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Microbial diversity on the leaf litter of Bhindi [*Abelmoschus esculentus* (L.) Moench] crop fields at the different growth stages of the plants in Barpeta, Assam

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The leaf-litter microflora in Bhindi crop fields at the different growth stages of the plants was analyzed. The results showed that the greater abundance of microorganisms (bacteria and fungi) were found in the leaf litter of Mandia Bhindi crop field than Goremari Bhindi crop field and the number of microflora in different days of decomposition of leaf litter differ at different growth stages of the plants. The maximum numbers of microorganism were recorded from the leaf litter in 45 days of decomposition at after harvesting stage.

A total of 24 different fungal types belonging to 17 genera were isolated qualitatively from the different days of decomposition of leaf litter at different growth stages of the plants in Mandia and Goremari Bhindi crop fields. Some of the most important dominant fungal species isolated were *Aspergillus fumigatus, A. flavus, A. niger, Fusarium oxysporum, Mucor hiemalis, Penicillium citrinum, P. oxalicum, Rhizopus nigricans* and *Trichoderma viride.* It was found that the number of microorganisms increases with the increase in days of decomposition and the appearance of fungal types vary according to the stages of decomposition of leaf litter. The variation of the abundance of microflora between the two Bhindi crop fields may be due to the variation of the physico-chemical properties of the crop field soils. The experimental results revealed that the decomposition of leaf litter and nutrient recycling by a wide range of microorganisms which is useful in soil and manure formation, soil structure and improvement of any habitat.

Key words: Leaf litter, microfungi, bacteria, diversity, decomposition, Bhindi crop

INTRODUCTION

Bhindi [*Abelmoschus esculentus* (L.) Moench] is one of the most important vegetable crops of the world from its nutritional, medicinal and export point of view. It is a popular vegetable in India grown extensively all the year round. Bhindi or Okra, an herbaceous hairy annual plant of the mallow family (*Malvaceae*) of the world tropics and widely cultivated or naturalized in the tropical and sub-tropical countries. It is grown from seed in tropical and sub-tropical parts of the world. The cultivated Bhindi is old world origin. Bhindi or Okra or Gumbo is a half hardy plant introduced into the USA and the West Indies from Africa and cultivated for its fruit pods which are used in soups, stews, catsups and the like. The phylloplane or leaf surface represents an important terrestrial habitat that harbours a wide range of microorganisms. The leaf surface is a suitable environment for microbial growth because of a thin film of nutrients deposited on the leaf. The phylloplane microflora of Bhindi has been studied by Ogwu and Osawaru (2014) and they have reported a wide range of predominant microflora of the members *Rhodotorula, Mucor, Aspergillus* and *Penicillium* for the fungi while *Micrococcus, Staphylococcus* and *Serratia* for the bacteria. The phyllosphere and phylloplane microflora take active part in the decomposition of plant material after leaf fall. The decomposition of leaf litter is an extremely complex processes and is controlled by multitude of organisms which inhabiting in soil. Leaf plays a major role in the succession of microorganisms in soil and enhances the fertility in soil. The sequence

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of fungal succession depends on the natural substratum which reflects a complex interaction of nutritional relationships between each microorganism and the substratum. Fungi are decomposers of plant materials and thus variety of organic constituents are given to the succession of the fungi of the ecological groups, an organism in the organic constituents until the complete decomposition. These organic constituents are the basic for the classification of fungi in to various ecological groups (Sumithra *et al*., 2016). The nutrient availability both for plant growth and ecosystem productivity is contributed directly by litter decomposition (Koukoura *et al*., 2003). The accumulation of litter and its decay are essential processes of the overall energy flow and nutrient cycling phenomenon within an ecosystem. This complex phenomenon of nutrient recycling by fungi in forest ecosystem as they elaborate an array of extracellular enzymes that deconstruct the different types of organic compounds in the litter (Baldrian and Lindahal, 2011) including lignocellulose which other organisms are unable to decompose (de Boer *et al*, 2005).

The leaves which fall on the soil decompose rapidly. It has been reported that competition plays a vital role in disappearance of some fungi during colonization of litter. The fungi present in plant litter influence the microbial population of the surface soil. It is known that the heterotrophic microorganisms play an active role in the initial stages of decomposition by producing extracellular enzymes to utilize the organic substrate present in the litter as energy and nutrient sources. Various intermediate substances are produced during the process of decomposition which is utilized by microbes very rapidly.

Leaves at the seedling stage of plants usually harbour the least number of microbes which increases as the plants age, reaching the maximum population only on yellowing leaves. It is found that fungal decomposition of teak leaf litter is carried out by weak parasitic and Phycomyceteous fungi in the initial stages and later replaced by cellulose and lignin decomposing Ascomycetes and Deuteromycetes. In a comparison of litter decomposition of *Shorea robusta* and *Eucalyptus camaldulensis* plantation of New Forest State it was found that the litter decomposition of *Eucalyptus* was much faster due to change of microclimatic conditions. It was also found that the leaf decomposition increased the fertility level of soil. The decomposition and nutrients recycling by diverse group of microfungi is useful in manure such as compost formation (Akare and Tagade, 2016). By contributing to nutrient and carbon cycling and the maintenance of ecosystems, fungi play an important role in soil formation, fertility, structure and improvement of any habitat (Pan *et al*., 2008). Studies on the microorganisms (bacteria and fungi) associated with potato litter at different days of decomposition showed that several types of fungi appeared during various stages of decomposition; some fungi were found to occur predominantly during the early stages of decomposition and some appeared during middle stage and some fungi were found at latter stages of decomposition.

Several workers have reported that the leaf litter is broken down by the combined action of the decomposer community consisting predominantly of micro-organisms specially bacteria and fungi (Shanthi and Vittal, 2010; Prakash *et al*., 2015; Akare and Tagade, 2016 and Sumithra *et al*., 2016).

The present investigation has therefore been undertaken to isolate and identify leaf litter micro-organisms in Bhindi crop fields at the different growth stages of the plants in Barpeta district of Assam.

MATERIALS AND METHODS

Study site

Barpeta District of Assam covers an area of 3245 square km and lies between latitude $26^0$5' North - $26^0$49' North and longitude $90^0$39' East - $91^0$17' East at an altitude of 53m mean sea level. The general topography of the district varies from low-lying plains to highland having small-hillocks in the South-West-corner of the District. The climate of Barpeta remains mild and pleasant round the year.

The leaf litter of Bhindi required for the experiment was collected from the Bhindi (local variety) cultivation fields of Mandia and Goremari, Barpeta district of Assam at the different growth stages of the plants. These were collected by laying 10 quadrates of 0.5m´0.5m at different litter fall stages i.e., flowering, harvesting and after harvesting stages of growth of the plants. For study of

decomposition, number of nylon net bags were taken, each containing 30 g of air dried litter samples. The study was conducted under cultural condition in the laboratory by making a trench and filling it with litter collected from the experimental fields to stimulate natural conditions for decomposition as the soil mixed with litter put in the trench does not mix with the crop field soil in course of the investigation. Analysis of the litter was carried out at the different growth stages of the plants i.e., 15 days, 30 days and 45 days. The experimental work was done in the Microbiological laboratory, Post Graduate Department of Botany, Madhab Choudhury College, Barpeta, Assam during April-June, 2019.

Isolation of microorganisms from the leaf litter

Direct observation method

This method was suggested by Sharma and Garg (1979) and in this method; an impression film was prepared by placing adhesive cello tape over the leaf surface. Such tape segments were stained with cotton blue and examined for the presence of fungal spores under a compound microscope.

Dilution plate method

The qualitative analysis of leaf litter microflora was studied by the dilution plate method (Warcup, 1960) using Nutrient agar and Potato Dextrose agar media. The litter sample was powdered. 1g of this powder was suspended into 100ml of sterile distilled water. Further dilution series were made (10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}). For the isolation of fungi 10^{-4} dilution and for bacteria 10^{-5} dilution were used. 1 ml of from10^{-4} dilution was inoculated on three Petriplates containing Potato Dextrose Agar medium supplemented with streptomycin (5mg/ 100ml of medium) for the inhibition of bacterial growth. Again 1 ml from 10^{-5} dilution was inoculated on three Petriplates containing Nutrient Agar medium for the isolation of bacteria.

The fungal culture plates were kept in an inverted position in an incubator at 27±1^{0}C for 7days and the bacterial culture plates were kept at 37±1^{0}C for 24 h. Then colony counts were made for individual fungi and bacteria with the help of a digital colony counter. The results were expressed as cfu/ g. Pure culture of individual fungus was made in slants containing PDA medium. On the basis of colony characters, morphological and reproductive structures, some of the fungi were identified up to generic level and some were identified up to species level and were confirmed as per the keys of the manuals of Gilman (1957); Barnett and Hunter (1972) and Funder (1968).

Analysis of soil of the experimental sites

The soil samples were analyzed for the estimation of the following:-

(i) Soil pH: Soil pH was measured at month wise interval by using a digital pH meter.

(ii)Soil temperature: Soil temperature was measured at month wise interval by inserting a soil thermometer into the soil.

(iii) Soil moisture: The moisture content of soil was estimated by Gravimetric method. The percentage of moisture content in the soil samples were calculated by using the following formula. (Jackson, 1973).

$$\% \text{ Moisture} = \frac{\text{Loss in weight}}{\text{Oven dry weight}} \times 100$$

(iv)Phosphorus (P_2O_5): Phosphorus in soil samples was estimated as the method described by Jackson (1973).

(v)Potash content (K_2O): Available potash in soil samples was determined flame photometrically after extracting the soil samples with neutral normal ammonium acetate as described by Jackson (1973).

(vi)Water holding capacity: Soil samples were collected by means of metal core cylinder, and placed in a flat pan. The weight of pan with the soil was taken. Water was gradually poured into the pan. After 24 h, the pan with sample was taken out and the excess water was drained off, dried in an oven at 110^{o}C and weighed. The weight of pan was taken and the difference of weight indicated the water holding capacity of the soil samples.

(vii)Soil organic carbon: Soil organic carbon was analyzed using the rapid titration method of Walkley and Black (1934) as described by Jackson (1973).

(viii) Nitrogen content in soil: The estimation of total nitrogen in soil was determined by use of Micro Kjaldhal apparatus.

(ix) Mechanical fraction and textural class of the sampled soil were determined by International pipette method (Piper, 1966).

RESULTS AND DISCUSSION

The quantitative analysis of leaf litter microorganisms from Mandia and Goremari Bhindi crop fields at the different growth stages of the plants is furnished in the Table 1 and Fig.1. The results showed that the greater abundance of microorganisms (bacteria and fungi) was found in Mandia Bhindi crop field than Goremari Bhindi crop field. It was observed that the number of microflora in the leaf litter differ at different growth stages of the plants and maximum number of micro-organisms (bacteria and fungi) was isolated from the leaf litter at after harvesting stage. The results also showed that the occurrence of bacterial and fungal population in the leaf litter from Mandia Bhindi field was maximum (58 millions/g) and 45 thousand/g respectively at 45 days of decomposition at after harvesting stages of the plants, while in case of Goremari field, these were 53 millions/g and 37 thousand/g respectively for bacterial and fungal population. The lowest occurrence of bacterial and fungal population were recorded as 18 millions/g and 20 thousand/g respectively in Mandia Bhindi crop field in flowering stage at 15 days of decomposition of leaf litter and in case of Goremari Bhindi field these were recorded as 15 millions/g and 18 thousand/g. The progressive changes in the state of the different stages of decomposing leaf can be related to the microflora associated with it. Microflora isolated from different stages of decomposing leaves may depend on the various growth stages of the plants. From the experimental results it was also observed that the number of micro-organisms gradually increases with increase in days of decomposition of leaf litter. The number of fungal and bacterial population was reduced at initial stages of decomposition but the numbers gradually increased from 15 days to 45 days of decomposition.

The results of the qualitative analysis of leaf litter microfungi from Mandia and Goremari Bhindi crop fields at different growth stages of the plants are furnished in Tables 2 and 3. It was observed from the results that a total of 24 different fungal types belonging to 17 genera were isolated from different days of decomposition of leaf litter at different growth stages of the plants in Mandia and Goremari Bhindi crop fields by dilution plate method. Some of the dominant fungal species isolated from the leaf litter of the two Bhindi crop fields were *Aspergillus fumigatus, A. niger, Fusarium oxysporum, Mucor hiemalis, Penicillium citrinum, Rhizopus nigricans* and *Trichoderma viride.* Some less occurring micro fungi from Mandia and Goremari fields isolated were *Aspergillus clavatus, A. flavus, Chaetomium* sp., *Mortierella subtilissima, Nigrospora* sp., *Phoma* sp., *Phytophthora* sp., *Pythium* sp. and *Rhizoctonia solani.* The results also showed that different kinds of fungi appeared during various stages of decomposition of the leaf. Some fungi are found to occur pre-dominantly during the early stages of decomposition (15 days) and some are during the middle stages of decomposition (30 days) whereas some are found at the latter stages of decomposition (45 days of decomposition). In the present investigation, it was observed that the appearance of fungal types vary according to the stages of decomposition of leaf litter. The role of primary fungi like *Alternaria, Cladosporium, Nigrospora, Fusarium, Curvularia,* etc. as initial colonist of fresh decaying plant parts have been reported by various investigators. Similar observations have also been found in the present investigation. Competition plays a vital role in disappearance of some fungi during colonization of litter as has already been emphasized by earlier workers (Akare and Tagade, 2016; Prakash *et al*., 2015). Akare and Tagade (2016) isolated 15 species of fungi belongs to Deuteromycetes, 8 species of Ascomycetes and 2 species of Zygomycetes from leaf litter of Umarzari forest (MS) India. Prakash *et al*., (2015) reported that fungi belonging to Ascomycota were invariably predominant in the litter of most plants in early stages of decay of tropical forest.

It was recorded (Tables 2 and 3) that maximum number of fungal colonies (60 no. of fungal colonies) at after harvesting stage in 45 days of

Table 1: Number of micro-organisms in different days of decomposition of leaf litter collected at different growth stages of the plants of Mandia and Goremari Bhindi crop fields (Fungi ×10^{-4}/g and Bacteria ×10^{-5}/g of leaf litter)

Growth stages of the plants →	Flowering stage						Harvesting stage						After harvesting stage					
Days of decomposition →	15		30		45		15		30		45		15		30		45	
Microorganisms ↓	Mandia	Goremari	Mandia	Goremari	Mandia	Goremari	Mandia	Goremari	Mandia	Goremari	Mandia	Goremari	Mandia	Goremari	Mandia	Goremari	Mandia	Goremari
Bacteria (Millions/g)	13.0	15.0	27.0	22.0	43.0	33.0	33.0	23.0	30.0	26.0	47.0	45.0	60.0	35.0	50.0	48.0	54.0	48.0
Fungi (Thousand/g)	20.0	18.0	27.0	24.0	36.0	33.0	33.0	22.0	30.0	27.0	40.0	36.0	23.0	24.0	32.0	28.0	45.0	37.0

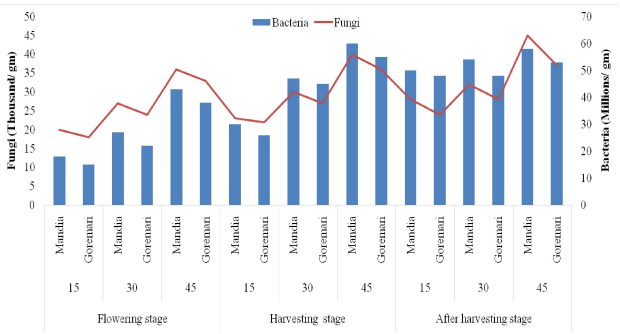

Fig. 1 : Graphical representation of number of micro-organisms in different days of decomposition of leaf litter collected at different growth stages of plants of Mandia and Goremari Bhindi crop fields

decomposition of leaf litter from Mandia Bhindi crop field while in case of Goremari Bhindi crop field, it was 53 nos. of fungal colonies. The lowest number of fungal colonies was recorded (20 nos. of fungal colonies) in flowering stage at 15 days of decomposition of Mandia crop field while in case of Goremari crop field it was recorded 19 no. of fungal colonies. From the results it was observed that the number of fungal colonies gradually increased with increased in days of decomposition from 15 days to 45 days. Some dominating fungi from different decomposed leaves at different growth stages of the plants from the two Bhindi crop fields isolated by dilution plate method were *Aspergillus fumigatus, A. niger, Fusarium oxysporum, Mucor hiemalis, Penicillium citrinum, Rhizopus nigricans* and *Trichoderma viride*. Various other unclassified, Phycomycetes and Basidiomycetes fungi were observed under direct observation through microscope. Similar findings were also reported by Shanthi and Vittal (2010) on cashew. The microfungal population in decomposed potato litter was studied at different days of decomposition which revealed that the presence of microfungi such as *Alternaria alternata, Aspergillus niger, A. flavus, Cladosporium herbarum, Penicillium citrinum, Nigrospora sp., Curvularia lunata, Fusarium semitectum, Trichoderma harzianum, Phoma sp.*

It has been known that favorable environmental condition stimulates the decomposition of leaf litter. The moisture content of soil is significant factor in soil population and it directly influence upon decomposition of leaf litter. From the experimental results (Tables 2 and 3), it was also observed that relatively a higher number of microflora isolated from the leaf litter in Mandia Bhindi crop field than

Table 2: Number of fungal colonies isolated from different days of decomposition of leaf litter collected at different stages of growth of the plants of Mandia Bhindi crop field by dilution plate method. (Results represent the average number of fungal colonies per 0.1 mg leaf litter)

Fungal types isolated	Flowering stage Days of decomposition			Harvesting stage Days of decomposition			After harvesting stage Days of decomposition		
	15	30	45	15	30	45	15	30	45
Alternaria alternata	-	2	2	-	-	3	-	5	3
Aspergillus candidus	-	-	2	-	-	-	2	-	-
A. clavatus	-	-	2	-	-	2	-	-	2
A. fumigatus	2	4	-	-	3	5	-	3	5
A. flavus	-	2	4	2	-	3	2	-	3
A. niger	4	5	6	5	6	6	4	7	9
Ascochyta abelmoschi	-	-	-	-	-	3	-	3	2
Chaetomium sp.	-	-	-	2	-	-	-	2	-
Cladosporium cladosporoides	2	-	-	-	3	3	2	-	3
Curvularia lunata	2	4	3	-	5	-	-	6	2
Fusarium moniliformae	-	2	-	2	-	-	2	-	2
F. oxysporum	3	4	4	4	5	5	-	3	4
F. semitectum	-	-	2	-	2	-	-	-	2
Mucor hiemalis	2	3	3	2	-	3	2	2	-
Mortirella subtilissima	-	-	-	-	2	2	-	-	3
Nigrospora sp.	-	-	-	2	-	-	2	-	-
Penicillium citrinum	-	2	2	2	3	3	-	5	4
P. oxalicum	2	-	-	-	3	2	-	2	-
Phoma sp.	-	-	2	-	-	-	-	3	-
Phytophthora sp.	-	2	-	-	-	-	2	-	2
Pythium sp.	-	-	-	-	-	2	-	-	2
Rhizopus nigricans	-	2	-	2	3	-	4	2	-
Rhizoctonia solani	-	-	-	-	-	2	2	-	2
Trichoderma viride	3	2	2	-	2	-	3	-	5
Unclassified group	-	-	3	2	3	2	4	2	5
Total	20	34	37	25	40	46	31	45	60

Goremari Bhindi crop field which may be due to the variation of the physico-chemical properties of soils (Tables 4 and 5).

The results revealed that the process of decomposition is not a simple but an extremely complex process and pre dealing. It is controlled

Table 3: Number of fungal colonies isolated from different days of decomposition of leaf litter collected at different stages of growth of the plants of Goremari Bhindi crop field by dilution plate method. (Results represent the average number of fungal colonies per 0.1 mg leaf litter).

Fungal types isolated	Flowering stage Days of decomposition			Harvesting stage Days of decomposition			After harvesting stage Days of decomposition		
	15	30	45	15	30	45	15	30	45
Alternaria alternata	2	2	-	2	-	2	-	3	3
Aspergillus candidus	-	-	2	-	-	2	2	-	2
A. clavatus	-	-	-	-	3	-	-	3	-
A. fumigatus	3	3	4	-	4	5	3	3	4
A. flavus	-	2	-	2	2	3	-	3	2
A. niger	3	4	6	3	4	5	5	4	6
Ascochyta abelmoschi	-	-	2	-	-	2	-	2	-
Chaetomium sp.	-	-	-	-	2	-	2	-	2
Cladosporium cladosporoides	-	-	3	-	-	2	-	-	-
Curvularia lunata	-	2	-	2	3	3	3	2	2
Fusarium moniliformae	-	2	2	-	-	2	-	-	-
F. oxysporum	3	3	3	3	4	3	-	3	4
F. semitectum	-	-	-	2	-	-	2	-	2
Mucor hiemalis	3	-	3	-	3	2	3	3	3
Mortirella subtilissima	-	-	2	-	2	-	-	-	2
Nigrospora sp.	-	-	-	-	-	2	-	-	2
Penicillium citrinum	3	2	-	3	-	3	-	3	2
P. oxalicum	-	2	-	-	-	-	-	2	-
Phoma sp.	-	-	2	-	2	-	-	2	2
Phytophthora sp.	-	-	-	-	-	-	2	-	2
Pythium sp.	-	-	-	-	-	2	-	-	-
Rhizopus nigricans	-	-	3	2	2	-	-	2	4
Rhizoctonia solani	-	-	-	-	-	2	-	-	2
Trichoderma viride	-	2	3	3	4	2	4	3	4
Unclassified group	2	-	2	-	3	-	2	3	3
Total	19	24	37	22	38	42	28	41	53

by the multitude of organisms wherein fungi and bacteria play an important role. It may be assumed from the experiment that the microbial diversity indicates the decomposition of leaf litter and nutrient recycling which is useful in soil and manure formation,soil structure and improvement of any habitat.

Table 4: Physico-Chemical characteristics of the soil sample taken from the experimental site of Mandia, Barpeta district, Assam

Months	pH	Moisture %	Temperature (°C)	Water holding capacity	Organic carbon (%)	Total nitrogen (%)	Available P_2O_5 (kg ha^{-1})	Available K_2O (Kg ha^{-1})	Textural class	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)
April	6.1	32.46	22.0°C										
May	5.9	33.07	26.0°C										
June	5.8	43.60	27.8°C										
				72%	0.84	0.12	19.12	128.00	Sandy Loam	8.30	58.75	17.95	15.30

Table 5: Physico-Chemical characteristics of the soil sample taken from the experimental site of Goremari, Barpeta district, Assam

Months	pH	Moisture %	Temperature (°C)	Water holding capacity	Organic carbon (%)	Total nitrogen (%)	Available P_2O_5 (kg ha^{-1})	Available K_2O (Kg ha^{-1})	Textural class	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)
April	5.2	33.76	20°C										
May	5.6	38.71	22°C										
June	5.5	45.79	26°C										
				75%	0.79%	0.08	24.03	98.00	Clay loam	6.50	25.30	28.20	40.00

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