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Degradation of Biopolymers of Khair *(Acacia catechu)* plant litter of Datia Forest through Fungi

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ABSTRACT

Biopolymers like Cellulose, hemicelluloses and lignin are major source of energy and nutrients for microbial communities in forest ecosystem. Plant biomass is degraded by variety of microorganisms living in the soil. Fungi are major decomposers and recyclers of organic matter by secreting various enzymes. The objective of the study was to determine cellulose, hemicellulose and lignin content of *Acacia catechu* leaf litter during different stages of its decomposition. Leaf litter was collected and litter bag technique was applied for its decomposition. Analysis of biopolymer degradation done after removing litter bags from pits at 15, 30, 45, 60, 75, 90, 120, 150, and 180 days of incubation. No change observed in cellulose, hemicellulose and lignin content in early stages of decomposition. Gradual reduction in litter biomass was observed from 45 to 180 days of decomposition. In early stages of decomposition, amount of cellulose, hemicellulose and lignin was remarkably high but as the decomposition progressed, these biopolymers were degraded at very faster rate.Maximum decomposition of biopolymers was observed on 180 days. During the process of decomposition fungi produce various enzymes that work cooperatively to hydrolyse these macromolecules.

Figures : 06 References : 14 Tables : 04
KEY WORDS : *Acacia catechu*, Biomass, Biopolymer, Decomposition, Degradation, Lignocellulose, Litter

Introduction

Plant litter represents a major source of organic carbon in terrestrial ecosystem and most of the vascular plants drop litter in the form of leaves, stems, twigs, branches, flowers, fruits, seeds, barks *etc.* Dead and fallen plant material is a main source of nutrition and energy of soil microbiota.

Lignocellulose is a predominant component of plant litter which mainly consists of Cellulose, hemicelluloses and lignin[11].

Cellulose is composed of linear homopolymer chain of glucose units.Unlike cellulose, hemicelluloses are heterogeneous polysaccharides mainly composed of xylan, xyloglucan, glucomannan, manna, galactomannan, callose and so on[14]. Lignin is a highly complex component that provides mechanical strength to plant cell wall. Lignin synthesized from phenyl propanoid precursors and mainly composed of three different monomers, syringyl lignin, guaiacyl lignin and hydroxyl-phenyl lignin. Gymnosperms mainly contain guaiacyl lignin, dicotyledon angiosperms contain guaiacyl- syringyl and the monocotyledon angiosperms mainly contain guaiacyl- syringyl-hydroxyl phenyl lignin[13].

In nature, microorganisms specially,saprophytic fungi is an integral component of forest ecosystem where they obtain their organic carbon from dead and decaying wood and leaves. These fungi produce enzymes capable of degrading biopolymer such as cellulose, hemicellulose and lignin and fulfil their energy and nutritional requirements fromthem, thus maintain carbon balance of the Earth. In comparison of bacteriam fungi are more suitable to decompose biopolymer due to their metabolism, production of extracellular digestive enzymes and filamentous (hyphal) growth.

The enzymes produced by fungi breakdown large and selectively soluble molecules such as carbohydrates, proteins and lipids into smaller and more soluble molecules. These enzymes allow them to

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TABLE- 1: Statistical analysis of biopolymer degradation

Days	Cellulose (mg)	Hemicellulose (mg)	Lignin (mg)
Control	0.329	0.267	0.378
15	0.329	0.267	0.378
30	0.329	0.267	0.378
45	0.308	0.252	0.378
60	0.176	0.138	0.369
75	0.126	0.098	0.208
90	0.096	0.073	0.187
120	0.058	0.049	0.134
150	0.026	0.018	0.072
180	0.012	0.006	0.023

efficiently attack the lignocellulosic material of plant that other organisms are unable to decompose[3,6].

Degradation of biopolymer is a sequential process that initially involves the loss of simple components of lignocellulosic matrix for example, cellulose and hemicelluloses followed by more complex component, lignin[4]. The objective of the study is to quantitatively analyse loss of biopolymer amount of *Acacia catechu* plant litter by fungi during degradation.

TABLE- 2: Statistical analysis (per cent decrease) in cellulose

Days	(%) Decrease
Control	0
15	0
30	0
45	6
60	47
75	61
90	71
120	82
150	92
180	97

Material and Methods

Study site:

Study site is located at25^0 28' to 26^0 20' N latitude and 78^0 10' to 78^0 45' E longitudein MP, India (Figs. 1a &1b).

The average annual rainfall of district Datia is

Fig. 1(a) : Forest of study sit

Fig. 1 (b) : Tree species *Acacia catechu*

TABLE- 3: Statistical analysis (per cent decrease) in hemicellulose

Days	(%) Decrease
Control	0
15	0
30	0
45	7
60	48
75	63
90	72
120	81
150	93
180	98

TABLE-4 : Statistical analysis (percent decrease) in lignin

Days	(%) Decrease
Control	0
15	0
30	0
45	0
60	2
75	45
90	50
120	64
150	80
180	94

825.93 mm., average maximum temperatures is 32.64ºC and minimum of 18.45ºC.The total forest cover of the Datia district is 291.04 sq. km and the forest blocks are mostly located on the plateau. Kardhai (*Anogeissus pendua*) and Khair (*Acacia catechu*) is a special vegetation of Datia forests, mostly found in northern part of river bank of Sindh near Ratangarh Mata mandir in Seodha block of Datia district (Fig. 1a).

Fig. 2 (a) : Sampling pits

Collection of samples:

The genus *Acacia* is mostly found in Balaghat, Bhopal, Chindwada, East Nimar, Gwalior, Hoshangabad, Indore, Jabalpur, Mandala, Panna, Raisen, Sagar, Satna, Shivpuri, Sidhi and Datia district of MP. The tree species of *Acacia catechu*(*L.F.*) willed commonly known as 'Khair' was selected for study purpose (Fig. 1b).

It is a small or medium sized thorny tree grows mixed with *Anogeissus pendula, Zizyphus xylopyrus* and *Butea monosperma*etc. It is common in the drier region and sandy soil of river banks and water shed. It is a hooked spine tree about 15 m high with dark grey to black bark, splitting irregularly and red inside. Leaves bipinnate; rachis long with a large gland at the base of the petiole, pinnae 7-30 pairs, leaflets 6-50 pair, linear, glabrous or pubscent. Flowers white, axillary pedunculate with long spikes. Pods 5-10 cm long brown beaked. Seeds 3-10, flowering: July to August, fruiting: October to January.

The leaves and timber of *Acacia catechu* are used as fodder, furniture and other household articles.'Kattha' used in pan is extracted from its wood.

The freshly fallen (immediately after the

abscission)and senescent leaves were collected in sterile polythene bags at regular intervals from the forest floor of study site. These sealed plastic bags were brought to the laboratory within four hours for further investigation. Approximately 20g air dried leaf material was placed within 9 different nylon mesh bag[2] and randomly placed inside each of the 9 pits of 2ft (Fig. 2a) for degradation at college campus.

To prevent extensive desiccation the field here the litter bag kept was protected, overlaid with freshly fallen leaves of *Tectona grandis.*

For isolation and identification of fungi and analysis of amount loss of cellulose, hemicellulose and lignin,decomposed litter bag was removed after the incubation period of 15, 30, 45, 60, 75, 90, 120, 150,180 days in sampling pits (Fig. 2b).

Removed bag immediately brought to laboratory for further investigation.

Isolation of fungi:

The fungi were isolated by using serial dilution method[12]. The suspension was diluted to $1:10^3$ to $1:10^4$ times. One ml of this aliquot was inoculated separately into each of the five Petri plates containing 20 ml Potato Dextrose Agar (PDA) medium. To avoid any contamination streptomycin was added in PDA medium. The petri plates were placed in incubator at the temperature of 28± 2ºC.

Estimation of litter biomass: The loss in biopolymer content was Analysed by using the method[9]. In this method, approximately 2g powder of decomposed leaf litter was boiled in ethanol (4 times) in water bath for 15 min. washed thoroughly with distilled water,material kept in oven for dry weight at 40<"C overnight. Dried material again treated with diastase enzyme for 30 min.

Fig. 2 (b) : Litter bag

Material kept in oven for dry weight at 40<"C overnight, then divided into two equal parts which one part was considered as A fraction. Second part of the material was treated with 24% KOH (w/v)for 4 hrs at 25<"C,washed thoroughly with distilled water, dried at 80<"C overnight and the dry weight taken as B fraction. The same samples were again treated with 72% H_2SO_4 at room temperature for 3 hrs to hydrolyse the cellulose and refluxed again with 5% H_2SO_4 for 2 hrs.Washed with distilled water, $H_2SO_4$was removed completely and residue was dried at 80<"C in oven at for overnight and dry weight taken as C fraction. The amount of Cellulose, hemicelluloses and lignin was calculated as:

Cellulose = B-C

Hemicellulose= A-B

Lignin = C itself

Results and Discussion

A total of 8 genera and 14 species colonised on *Acacia catechu* leaf litter during 6 months of decomposition. Among the identified species, 2 species such as *Mucor hiemalis* and *Rhizopus stolonifer* belong to Zygomycota phylum and rest of the 12 species assigned to Ascomycota phylum. The result of the present study also showed that micro fungi colonised during degradation were capable to decompose biopolymer of litter cellulose, hemicellulose and lignin.

The results presented in Tables 1, 2, 3, & 4 showed that when samples of 15 and 30 days analysed, amount of litter biomass remained unchanged This period of decomposition was dominated by 'sugar fungi' which restricted only on simple sugar.

When 45 to 180 days old litter samples were analysed, cellulose and hemicellulose content found to reduce from 0.308 mg to 0.012 mg and 0.252 mg to 0.006 mg respectively while lignin content remained unchanged in this period of degradation. Lignin content was found to reduce from 0.369 mg to 0.023 mg, when the litter samples of 60 to 180 days were analysed.

Approximately 97% amount loss of cellulose, 98% hemicellulose have been observed from 45 to 180 days period of decomposition. The amount loss of lignin was observed about 94% from 60 to 180 days of decomposition (Figs. 3, 4 & 5). In statistical analysis, we observed that the degradation of biopolymer (cellulose, hemicellulose and lignin) was positively correlated and degrade in a same pattern (Fig. 6).

Decrease in the amount of cellulose, hemicellulose and lignin indicate that fungi colonised on litter during decomposition might have actively utilise these compounds through extracellular enzymes

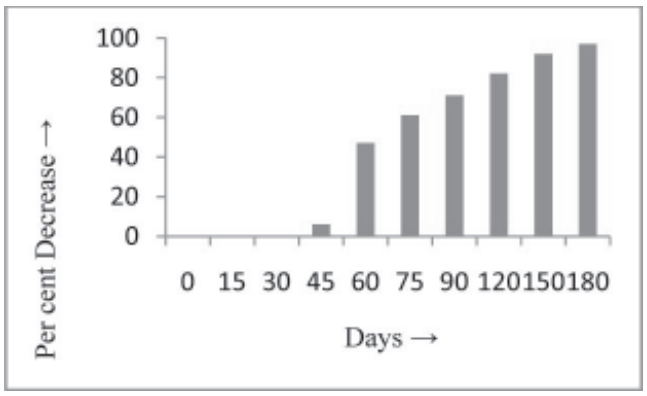

Fig. 3 : Content of cellulose of leaf litter (*Acacia catechu*) indicate significant decrease (percent) in different stages of decomposition

produced by fungi. Decrease in lignin amount indicate that the fungal communities progress from soluble carbohydrates to more complex compound such as cellulose, hemicellulose and lignin.

The first two months of study were characterised by a relatively slow decrease in cellulose, hemicellulose content and no change in lignin content. This period of litter decomposition was dominated by cellulolytic fungi which is generally known to decompose cellulose and hemicellulose over lignin. Rapid loss of biopolymer observed in last 4 months of the litter degradation. The genera colonised on *Acacia catechu* leaf litter are capable to degrade the biopolymer of leaf litter. The majority of fungal species involved in the degradation process belong to the Ascomycota phylum was dominant in early stage of degradation. Fungi belonging to Basidiomycota were detected on litter beginning of at 3 months and their abundance gradually increase as decomposition of leaf litter progresses. The rate of mass loss are relatively slow at this period and the activity of cellulolytic fungi decreased, which indicate that the non-lignified mass of litter was depleted.

The finding of present work was similar to that of another research[5]. Most of the fungal communities follow the similar pattern as observed in previous study.

Fungal communities colonised on leaf litter of *Acacia catechu* produce specific enzymes that hydrolyse lignocellulosic material of plant. The extracellular enzymes produce by these fungi perform both hydrolysis and oxidation action. The hydrolytic enzyme hydrolyses and degrade cellulose, hemicellulose while the oxidative enzyme oxidises lignin.

Cellulases are key enzymes that synergetically act on cellulose to hydrolyse it to its monomers (simple sugars). These enzymes were produced by fungi colonising on cellulosic material during decomposition[8]. The Fungal genera such as *Trichoderma*, *Penicillium* and *Aspergillus* isolated during the investigation were highly cellulolytic in nature. *Trichoderma reesei* produces two cellobiohydolases, five endoglucanases, and two â-glucosidases which hydrolyse cellulose[10].

Fungi such as *Trichoderma reesei* and *Aspergillusniger* produces xylanases requires for hydrolysis of hemicelluloses. Xylan is the main component of hemicellulose, its long chain cleaved by endo-cleaving enzymes[1].

After the significant decomposition of cellulose and hemicellulose fungi advanced to utilise the most complex compound of litter, lignin. Fungi from Basidiomycota produce different enzymes that hydrolyse the more complex component of litter. Some fungi of Basidiomycota produce lignin degrading enzymes that act biocatalyst for oxidation of lignin. Versatile peroxidases, Laccases, Manganese dependent peroxidase and Lignin peroxidase are the main enzymes produced by white rot fungi[7].

Conclusion

Degradation of biopolymer is a sequential process

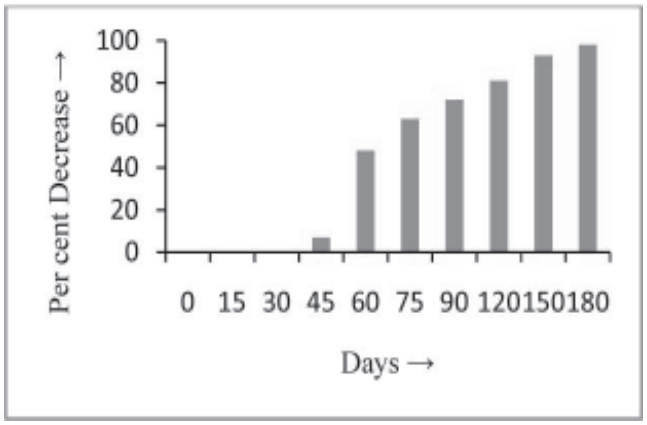

Fig. 4 : Content of hemicellulose of leaf litter (*Acacia catechu*) indicate significant decrease (per cent) in different stages of decomposition

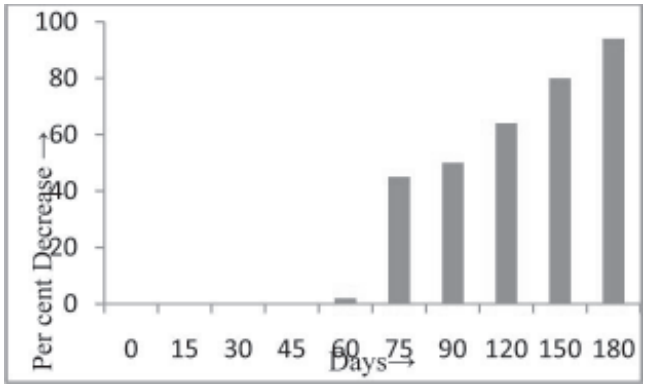

Fig. 5: Content of lignin of leaf litter (*Acacia catechu*) indicate significant decrease (per cent) in different stages of decomposition

that initially involves the loss of simple or soluble polysaccharides (cellulose and hemicelluloses) followed by the more complex biopolymer, lignin. Fungi belong to Ascomycota and Basidiomycota phylum produce enzymes that hydrolyse the biopolymer of *Acacia catechu* leaf litter. These enzymes after isolation and characterization can be used in various industrial purposes such as food processing, brewery and wine making, pulp and paper, textile and laundry, animal feed, agriculture, biomass refining, bioconversion in biofuel *etc.* Recently, many laboratories around the world are searching lignocellulosic wastes as an alternative source of biofuels other than food crops.

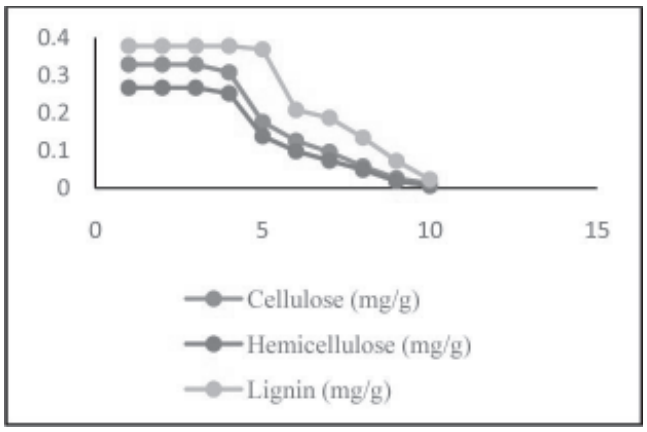

Fig. 6: Statistical analysis (correlation) of biopolymer degradation

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