Plant Archives Volume 20 No. 2, 2020 pp. 3681-3686 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

BIODEGRADATION AND ESTIMATION OF CELLULOSE, HEMICELLULOSE AND LIGNIN CONTENT OF *ANOGEISSUS PENDULA* LEAF LITTER IN DATIA, MADHYA PRADESH, INDIA

Brijesh Kumar Jatav, Tirthesh Kumar Sharma* and Sippy Dassani

Department of Botany and Industrial Microbiology, Bipin Bihari College, Jhansi-284001 (U.P.), India

Abstract

Cellulose, hemicellulose and lignin are major components of lignocellulose and comprise the main composition of plant cell wall. Lignocellulosic waste not only provides alternative source of energy but also reduces our environmental concerns associated with traditional food supplies. The purpose of the study was to isolate and identify the fungal species involved in litter decomposition and determine the content of cellulose, hemicellulose and lignin during different stages of decomposition. *Anogeissus pendula* (Combretaceae) a dominant tree species of study area was selected for study. Leaves were collected at monthly intervals. Isolation and identification was done after removing bag from pit at 15, 30, 45, 60, 75, 90, 120, 150, and 180 days of interval following serial dilution method and PDA as culture media. A total of 16 fungal species belonging to 7 genera were isolated and identified. Among these 3 species belongs to Zygomycota and 13 species belongs to Ascomycota and their anamorphs. *Mucor varians, Mucor hiemalis, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus japonicus* were early colonizers and *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus versicolor, Aspergillus flavipes, Geotrichum candidum, Penicillium chrysogenum, Penicillium aurantiogriseum, Trichoderma reesei, Trichoderma viride* and *Chaetomium osmoniae* found to be as late colonizers. *Aspergillus, Penicillium, Trichoderma,* and *Chaetomium* were the main decomposers of lignocellulose material. Biochemical estimation of cellulose, hemicellulose and lignin content showed that no change in lignocellulose content in early stages of decomposition. Gradual reduction in lignocellulose content was observed from 45 to 180 days due to the colonization of different fungal species which produce a large set of extracellular enzymes that works cooperatively to hydrolyze these compounds. The contents of cellulose, hemicellulose and lignin were remarkably high in the initial stages of decomposition and decreases as the decomposition progresses. It can be concluded that the lignocellulose products and enzymes produced by fungi utilized in various industrial processes.

Key words: *Anogeissus pendula*, Litter, Lignocellulose, Colonization, Decomposition, Estimation, Hydrolyze

Introduction

In recent years, due to the increasing demand for second generation biofuels and biobased products, lignocellulosic biomass attract research attention around the world. This not only provides alternative source of energy but also reduces our environmental concerns associated with traditional food supplies. Lignocellulose is consists of three types of polymers and comprise the main composition of plant cell wall. Cellulose is a linear homopolymer composed of D-glucopyranosic units linked by ß-1,4-glycosidic bonds with cellobiose as its repeating unit (Sun *et al.*, 2014a). Hemicellulose (second only to the cellulose) is an amorphous branched heteropolymer of pentose and hexose sugars (Pu *et al.*, 2008). These sugar units are bound together with glycosidic linkage and are easy to hydrolyze to monosaccharides in hot, dilute mineral acid or cold 5% NaOH solution (Zhang *et al.*, 2006). Lignin is an amorphous phenolic polymer of cross-linked phenyl propane units (*i.e.* syringyl, guaiacyl, and p-hydroxy phenyl) (Ragauskas *et al.*, 2014). Together with hemicellulose, lignin is found in the primary wall, the secondary wall and in the middle lamella of voids of the cellulose-microfibrils (Vanholme *et al.*, 2010). A great variety of microorganisms living in the soil degrade these macromolecules. Saprotrophic wood-decaying and litter decomposing fungi play a crucial role in degradation of natural biopolymers. Fungi are better adapted for plant litter decomposition than bacteria, due to their hyphal

Author for correspondence : E-mail: tirtheshk@gmail.com

growth pattern, production of vegetative spores, specific survival strategies, and capacity to produce a variety of enzymes.

Materials and Methods

Study site and collection of samples

For study and sampling purpose, the forest area of Ratangarh in Seondha block is selected. This place is 65 Km away from Datia city of Madhya Pradesh. The district is located in the north eastern part of M.P., India (25^0 28' to 26^0 20' N latitude and 78^0 10' to 78^0 45' E longitude). The forest land with an area of about 291.04 sq. km is famous for its 'Kardhai' and 'Khair' trees. The average maximum and minimum temperature is 32.64°C and 18.45°C with an average annual rainfall of 825.93 mm.

Anogeissus pendula Edgew (Roxb. ex De) known as kardhai or dhoy in the region, is a tree species of family combretaceae comprising about 600 species, 17 in Madhya Pradesh, mostly found in scrub and dry deciduous forests of Gwalior, Hoshangabad, Indore, Jabalpur, Panna, Raisen, Rewa, Sagar, Satna, Shivpuri, Tikamgarh, Vidisha and Datia. It is a deciduous, moderate sized tree up to 8 cm high and grows as a shrub or small tree with pendulous branches and is an important source of fodder, gum, timber and tannins. The aim of present work was to determine cellulose, hemicellulose and lignin content in the leaf litter of *Anogeissus pendula*.

Freshly fallen and senescent leaves of Anogeissus pendula were collected from the forest floor in sterile polythene bags from January to June, 2017 at monthly intervals. The collected leaves were brought to the laboratory in sealed plastic bags within four hours. Approximately 20 g leaves were enclosed in nylon mesh bag (Crossley and Hoglund, 1962) and placed randomly in each of the nine 2ft x 2ft x 2ft pits (one bag in each pit) for decomposition. One bag from each pit was randomly removed at the interval of 15, 30, 45, 60, 75, 90, 120, 150 and 180 days after the placement. Each bag was placed in a separate paper bag and transported to the laboratory for further work.

Isolation and identification

Fungi were isolated from the *Anogeissus pendula* leaf litter following serial dilution method of Waksman (1916). In this method, 10 g of decaying leaves were transferred into 250 ml Erlenmeyer flask containing 100 ml of distilled water. The flask was shaken thoroughly for 15 minutes on mechanical shaker at 100 rpm for fungal suspension. The fungal spores attached to the surface of decaying leaves come into the water. The filtered spore

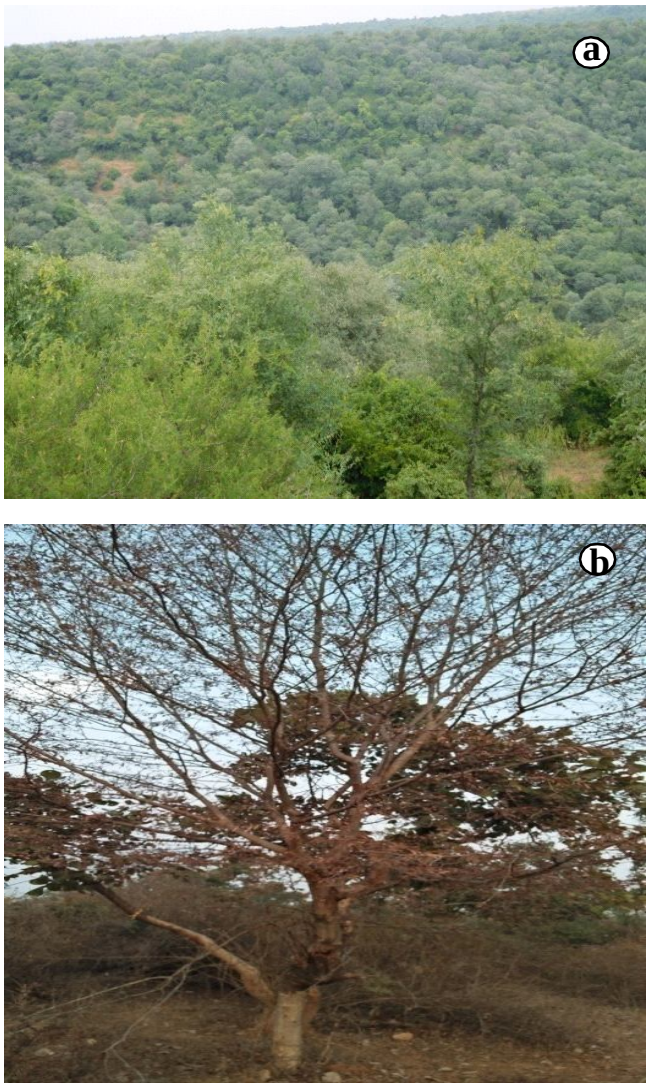

Fig. 1: (a) Forest Area selected for study, (b) Selected plant *Anogeissus pendula*

suspension was further diluted to10^3 to 10^4 times. One ml of this spore suspension was streaked over Petri plate containing potato dextrose agar medium (PDA) which is composed of 200g Potato, 20g Dextrose, 15g Agar, 1000ml Distilled water, pH 5.5 and supplemented with tetracycline. The plates were incubated at 28± 2ºC and the development of fungal colonies was noticed from 48 hrs of incubation. The heterogeneous or mixed fungal colonies were appeared after 72 hrs of incubation. The desired colonies were transferred to freshly poured PDA medium to obtain pure and mono culture. The fungi were isolated and identified on the basis of colony appearance, growth rate, colour, presence of hyphae, length of hyphal fragments, sporulation and recorded with the help of standard text and keys (Gilman, 1957; Barnett and Hunter, 1972; Nagmani *et al.*, 2006).

Estimation of Cellulose, Hemicellulose and Lignin content

Analysis of cellulose, hemicellulose and lignin content was performed by Moubasher *et al.*, (1982). In this method, 2 gm powder of leaves (freshly fallen and decomposed) boiled in 20 ml absolute ethyl alcohol, four times for 15 minutes. The alcohol was removed after last treatment and resultant residues were dried in an oven at 40^0C overnight. Material obtained was treated with 30 ml of diastase enzyme (1mg/ml) for 30 minutes after incubation and washed with distilled water several times by vacuum filtration technique. The remaining material considered as free from sugars. This was kept in an oven for dry weight at 40^0C and then divided into two equal parts. The first part was kept at 80^0C for taking dry weight (A fraction). The second part was treated with 24% (w/v) potassium hydroxide for 4hr at 25^0C and then washed thoroughly with distilled water and KOH was removed by suction and kept in an oven at 80^0C overnight for taking dry weight (B fraction). The B fraction was treated with 72% (v/v) H_2SO_4 for 3hr at room temperature to hydrolyze cellulose and refluxed with 5% (v/v) H_2SO_4 for 2hr, then H_2SO_4 was removed by washing residue with distilled water and then kept in oven for dry weight at 80^0C (C fraction). Cellulose amount was calculated as B-C, hemicelluloses as A-B and the fraction C itself indicated the amount of lignin.

Calculation

Amount of cellulose (mg/g) = fraction B - C

Amount of hemicelluloses (mg/g) = fraction A - B

Amount of lignin (mg/g) = fraction C itself

Results and Discussion

A total of 16 fungal species belonging to 7 genera were isolated from *Anogeissus pendula* leaf litter (Table 1). Among these 3 species belongs to Zygomycota and 13 species belongs to Ascomycota and their anamorphs. Fungal species such as *Mucor varians, Mucor hiemalis Rhizopus stolonifer, Aspergillus niger, Aspergillus japonicus, Aspergillus flavus, Aspergillus nidulans, Aspergillus fumigatus, Aspergillus versicolor, Aspergillus flavipes, Geotrichum candidum, Penicillium chrysogenum, Penicillium aurantiogriseum, Trichoderma viride, Trichoderma reesei* and *Chaetomium osmoniae* have been recorded. The species of *Mucor varians, Mucor hiemalis* and *Rhizopus stolonifer* colonize first on available substrate followed by *Aspergillus flavus, Aspergillus nidulans, Aspergillus fumigatus, Aspergillus versicolor, Aspergillus flavipes, Geotrichum candidum, Penicillium chrysogenum, Penicillium aurantiogriseum, Trichoderma viride, Trichoderma reesei* and *Chaetomium osmoniae*. The species of *Aspergillus niger* and *Aspergillus japonicus* was dominant in all the stages of litter decomposition.

Experimental results are presented statistically in table 2, 3, 4, 5 and figure 2, 3, 4 showed significant variations in cellulose, hemicellulose and lignin content. When the

Table 1: Fungal species isolated from *Anogeissus pendula* leaf during different stages of decomposition (in order of appearance)

Fungal species	Decomposition period (days)								
	15	30	45	60	75	90	120	150	180
Mucor varians Povash, *Bull*	+	+	+	+	–	–	–	–	–
Mucor hiemalis Wehmer	+	+	+	+	–	–	–	–	–
Rhizopus stolonifer Ehrenberg	+	+	+	+	–	–	–	–	–
Aspergillus niger Tiegh	+	+	+	+	+	+	+	+	+
Aspergillus japonicus Saito	+	+	+	+	+	+	+	+	+
Aspergillus flavus Link	–	–	+	+	+	+	+	–	–
Aspergillus nidulans Fennell and Raper	–	–	+	+	+	+	+	–	–
Aspergillus fumigatus Fresen	–	–	–	+	+	+	+	–	–
Aspergillus versicolor Vuill	–	–	–	+	+	+	+	–	–
Aspergillus flavipes Bainier and Sartory	–	–	–	+	+	+	+	–	–
Geotrichum candidum Link	–	–	–	–	+	+	+	–	–
Penicillium chrysogenum Thom. Bull	–	–	–	–	+	+	+	+	–
Penicillium aurantiogriseum Dierekx	–	–	–	–	+	+	+	+	–
Trichoderma reesei E. G. Simmons	–	–	–	–	+	+	+	+	–
Trichoderma viride Pers	–	–	–	–	+	+	+	+	+
Chaetomium osmoniae Rama Rao and Ram Reddy	–	–	–	–	–	+	+	+	+

(+) = Presence, (–) = Absence

Table 2: Reduction in cellulose, hemicellulose and lignin content of *Anogeissus pendula* leaf litter during different stages of decomposition

No. of Days	Cellulose content (mg/g)	Hemicellulose content (mg/g)	Lignin content (mg/g)
Control	0.371	0.220	0.280
15	0.371	0.220	0.280
30	0.371	0.220	0.280
45	0.364	0.157	0.280
60	0.180	0.096	0.180
75	0.136	0.056	0.118
90	0.086	0.032	0.098
120	0.036	0.018	0.065
150	0.009	0.008	0.028
180	0.006	0.004	0.013

Table 3: Percent decrease in cellulose content of *Anogeissus pendula* leaf litter during different stages of decomposition

No. of days	Per cent decrease
Control	0
15	0
30	0
45	1
60	51
75	63
90	76
120	90
150	97
180	98

Table 4: Per cent decrease in hemicellulose content of *Anogeissus pendula* leaf litter during different stages of decomposition

No. of days	Per cent decrease
Control	0
15	0
30	0
45	28
60	56
75	74
90	85
120	91
150	96
180	98

leaf litter samples of 15 and 30 days was analyze for biochemical estimation, no change in content of cellulose, hemicelluloses and lignin was observed. The gradual reduction in lignocellulose content was observed from 45 to 180 days of decomposition. When the litter samples of

Table 5: Per cent decrease in lignin content of *Anogeissus pendula* leaf litter during different stages of decomposition

No. of days	Per cent decrease
Control	0
15	0
30	0
45	0
60	35
75	57
90	65
120	76
150	90
180	95

45 to 180 days were analyzed for biochemical estimation, the content of cellulose ranged from 0.371 to 0.006 mg, hemicellulose 0.220 to 0.004 mg and lignin 0.280 to 0.013 mg respectively.

The results showed that the leaves of Anogeissus pendula contained significant percentage of cellulose, hemicellulose and lignin. Different fungal species colonizing on leaf litter produced a large set of extracellular enzymes that hydrolyze the lignocellulose material. In early stages of degradation, no significant changes have been observed. From 45 to 90 days rapid decrease in cellulose and hemicellulose with imperceptible amount loss of lignin was observed. The decrease in the content of leaf litter was continued up to 120 days and slows down there after from 120 to 180 days of study. The changes in the content of cellulose, hemicellulose and lignin during decomposition, is due to leaching, microbial processing and digestion by saprophagous soil animals.

Early colonizers (*i.e.* sugar fungi) utilise simple soluble nutrients of leaf litter followed by the more specialised polymer degraders such as ascomycetes and imperfect fungi. These fungi consume cellulose and hemicellulose present in the leaf tissue, but there is no evidence that lignin is degraded during this early stage of decay. The remaining lignified organic matter of litter is further modified by actinomycetes and protozoans. Bacteria and fungi in the guts of these invertebrates then assist in the breakdown of this cellulose, but they do not degrade the lignin component appreciably. Finally, the modified but still lignified litter colonized by Basidiomycetes that degrade it further. Generally, white rot fungi produce specific enzymes such as Mn P, Li P, and laccases, collectively known as ligninase.

Enzymes involved in hydrolysis of cellulose, hemicellulose

In our study, except the non-cellulolytic "Sugar fungi"

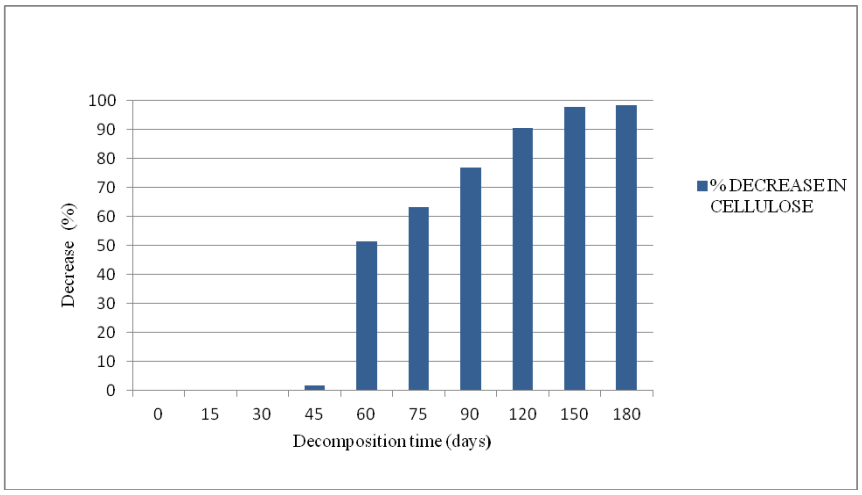

Fig 2: Per cent decrease in cellulose content of *Anogeissus pendula* leaf litter during different stages of decomposition

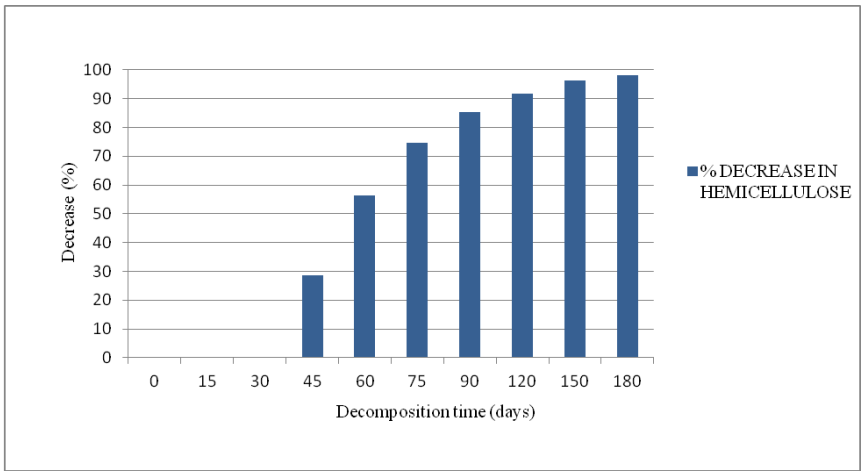

Fig 3: Per cent decrease in hemicellulose content of *Anogeissus pendula* leaf litter during different stages of decomposition

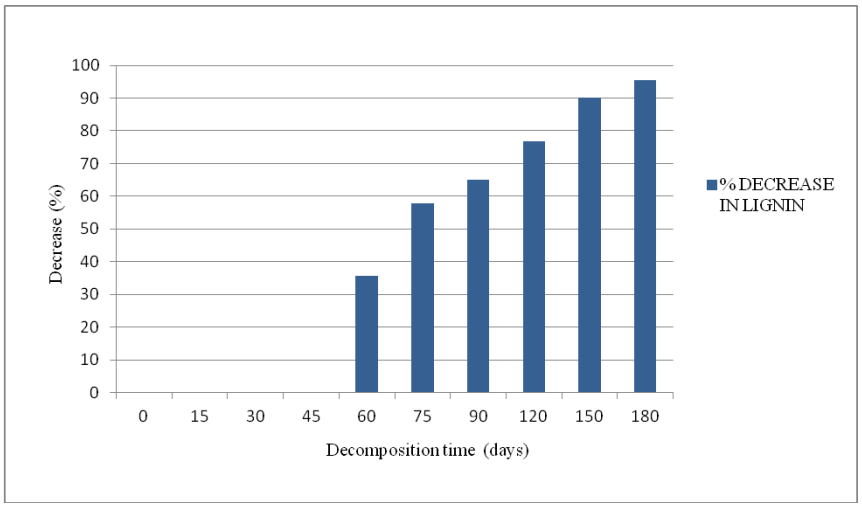

Fig 4: Per cent decrease in lignin content of *Anogeissus pendula* leaf litter during different stages of decomposition

most of the observed genera have been capable to degrade the lignocelluloses material. Aspergillus, Penicillium, Trichoderma, and Chaetomium were the main genera involved in decomposition. These have the enzymatic capability to use the structural compounds of leaves. Cellulases are a class of enzymes produced by fungi involved in converting cellulose into simple sugars. It includes endoglucanase (endo-1, 4-ß-D-glucanase), exoglucanase (exo-1, 4-ß-D-glucanase), and ß-D-glucoside glucanohydrolase (ß-D-glucosidase) (Sajith *et al.*, 2016). Endoglucanase hydrolyze long chains carboxymethyl cellulose (CMC) in a random fashion and converts the cellodextrins (intermediate product of cellulose hydrolysis) into cellobiose and glucose. They are also referred as CMCase. Accordingly, the decrease in the length of polymer results in the rise of reducing sugar concentration. Exoglucanase or cellobiohydrolase degrades or splitting the cellobiose units present in the non-reducing end of the chain. The cellobiose molecules can be further hydrolyzed by ß-glucosidases into two glucose units. The resulting cellobiose and glucose molecules can be absorbed by fungal mycelium as a sole source of carbon and energy (Sajith *et al.*, 2016).

Hemicellulose degradation is similar to cellulose hydrolysis but it requires a large set of different enzymes, because of the complex structure of hemicellulose. Hemicellulose degradation requires the cooperative action of hydrolytic enzymes. Endo-1, 4-ß-xylanase or Endo-cleaving enzymes cleave long hemicellulose chains and release shorter fragments of xylo-oligosaccharides which are further degraded by ß-xylosidase or Exo-cleaving enzymes into small soluble compounds, xylose (Baldrian, 2008).

Lignin degrading enzymes is composed of oxidases, peroxidases and laccases along with their accessory enzymes (Hatakka, 1994; Kirk and Cullen, 1998; Hammel and Cullen, 2008). Class II peroxidases are secreted by several groups of basidiomycetous fungi (Hatakka, 1994) and include lignin peroxidase or ligninase, manganese peroxidase and versatile peroxidase. These all are heme-containing glycoproteins that catalyze oxidation of wide variety of aromatic macromolecules including lignin and its related compounds (Martinez, 2002). Laccases or p-diphenol:oxygen oxidoreductase are copper containing oxidases that catalyzes oxidation of phenolic compounds. These enzymes are found in many fungal taxa (Baldrian, 2006). In addition, accessory enzymes such as glyoxalate oxidase, glucose-1-oxidase and aryl alcohol oxidase generating hydrogen peroxide required by peroxidases have been found to be involved in lignin degradation (Martinez *et al.* 2005).

The degradation of lignocellulosic waste by microbes is the coordinated action of several enzymes in which the cellulases, peroxidases and laccases are the most prominent enzymes. These enzymes utilized in various industrial processes. The isolated fungal strains might provide economic feasibility to second generation biofuels, those obtained from lignocellulosic wastes, which is an important alternative to biofuels obtained from food crops. Many laboratories around the world are searching for the different aspect of natural biodegradation of these macromolecules. Despite all the progresses achieved, the results of this study indicate that further researches are needed regarding lignocellulose biodegradation by fungi.

Acknowledgement

The authors are thankful to Principal, Head of the Department of Botany and Industrial Microbiology, Jhansi and the authorities of Govt. P.G. College, Datia for providing necessary facilities throughout the course of this investigation.

References

Baldrian, P. (2006). Fungal laccases- occurrence and properties, *FEMS Microbiol Rev,* **30:** 215-242.

Baldrian, P. (2008). Enzymes of Saprotrophic Basidiomycetes. In: Ecology of Saprotrophic Basidiomycetes (Boddy, Watkinson and van West, eds.), Academic Press, USA: 19-41.

Baldrian, P. (2017). Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiology reviews*, **41 (2):** 109-130.

Barnett, H.L. and B.B. Hunter (1972). *Illustrated Genera of Imperfect Fungi*, (3rd edition). Burgess Publishing Company, Minneapolis. p.331.

Berg, B. and C. McClaugherty (2003). Plant litter: Decomposition, humus formation, carbon Sequestration. Berlin, Germany. *Springer* Verlag, 286.

Crossley, D.A. and Hoglund (1962). A litter bag method for the study of microarthropods inhabiting leaf litter. *Ecology,* **43:** 571-573.

Gilman, J.C. (1957). A manual of soil fungi 2nd ed. Iowa, The Iowa State College Press, 450.

Hammel, K.E. and D. Cullen (2008). Role of fungal peroxidases in biological ligninolysis. *Curr Opin Plant Biol*, **11:** 349-355.

Hatakka, A. (1994). Lignin-modifying enzymes from selected white-rot fungi – production and role in lignin degradation. *FEMS Microbiol Rev*, **13:** 125-135.

Kirk, T.K. and D. Cullen (1998). Enzymology and molecular genetics of wood degradation by white-rot fungi. In: Young, R.A., Akhtar, M (eds) Environmentally friendly technologies for the pulp and paper industry. Wiley, New York, 273-307.

Martinez, A.T. (2002). Molecular biology and structure-function of lignin-degrading heme peroxidases. *Enzyme Microb Technol*, **30:** 425-444.

Martinez, A.T., M. Speranza, F.J. Ruiz-Duenas, P. Ferreira and S. Camarero (2005). Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *Int. Microbiol* **8:** 195-204.

Moubasher, A.H., S.I.I. Hafez, H.M. Aboelfattah and A.M. Moharrarh (1982). Fungi of wheat and broad bean straw compost. II. Thermophilic fungi. *Mycopathologia*, **783:**169-176.

Nagmani, A., I.K. Kunwar and C. Manoharachary (2006). Handbook of soil fungi I. K International Pvt. Ltd. New Delhi. India.

Pu, Y., D. Zhang, P.M. Singh and A.J. Ragauskas (2008). "The new forestry biofuels sector", *Biofuel. Bioprod. Bior,* **2:** 58-73.

Ragauskas, A.J., G.T. Beckham, M.J. Biddy, R. Chandra, F. Chen (2014). Lignin valorization: improving lignin processing in the biorefinery. *Science* 344 (6185), 709-718.

Sajith S, P. Priji, S. Sreedevi and S. Benjarnin (2016). An overview on fungal cellulases with an industrial perspective. *J. Nut. Food Sci*. **6:** 1-13.

Sun, Q., M. Foston, X. Meng, D. Sawada, S.V. Pingali (2014a). Effect of lignin content on changes occurring in poplar cellulose ultra structure during dilute acid pretreatment *Biotechnol. Biofuels.* **7 (1):** 150-163.

Vanholme, R., B. Demedts, K. Morreel, J. Ralph and W. Boerjan (2010). Lignin biosynthesis and structure. *Plant Physiology*, **153:** 895-905.

Waksman, S.A. (1916). Do fungi live and produce mycelium in the soil? *Sci. N. S.* **44:** 320-322.

Zhang, P., H.R. Hu and S.L. Shi (2006). Application of hemicellulose. Tianjin Pap Mak. **2:** 16-8.