and *Alternaria alternata*. Rare occurring fungi were *Epicoccum purpurascens*, *Torula graminis*, *Mortirella subtilissima* and *Choanephora cucurbitarum*. Some decomposing mycobiota viz., *Aspergillus niger*, *A. flavus*, *Penicillium rubrum*, *Trichoderma harzianum*, *Cladosporium cladosporioides*, *Alternaria alternata*, *Curvularia lunata* and *Fusarium semitectum* were found throughout the decomposing period. These fungi were designated as dominant decomposing mycobiota. Data contained in Table 5 revealed the class wise distribution of fungal communities involved in the decomposition of *Sesbania aculeata* L. Among the recorded fungal communities the Deuteromycetous fungi constituted 74.47% of total fungal population followed by Zygomycotina, Mastigomycotina and Ascomycotina, respectively.

Table 4: Fungi recorded during decomposition of green manure of *Sesbania aculeata* L.

Fungi	Methods		
	D.O. ¹	D.C. ²	D.P. ³
Mastigomycotina			
Oomycetes			
Peronosporales			
Pythiaceae			
<i>Pythium aphanidermatum</i> (Edson) Fitzpatrick	-	+	+
Zygomycotina			
Zygomycetes			
Mucorales			
Mucoraceae			
<i>Rhizopus stolonifer</i> (Ehrenberg ex. Fresenius) Lind	+	+	+
<i>Mucor racemosus</i> Fresenius	-	+	+
Mortierellaceae			
<i>Mortierella subtilissima</i> Oudemans	-	+	-
Choanephoraceae			
<i>Choanephora cucurbitarum</i> Currey	+	-	-
Ascomycotina			
Pyrenomycetes			
Sphaeriales			
Chaetomiaceae			
<i>Chaetomium globosum</i> Kunze	-	+	+
Deuteromycotina			
Coelomycetes			
Sphaeropsidales			
Sphaerioidaceae			
<i>Phoma hibernica</i> Grimes, O' Connor and Cummins	-	+	+
<i>Macrophomina phaseoli</i> (Maubl.) Ashby	-	-	+
Melanconiales			
Melanconiaceae			
<i>Pestalotia mangiferae</i> Pat.	-	+	+
<i>Colletotrichum falcatum</i> Went	+	-	-
Hyphomycetes			
Moniliales			
Moniliaceae			
<i>Aspergillus fumigatus</i> Fresenius	+	-	+
<i>Aspergillus flavus</i> Link.	-	+	+
<i>Aspergillus niger</i> van Tieghem	+	+	+
<i>Aspergillus luchuensis</i> Inui	-	-	+
<i>Aspergillus candidus</i> Link	-	-	+
<i>Aspergillus sydowi</i> Bainier and Sartory	-	-	+
<i>Aspergillus sulphureus</i> (Fresenius) Thom and Church	-	-	+
<i>Aspergillus terreus</i> Thom	-	-	+
<i>Penicillium rubrum</i> Stoll	-	-	+
<i>Penicillium citrinum</i> Thom	+	+	+
<i>Penicillium chrysogenum</i> Thom	-	-	+
<i>Trichoderma harzianum</i> Rifai aggr	+	+	+
<i>Trichoderma viride</i> Persoon ex Fr.	-	-	+
<i>Trichoderma koningii</i> Oudemans	-	-	+
<i>Gliocladium roseum</i> (Link)Thom	-	-	+

Table 4: Continued

Fungi	Methods		
	D.O. ¹	D.C. ²	D.P. ³
Dematiaceae			
<i>Alternaria alternata</i> (Fr.) Keissler	+	+	+
<i>Alternaria solani</i> Sorauer	-	-	+
<i>Curvularia lunata</i> (Walker) Boedijn	+	+	+
<i>Curvularia pallescens</i> Boedijn	-	-	+
<i>Drechslera avenacea</i> (Centis ex. Cooke) Shoemaker	-	+	+
<i>Humicola grisea</i> Traaen	+	-	+
<i>Nigrospora sphaerica</i> (Sacc.) Mason	+	+	+
<i>Torula graminis</i> Desm	+	+	+
<i>Diplococcium spicatum</i> Grove	-	-	+
<i>Helminthosporium oryzae</i> Breda de Haan	-	-	+
<i>Cladosporium cladosporioides</i> (Fresenius) de Vries	+	+	+
<i>Epicoccum purpurascens</i> Ehrenberg ex Schlecht	+	+	+
Tuberculariaceae			
<i>Fusarium semitectum</i> Berkeley and Ravenel	+	+	+
<i>Fusarium solani</i> (Martius) Appel and Wollenweber	-	-	+
<i>Fusarium moniliforme</i> Sheldon	-	+	+
<i>Myrothecium roridum</i> Tode	-	-	+
Mycelia Sterilia			
Sterile dark mycelium	+	+	+
Sterile white mycelium	-	-	+
Sterile pink mycelium	-	+	+
Unidentified -	+	+	

¹Direct observation method; ²Damp chamber method; ³Dilution plate technique. +: Present, -: Absent

Table 5: Class wise occurrence of fungi and per cent occurrence of various classes colonizing the decomposing *Sesbania aculeata* L. under experimental conditions

Class of fungi	No. of species isolated	Occurrence (%)
Mastigomycotina	1	2.13
Oomycetes	1	2.13
Peronosporales	1	
Pythiaceae	1	
Zygomycotina	4	8.51
Zygomycetes	4	8.51
Mucoraceae	2	
Mortierellaceae	1	
Choanephoraceae	1	
Ascomycotina	1	2.13
Pyrenomycetes	1	2.13
Sphaeriales	1	
Chaetomiaceae	1	
Deuteromycotina	35	74.47
Coelomycetes	4	8.51
Sphaeropsidales	2	
Sphaerioidaceae	2	
Melanconiales	2	
Melanconiaceae	2	

Table 5: Continued

Class of fungi	No. of species isolated	Occurrence (%)
Hyphomycetes	31	65.95
Moniliales 31		
Moniliaceae	15	
Dematiaceae	12	
Tuberculariaceae	4	
Mycelia Sterilia	3	6.38
Unidentified	3	6.38
Total No. of fungi isolated	47	

DISCUSSION

Maximum weight loss was recorded in January, 2009. It may be attributed to increased microbial activity due to favourable atmospheric temperature (Table 1), optimum soil moisture and soil pH (Table 2) condition owing to rainfall towards end of January. The significant correlation with weight loss and rate of decomposition owing to environmental factors were reported earlier by Cookson *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 Beare *et al.* (2002). Whereas, pH increases in soil due to 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 July. It may be attributed to senescent residues provided enough moribund tissues and the surface area for the activities of initial colonizers to allow the succession which are unable to appear on fresh decaying tissue and narrow C:N ratio. Rate of decomposition of substrate in soil after incorporation remained higher in the first and second week, which gradually slowed down and finally become steady owing to decline in fungal population (Berkenkamp *et al.*, 2002). Sariyildiz and Anderson (2003) reported that the decomposition of organic amendment were initially rapid and then plateaued. 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 (Ambus and Jensen, 1997; Cookson *et al.*, 1998; Osono, 2005; Gusewell and Gessner, 2009).

The distribution of higher percentage of Deuteromyceteous fungi suggested that the fungi belonging to this class are strong colonizers 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 several workers (Rai *et al.*, 2001; De Santo *et al.*, 2002; Vibha and Sinha, 2007). These reports support the experimental findings of the present study. 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 (Hobbie *et al.*, 2003; Kodsueb *et al.*, 2008).

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