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Biodegradation of *Triplochiton scleroxylon* K. Schum *Tectona grandis* Linn, *Gmelina arborea* Roxb, *Nauclea diderichii* Linn and *Terminalia ivorensis* A. Chev by *Lenzites palisoti* Fr.

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Abstract: *Triplochiton scleroxylon, Tectona grandis, Gmelina arborea, Nauclea diderichii* and *Terminalia ivorensis* wood blocks were biodegraded *in situ* in the laboratory by *Lenzites palisoti* a white rot fungus. The test blocks were cut into 10×10×20 mm and exposed into the infection region of pure culture of *Lenzites palisoti* for 16 weeks. The test blocks were examined for weight loss at 4 weeks interval. The percentages of weight loss obtained after 4, 8, 12 and 16 weeks of incubation of the test blocks in *Lenzites palisoti* indicated that decay occurred at a relatively constant rate. After 16 weeks, micro morphological analysis of the decayed wood showed that the action of the cellulolytic enzymes of *Lenzites palisoti* was restricted to cell wall surface.

Key words: Cellulose, hemicellulose, lignin, enzymes, mycelia, sectioning

INTRODUCTION

White rot fungus is thought of as those organisms which is able to decompose all of the structural wood components (cellulose, hemicellulose and lignin) (Deacon, 2005). Records have shown that it is in part the removal of lignin which gives white-rotted wood its whitened appearance, although oxidative bleaching reaction also cause whitening (Eaton and Hale, 1993)

Some white rot fungi have the ability to selectively degrade lignin and hemicellulose without degrading extensive amount of cellulose (Otien and Blanchette, 1985).

Special attention has been placed on understanding the decay processes as a major biological event considering the microecological factors affecting the colonization of wood by sequences of wood-rotters (Rayer and Boddy, 1988) and considering the environmental impact and alternatives to the traditional chemical approaches to decay control (Eriksson *et al*., 1990).

Otien and Blanchette (1985) reported that white rot fungi are known to degrade lignin in two micro morphological distinct ways. One type of decay is selective for lignin and hemicellulose removal. The other type of decay is non selective where all the cell wall components are removed simultaneously either directly around fungal hyphae causing erosion troughs and bore holes or uniformly from the cell lumen outward causing a gradual thinning of the cell walls. The purpose of this study was to observe the decay process caused by *Lenzites palisoti* Fr. a white rot fungus on test blocks of *Triplochiton scleroxylon* K. Schum, *Tectona grandis* Linn, *Gmelina arborea* Roxb, *Nauclea diderichii* Linn and *Terminalia ivorensis* A. Chev *in vitro* at 4 weeks interval for 16 weeks.

MATERIAL AND METHODS

Preparation of test blocks: The protocol of Adetogun and Adegeye (2002) was adopted. Sapwood of good quality and straight grained stock was taken from the bole the wood species, the cutting was so arranged that the grains of the wood followed the long axis. The five wood species were planned accurately to 10×20 mm cross section and cross cut to 10 mm numbering 1-450. Four hundred test blocks were selected for the test. The test blocks were oven dried in the oven for 18 h at 103°C and the weight obtained was taken as initial oven dry weight (odwi).

Procurement of test fungi: Innoculum was obtained from the freshly collected fruiting bodies of *Lenzites palisoti* by taking portions asceptically with sterile surgical blade from the inner portion of the cap. The inocula were initially grown on PDA slant in McCartney bottles.

Stock culture of pure *Lenzites palisoti* inside McCartney bottles were sub-cultured by transferring bits

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of the fungi with sterilized picker into clean Petri plates containing Potato Dextrose Agar (1000 mL distilled water, 20 g agar and 17 g dextrose) and incubated at 25±2°C for 7 days.

Test block infection: The test blocks were wrapped in aluminum foil and sterilized for 10 min at 115°C at 1.06 kg cm^{-2} before introduction into the Petri-dishes containing Lenzites palisoti. About 60 test blocks were inoculated with the test fungus at the rates of four test blocks per Petri-dish. All the test blocks inoculated with the fungal organisms were incubated at 25±2°C. Four blocks were introduced into the petri plate containing no fungus served as the control. The blocks were incubated for a period of 16 weeks. The experiment was replicated five times over a period of 16 weeks.

Statistical analysis: All the data obtained were subjected to an analysis of variance and statement of significance are based on $p \leq 0.05$ (Gomez and Gomez, 1984).

Weight loss determination: Twenty test blocks were removed per species from the infection region at 7 days interval. The adhering mycelia mats on the test blocks were cleaned with dry cotton wool and weighed to determine the wet weight (odw 2). The blocks were then dried in the oven for 18 h at 103°C and weighed to obtain the final oven dry weight (odw 3). The weight loss was determined with the formula below:

$$\frac{Odw1 - Odw3}{Odw1} \times \frac{100}{1}$$

Wood sectioning: The distribution of fungal hyphae in decomposing wood can readily be ascertained using routine sectioning and staining methods (Rayer and Boddy, 1988). Four wood blocks exposed to *Lenzites palisoti* were removed after 16 weeks of incubation for sectioning so as to locate the hyphae in the wood and to study the level of penetration and part of the wood most affected.

The observations were performed on cross section of decayed test blocks of *Triplochiton scleroxylon, Tectona grandis, Gmelina arborea, Nauclea diderichii* and *Terminalia ivorensis* that had been embedded in hot distilled water prior to sectioning with slide microtome. Changes in the test blocks due to the effect of the fungus were studied by first staining with safranin red in order to stain the lignin and then with fast green to stain the cellulose. The sections were then mounted on the slide with clove oil. Filter paper was used to drain off excess clove oil. The slides were covered and then smeared with Canada balsam. The slides were heated to allow the Canada balsam to spread in order to remove all air bubbles. A light microscope was used to detect the micro morphological changes in the wood due to decay by *Lenzites palisoti.*

RESULTS AND DISCUSSION

The percentages of weight loss of the test blocks of *Triplochiton sclerexylon, Tectona grandis, Gmelina arborea, Nauclea diderichii* and *Terminalia ivorensis* exposed to the infection region of *Lenzites palisoti* are shown in Table 1. Based on observations of the ranges of weight loss values, degradation of the test blocks appeared to occur steadily during the incubation periods. The mean percentage of weight loss of the test blocks inoculated with the test fungus ranged from 4.29-21.41% after 1 month. This low percentage weight loss may be due to the method of inoculation since the innoculum used in the study consisted of large mats of mycelium, it is possible that the fungus was still utilizing the free nutrients present in the mycelial mat during the early stage of decay.

When the blocks were stained with safranin red and fast green, a stain that reacts with lignin to produce a red colour indicated a reaction with lignin. The affected areas of the blocks remained white indicating an absence of lignin. Microscopic examination of the decayed wood showed that lignin removal was restricted to the periphery of the wood blocks producing a pattern similar to the degradation patterns observed in wood blocks of Aspen decayed by *Ischnorderma resinonsum* and *Poria medulla-panis* (Otien and Blanchette, 1985; Chirkova *et al*., 2004). No decomposition was apparent in any of the control blocks used in the study.

The micro morphological studies of the decayed wood indicated that *Lenzites palisoti* eroded the cell wall. Large voids, resulting from the enzymatic erosion of cell walls were observed Bore holes and pit canals, the

Table 1: Percentage weight loss of *Triplochiton scleroxylon, Tectona grandis, Gmelina arborea, Nauclea diderichii* and *Terminalia ivorensis* wood block in *Lenzites palisoti* after 16 weeks of incubation

	Weight loss (%)				
	Incubation period (week)				
Wood species	4	8	12	16	Control
Nauclea diderichii	13.04	14.22	18.18	20.15	0.0
Triplochiton scleroxylon	21.41	27.50	61.42	85.22	0.0
Tectona grandis	4.29	6.35	11.23	15.22	0.0
Gmelina arborea	21.05	23.40	40.42	65.21	0.0
Terminalia ivorensis	13.64	17.30	31.62	56.22	0.0

Significant ranges at $p<0.05$, 4.17, 2.27, 2.69, 3.32; *all values are % of original wt

avenues by which the hyphae advanced from cell to cell, underwent a progressive enlargement. This is in consonance with the reports of Wilcox (1968) and Otien and Blanchette (1985) that attack by the white rot fungus produce a gradual thinning of the wood cell walls progressing from the lumen outward. Evidence from the present study showed that the thinning of the secondary walls of *Triplochiton sclerexylon*, *Tectona grandis*, *Gmelina arborea*, *Nauclea diderichii* and *Terminalia ivorensis* did not follow the usual simultaneous removal of cellulose from the various regions of the cell wall but rather was characterised by essentially complete removal of the cellulose from one layer at a time beginning with the S3 adjacent to the lumen. Thus, the cellulose of the S3 layer was attacked until it disappeared, then that of the S2 and finally the S1. This suggests that the action of the cellulolytic enzyme of *Lenzites palisoti* a white rot fungus was restricted to cell wall surfaces exposed to the lumen or other confluent cavities and it is in line with the study of Zabel and Morrell (1992).

CONCLUSION

Micro morphological evidence from this study indicated that the action of the cellulolytic enzymes of *Lenzites palisoti* a white-rot fungus was restricted to exposed wood cell wall surfaces. However, effort should be geared towards the action of the lignin-destroying enzymes of the white-rot fungus in further studies.

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