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Abundance, frequency and occurrence based comparison between mycoflora involved in the decomposition of *Sesbania aculeata* L. under experimental and natural conditions

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Abstract
Green manuring is a cost-effective technology to sustain soil fertility and enhance activities of soil micro-flora and micro-fauna. The comparison between mycoflora associated with the decomposition of *Sesbania aculeata* L. green manure under nylon net bag and mycoflora inhabiting soil mixed green manure at experimental site was investigated. During the study abundance, frequency and occurrence of both type of mycoflora was investigated. Maximum moisture content was recorded in the month of July and thereafter it decreased. The average number of fungi involved in the decomposition of *Sesbania aculeata* L. was found to be dependent on physical climate *viz.,* moisture content, rainfall, temperature and relative humidity. The highest occurrence of mycoflora in experimental condition (48.95×10^4 per g green manure) and natural condition (47.85×10^4 per g green manure) was found in the month of July, whereas in the month of June lowest occurrence of mycoflora in both conditions was recorded. The number of fungi slightly decreased in August and thereafter declined gradually up to December. From the above finding, pattern of fungal succession on decomposing green manure was deciphered that may enable rapid decomposition of agricultural wastes.

Keywords: Decomposition, green manure, Mycoflora, *Sesbania aculeata* L. and soil

Introduction
The excessive application of synthetic agrochemicals has jeopardized the environment through soil, water and air contaminations and pollutions and has adversely affected the soil microflora by altering the chemical and physical properties of the soil. The pesticides residues in food chain have endangered the whole life sustaining system around the world (Singh *et al.,* 2018) [30]. In recent years, however, greater regulation of agrochemicals and increasing costs of application are motivating the development of alternative crop management strategies. The use of green manuring may be a viable option to restrict the excessive application of fertilizers and other agrochemicals. Hence, there have been renewed research efforts during the past two decades on green manures (Sinha *et al.,* 2009, Kumar *et al.,* 2014a, b; Kumar *et al.,* 2017; Kumar *et al.,* 2020) [31, 20, 19, 17]. However, crop intensification urgently needs a rapid decomposition of crop residues, green manures or organic bulks of agricultural wastes in the soil. As an alternative, burning of the crop residues is hazardous to soil health and polluting our environment drastically and thereby the decomposition of this material is one of the leading environmental concerns of today's world (Kumar *et al.,* 2014a) [20]. Soil organisms carry a wide range of processors that are important for soil health in both natural and managed agricultural system. The population, diversity and activity of soil-biota will fluctuate as soil environment changes (Kumar *et al.,* 2010) [18, 24]. Therefore, the present study was undertaken to understand the changes in abundance, frequency and occurrence of fungal community involved in the decomposition of *Sesbania aculeata* L. green manure and their efficacy in the decomposition.

Material and Methods
A. Decomposition of *Sesbania aculeata* L. under experimental conditions
Sesbania aculeata L. was sown to generate the research material at experimental farm of Institute of Agricultural Sciences, B.H.U. and at 45 DAS, the crop was cut down.

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50 nylon net bags (mesh size of 1 mm^2) were prepared containing 50 g of air-dried green manure in each bag (size 30x25cm) to facilitate the microbial decomposition and reduced the macro faunal disturbances.

These bags were buried in a trench with an area of 4x4x0.1m in the field. Samples (4 bags) were drawn randomly from July, 2008 to June, 2009 at monthly intervals for isolation of mycoflora.

In natural condition, the green manure was allowed for decomposition after cutting down and mixing of the crop in the field.

The sampling programme was run monthly for litter analysis in both experimental and natural conditions.

Weight Loss: The weight loss of the green manure was determined by means of litter nylon net bag weight technique (Bocock and Gilbert, 1957) [4].

In each month three bags were picked and fresh weight of the content was taken. The material was oven dried at 60˚C for dry weight determination.

pH: The decomposing green manure mixed in distilled water in the ratio of 1:5 and thereafter the pH was determined with the help of Elico-Electric pH meter.

Moisture content: The samples of the decomposing green manure from the bags were oven dried at 60 ºC for 24 hours and moisture content was determined on the basis of losses in dry weight.

Meteorological data: Meteorological data regarding maximum and minimum temperatures, relative humidity and rainfall were obtained from Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (UP), India (Fig. 1).
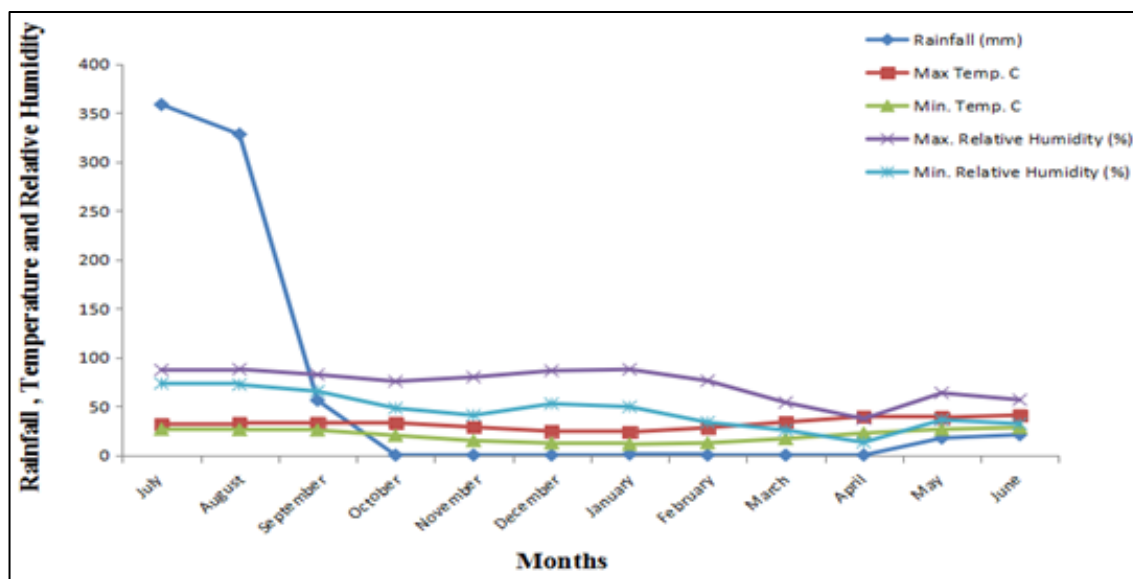

Fig 1: Meteorological data standard (month wise) of Varanasi during 2008-2009.

Isolation of fungi: Fungal populations involved in decomposition of *Sesbania aculeata* L. green manure were studied and isolated by following methods:

1. **Direct observation of green manure samples:** The fungi on the decomposing green manure were observed under a binocular microscope (Garrett, 1981) [8].
2. **Damp chamber incubation method:** In the initial stages of decomposition process, the green manure inhabiting fungi were observed by damp chamber incubation method (Boedijn, 1956) [5]. The litter sample was cut into 0.5-1.0 cm pieces and placed on sterilized blotting paper in Petri-dishes and incubated at 25+ 2˚C for 15 days for observation.
3. **Dilution plate technique:** For the determination of population of mycoflora, their isolation was done by dilution plate technique (Warcup, 1960) [36]. In this method, 1 g green manure sample from the nylon net bag was suspended into 10 ml sterilized distilled water. Further dilution series (1:10^3, 1:10^4, 1:10^5, 1:10^6) were prepared from it. 1 ml of each dilution was poured individually in sterilized Petri-dishes. Then 15-20 ml of sterilized cool molten potato dextrose agar medium was added. Five replicates of each dilution were prepared and all plates were incubated at 25+ 2ºC for a week. The isolated fungi were identified with the help of literature (Thom and Raper, 1945 [34]; Raper and Thom, 1949 [28];

Ellis, 1971 [7]; Subramanium, 1971 [33]; Barnett and Hunter, 1972 [1]; Gilman, 1975 [9]; www.mycobank.org and www.forestryimages.org).

Determination of number of fungi: Total number of fungi/g of oven dried litter (green manure) was calculated using following formula:

$$\text{No of fungi/g oven dry litter} = \frac{\text{Total number of fungi} \times \text{Concentration of the solution}}{\text{Oven dry weight of the litter}}$$

Determination of frequency and abundance of fungi: Frequency and abundance of fungi were determined following Saksena (1955) [29].

Frequency: Percent occurrence of a species in the units was calculated in the usual manner and frequency classes were expressed on 1-5 scale as follows:

Class 1: Species occurring in 1-20% of Petri-dishes;
Class 2: Species occurring in 21-40% of Petri-dishes;
Class 3: Species occurring in 41-60% of Petri-dishes;
Class 4: Species occurring in 61-80% of Petri-dishes;
Class 5: Species occurring in 81-100% of Petri-dishes.
Abundance: Numerical abundance was calculated from the species on following scale:

Class 1: Species occurring in 1-3% of the total species;
Class 2: Species occurring in 4-8% of the total species;
Class 3: Species occurring in 9-15% of the total species;
Class 4: Species occurring in 16-25% of the total species;
Class 5: Species occurring in 26-100% of the total species.

(B) Decomposition of *Sesbania aculeata* L. under natural conditions: One set of experimental material *Sesbania aculeata* L. after cutting at 45 DAS was mixed in the soil and left for decomposition. The decomposing green manure samples were collected at monthly intervals and the pH, moisture content and mycoflora population dynamics (quantitative and qualitative) were estimated by standard methods described above.

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

Results and Discussion
(A) Decomposition of *Sesbania aculeata* L. under experimental conditions:
Weight Loss: The weight loss was observed maximum (30.60%) in the month of January because of the maximum rainfall in the months of rainy season, which enhanced decomposition process by increased microbial activities. The loss in weight of decomposing green manure was rapid during initial months (Table 1). The process of decay started by the action of decomposers and abiotic environment after the green manure bags were kept in the soil (Kumar *et al.,* 2011) [23]. Decomposition results in the change of state of the substrates under the influence of a number of biotic and abiotic factors. Weight loss of substrate has been regarded as the simplest expression of decomposition process which was increased with months perhaps with increased enzymatic production by

fungal colonizers (Kumar *et al.,* 2018) [21]. This could be meant that different microbial species have different rates of decomposition as reported by Kumar (2010) [18, 24] and Herzog *et al.* (2019) [10]. A small percentage of original weight remaining at last and this may due to there was always litter remaining before new crop (Oladoye *et al.* 2007) [26].

Table 1: Weight loss of green manure (*Sesbania aculeata* L.) litter during decomposition

Months	Dry Weight of litter (g)	Loss of Weight (g)		Per cent 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
SEm ±	0.14	-	-	0.01	0.01
CD(P=0.05)	0.42	-	-	0.02	0.03

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

pH: The pH of decomposing green manure (*Sesbania aculeata* L.) varied from 5.69±0.01 to 7.21±0.02 with no definite trend (Fig. 2). The increase in pH during decomposition was probably due to higher calcium content, rapid loss in citric acid and malic acid through leaching (Xu and Coventry, 2003; Batty and Younger, 2007) [38, 2].
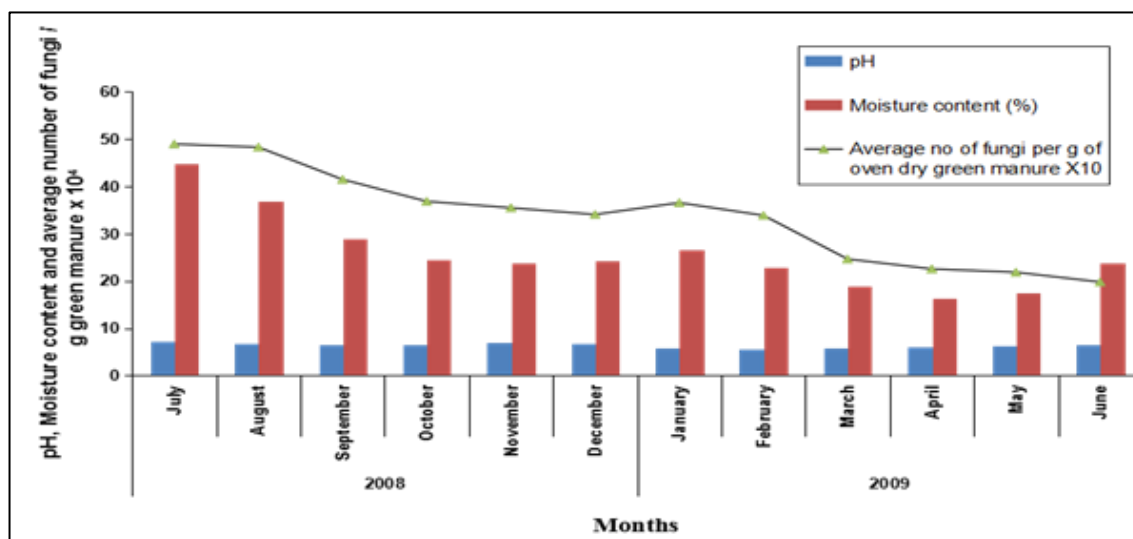
Fig 2: pH, moisture content and average number of fungi per g oven dried decomposing green manure (*Sesbania aculeata* L.) under experimental conditions

Moisture content: Maximum moisture content was recorded in the month of July (44.74%) and thereafter it decreased. The summer months remain almost dry when moisture content gradually decreases in the months of March and April resulting in slower rate of decomposition. The population of fungi in terms of both quantity and quality was found highest in the rainy season. Moisture content is one of the key factors that govern decomposition process (Krishna and Mohan,

2017) [15]. High moisture content helps in increasing microbial activities, which could be limited by low moisture content in soil (Manzoni *et al.*, 2012) [25] as moisture content is chiefly responsible for colonization of substrate by microorganisms (Pandey and Sinha, 2008; Kumar, 2010; Krishna and Mohan, 2017) [27, 18, 24].

Population dynamics of *Sesbania aculeata* L. litter inhabiting mycoflora:

Quantitative nature of the green manure inhabiting mycoflora: The maximum number (48.95×10^4) of fungi/g litter was recorded in the month of July, whereas minimum was in the month of June (19.78×10^4) (Fig. 2). The average number of fungi involved in the decomposition of *Sesbania aculeata* L. was found to be dependent on physical climate *viz.,* moisture content, rainfall, temperature and relative humidity. The number of fungi slightly decreased in August and thereafter declined gradually up to December, 2008. After this there was slight increase in the average number of fungi/g of litter in the month of January perhaps due to sporadic rainfall in this month. Thereafter, the fungal population declined heavily up to June. Despite of rainfall in the month of May and June, 2009, the average number of fungi continued to decline may be due to insufficient availability of green manure substrate for further fungal colonization (Kumar, 2010) [18, 24].

It was observed in the rainy season, the population of fungi was highest. The decomposition of organic matter started after the residue incorporation (Berkenkamp *et al.* 2002) [3]. Temperature and relative humidity might be responsible for the decline in the number of fungi during summer season. The significance of increasing temperature and decreasing relative humidity of atmosphere as resulted in the decline of fungal population during summer months has also been reported by several workers (Jing *et al.,* 2007; Pandey and Sinha, 2008; Kumar *et al.*, 2013) [27, 12, 16].

The higher number of the fungi/g of substrate in initial stages of decomposition was due to availability of the maximum substrate residue in the soil, whereas the fungal population declined due to physiological constraints in the later stage of decomposition (Sinha *et al.*, 2010) [32]. While on the other hand favourable climatic conditions *viz.* high relative humidity, moderate temperature and high moisture content of decaying tissues also favoured the better colonization during rainy season.

During the winter season the colonization found to be in declining order because the temperature below optimum level does not favour sporulation of fungi (Jing *et al.,* 2007; Kumar, 2010) [12, 18, 24]. The minimum number of fungi was found in the end of experimental period may be due to unavailability of the substrate. Same pattern of fungal succession was also reported by several workers (Pandey and Sinha, 2008; Wardle *et al.,* 2009; Krishna and Mohan, 2017) [27, 37, 15].

Qualitative nature of the green manure inhabiting mycoflora: Green manures are initially colonized by various saprophytic and weak parasitic fungal species before they are incorporated in the soil and further colonization of litter by fungi depends on the climatic conditions and the nature of substrate (Pandey and Sinha, 2008) [27]. The fungal species were divided into early and late colonizers according to their occurrence during experiment. Different techniques *viz.,* direct observation method, damp chamber incubation method and dilution plate technique were used for the isolation with the expectation that they would reduce the bias associated with single technique.

Direct observation method: Sixteen fungal species were observed directly under the binocular microscope. Out of these, *Aspergillus niger, A. fumigatus, Penicillium citrinum, Trichoderma harzianum, Alternaria alternata, Curvularia lunata, Cladosporium cladosporioides, Fusarium semitectum* and dark sterile mycelium were observed as dominant fungi (Table 2).

Table 2: Direct observation of fungal species from decomposing *Sesbania aculeata* L. by binocular microscope under experimental conditions

Fungal Species	2008						2009					
	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rhizopus stolonifer	-	+	-	+	-	-	-	-	-	-	-	-
Choanephora cucurbitarum	+	+	-	-	-	-	-	-	-	-	-	-
Colletotrichum falcatum	+	-	-	-	-	-	-	-	-	-	-	-
Aspergillus fumigatus	+	+	+	-	+	-	-	+	-	-	-	-
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+
Penicillium citrinum	+	+	+	-	+	+	+	+	-	+	+	+
Trichoderma harzianum	-	+	+	+	+	+	+	+	-	+	+	+
Alternaria alternata	-	-	+	+	+	+	+	+	-	+	-	-
Curvularia lunata	-	-	+	+	-	+	-	+	+	+	+	+
Humicola grisea	-	-	-	-	-	-	-	+	+	-	-	+
Nigrospora sphaerica	-	-	-	+	+	-	-	-	-	-	-	-
Torula graminis	+	-	-	-	-	-	-	-	+	-	-	-
Cladosporium cladosporioides	-	-	+	+	+	+	+	+	+	-	+	+
Epicoccum purpurascens	+	-	-	-	+	-	-	-	-	-	-	-
Fusarium semitectum	+	+	+	-	+	+	+	+	+	+	-	+
Dark sterile mycelium	+	+	-	+	+	+	+	+	+	+	-	+

+=Present, - = Absent

Damp chamber incubation method: By this method sampling was performed from July 2008 to September 2008 because the green manure was broken down into small pieces after this period which was very difficult to handle for sampling. A total number of 23 fungal species were isolated by this method (Table 3). *Aspergillus flavus, A. niger, Penicillium citrinum, Trichoderma harzianum, Alternaria alternata, Curvularia lunata, Cladosporium cladosporioides, Fusarium semitectum* and dark sterile mycelium were recorded as dominant species, whereas *Mortierella subtillissima, Phoma hibernica, Drechslera avenacea, Nigrospora sphaerica, Epicoccum purpurascens* and pink sterile mycelium were observed only once by this method.

Table 3: Fungi recorded from decomposing *Sesbania aculeata* L. by damp chamber incubation method under experimental conditions

Fungal Species	Months		
	July	August	September
Pythium aphanidermatum	+	+	-
Rhizopus stolonifer	+	+	-
Mucor racemosus	+	+	-
Mortierella subtilissima	+	-	-
Chaetomium globosum	+	+	-
Phoma hibernica	-	-	+
Pestalotia mangiferae	+	-	+
Aspergillus flavus	+	+	+
Aspergillus niger	+	+	+
Penicillium citrinum	+	+	+
Trichoderma harzianum	+	+	+
Alternaria alternata	+	+	+
Curvularia lunata	+	+	+
Drechslera avenacea	-	-	+
Nigrospora sphaerica	-	-	+
Torula graminis	+	-	+
Cladosporium cladosporioides	+	+	+
Epicoccum purpurascens	+	-	-
Fusarium semitectum	+	+	+
Fusarium moniliforme	+	+	-
Dark sterile mycelium	+	+	+
Pink sterile mycelium	-	+	-
Unidentified	+	-	-

+=Present, - = Absent

Dilution plate technique: A total number of 42 fungal species was isolated by this method (Table 4). *Aspergillus luchuensis, A. candidus, A. sydowi, A. sulphureus, A. terreus, Penicillium rubrum, P. chrysogenum, Trichoderma viride, T. koningii, Gliocladium roseum, Alternaria solani, Curvularia pallenscens, Diplococcum spicatum, Helminthosporium oyzae, Fusarium solani, Myrothecium roridum* and white sterile mycelium were observed by only this technique.

The diverse fungal population appeared at different stages of decomposition may be classified into four different groups depending upon their occurrence during experimental period.

Table 4: Frequency and Abundance of mycoflora associated with the decomposing *Sesbania aculeata* L. under experimental conditions

Fungal Species	2008												2009											
	Jul		Aug		Sept		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun	
	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A
Pythium aphanidermatum	2	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus stolonifer	4	3	3	4	3	2	2	1	-	-	-	-	5	4	4	3	-	-	-	-	2	2	-	-
Mucor racemosus	2	2	4	3	-	-	-	-	4	3	3	3	-	-	-	-	-	-	-	-	-	-	-	-
Chaetomium globosum	-	-	-	-	-	-	2	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phoma hibernica	-	-	-	-	1	1	2	2	3	2	-	-	2	3	3	3	-	-	-	-	-	-	-	-
Macrophomina phaseoli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	3	3	3	2	-	-	-	-
Pestalotia mangiferae	1	1	1	1	2	1	-	-	2	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus fumigatus	5	5	4	5	3	4	5	5	4	3	-	-	3	3	1	1	-	-	-	-	-	-	-	-
Aspergillus flavus	5	3	5	4	2	3	1	1	3	3	4	3	4	3	2	1	1	2	3	4	2	1	1	1
Aspergillus niger	4	4	4	5	5	5	5	4	5	3	3	2	4	3	2	2	4	5	2	2	1	2	2	1
Aspergillus luchuensis	-	-	-	-	-	-	-	-	-	-	4	2	-	-	-	-	-	-	2	2	-	-	-	-
Aspergillus candidus	-	-	-	-	1	1	-	-	-	-	2	1	2	2	-	-	1	1	-	-	-	-	-	-
Aspergillus sydowi	-	-	-	-	3	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus sulphureus	1	1	-	-	-	-	-	-	1	1	-	-	2	1	2	2	2	2	-	-	-	-	-	-
Aspergillus terreus	-	-	1	1	-	-	2	1	-	-	2	2	3	2	2	2	-	-	-	-	-	-	-	-
Penicillium rubrum	-	-	1	1	3	2	5	3	2	1	5	4	4	3	3	5	5	5	2	2	3	1	1	1
Penicillium citrinum	5	3	4	2	3	2	5	5	2	3	4	3	5	5	4	2	5	3	5	4	2	2	1	1
Penicillium chrysogenum	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Trichoderma harzianum	1	1	1	1	2	2	1	1	4	3	5	4	5	5	4	2	2	3	5	2	2	1	1	1
Trichoderma viride	-	-	-	-	-	-	-	-	-	-	1	2	-	-	1	1	1	3	1	1	1	1	-	-
Trichoderma koningii	-	-	-	-	4	3	4	5	3	2	-	-	-	-	2	2	-	-	-	-	-	-	-	-
Gliocladium roseum	-	-	-	-	4	3	2	2	3	4	-	-	-	-	2	2	-	-	-	-	-	-	-	-
Alternaria alternata	-	-	-	-	3	2	2	1	1	1	3	2	2	1	1	1	-	-	2	2	-	-	-	-
Alternaria solani	-	-	-	-	1	2	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	-	-	-	-	1	1	2	2	-	-	1	1	-	-	2	3	5	4	5	5	3	4	5	2
Curvularia pallescens	1	1	1	1	-	-	-	-	1	2	2	2	-	-	-	-	-	-	-	-	-	-	-	-
Drechslera avenacea	2	1	3	2	3	2	2	2	-	-	-	-	-	-	1	1	2	2	-	-	-	-	-	-
Humicola grisea	-	-	-	-	-	-	-	-	1	1	-	-	2	2	-	-	-	-	-	-	-	-	-	-
Nigrospora sphaerica	-	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	-	1	1	-	-	-	-

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Fungi	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A
Torula graminis	1	1	2	2	1	1	-	-	-	-	-	-	-	-	-	-	1	1	1	1	-	-	-	-
Diplococcium spicatum	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Helminthosporium oryzae	1	1	1	1	2	1	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	1	-	-
Cladosporium cladosporioides	-	-	-	-	-	-	-	-	5	5	5	5	4	5	4	4	4	3	3	2	1	1	-	-
Epicoccum purpurascens	2	2	1	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium semitectum	5	5	5	4	5	3	5	4	4	2	2	3	1	1	-	-	2	2	5	4	-	-	-	-
Fusarium solani	-	-	2	2	1	1	-	-	-	-	3	4	5	4	-	-	-	-	-	-	1	1	-	-
Fusarium moniliforme	-	-	2	1	1	1	3	2	3	2	1	1	-	-	1	1	-	-	-	-	-	-	-	-
Myrothecium roridum	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dark sterile mycelium	5	4	3	2	5	4	-	-	5	3	5	2	3	4	3	5	-	-	2	2	1	1	-	-
White sterile mycelium	-	-	-	-	2	2	-	-	3	2	-	-	-	-	2	2	-	-	1	1	-	-	-	-
Pink sterile mycelium	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	1	-	-	-	-
Unidentified	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-

F= Frequency, A= Abundance

Group I: Dominant Fungi: The fungi which appeared in more than six months during isolation period were included in this group. *Rhizopus stolonifer, Aspergillus fumigatus, A. flavus, A. niger, Penicillium rubrum, P. citrinum, Trichoderma harzianum, Alternaria alternata, Curvularia lunata, Cladosporium cladosporioides, Fusarium semitectum* and dark sterile mycelium were found as dominant fungi.

Group II: Common Fungi: The group of common fungi occurred in five to six months of experimental period with less frequency and abundance than the dominant ones. The common fungi isolated were *Phoma hibernica, Pestolotia mangiferae, Aspergillus sulphureus, A. terreus, Trichoderma viride, Drechslera avenacea, Torula graminis, Fusarium solani* and *F. moniliforme.*

Group III: Frequent Fungi: This group includes the mycoflora which appeared in at least three to four months of experimental period with normal frequency and abundance. *Mucor racemosus, Aspergillus candidus, Trichoderma koningii, Gliocladium roseum, Curvularia pallenscens* and white sterile mycelium were found to be frequent fungi.

Group IV: Rare Fungi: The fungi of this group appeared only once or twice with very low frequency and abundance. *Pythium aphanidermatum, Chaetomium globosum, Aspergillus luchuensis, A. sydowi, Penicillium chrysogenum, Alternaria solani, Humicola grisea, Nigrospora sphaerica, Diplococcum spicatum, Myrothecium roridum* and pink sterile mycelium were recorded as member of this group.

(B). Decomposition of *Sesbania aculeata* L. mixed in soil under natural conditions
pH: The pH of decomposing green manure (*Sesbania aculeata* L.) litter mixed in soil varied from 6.70±0.20 to 7.20±0.70 with no definite trend (Table 5). The increase in pH during decomposition was probably due to higher calcium content, rapid loss in citric acid and malic acid through leaching (Xu and Coventry, 2003; Batty and Younger, 2007) [38, 2].

Moisture content: Maximum moisture content was recorded in the month of July (46.20%) and thereafter it decreased (Table 5). Moisture content is one of the key factors that govern decomposition process (Krishna and Mohan, 2017) [15].

Table 5: pH, moisture content and average number of fungi per g oven dry decomposing green manure (*Sesbania aculeata* L.) under natural conditions.

Months	pH	Moisture content (%)	Average no of fungi per G of oven dry green manure X10^4
2008			
July	6.70+0.02	46.20+0.30	47.85+0.20
August	6.90+0.05	38.76+0.85	46.96+0.94
September	7.18+0.10	29.72+0.25	40.84+0.15
October	6.88+0.06	26.40+0.95	37.50+0.56
November	6.85+0.09	23.45+0.20	36.64+0.10
December	7.15+0.12	23.80+0.64	34.86+0.20
2009			
January	7.00+0.15	27.18+0.25	35.54+0.02
February	7.20+0.70	22.50+0.90	32.68+0.26
March	6.82+0.02	17.58+0.46	23.80+0.30
April	6.90+0.17	15.98+0.65	21.90+0.16
May	7.00+0.04	17.85+0.34	20.48+0.45
June	7.10+0.06	24.20+0.40	21.32+0.20

Values are mean (n=3) + SD

Population dynamics of *Sesbania aculeata* L. litter (mixed in soil) inhabiting mycoflora:
Quantitative nature of green manure inhabiting mycoflora: The maximum fungal population (47.85×10^4) per gram decomposing dry litter incorporated in the field soil was in the month of July, 2008 (Table 5), approximately after 30 days of litter amendment in soil during decomposition period. The minimum fungal population was recorded in the month of May, 2009 (20.48×10^4).

The dynamics of fungal population can be attributed to abiotic variables *viz.,* temperature, moisture content, type of soil and nature of the substrate (Kumar, 2010) [18, 24].

Qualitative nature of green manure litter inhabiting mycoflora: The mycoflora were classified into four groups depending upon frequency, abundance, sporulation and time of appearance in different months.

Direct observation method: Eighteen fungal species were observed directly under the binocular microscope (Table 6). Out of these, *Aspergillus niger, Penicillium citrinum,* *Trichoderma harzianum, Alternaria alternata, Curvularia lunata, Cladosporium cladosporioides, Fusarium semitectum* and dark sterile mycelium were observed as dominant fungi.

Table 6: Direct observation of fungal species from decomposing *Sesbania aculeata* L. by binocular microscope under natural conditions

Fungal Species	2008			
	July	Aug	Sept	Oct
Rhizopus stolonifer	-	+	-	+
Choanephora cucurbitarum	+	+	-	-
Colletotrichum falcatum	+	-	-	-
Aspergillus fumigatus	+	-	+	-
Aspergillus niger	+	+	+	+
Penicillium citrinum	+	+	+	+
Trichoderma harzianum	-	+	+	+
Alternaria alternata	+	-	+	+
Curvularia lunata	+	-	+	+
Humicola grisea	-	-	-	+
Nigrospora sphaerica	-	-	-	+
Torula graminis	+	-	-	-
Cladosporium cladosporioides	-	+	+	+
Epicoccum purpurascens	+	+	-	-
Fusarium semitectum	+	+	+	-
Drechslera avanacea	+	+	-	-
Diplococcum spicatum	+	-	-	+
Dark sterile mycelium	+	+	-	+

Damp chamber incubation method: By this method sampling was performed from July 2008 to September 2008 because the green manure was broken down into small pieces after this period which was very difficult to handle for sampling. A total number of 21 fungal species were isolated by this method (Table 7). *Pythium aphanidermatum, Aspergillus flavus, A. niger, Penicillium citrinum,* *Trichoderma harzianum, Curvularia lunata, Cladosporium cladosporioides, Fusarium semitectum* and dark sterile mycelium were recorded as dominant species, whereas *Phoma hibernica, Drechslera avenacea, Humicola grisea* and pink sterile mycelium were observed only once by this method.

Table 7: Fungi recorded from *Sesbania aculeata* L. litter by damp chamber incubation method under natural conditions

Fungal Species	Months		
	July	August	September
Pythium aphanidermatum	+	+	+
Rhizopus stolonifer	+	+	-
Chaetomium globosum	+	+	-
Phoma hibernica	-	+	-
Pestalotia mangiferae	+	-	+
Aspergillus flavus	+	+	+
Aspergillus niger	+	+	+
Aspergillus sulphureus	+	+	-
Penicillium citrinum	+	+	+
Trichoderma harzianum	+	+	+
Alternaria alternata	-	+	+
Curvularia lunata	+	+	+
Drechslera avenacea	-	-	+
Diplococcium spicatum	+	-	-
Torula graminis	+	-	+
Cladosporium cladosporioides	+	+	+
Humicola grisea	+	-	-
Fusarium semitectum	+	+	+
Fusarium moniliforme	+	+	-
Dark sterile mycelium	+	+	+
Pink sterile mycelium	-	-	+

+=Present, - = Absent

Dilution plate technique: A total number of 37 fungal species were isolated by this method (Table 8). *Mucor racemosus, Phoma glomerata, Macrophomina phaseoli, Aspergillus luchuensis, A. sydowi, A. terreus, Penicillium rubrum, Trichoderma viride, T. koningii, Gliocladium roseum, Alternaria solani, Fusarium solani, Myrothecium* *roridum* and white sterile mycelium were observed by only this technique.

The diverse fungal population appeared at different stages of decomposition under natural conditions may be classified into four different groups depending upon their occurrence during experimental period.

Table 8: Frequency and Abundance of fungal species associated with the decomposing *Sesbania aculeata* L. litter mixed in soil under natural conditions

Note: Months Jul–Dec belong to 2008; Jan–Jun belong to 2009. For each month, F = Frequency, A = Abundance.

Fungal Species	Jul F	Jul A	Aug F	Aug A	Sept F	Sept A	Oct F	Oct A	Nov F	Nov A	Dec F	Dec A	Jan F	Jan A	Feb F	Feb A	Mar F	Mar A	Apr F	Apr A	May F	May A	Jun F	Jun A
Pythium aphanidermatum	-	-	1	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus stolonifer	2	4	3	4	3	2	-	-	-	-	-	-	5	4	4	3	-	-	-	-	1	1	1	1
Mucor racemosus	-	-	2	1	2	3	-	-	-	-	1	2	2	3	-	-	-	-	-	-	-	-	-	-
Chaetomium globosum	-	-	-	-	2	3	2	2	1	1	-	-	-	-	-	-	-	-	1	1	-	-	-	-
Phoma glomerata	5	3	3	1	-	-	3	4	5	4	2	4	2	2	4	3	1	1	-	-	-	-	-	-
Macrophomina phaseoli	-	-	-	-	-	-	-	-	-	-	-	-	1	2	1	1	1	2	-	-	-	-	-	-
Pestalotia mangiferae	-	-	-	-	2	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	1	1	-	-
Aspergillus flavus	2	3	4	3	5	4	2	3	1	1	3	2	4	3	4	2	2	1	1	2	-	-	-	-
Aspergillus fumigatus	5	4	4	5	3	4	5	2	4	2	-	-	2	1	1	1	-	-	-	-	1	1	-	-
Aspergillus niger	4	3	4	5	5	2	5	5	4	5	3	2	4	1	2	2	4	3	2	1	-	-	-	-
Aspergillus luchuensis	-	-	-	-	-	-	-	-	-	-	4	1	-	-	-	-	-	-	2	1	-	-	-	-
Aspergillus sydowi	-	-	-	-	4	2	1	1	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus sulphurous	1	1	1	2	-	-	-	-	1	1	2	1	2	3	2	1	-	-	-	-	1`	1	-	-
Aspergillus terreus	-	-	1	1	-	-	-	-	2	1	1	1	3	2	4	3	-	-	1	1	-	-	-	-
Penicillium citrinum	5	4	4	2	3	2	5	4	3	2	3	4	4	2	3	1	5	3	5	4	2	1	1	1
Penicillium rubrum	2	3	2	1	-	-	1	1	3	2	5	4	2	3	4	3	1	2	-	-	-	-	1	3
Trichoderma harzianum	-	-	1	1	1	2	3	2	4	3	5	3	4	2	4	5	2	1	2	4	2	1	1	1
Trichoderma koningii	1	1	-	-	-	-	3	4	4	5	3	2	-	-	-	-	2	1	3	2	-	-	-	-
Gliocladium roseum	2	3	4	2	5	4	1	2	1	1	-	-	-	-	2	1	3	2	4	5	-	-	-	-
Alternaria alternata	-	-	-	-	3	2	2	1	1	1	3	2	2	1	1	1	-	-	2	3	-	-	-	-
Alternaria solani	-	-	-	-	4	3	-	-	1	2	2	1	-	-	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	5	3	5	2	-	-	1	3	4	2	-	-	1	1	-	-	2	3	5	4	5	5	3	4
Drechslera avenacea	2	1	3	2	3	1	2	1	-	-	-	-	-	-	1	2	2	3	-	-	-	-	-	-
Humicola grisea	-	-	-	-	-	-	1	1	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-
Nigrospora sphaerica	-	-	-	-	-	-	-	-	-	-	-	-	2	1	1	1	-	-	-	-	-	-	-	-
Torula graminis	1	2	2	2	1	1	-	-	-	-	-	-	-	-	-	-	1	1	1	1	1	1	-	-
Diplococcium spicatum	1	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium cladosporioides	-	-	-	-	-	-	5	4	5	3	4	5	4	4	3	2	3	1	1	1	-	-	-	-
Epicoccum purpurascens	2	1	1	2	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium semitectum	5	4	5	3	4	3	5	2	4	1	2	3	1	1	-	-	-	-	2	1	-	-	-	-
Fusarium solani	-	-	2	1	1	1	-	-	-	-	3	4	5	3	-	-	-	-	-	-	1	1	-	-
Fusarium moniliforme	-	-	2	3	1	1	3	2	4	1	1	2	-	-	1	1	-	-	-	-	-	-	-	-
Myrothecium roridum	-	-	-	-	-	-	1	1	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-
Dark Sterile mycelium	5	4	3	2	5	3	-	-	5	3	4	2	3	4	3	5	-	-	2	1	1	1	-	-
White Sterile mycelium	-	-	2	1	-	-	3	2	-	-	-	-	2	2	-	-	1	1	-	-	-	-	-	-
Unidentified I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	2	-	-	-	-
Unidentified 2	-	-	-	-	-	-	1	1	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-

Groups I: Dominant Fungi: This group includes only those decomposing mycoflora which appeared in more than six observations. *Rhizopus stolonifer, Phoma glomerata, Aspergillus flavus, A. fumigatus, A. niger, A. sulphureus, Penicillium citrinum, P. rubrum, Trichoderma harzianum, Gliocladium roseum, Alternaria alternata, Curvularia lunata, Cladosporium cladosporioides, Fusarium semitectum* and Dark sterile mycelium were found to be dominant fungi during isolation period.

Group II: Common Fungi: This group includes the fungi which occurred in five to six months during experimental period with less frequency and abundance. The common fungi isolated were *Aspergillus terreus, Trichoderma koningii, Drechslera avanacea, Torula graminis, Fusarium solani* and *F. moniliforme.*

Group III: Frequent Fungi: This group includes the mycoflora which appeared in at least 3-4 observations with normal frequency and abundance. *Mucor racemosus, Chaetomium globosum, Macrophomina phaseoli, Pestolotia mangiferae, Aspergillus sydowi, Alternaria solani, Epicoccum purpourascens* and white sterile mycelium were recorded as frequent fungi.

Group IV: Rare Fungi: The fungi of this group appeared only once or twice with very low frequency and abundance. *Pythium aphanidermatum, Aspergillus luchuensis, Humicola grisea, Nigrospora sphaerica, Diplococcum spicatum* and *Myrothecium roridum* were recorded to be the member of this group.

In present study, the fungi recorded from decomposing green manure substrate both in experimental and natural conditions were mostly the members of Dueteromycetes suggesting that the fungi belonging to this class are strong colonizers of the decaying substrate with better adaptability, high competitive ability, whereas those of Phycomycetes and Ascomycetes were found weak colonizers. These results are in accordance with De Santo *et al.* (2002) [6] and Vibha and Sinha (2007) [35]. The dominance and predominance of *Aspergillus* spp., *Penicillium citrinum, Fusarium* spp, *Trichoderma* spp. were observed and obtained results were similar with the findings of Kara and Asan (2007) [13], Pandey and Sinha (2008) [27] and Kumar *et al.* (2011) [23]. 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; Sinha *et al.*, 2010) [11, 14, 32].

Conclusion

The dominant decomposing fungi selected after screening based on several techniques, contribute efficiently in the decomposition process. Several fungal communities ensured their stage specific occurrence during the whole process of the decomposition. Their occurrence was fluctuated with the varying substrate of the decomposing green manure (*Sesbania aculeata* L.). Some of the dominant decomposing fungi *viz., Aspergillus niger, Penicillium citrinum, Trichoderma harzianum, Curvularia lunata* and *Cladosporium cladosporioides* contributed potentially in the decomposition of the *Sesbania aculeata* L. These fungi were already present in the soil but after getting their substrate in the form of *Sesbania aculeata* L. they enhanced their growth and reproduction by colonizing the substrate rapidly. These fungi can be used for the purpose of biodegradation of the agricultural waste and agro-industrial wastes.

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