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

Asha Sinha, Ravindra Kumar and Seweta Srivastava

DOI: https://doi.org/10.22271/chemi.2020.v8.i5ac.10613

Abstract

Green manuring is a cost-effective technology to sustain soil fertility and enhance activities of soil microflora 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 (48.95×10⁴ per g green manure) and natural condition (47.85×10⁴ 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) [10]. 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) [11, 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.
50 nylon net bags (mesh size of 1 mm²) 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 °C 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).

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.

**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 (1:10³, 1:10⁴, 1:10⁵, 1:10⁶) 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+ 2°C 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]; Subramaniam, 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:

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

**Determination of frequency and abundance of fungi:** Frequency and abundance of fungi were determined following Saksena (1955) [20].

**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:

![Fig 1: Meteorological data standard (month wise) of Varanasi during 2008-2009.](image-url)
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 |
|--------|--------------------------|--------------------|-------------------------|
| 2008   |                          |                    |                         |
| July   | 50.00                    | -                  | -                       |
| August | 46.78                    | 3.22               | 6.44                    |
| September | 37.99            | 8.79               | 23.40                  |
| October | 29.78                    | 8.21               | 27.40                   |
| November | 21.26                   | 8.52               | 39.58                   |
| December | 16.40                    | 4.86               | 29.60                   |
| 2009   |                          |                    |                         |
| January | 11.38                    | 5.02               | 36.62                   |
| February | 10.08                   | 1.30               | 11.42                   |
| March   | 8.82                     | 1.26               | 13.20                   |
| April   | 8.15                     | 0.67               | 7.94                    |
| May     | 7.40                     | 0.75               | 9.20                    |
| June    | 6.41                     | 0.99               | 13.37                   |
| SEm±    | 0.14                     | -                  | 0.01                   |
| CD(P=0.05) | 0.42                 | -                  | 0.02                   |

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].

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].

Fig 2: pH, moisture content and average number of fungi per g oven dried decomposing green manure (Sesbania aculeata L.) under experimental conditions
Population dynamics of *Sesbania aculeata* L. litter inhabiting mycoflora:

Quantitative nature of the green manure inhabiting mycoflora: The maximum number (48.95×10⁴) of fungi/g litter was recorded in the month of July, whereas minimum was in the month of June (19.78×10⁴) (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*              | -         | +         | -         | -         | -         | -         | -         | -         | -         | -         | -         | -         |
| *Chonemephora cucurbitarum*        | +         | +         | -         | -         | -         | -         | -         | -         | -         | -         | -         | -         |
| *Colletotrichum falcatus*          | +         | +         | -         | -         | -         | -         | -         | -         | -         | -         | -         | -         |
| *Aspergillus fumigatus*            | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         | -         |
| *Aspergillus niger*                | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         |
| *Penicillium citrinum*             | +         | -         | +         | -         | -         | -         | -         | -         | -         | -         | -         | -         |
| *Trichoderma harzianum*            | -         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         |
| *Alternaria alternata*             | -         | -         | +         | +         | +         | +         | +         | +         | +         | +         | +         | -         |
| *Curvularia lunata*                | -         | -         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         |
| *Hemicola grisea*                  | -         | -         | -         | -         | -         | -         | -         | -         | -         | -         | -         | -         |
| *Nigrospora sphaerica*             | -         | -         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         |
| *Torula graninis*                  | +         | -         | -         | -         | -         | -         | -         | -         | -         | -         | -         | -         |
| *Cladosporium cladosporioides*     | -         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         | +         |
| *Epichoccum 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 subtilissima*, *Phoma hibernica*, *Drechslera avenacea*, *Nigrospora sphaerica*, *Epichoccum purpurascens* and pink sterile mycelium were observed only once by this method.
Fungi recorded from decomposing *Sesbania aculeata* L. by damp chamber incubation method under experimental conditions

| Fungal Species          | 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 pallescens*, *Diplodococcus spicatum*, *Helminthosporium oryzae*, *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 3

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

| Fungal Species          | 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    | -    | -      | +         |

++=Present, - = Absent

### Table 4

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

| Fungal Species          | 2008  | 2009  |
|-------------------------|-------|-------|
| Pythium aphanidermatum  | 2 1 3 2 | 2 1 3 2 |
| Rhizopus stolonifer     | 4 3 3 4 | 4 3 3 4 |
| Mucor racemosus         | 2 2 4 3 | 2 2 4 3 |
| Chaetomium globosum     | - - - - | - - - - |
| Phoma hibernica         | - - 1 1 2 2 3 2 | - - 1 1 2 2 3 2 |
| Macrophomina phaseolii  | - - - - | - - - - |
| Pestalotia mangiferae   | 1 1 1 1 2 1 | 1 1 1 1 2 1 |
| Aspergillus flavus      | 5 3 5 4 | 5 3 5 4 |
| Aspergillus niger       | 4 4 4 5 | 4 4 4 5 |
| Aspergillus luchuensis  | - - - - | - - - - |
| Aspergillus candidus    | - - - - | - - - - |
| Aspergillus sydowi      | - - - - | - - - - |
| Aspergillus sulphureus  | 1 1 - - | 1 1 - - |
| Aspergillus terreus     | - - 1 1 3 2 5 | - - 1 1 3 2 5 |
| Penicillium rubrum      | - 1 1 3 2 5 | - 1 1 3 2 5 |
| Penicillium citrinum    | 5 3 4 2 3 2 5 | 5 3 4 2 3 2 5 |
| Penicillium chrysogenum | - - - - | - - - - |
| Trichoderma harzianum   | 1 1 1 1 2 1 | 1 1 1 1 2 1 |
| Trichoderma viride      | - - - - | - - - - |
| Trichoderma koningii    | - - - - | - - - - |
| Gliocladium roseum     | - - - - | - - - - |
| Alternaria alternata    | - - - - | - - - - |
| Alternaria solani       | - - - - | - - - - |
| Curvularia lunata       | - - 1 1 2 1 | - - 1 1 2 1 |
| Curvularia pallescens   | 1 1 1 1 | 1 1 1 1 |
| Drechslera avenacea     | 2 1 3 2 3 2 2 | 2 1 3 2 3 2 2 |
| Humicola grisea         | - - - - | - - - - |
| Nigrospora sphaerica    | - - - - | - - - - |
The minimum fungal population was recorded in the month of July, 2008 (Table 5), and pink sterile mycelium were recorded as member of this group.

### 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*, *Pestalotiopsis 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*, *Diplococcium 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 X10¹⁴ |
|--------------|-----------|----------------------|----------------------------------------------------------|
|              |           |                      | 2008                                                      | 2009                                                      |
| 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                                               |
| 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 (47.85×10¹⁴) 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 (20.48×10¹⁴).

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.

| Fungal Species                        | 2008 |       |       |
|---------------------------------------|------|-------|-------|
|                                       | July | Aug   | Sept  |
| Rhizopus stolonifer                   | -    | +     | -     |
| Choanephora cucurbitarum              | +    | +     | -     |
| Colletotrichum falcum                 | +    | -     | -     |
| 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 avenacea                   | +    | +     | -     |
| 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.

| Fungal Species                        | 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*                 | -    | -      | +         |
| *Diplococcum spicatum*                | +    | -      | -         |
| *Torula graminis*                     | +    | -      | +         |
| *Cladosporium cladosporioides*        | +    | +      | +         |
| *Humicola grisea*                     | +    | -      | -         |
| *Fusarium semitectum*                 | +    | +      | +         |
| *Fusarium moniliforme*                | +    | +      | -         |
| *Dark sterile mycelium*               | +    | +      | +         |
| *Pink sterile mycelium*               | -    | -      | +         |

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

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

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

| Fungal Species                      | 2008                              | 2009                              |
|-------------------------------------|-----------------------------------|-----------------------------------|
|                                     | Jul  | Aug | Sept | Oct  | Nov  | Dec  | Jan  | Feb  | Mar  | Apr  | May  | Jun  |
| Pythium aphanidermatum              | -    | -   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Rhizopus stolonifer                 | 2    | 4   | 3    | 4    | 3    | 2    | 3    | 4    | 3    | 4    | 3    | 1    |
| Mucor racemosus                     | -    | -   | 2    | 1    | 2    | 3    | 2    | -    | 1    | 2    | 3    | -    |
| Chaetomium globosum                 | -    | -   | -    | 2    | 3    | 2    | 1    | 1    | 1    | -    | -    | -    |
| Phoma glomerata                     | 5    | 3   | 3    | 1    | -    | -    | -    | 1    | 1    | 1    | 1    | 1    |
| Macrophomina phaseoli               | -    | -   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Pestalotia mangifera                | -    | -   | -    | 2    | 1    | -    | -    | 1    | 1    | 1    | 1    | -    |
| Aspergillus flavus                  | 2    | 3   | 4    | 5    | 3    | 4    | 2    | 3    | 2    | 1    | 1    | 1    |
| Aspergillus fumigatus               | 5    | 4   | 4    | 5    | 4    | 5    | 2    | 4    | 2    | 3    | 1    | 1    |
| Aspergillus niger                   | 4    | 3   | 4    | 5    | 4    | 5    | 3    | 4    | 3    | 2    | 3    | 1    |
| Aspergillus luchuensis              | -    | -   | -    | 2    | 3    | 4    | 3    | 2    | 1    | 1    | -    | -    |
| Aspergillus sydowi                  | -    | -   | -    | 4    | 2    | 1    | 1    | -    | -    | -    | -    | -    |
| Aspergillus sulphures               | 1    | 1   | 1    | 2    | -    | -    | -    | 1    | 2    | 3    | 2    | 1    |
| Aspergillus terreus                 | -    | -   | 1    | 1    | -    | -    | 2    | 1    | 1    | 1    | 2    | -    |
| Penicillium citrinum                | 5    | 4   | 4    | 2    | 3    | 2    | 5    | 4    | 3    | 2    | 3    | 4    |
| Penicillium rubrum                  | 2    | 3   | 2    | 1    | -    | -    | 3    | 2    | 3    | 4    | 3    | 2    |
| Trichoderma harzianum              | -    | -   | 1    | 1    | 2    | 3    | 2    | 4    | 3    | 3    | 1    | 2    |
| Trichoderma koningii               | 1    | 1   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Gliocladium roseum                 | 2    | 3   | 4    | 2    | 5    | 4    | 1    | 2    | 1    | 1    | -    | -    |
| Alternaria alternata                | -    | -   | -    | 3    | 2    | 1    | 1    | 1    | 3    | 2    | 1    | -    |
| Alternaria solani                  | -    | -   | 4    | 3    | -    | -    | -    | 1    | 2    | 2    | 1    | -    |
| Curvularia lunata                  | 5    | 3   | 5    | 2    | -    | -    | 1    | 3    | 4    | 2    | -    | -    |
| Drechslera avanaeacea              | 2    | 1   | 3    | 2    | 3    | 1    | 2    | 1    | -    | -    | -    | -    |
| Humicola grisea                    | -    | -   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Nigrospora sphaerica               | -    | -   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Torula graminis                    | 1    | 2   | 2    | 2    | 1    | 1    | -    | -    | -    | -    | -    | -    |
| Diplodococcum spicatum             | 1    | 2   | 1    | 1    | -    | -    | -    | -    | -    | -    | -    | -    |
| Cladosporium cladosporoides        | -    | -   | -    | -    | 5    | 4    | 3    | 4    | 5    | 4    | 4    | 3    |
| Epicoccum purpurascens             | 2    | 1   | 1    | 2    | -    | -    | 1    | 1    | -    | -    | -    | -    |
| Fusarium semitectum                | 5    | 4   | 5    | 3    | 4    | 5    | 2    | 4    | 1    | 2    | 3    | 1    |
| Fusarium solani                    | -    | 2   | 1    | 1    | -    | -    | -    | 3    | 4    | 5    | 3    | -    |
| Fusarium moniliforme               | -    | 2   | 3    | 1    | 1    | 3    | 2    | 4    | 1    | 1    | -    | -    |
| Myrothecium roidur                 | -    | -   | 1    | 1    | -    | -    | -    | 1    | 2    | -    | -    | -    |
| Dark Sterile mycelium              | 5    | 4   | 3    | 2    | 5    | 3    | -    | 5    | 3    | 4    | 2    | 3    |
| White Sterile mycelium             | -    | 2   | 1    | -    | 3    | 2    | -    | 2    | 2    | -    | -    | -    |
| Unidentified 1                     | -    | -   | -    | -    | -    | -    | -    | 1    | 1    | 1    | 1    | 2    |
| Unidentified 2                     | -    | -   | -    | -    | -    | -    | -    | 1    | 1    | 1    | 1    | -    |

**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 cladosporoides, 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 avanaeacea, 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, Macroghromina phaseoli, Pestalotia mangifera, Aspergillus sydowi, Alternaria solani, Epicoccum purpurascens* 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, Diplodococcum spicatum and Myrothecium roidur* 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 Dueterozymes suggesting that the fungi belonging to this class are strong colonizers of the decaying substrate with better adaptability, high competitive ability, whereas those of Phycomyces and Ascomycetes were found weak colonizers. These results are in accordance with De Santo et al. (2002) and Vibha and Sinha (2007) and Kumar et al. (2011). 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; Kodseue et al., 2008; Sinha et al., 2010).
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.

References
1. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi, Burges Publishing Co., Minneapolis, Minnesota, USA, 1972.
2. Batty LC, Younger PL. The effect of pH on plant litter decomposition and metal cycling in wetland mesocosms supplied with mine drainage. Chemosphere. 2007; 66:158-164.
3. Berkenkamp A, Priesack E, Munch JC. Modeling the mineralization of plant residue on the soil surface, Agronomie. 2002; 22:711-722.
4. Bocock KL, Gilbert OJW. The disappearance of leaf litter under woodland condition, Plant and Soil. 1957; 9:179-185.
5. Boedijn KB. Trypon blue as a stain for fungi, Stain Technology. 1956; 31:115-116.
6. De Santo AV, Rutigliano FA, Berg B, Fioretto A, Pappi G, Alfuni A et al. Fungal mycelium and decomposition of needle litter in three contrasting coniferous forests, Acta Oecologia. 2002; 23(4):247-259.
7. Ellis MB. More Dematiaceous Hyphomycetes, CMI, Kew, England, 1971.
8. Garrett SD. Soil Fungi and soil Fertility, The Macmillan Company, New York, 1981, 66-77.
9. Gilman JC. A Manual of soil Fungi, Oxford and IHB Publishing Co, Calcutta, 1975.
10. Herzog C, Hartmann M, Frey B, Stierli B, Rumpel C, Buchmann N et al. Microbial succession on decomposing root litter in a drought-prone Scots pine forest. ISME Journal. 2019; 13:2346-2362, doi: 10.1038/s41396-019-0436-6
11. Hobbie EA, Watrud LS, Maggard S, Shiroyama T, Rygiewicz PT. Carbohydrate use and assimilation by litter and soil fungi assessed by carbon isotopes and BIOLOG (R) assays. Soil Biology and Biochemistry. 2003; 35(2):303-311.
12. Jing T, Shibata A, Zhou Q, Katayama A. Effect of temperature on reaction rate and microbial community in composting of cattle manure with rice straw, Journal of Bioscience and Bioengineering. 2007; 104(4):321-328.
13. Kara O, Asan A. Microfungal community structure from forest soils in Northern Thrace Region, Turkey. Annals of Microbiology. 2007; 57(2):149-155.
14. Kodsueb R, McKenzie EHC, Lumyong S, Hyde KD. Fungal succession on woody litter of Magnolia nitida (Magnoliaceae). Fungal Diversity. 2008; 30:55-72.
32. Sinha A, Srivastava M, Kumar R, Srivastava S, Mishra HM. Mycoflora of decomposing kitchen waste in relation to different climatic factors and its effect on soil borne plant pathogens. Environment and Ecology. 2010; 28(3):1458-1462.

33. Subramanium CV. Hyphomycetes, ICAR, New Delhi, 1971.

34. Thom C, Raper KB. Manual of Aspergilli. Williams and Wilkins Co., Baltimore, USA, 1945.

35. Vibha, Sinha A. Variation of soil mycoflora of rice stubble from rice wheat cropping system. Mycobiology. 2007; 35(4):191-195.

36. Warcup JH. Method for isolation and estimation of activities of fungi in soil. In: Parkinson D., Waid S., (Eds) Ecology of Soil Fungi, The University Press, Liverpool, 1960, 3-21.

37. Wardle DA, Bardgett RD, Walker LR, Bonner KI. Among and within species variation in plant litter decomposition in contrasting long-term chronosequences. Functional Ecology. 2009; 23(2): 442-453.

38. Xu RK, Coventry DR. Soil pH changes associated lupin and Wheat plant materials incorporated in a red-brown soil. Plant and Soil. 2003; 250(1):113-119.

39. Zaller JG, Koepke U. Effect of traditional and bio dynamic farmyard manure amendment on yields, soil chemical, biochemical and biological properties in a long term field experiment. Biology Fertility of Soils. 2004; 40:222-229.