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# Fungal degradation of bamboo treated with crude lake salt and a mixture of borax and boric acid

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## ABSTRACT

This study compared the effectiveness of crude lake salt, a traditional preservative used by artisans in Uganda, and borax-boric acid, a conventional preservative, against fungal degradation. Using the European standard, an experiment was set up to determine the durability of *Oxytenanthera abyssinica, Oldeania alpina* and *Bambusa vulgaris* treated with 2 % and 6 % crude lake salt and borax-boric acid against *Gloeophyllum trabeum, Coniophora puteana* and *Trametes versicolor*. Mass loss comparisons between treated and untreated bamboo samples were made. Durability classes were assigned according to the amount of mass lost. Bamboo samples exposed to *G. trabeum* had lower surface hyphal coverage compared to those exposed to *C. puteana and T. versicolor* irrespective of the preservative used. Samples treated with borax-boric acid were in durability class I compared to those treated with crude lake salt that were between class II and III according to European standard. The lowest mass loss for bamboo treated with crude lake salt was 14.24 % in *B. vulgaris* exposed to *T. Versicolor*. The study confirmed that while artisans use crude lake salt as a traditional preservative, it does not protect against white and brown rot while all concentrations of borax-boric acid provided protection against all fungi.

## Introduction

Bamboo is a major non-wood forest product with properties comparable to those of trees. Bamboo as a plant has a low weight-to-height ratio, high productivity, is adaptable to a wide range of site conditions and is fast-growing, giving it a competitive edge against slow-growing trees (Kaminski et al. 2016; Mehramiz et al. 2020). Although bamboo is well known for its fast growth, its use may not immediately solve the current wood crisis unless its resistance to multiple biodegradation agents is enhanced (Hamid et al. 2012). Bamboo, in general, is a lignocellulosic material with high amounts of starch and limited quantities of resins, waxes, and tannins, increasing its susceptibility to biodeterioration agents (Liese and Köhl, 2015). Different bamboo species may vary in resistance to biodeterioration due to differences in chemical composition (Suprapti, 2010). However, even with variations in resistance of individual bamboo species, generally, most species rapidly deteriorate when exposed to high relative humidities with inadequate air circulation or when used in contact with soil (Schmidt et al. 2020; Tomak, 2022).

Among the bio-deteriorators of bamboo are fungi, classified as soft rot, white rot and brown rot. Fungi cause degradation through discoloration and, in extreme cases, reduction in the strength of bamboo products. The decrease in the quality of bamboo products often leads to financial losses through the reduction of value and, in different scenarios, the requirement of replacement costs (Huang et al. 2014; Möller and Mild, 2018). Fungal degradation occurs during storage, processing and transportation especially in areas with high relative humidity above 70 % (Kaur et al., 2016a; Mehramiz et al., 2020; Sun et al., 2012; Tang et al., 2012). Fungal degradation usually occurs in split bamboo or at cut culms ends that do not have the outer cuticula (Tang, 2009). Therefore,

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during bamboo processing, as the culms are split, there is need for modification to change bamboo chemical composition or use of preservative treatment to increase products service life beyond five years (Gauss, 2020; Gauss et al., 2019; Kaur et al., 2016a).

Several methods have been used to protect bamboo from biodegradation successfully. These methods include the use of pressure to force toxic preservatives into bamboo cells (Kent, 2007; Meena, 2022).The conventional preservatives used in the pressure treatment include copper chrome arsenic (CCA), sodium pentachlorophenol, borax-boric acid, Cu/Zn naphenates/ abietates, tebuconazole, IPBC (3-iodo 2- propanyl butyl carbamate), chlorothalonil, isothiozolones, synthetic pyrethroides, tanalith, coal tar creosote (Helsen et al., 2007; Kaur et al., 2016a; Kent, 2007; Meena, 2022). Apart from pressure, non- pressure methods commonly used are water leaching, painting, smoking and soaking in chemicals such as crude lake salt. In soaking, for example, the bamboo is submerged in a solution of the preservative for a certain period of time, allowing the solution to penetrate the bamboo's cell walls and protect it from decay and insect infestation.

Most of the non- pressure methods are referred to as traditional methods. The traditional methods are considered environmentally friendly and relatively less expensive than the non-traditional methods (Belt et al. 2023; Hill et al. 2021; Mwanja et al. 2023; Tripathi et al. 2014). The increased resistance of bamboo towards decay has been reported in some of the studies involving these traditional methods such as water leaching treatment (Ashaari and Mamat, 2000; Kaur et al. 2013; Mehramiz et al. 2020) and smoking of bamboo (Kaur et al.2016b).

In Uganda, 31 % of the bamboo artisans were found to use crude lake salt as a preservative against biodegradation agents (Mwanja et al. 2023; Zuraida & Larasati, 2015). Crude lake salt is highly alkaline and rich in sodium, chloride, carbonate, and hydrocarbonates with lesser quantities of potassium, magnesium, calcium, bromine and fluorine ions (Kasedde et al. 2013). Crude lake salt is readily available and easily affordable to many artisans. Although, widely used in Uganda, little is known about the effectiveness of crude lake salt as a preservative against bamboo decay compared with other conventional preservatives. Studies recommend assessing traditional preservative methods to determine their efficacy against deteriorating organisms, taking advantage of their easy-to-apply and sustainable procedures (do Amaral et al. 2023).

This study compared the effectiveness of crude lake salt, a traditional preservative used by artisans in Uganda, and borax-boric acid, a conventional preservative, against fungal degradation. The study also hypothesized that bamboo samples treated with borax-boric acid would be more resistant to fungal degradation than those treated with crude lake salt. The study provides useful insights for artisans and researchers working on sustainable and effective bamboo preservation methods.

## Material and methods

Mature (four year old) culms of Oxytenanthera abyssinica Munro, Oldeania alpina K.Schum and Bambusa vulgaris Schrad. ex J.C.Wendl were marked and collected from Metu at 3.67° N, 31.76° E, Echuya at 1.28° S, 29.81° E and Kifu at 0.43° N, 32.73° E forests respectively in Uganda. The culms were harvested at approximately 20 cm above ground level. Each culm was subdivided into three equal sections corresponding to the basal, middle, and top sections following International Standard - (ISO 22157, 2004). The marked sections were transported to the National Forestry Resources Research Institute. Samples of  $50 \times 25$  $\times$ 15 mm (length  $\times$  width  $\times$  thickness) were extracted from the middle section (Fig. 1). The middle section was preferred for sample extraction because it is more representative of the overall quality and properties of the bamboo culm. Bamboo samples were conditioned to 12 % moisture content at 65  $\pm$  5 % relative humidity (RH) and 20  $\pm$  2 °C for four weeks before preservation. For each bamboo species 40 samples were prepared.

Crude lake salt (CLS) locally mined from Lake Katwe located at  $0.13^{\circ}$ S, 29.87° E was diluted with water as a solvent to make 2 % and 6 % concentrations as used by artisans (Mwanja et al. 2023). Disodium octaborate tetrahydrate (Na<sub>2</sub>B<sub>8</sub>O<sub>13</sub>·4 H<sub>2</sub>O), commonly referred to as Borax-Boric Acid (BBA) mixture, was diluted to 2 % and 6 % concentration.

Two impregnation methods used in this study were soaking and pressure, they are the most commonly used bamboo impregnation methods in Uganda (Mwanja et al. 2023). With the soaking method, the bamboo samples were placed in the preservative solution for seven days and ballasted with a weight to ensure they did not float. For the pressure method, an impregnation schedule was adopted from (Wahab, 2006). An initial vacuum of -600 mmHg was applied for 30 minutes to expel air from the bamboo cells. The preservative solution was let into the cylinder, and a pressure of 5.09 kgcm<sup>-2</sup> was applied for 2 h to force the preservative solution into the bamboo. A final vacuum of -600 mmHg for 30 minutes was introduced to remove the excess preservative solution. Preservative retentions in the treated samples were determined as described by Gauss et al. (2019). After preservation, the samples were conditioned to 12 % moisture content at  $65 \pm 5$  % RH and  $20 \pm 2$  °C for

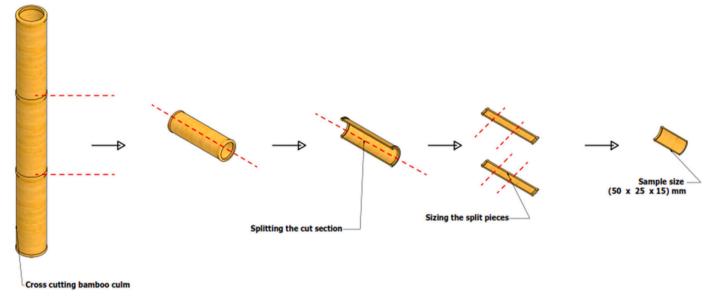


Fig. 1. Illustration of the sample extraction process.

two weeks before carrying out the decay tests.

The decay tests were carried out following European Standard - EN 113 (1996). A culture media consisting of 40 g of malt agar was dissolved in 1000 ML of deionized water by warming the agar in a boiling water bath. A sufficient quantity of the media (minimum depth of 4 mm) was placed in the culture jars with a capacity of 500 ML and a flat surface area of approximately 85 cm<sup>2</sup>. The culture jars with media were closed with leakproof lids and sterilized in an autoclave at 121°C for 20 min to kill any microorganisms present. The jars with the sterilized media were left to cool in their in-use position. After two days, the sterilized culture media was inoculated with fresh 8 mm<sup>2</sup> mycelial agar plugs of basidiomycete monocultures Table 1. The inoculated jars were left in a dark culture chamber for the fungi to fully cover the surface of the growth medium. The chamber was maintained at  $22 \pm 2^{\circ}$ C and  $70 \pm 5$  % RH. For each fungus,120 samples totaling to 360 samples for the three fungi were set up.

After two weeks, bamboo test samples were dried at  $103 \pm 2^{\circ}$ C, weighed (m<sub>0</sub>), and autoclaved at  $121^{\circ}$ C for 20 min to sterilize the samples. The sterilized bamboo samples and specimen supports were aseptically introduced into the culture jars, the surfaces of which were completely covered with mycelium (Schmidt et al. 2011). The jars were covered with leakproof lids with a hole in the center plugged with cotton wool to allow air circulation. These jars were placed in the culture chamber for 16 weeks (Fig. 2). They were routinely observed for any sign of contamination and water logging. Bamboo samples were also placed into culture jars without fungi to determine weight losses caused by non-biological factors. The weight loss from non-biological factors were used for the calculation of correction factors. Virulence tests were carried out for all fungi using *Pinus caribaea* to confirm the effectiveness of the colonies.

At the end of 16 weeks, the test specimen were removed from the culture jars and adhering mycelium cleaned. The test samples were weighed  $(m_2)$  and placed to dry in an oven at  $103 \pm 2^{\circ}$ C till constant mass, they were left to cool at room temperature in desiccators and weighed again  $(m_3)$ . The following measurements were taken on all samples: (i) moisture content, (ii) mass loss, and (iii) hyphal coverage. The moisture content of each specimen was calculated by expressing its water content  $(m_2-m_3)$  as a percentage of its final dry mass  $(m_3)$ . The mass loss was calculated by expressing the loss in mass  $(m_0-m_3)$  as a percentage of the initial dry mass  $(m_0)$ . The fungal development on the specimens was visually evaluated and scored by one technician to minimize errors. This involved examining the sample and estimating the percentage of the sample covered by hyphal filaments. Hyphal coverage and durability scores were assigned according to Table 2.

## Data analysis

To evaluate the fungal degradation of bamboo treated with preservatives, a randomized design with a factorial arrangement was used for each fungus in which the following factors were evaluated: three bamboo species, *O. abyssinica, O. alpina* and *B. vulgaris*, preservatives at four levels, CLS 2 %, CLS 6 %, BBA 2 %, BBA 6 % and a control, and two impregnation methods, soaking and pressure giving a factorial pattern of  $3 \times 5 \times 2$ . Four repetitions by factor were performed totaling to 120 observations for each fungus and 360 for the three fungi. The data were checked for normality using Kolmogorov- Smirnov test and Levene's test

## Table 1

Screened basidiomycetes, strain, rot type.

Fungus	Strain	Rot type		
Coniophora puteana	BAM Ebw. 15	Brown Rot		
Gloeophyllum trabeum	BAM Ebw. 109	Brown Rot White Rot		
Trametes versicolor	CTB 863 A	white Rot		

Source of cultures: Laboratory of Forest Biomaterials and Technology, Swedish University of Agricultural Sciences



Fig. 2. Experiment setup in a culture chamber.

## Table 2

Evaluation of Hyphal coverage and durability rating.

Parameter	Scores	Description
Hyphal coverage (HC)	0	No coverage
	1	1–33 % coverage
	2	34-66 % coverage
	3	67–99 % coverage
	4	total coverage
Durability class (DC)	I	Very durable (mass loss - ML<5 %)
-	II	Durable (5 $<$ ML $\le$ 10 %)
	III	Moderately durable ( $10 < ML \le 15$ %)
	IV	Slightly durable ( $15 < ML \le 30$ %)
	v	Not durable (30 < ML)

Adapted from European standard - EN 113, 1996; EN 350, 2016 and Wei et al. (2013)

was used to test for homogeneity of variance before data were subjected to analysis of variance (ANOVA). Factorial analysis of the mean ML was conducted at 95 % level of significance All analyses were performed using Minitab statistical packages.

## **Results and discussion**

The preservative retention varied from  $4.6 - 20.6 \text{ kg/m}^3$  across the bamboo species Table 3. The pressure-impregnated samples retained more preservatives than the soaked samples. Preservative retention increased with increasing concentration of preservatives. The moisture content of the bamboo samples was found to range from 25 % to 98 %, which is generally suitable for fungal degradation. The availability of moisture is a prerequisite for fungal degradation, and exclusion of moisture can be used as a protection method (Schmidt, 2007). Different moisture content ranges have been reported as suitable for fungal

#### Table 3

Retention of preservatives in the bamboo samples.

Species	Preservative	Borax-boric acid		Crude lake salt		
	Method of impregnation	2 %	6 %	2 %	6 %	
O. abyssinica	Pressure	5.2 (0.5)	18.3 (1.1)	10.5 (1.5)	14.7 (1.9)	
	Soaking	4.6 (0.6)	13.7 (1.7	9.3 (1.5)	12.7 (1.4)	
O. alpina	Pressure	6.0 (1.2)	20.6 (1.8)	13.3 (2.4)	18.8 (3.4)	
	Soaking	5.4 (1.2)	15.9 (2.3)	10.2 (1.3)	16.3 (2.4)	
B. vulgaris	Pressure	5.5 (1.1)	19.1 (2.2)	13.1 (1.5)	18.8 (1.8)	
	Soaking	4.9 (0.9)	16.0 (2.5)	9.5 (1.5)	16.1 (2.1)	

Standard deviations are shown in parenthesis; Retention of preservatives in kg/m<sup>3</sup>

degradation in previous studies. Wei et al. (2013) reported moisture content range of 21–187 % as suitable for fungal growth of white, brown and soft rot on five bamboo species according European standards - EN 350–1, EN 350–2 and EN 113. Schmidt, (2007) reported 36 – 210 % for *C. puteana* and 46 – 179 % for *G. trabeum* as optimum moisture content ranges for decay.

Decay requires availability of moisture above fiber saturation point (FSP) to have successful colonization of the bamboo samples (Candelier et al. 2017; Schmidt et al. 2020; Thybring et al. 2018). Even with moisture as a factor that influences fungal degradation, some studies have reported fungal degradation by *C. puteana* at 22 % which is below fiber saturation point (Schmidt, 2007).

Table 4, shows that samples treated with CLS showed hyphal coverage of 30–85 %, scoring between 1 and 3, while those treated with BBA had coverage of 0–37 % with a score of 0–2. Low hyphal surface coverage was noted in most samples exposed to *G. trabeum* compared to those exposed to *C. puteana and T. versicolor* irrespective of the preservative and impregnation method used. While hyphal surface coverage is a specific feature of many fungi it does not explain the intensity of the fungal activity. Studies have reported that some strains of fungi do not show surface hyphal coverage but degradation still takes place.

Brown rot (*G. trabeum*) in wood has limited hyphal development but widespread decay (Daniel, 2016). Wei et al. (2013) reported that some strains of *G. trabeum* fungi did not show hyphal coverage when exposed to *Bambusa maculata, Dendrocalamus asper* and *Guadua angustifolia* although there was degradation. Among the three fungi strains, *G. trabeum* did show limited hyphae coverage on the surface of some samples even when the fungi produced degradation in form of mass loss. This mass loss was as a result of substrate mycelium and not surface hyphal growth. Therefore, it's important to consider other factors besides hyphal coverage in assessing fungal activity.

The percentage corrected mass loss (ML) and decay resistance classes of the bamboo samples exposed to the different fungi are presented in Fig. 3. The study found that all the three bamboo species were susceptible to fungal degradation, with untreated *B. vulgaris* exhibiting the highest ML across all the bamboo species. The ML of untreated samples across the bamboo species ranged from 10.3 - 15.4 %, 6 - 7.5 %, and 8.5-9.8 % for samples exposed to *T. versicolor*, *G. trabeum* and *C. puteana* basidiomycetes respectively. The ML for the CLS treated bamboo samples ranged from (8.8 - 14.5 %), (5.5 - 7.2 %) and (7.2 - 9.6 %) when exposed to *T. versicolor*, *G. trabeum* abasidiomycetes, respectively. The study also revealed that bamboo samples treated with BBA were more durable and resistant to fungal degradation than those treated with CLS. Classification according to durability classes shows that bamboo samples treated with 2 \% and 6 % of BBA were in class I which is very durable compared samples treated with CLS which belong to class II and III.

Table 5 shows factorial analysis of variance results for samples exposed to *T versicolor*, *C. puteana* and *G. trabeum*. For all the three fungi, the average ML was significantly different across preservatives. *T. versicolor* and *G. trabeum* registered significant differences in ML across bamboo species while for samples exposed to *C. puteana*, the ML was not significantly different. Across all fungi the mean ML as a result of the impregnation method was not significantly different

The profile plots in Fig. 4 illustrate variations in the mean ML for samples exposed to each fungus. There is decreasing ML with from control samples to those treated with CLS to BBA. There variations in ML with the different concentrations with in each preservative. Samples treated with concentration 6 % had lower ML than those treated with 2 %. There variations in impregnation method with pressure method registering lower ML than the soaking method. For variations across bamboo samples *T. versicolor* and *C. puteana* had the same tread with *B. vulgaris* registering the highest ML, to *O. alpine* lowest. This tread changes with *G. trabeum* where *B. Vulgaris* had the highest ML, to *O. alpine and O. abyssinica*.

The decay of bamboo due to fungal attack is a complex process

#### Table 4

The hyphal coverage scores of bamboo samples after test period

Preservative	Conc %	Method of impregnation	Trametes versicolor			Gleophyllum trabeum			Coniophora puteana		
			O. abyssinica	B. vulgaris	O. alpina	O. abyssinica	B. vulgaris	O. alpina	O. abyssinica	B. vulgaris	O. alpina
Untreated			4	4	3	1		1	2	2	2
							2				
CLS	2	Pressure	3	2	3	1		1	2	2	2
							1				
		Soaking	3	3	3	1		1	2	2	2
							1				
	6	Pressure	2	2	2	1	1	1	1	1	1
		Soaking	3	3	2	1	1	1	2	2	2
BBA	2	Pressure	1	1	1	1		1	1	1	1
							1				
		Soaking	2	2	2	1		1	1	1	1
							1				
	6	Pressure	1	1	1	0	0	0	1	1	1
		Soaking	1	1	1	0	0	0	1	1	1

Hyphal coverage scores: 0, no coverage; 1, 1–33 % coverage; 2, 34–66 % coverage; 3, 67–99 % coverage; 4, total coverage CLS: Crude Lake Salt; BBA: Borax–boric acid

Table 5

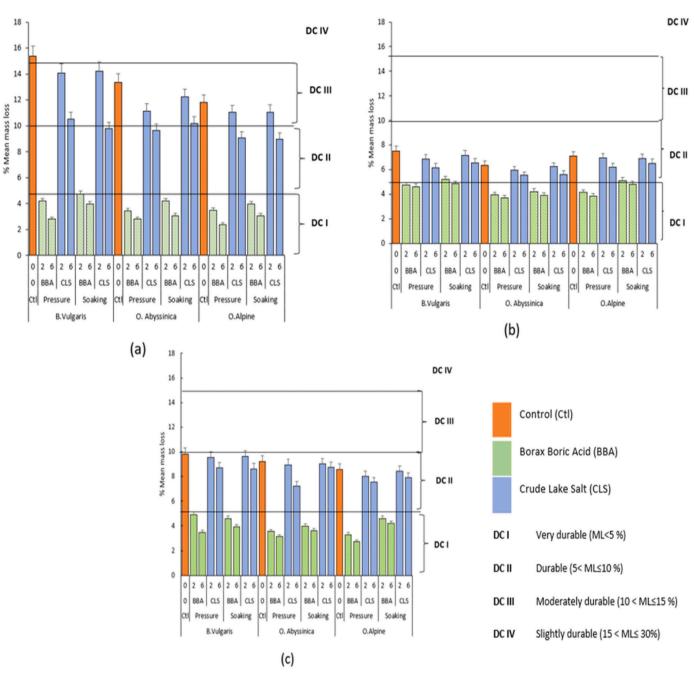


Fig. 3. Mean mass loss and durability classes for untreated and treated bamboo samples with borax-boric acid and crude lake salt after 16-week incubation with a) *T. versicolor, b) G. trabeum, c) C. puteana.* 

	DF	T versicolor		C puteana		G trabeum	
Source of Variation		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Main Effects							
Preservative	4	197.32	0.000 *	38.44	0.000 *	13.6	0.000 *
Impregnation method	1	1.12	0.292	1.07	0.304	0.96	0.329
Species	2	12.49	0.000 *	1.89	0.158	3.48	0.035 *
Interactions							
2-Way Interactions	14	0.14	1.000	0.14	1.000	0.14	1.000
3-Way Interactions	8	0.11	0.999	0.11	0.999	0.11	0.999
Error	90						
Total	119						

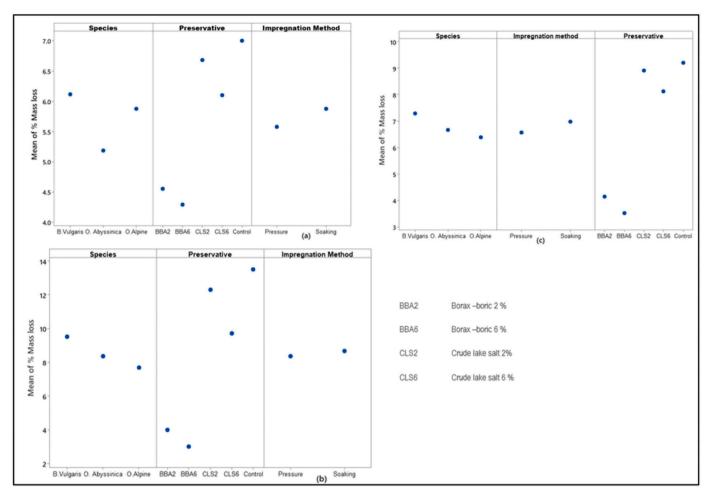


Fig. 4. Profile plot for mass loss experiment of bamboo treated with the different preservatives under (a) G. Trabeum, (b) T. versicolor (c) C. puteana.

influenced by different factors such as moisture content, type of fungi, bamboo species, and the presence of toxic compounds from preservative treatment. Studies have shown that during the decay process, the primary components of bamboo (cellulose, hemicelluloses, and lignin) are broken down or altered to provide energy for the growth and development of fungi (Boonstra et al. 2007). Several studies have reported variations in ML of bamboo when exposed to different fungi.

Our results are in agreement with other studies that have reported ML ranges in untreated bamboo of 11.2–16.8 % in *B. vulgaris* and 13.6 % in *Phyllostachys pubescenes* when exposed to *T. versicolor* (Ogunsanwo et al. 2015; Zhang et al. 2007). However, some studies have reported higher ML of up to 62.5 % when *P. pubescens, Bambusa maculate* and *Gigantochloa* were exposed to *T. versicolor* (Schmidt et al. 2011). Other studies reported ML of up to 35 % in *P. edulis* and 25 % in *P. pubescens* when exposed to *G. trabeum* (Cho et al. 2008; Ma et al. 2010). The variations in ML across different bamboo species can be attributed to differences in their chemical composition.

Several studies have reported variations in ML across different bamboo species with some being more resistant to fungal attack compared to others (Schmidt et al. 2011; Tang et al. 2012). Studies have reported bamboo species such as *Goniothalamus scortechinii* are naturally resistant to degradation by T. *versicolor* and *C. puteana* (Hamid et al. 2012). Suprapti (2010) showed *B. vulgaris, Gigantochloa apus* and *Gigantochloa atroviolacea* to be moderately resistant, whereas *Gigantochloa pseudoarundinacea* and *D. asper* were not resistant.

White rot fungus (*T. versicolor*) was found to be more effective in decaying bamboo compared to brown rot fungi (*G. trabeum* and *C. puteana*). Degradability by white rot is higher because *T. versicolor* simultaneously degrades cellulose, hemicellulose, and lignin while

brown rot only attacks cellulose and hemicellulose and modifies lignin. (Daniel, 2016; De Melo et al. 2015; do Amaral et al. 2023; Liese and Tang, 2015; Schmidt et al. 2011; Tang et al. 2012). However, there were variations in the rate of degradation not only between white rot and brown rot, but also within different species of brown rot fungi with *C. puteana* registering higher ML in relation to *G. trabeum*. The ability of *C. puteana* to degrade more than *G. trabeum* is probably due to different degradation mechanisms, with *C. puteana* has cellobiohydrolases (Daniel, 2016).

Different types of preservatives can have varying effects on fungal degradation. In our study, we found that two concentrations of BBA were effective in protecting bamboo against fungal decay. The protection against fungal decay increased with increasing concentration of BBA. This means that the anti-fungal properties, which inhibit the oxidative metabolisms of fungi, increase with concentration in the chemical. This leads to cytoskeletal disruption and affects hyphal growth (Estevez-Fregoso et al. 2021; Gavilanes-Martínez et al. 2021). Our results are consistent with other research, such as the study conducted by Priadi et al. (2021) which found that borax and boric acid solutions can provide protection against decay in wood. Similarly Tomak et al. (2011) reported that boron-containing compounds can protect wood to a ML of less than 3 %. However, Temiz et al. (2008) reported that boric acid offers resistance to fungal degradation. But it should be noted that borax and boric salts are highly soluble in water, which makes them easily leachable. It is therefore recommended to use products treated with borax and boric salts for indoor applications to avoid leaching out of the preservative.

On the other hand, the two concentrations of CLS did not provide protection against both white and brown rot degradation. Our study

results are in agreement with Yanagawa (2016) who found that sea salt did not have enough concentrations of salt to inhibit the growth of white rot (*T. versicolor*) in wood. Jones et al. (2022) reported that some fungi were found to evolve in order to tolerate saline conditions. Our research contradicts the findings of Tresner and Hayes (1971) and Sato et al. (2014), who reported that certain basidiomycetes cannot tolerate NaCl salt concentrations of 5 % and 12 %.

## Conclusion

In this study, the effectiveness of crude lake salt was compared with borax-boric acid as preservatives against fungal degradation of bamboo. The results showed that whereas borax-boric acid was effective, crude lake salt was not effective in protecting bamboo against fungi. This suggests that crude lake salt is ineffective against agents that attack cellulose, hemicellulose and lignin. Further research could be conducted to explore the effectiveness of crude lake salt against agents that attack starch-based food sources.

## CRediT authorship contribution statement

Christine Kalembe Mwanja: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Fred Kalanzi: Writing – review & editing. Abwoli Banana: Writing – review & editing, Supervision. Romanus Ishengoma: Writing – review & editing, Supervision. Nasko Terziev: Writing – review & editing, Supervision, Methodology.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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