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THE IDENTIFICATION OF XYLOPHAGOUS FUNGI AND EVALUATION OF MECHANICAL DAMAGE IN PRESERVED WOOD

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ABSTRACT

In this study, seven xylophagous fungi (*Trametes*; *Trichoderma* A1; *Trichoderma* A2; *Aspergillus*; *Paecilomyces*; *Coniochaeta*; *Gloeophyllum*) were exposed to wood preserved with CCA salts, boric acid, and a polyphenolic extract (ExPol). The results showed a prevalence of xylophagous fungi that affects the use of wood. Changes in physical parameters such as mass are accompanied by changes in mechanical properties; variation in weight losses went from 12.69-20.42 %, 6.13-19.70 % and 6.38-23.68% for CCA, H_3BO_3 and ExPol respectively. In conclusion, variations in response to the preservatives were shown for each microorganism due the fact that metabolism is not the same because they use the substrate components or polymers in different ways for growth, being Expol the best option to inhibit *Trametes*, *Trichoderma* A2 and *Aspergillus*, there's not much difference between CCA and H_3BO_3 for the control of the rest of the organisms but both are better alternative than ExPol to inhibit their growth.

KEYWORDS: Wood preservative, mechanical damage, wood decay, CCA.

INTRODUCTION

In natural and urban environments, xylophagous fungi are a group of organisms that cause the deterioration and degradation of wood's lignocellulose, assimilating its structural polymers (Kracher et al. 2016). This degradation has significant repercussions for the physical and chemical stability of wood, compromising its utility. In the last century, the wood preservation industry has created techniques like chemical treatments and thermal modification (Sandberg et al. 2017). Conventional wood preservatives are categorized as chemical compounds with fungistatic or fungicidal activity. These include oil-based chemicals such as pentachlorophenol or creosote, and water-based compounds like copper chromate arsenate (CCA) and other salts. In developed countries, CCA is a restricted chemical because of the health risks, environmental dangers and residual waste (Zelinka et al. 2022). Environmental and health restrictions on

the use of conventional preservatives have led to the search for alternatives to extend the useful life of lignocellulosic biomaterials and their derivatives. Essential oils and plant extracts have shown positive biological impacts, which can help in developing novel wood preservatives (Ohgami et al. 2015; Woźniak 2022).

However, despite decades of development in the wood preservation industry, there are still significant opportunities for the generation of new knowledge in basic and applied science. In México, for instance, a wood preservation standard was created in 2014 by the National Agency for Standardization and Certification of Construction and Building S.C. (ONNCCE), NMX-C-419-ONNCCE-2001 Construction industry-pressure-preserved wood-classification and requirements (NMX-C-419-ONNCCE-2001), which establishes the retention and penetration parameters of preservatives in wood according to its use and expected risk level in service. This standard does not coincide with any other international standard; however, it is partially harmonized with important international regulations made by the American Wood Protection Association (AWPA 2011); the Department of Agriculture, Forest Service, Forest Product Laboratory USA (FPL-GTR-19011) and the National Institute of Research and Standardization Chile (1977) (NOM: NCh819:2019). These standards focus on *Pinus* sp. wood and set preservative/active ingredient retention levels based on wood exposure risk (R1-R5). It is also important to note that these retention levels primarily consider the environmental conditions of the exposed wood, without considering the type of pest. Even though it is difficult to estimate the exact conditions of parameters like humidity or temperature in each specific environment, wood-eating organisms are categorized via attack methods and damage. It is important to establish the best conditions for the use of preservative compounds, considering their effectiveness based on the type of organism.

To prevent xylophagous fungi from adapting to widely used preservatives, it is necessary to monitor the concentrations of their compounds, ensuring they do not exceed the levels required for pest control. Hadi et al. (2025), conducted a study using 3% CCA salts, treating different tropical wood species and evaluating protection against termites. The–work shows a high level of CCA salt use (8.4 kg/m³), without considering the effective amount needed to combat termite damage. Hasan et al. (2010) conducted a study on the leaching of CCA, copper azole (CA), and alkaline copper quaternary (ACQ) salts, finding a relationship between leaching and concentration at the high concentrations tested (2.69 kg/m³ to 35.4 kg/m³). Finally, He et al. (2025) found a substitution of CCA salts by CA and ACQ, compounds that even present a higher leaching rate than the CCA salts. The purpose of this study is to evaluate the variability of damage to preserved wood caused by various xylophagous fungi.

MATERIAL AND METHODS

Collection and isolation of xylophagous fungi

Fungal samples were collected in situ under *(1)* natural forest conditions, *(2)* different areas of wood processing in a commercial sawmill, and *(3)* structural wood in use. The samples were collected according to Sánchez-Corzo et al. (2021) and taken to the Wood Microbiology Laboratory at the Universidad Michoacana de San Nicolás de Hidalgo, where cultivable species were grown using potato dextrose agar (PDA; BIOXON), Sabouraud dextrose agar (ADS;

BIOXON), malt extract agar supplemented with yeast extract (MEA+YEA; DIFCO), and a 30–70% PDA/ADS mixture. Samples were incubated for 5 days at 30°C. Colonies with distinct morphologies were then selected, isolated, and replated until pure cultures were obtained.

Qualitative of cellulase and laccase activity

For cellulase detection, bacteriological agar medium (BIXON) (10 g/L) plus yeast extract (CONDALAB) (26 g/L) enriched with 0.5% carboxymethylcellulose (CAS: 9004-32-4) was used. The mixture was sterilized at 120°C and 120 lbs of pressure for 15 min and then placed in 90 mm Petri dishes. An inoculum of the mycelium of each of the fungal isolates (5 mm) was placed in the center of the dish and incubated at 30°C for 72 h. Subsequently, 3-5 ml of 0.2% Congo red solution (CAS: 573-58-0) was added and incubated for 15 min. Then, it was decanted and rinsed with a 1 M NaCl solution, until the appearance of a light-colored halo with orange edges was observed around the mycelium. Using four replicates per organism, the halo diameter was measured to determine the enzymatic index (EI) using the Eq. 1. According to Florencio et al. (2012) strains with an EI greater than 1.50 are considered potential producers of cellulases:

$$EI = (\text{Hydrolysis diameter}) / (\text{Colony diameter}) \quad (1)$$

For laccase detection, bacteriological agar medium (BIXON) (10 g/L) plus yeast extract (CONDALAB) (26 g/L) enriched with 0.05 g of 1-naphtol L-1 (CAS: 90-15-3) was used. The mixture was sterilized at 120°C and 120 lbs of pressure for 15 min and then placed in 90 mm Petri dishes. An inoculum of mycelium from each of the xylophages (5 mm) was placed in the center of the dish and transferred to a microbiological incubator at 30°C for 72 and 120 h. The reaction of 1-naphtol with ligninolytic enzymes was recorded, resulting in a dark blue color change (four replicates were performed for each organism).

Taxonomic identification of xylophagous fungi

Pure cultures were characterized by morphological evaluation and genetic sequencing. For morphological evaluation, samples were observed under a LABOMED optical microscope at 10x, 40x, and 100x magnification, and photographic processing was performed using MOTIC IMAGES Plus 2.0 software. For genetic sequencing, the collected samples were extracted using the commercial QIAGEN DNEasy Power Soil Pro kit, following the manufacturer's instructions. Purity and DNA concentration were quantified using a nanodrop (Nanodrop 2000, Thermo Fisher) and the 260/280 ratio. The integrity of the extracts was assessed by electrophoresis on a 1% agarose gel. The extracted DNA was sent to a sequencing service (MR.DNA, Shallowater, TX, USA) for amplification of intergenic sequences (ITS). ITS sequence information was evaluated using FastQC and Trimmomatic software. Finally, the taxonomic category was obtained using the NCBI BLASTn platform (www.ncbi.nlm.nih.gov) (Prieto-Barajas et al. 2018).

Xylophagous fungi assay in *Pinus* sp. wood specimens

Ten-year-old *Pinus* sp. wood was pruned through 5 mm × 10 mm × 20 mm tangential cuts. The specimens were subsequently treated with 2% arsenic-copper-chromium salts (CCA), 2%

boric acid (H_3BO_3), and a 2% preservative polyphenol extract (ExPol). The preservation procedure was based on the method proposed by Bravery (1979), in which wood specimens were placed in a drying oven until they reached 13% moisture content. Then, wood specimens were distributed among various treatments containing *(a)* CCA, *(b)* H_3BO_3, and (c) ExPol, plus a control treatment. Wood specimens were immersed in their respective treatment for 48 h, before being reconditioned by air drying. The wood specimens (n = 378) from the treatments were placed in 90 mm diameter Petri dishes (3 wood specimens per Petri dish), with a base of moistened sterile filter paper.

Inoculation of the test probes

The Petri dish treatments were inoculated with 5 mm^3 propagules of each of the previously isolated and identified xylophagous fungi. The treatments were placed in a 30°C incubator for 20 weeks (the humidity of the filter paper was checked every 4 weeks).

Physical-mechanical testing on the wood specimens

After 20 weeks of exposure to the etiological agents, the wood specimens (5 mm × 10 mm × 20 mm) from the treatments were recovered and reconditioned until reaching 13% moisture content. Using a universal machine (Automatic Universal Testing Machine UTM300KN), wood specimens were placed with the surface of the end fibers parallel to each other and at right angles to the longitudinal axis, carrying them to failure, identified by the end of the elastic zone of the material, at a loading speed of 6 mm/min (261 wood specimens distributed among the treatments).

Total phenol quantification

Total phenol content from the preservative polyphenol extract (ExPol) was quantified using the Folin-Ciocalteu spectrophotometric method, where the reducing capacity was evaluated by the reaction of tungsten and molybdate salts with the reducing agents present in the sample forming a blue complex whose maximum absorbance was read at 765 nm. The reaction conditions were 100 µl of sample to which 10 µl of 100% Folin's reagent plus 70 µl of 7.5% w/v sodium carbonate and 20 µl of deionized water were added to a final volume of 200 µl. The mixture was stirred and kept in the dark at 25°C for 60 min, and its absorbance at 765 nm was measured using a BioTek Epoch Microplate mod UV-Vis spectrophotometer. Quantification was performed using a calibration curve with gallic acid as a standard, expressing the results as milliequivalents of gallic acid per ml of sample. All measurements were performed in triplicate.

Statistical analyses

Weight loss tests on wood specimens and mechanical tests on a universal machine were analyzed using MINITAB software, performing an analysis of variance (ANOVA) and subsequent analysis of means (Tukey) at a significance level of 0.05.

RESULTS

Obtaining fungal isolates

The selected sites showed an interesting presence of fungal organisms. Three organisms were isolated from site under natural forest conditions (isolates NFC1, NFC2, and NFC3). From a commercial sawmill, two fungal organisms were isolated (CS1 and CS2); and finally, from site a structural timber in service, two more were obtained (SW1 and SW2). The fungi obtained in pure culture are shown in Fig. 1, showing consistent growth across all seven isolates.

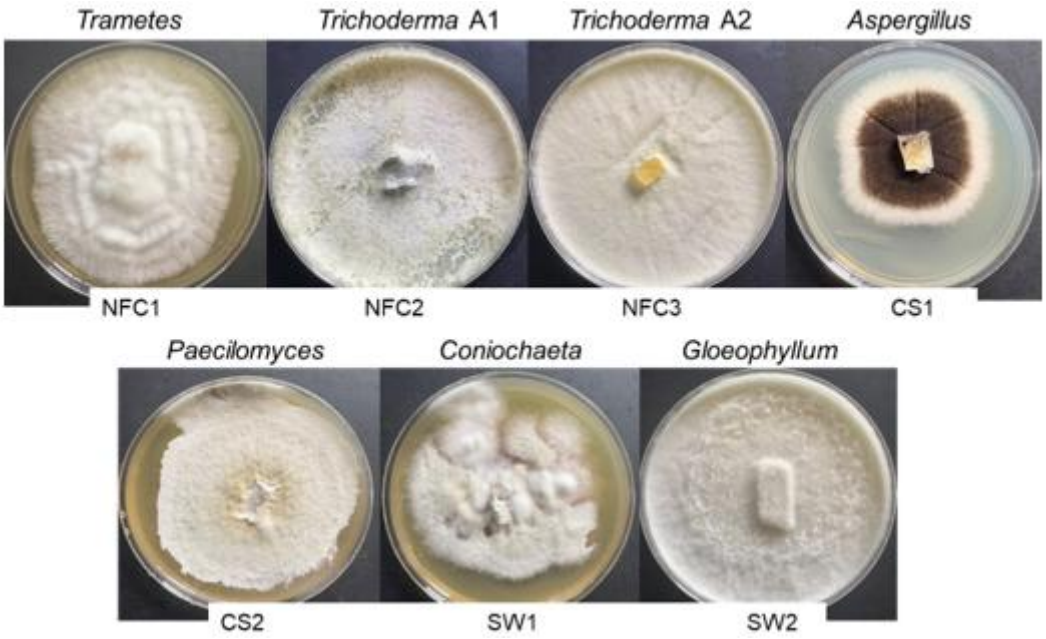

Fig. 1: Fungi isolates obtained, code keys meaning natural forest conditions (NFC1, NFC2 and NFC3), commercial sawmill (CS1 and CS2), in service structural wood (SW1 and SW2). Images taken 5 days after the fungus began to grow.

Taxonomic identification of xylophagous fungi

Isolate NFC1 showed the presence of septate hyphae, some of which were rhizomorphic or in the process of forming other bodies. Clamps characteristic of *Basidiomycetes*, such as *Trametes*, were observed, consistent with macroscopic growth in culture media (Fig. 2A). NFC2 and NFC3 isolates exhibited similar microscopic characteristics typical of the genus *Trichoderma*; however, the developmental forms showed conidia and chlamydospores, although they also exhibit different arrangements and sizes (Fig. 2B-C).

Conversely, the CS1 isolate displayed *Aspergillus*'s reproductive structures like sporangia and ascospores, with dark pigmentation (Fig. 2D). Isolate CS2 presented a characteristic chain-like clustering characteristic of the *Paecilomyces* genus, evident in abundance at various magnifications (40x and 100x) (Fig. 2E). Isolate SW1 exhibited conidiogenic cells and swollen hyphae, as well as haustoria-like structures, indicating that it belongs to the genus *Coniochaeta* (Fig. 2F). Isolate SW2 showed a hyphal framework, clamps, and developing basidioconidia. The arrangement of these elements and its macroscopic characteristics allow it to be classified in the genus *Gloeophyllum* (Fig. 2G).

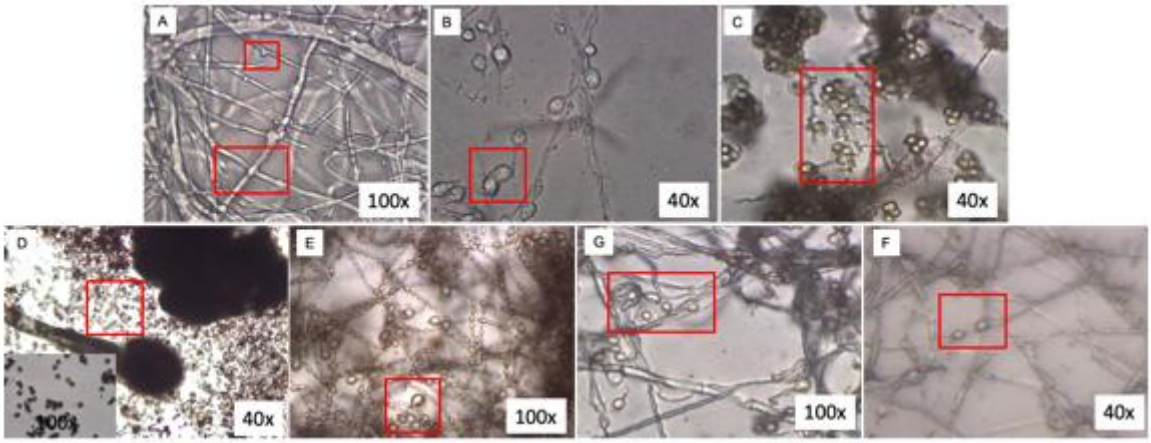

Fig. 2. Microscopic structures of the fungi isolate, pictures correspond to isolates (A) NFC1, (B) NFC2, (C) NFC3, (D) CS1, (E) CS2, (F) SW1 and (G) SW2. Panel A shows septate and rhizomorphic hyphae with presence of fibulae, B and C panels exhibit resistance structures development such as chlamydospores, D panel shows one sporangiophore next to double membrane ascospores, panel E depicts characteristic conidia of the *Paecilomyces* genus, F panel illustrates conidiogenic cells, septate and swollen hyphae as well as haustorium like structures and final panel G exhibit septate hyphae, generative framework with presence of developing basidioconidia. Images taken 5 days after the fungus began to grow.

BLASTn

Using the FASTA files obtained, a library was constructed to determine the taxonomic identity of the fungal isolates. Similarities were found above 97% and with a low number of Bootstrap errors. Among the fungal isolates, *Trichoderma citrinoviride* (NFC2) and *Trichoderma aquatica* (NFC3), *Aspergillus niger* (CS1) and *Paecilomyces variotii* (CS2), *Coniochaeta deborreae* (SW1), and *Gloeophyllum* spp. (SW2) were identified (Tab. 1). The information is stored in a database (https://www.ncbi.nlm.nih.gov) under the accession code PRJNA1305116.

Tab. 1: Taxonomic identity of the fungi isolates.

Isolate	ID	Result (BLAST)	Simility (%)	Taxonomic assignation
NFC1	*Trametes*	*Trametes*	N/A	*Trametes spp.*
NFC2	*Trichoderma A1*	*Trichoderma citrinoviride* *Trichoderma reesei* *Trichoderma pseudokoningii*	100 99.21 99.21	*Trichoderma spp.*
NFC3	*Trichoderma A2*	*Trichoderma aquatica* *Trichoderma reesei* *Trichoderma parareesei*	99.68 99.68 99.68	*Trichoderma spp.*
CS1	*Aspergillus*	*Aspergillus niger* *Aspergillus foetidus* *Aspergillus welwitschiae*	100 100 100	*Aspergillus spp.*
CS2	*Paecilomyces*	*Paecilomyces variotii* *Paecilomyces formosus* *Paecilomyces peniciliformis*	100 99.82 94.39	*Paecilomyces spp.*
SW1	*Coniochaeta*	*Coniochaeta deborreae*	99.82	*Coniochaeta spp.*

		Coniochaeta cipronana	93.85	
		Coniochaeta boothii	97.53	
SW2	*Gloeophyllum*	*Gloeophyllum sepiarium*	92.82	*Gloeophyllum spp.*
		Heliocybe villosa	87.85	
		Heliocybe fasciculata	84.44	

Data and sequences are deposited in the NCBI data base (https://www.ncbi.nlm.nih.gov) under the accession number: PRJNA1305116.

Qualitative of cellulase and laccase activity

Of the seven fungal organisms evaluated, all showed cellulase activity, revealing a characteristic yellow halo on the surface of the medium (Fig. 3A-C). The cellulolytic enzyme index values found are shown in Fig. 3B-CA, highlighting the *Trametes* isolate with an EI of 1.14, and *Gloeophyllum* with an EI of 3.28. Meanwhile, laccase activity was found only in the *Trametes* isolate, displaying a characteristic dark hue on the growth medium (Fig. 3A-LA).

B

Fungi Isolate	CA	LA
Trametes	+ IE 1.14 ± 0.06	+
Trichoderma A1	+ IE 1.10 ± 0.02	-
Trichoderma A2	+ IE 1.08 ± 0.03	-
Aspergillus	+ IE 1.24 ± 0.05	-
Paecilomyces	+ IE 1.25 ± 0.03	-
Coniochaeta	+ IE 1.36 ± 0.09	-
Gloeophyllum	+ IE 3.28 ± 0.3	-

Fig. 3: Panel A exhibits representative images of cellulase activity (CA) and laccase activity (LA) from isolated Trametes, Aspergillus and Gloeophyllum. Panel B shows enzymatic index obtained from fungi isolates (EI) (+ and – indicates presence and absence respectively).

Wood weight loss

The model used for the *Pinus* sp. wood test after 20 weeks of exposure to the fungal isolates, showed growth on the wood surface (Fig. 4A), which was also reflected in wood damage from the various treatments (Fig. 4C). For example, wood treated with CCA and exposed to *Trichoderma* and *Coniochaeta* showed significant damage that could compromise the structure. Consequently, when assessing weight loss in treated wood specimens, CCA-treated wood with *Trametes versicolor* showed a 12.69% weight loss, boric acid-treated wood lost 18.07%, and ExPol-treated wood lost 11.4%. However, at least three important facts can be highlighted: first, due to the weight loss values between the CCA and ExPol treatments, the latter is preferable (lower toxicity to the environment). Second, the fungus (*Trametes*) was able to grow in wood treated with CCA (a widely used preservative) and cause damage to the material. Third, boric acid was found to be ineffective in controlling *Trametes* (Tab. 2).

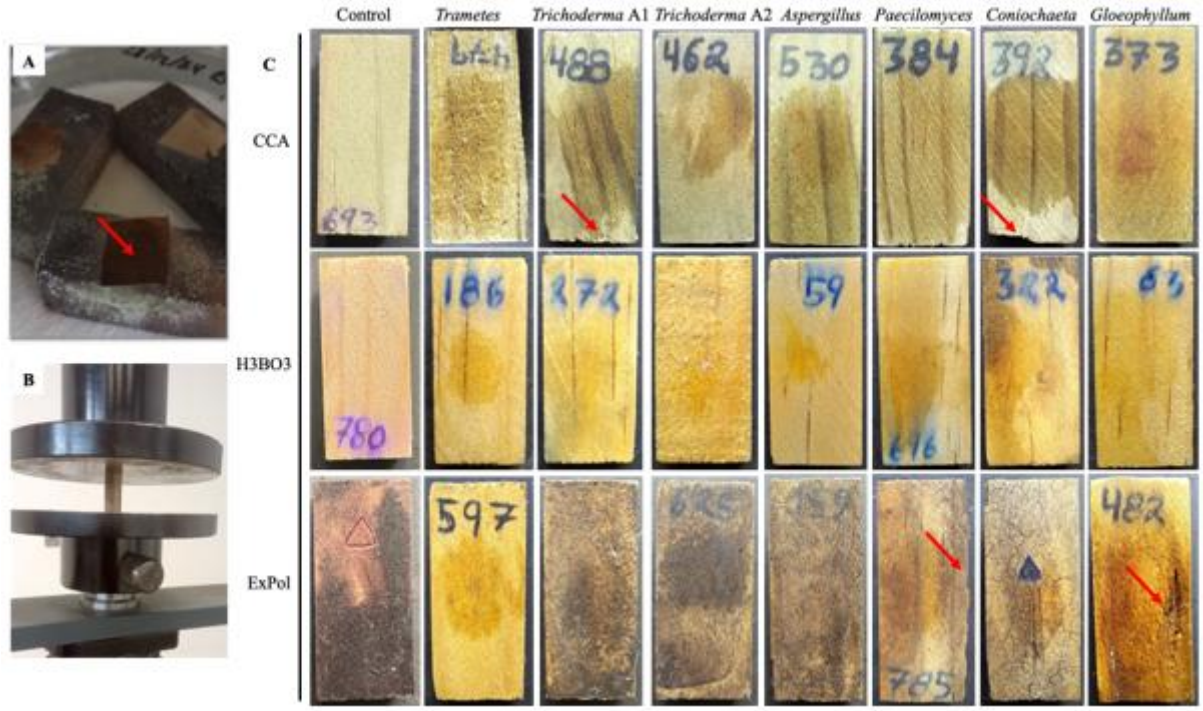

Fig. 4: (A) Petri dish assay, wood probes and fungi inoculum are shown (red arrow), (B) universal test machine assay (compressive strength of wood parallel to the grain on oriented probe) and (C) representative image of wood probes treated with CCA, H_3BO_3 and ExPol exposed and not exposed to fungi isolates (arrows indicate damage on the material).

Another important result is that *Paecilomyces* shows resistance to CCA, causing a 15.08% weight loss in the material; in the treatments with H_3BO_3 and ExPol, the results are 6.13% and 6.38%, respectively. This set of results demonstrates that the effectiveness of CCA in combating wood-boring insects in general is questionable, and that there are more effective and environmentally friendly alternatives.

Tab. 2: Weight losses on wood probes after 20 weeks exposed to fungi isolates.

Isolate	CCA			H3BO3			ExPol		
	C	**I**	**D (%)**	**C**	**I**	**D (%)**	**C**	**I**	**D (%)**
NFC1	3.31± 0.06	2.89± 0.07*	12.69 **B**	3.32± 0.05	2.72± 0.04*	18.07 **A**	3.07± 0.05	2.72± 0.03*	11.40 **B**
NFC2	3.82± 0.16	3.04± 0.09*	20.42 **B**	3.30± 0.05	2.65± 0.05*	19.70 **B**	3.63± 0.08	2.83± 0.06*	22.04 **A**
NFC3	3.47± 0.12	2.93± 0.10*	15.56 **A**	3.22± 0.11	2.69± 0.06*	16.46 **A**	3.07± 0.08	2.76± 0.07*	10.1 **B**
CS1	3.27± 0.07	2.82± 0.06*	13.76 **A**	3.11± 0.08	2.63± 0.07*	15.43 **A**	3.07± 0.06	2.73± 0.06*	11.07 **B**
CS2	3.58± 0.11	3.04± 0.09*	15.08 **A**	3.10± 0.09	2.91± 0.06*	6.13 **B**	2.82± 0.09	2.64± 0.09*	6.38 **B**
SW1	3.53± 0.09	3.02± 0.08*	14.45 **B**	3.28± 0.007	2.81± 0.05*	14.33 **B**	3.59± 0.06	2.74± 0.06*	23.68 **A**

SW2	3.57± 0.10	3.05± 0.09*	14.57 **A**	3.17± 0.11	2.78± 0.08*	12.30 **A**	3.15± 0.08	2.54± 0.09*	19.37 **A**

The labels mean: C= Control treatment, I= Inoculated treatment, D= Difference between treatments.

Universal machine testing

Universal machine testing (Fig. 4B) shows graphs where the material's yield point is observed when the line begins its descent. For example, when examining what happened with wood specimens exposed to *Trametes* (a lignin-degrading fungus), the material becomes more flexible and softer, tolerating less load under deformation. However, this same material, when preserved with CCA (Fig. 5A, green line), increases its density and stiffness, as the CCA is incorporated into its structure. Meanwhile, *Gloeophyllum* shows a more brittle material due to the loss of cellulose. A similar phenomenon occurs when CCA is incorporated into lignocellulosic tissue, which, by increasing its density and stiffness, also increases its maximum load-bearing capacity (Fig. 5G, green line).

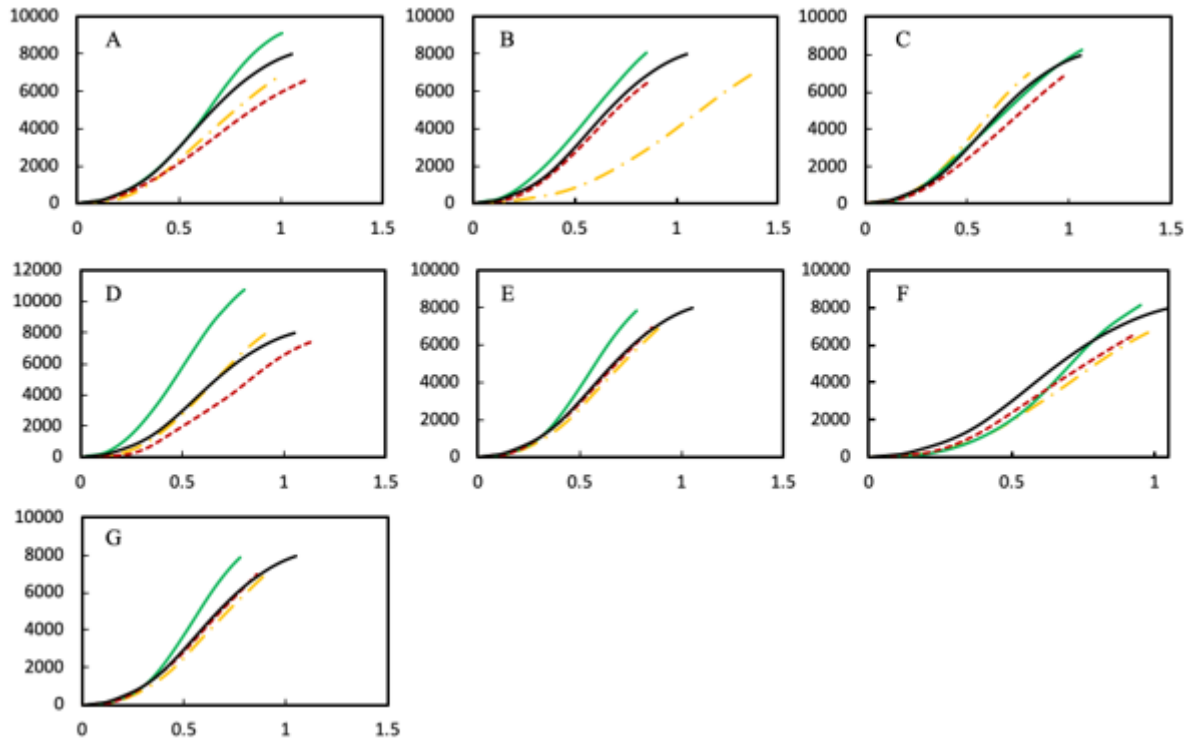

Fig. 5: Stress deformation graphs; X axis deformation (mm) and Y axis maximum load (N). Mechanical behavior of the treatments can be appreciated CCA (green), H3BO3 (yellow), ExPol (brown) and blank (black) exposed to isolates (A) Trametes, (B) Trichoderma A1, (C) Trichoderma A2, (D) Aspergillus, (E) Paecilomyces, (F) Coniochaeta and (G) Gloeophyllum.

In general, xylophagous organisms with enzymatic characteristics for degrading cellulose (*Trametes, Trichoderma* 1*, Trichoderma* 2*, Aspergillus, Paecilomyces, Coniochaeta, Gloeophyllum*) cause the material to have a premature yield point. When treated with CCA, the yield point can shift, reaching a later point (green line). Because this is an anisotropic and heterogeneous material, it is necessary to analyze the mechanical behavior of the material using stress-strain graphs, considering specific values of maximum load and displacement. The elastic

zone behaviour of the material its important because is the region where the polymers of the wood are still structurally organized explaining their mechanical properties, changes in this zone may evidence the effect of the preservatives and the fungi metabolism on the wood matrix.

DISCUSSION

In México, the state of Michoacán, and specifically its eastern region, is considered a high-risk phytosanitary area due to various wood-eating organisms and a high prevalence of uncharacterized fungal species. Although there are reports monitoring incidences in this region, there is no properly established follow-up, nor has the identification of fungal organisms been achieved, especially those that escape the sampling criteria of the surveillance system of institutions such as the Planning Institute of the State of Michoacán de Ocampo and the Forestry Commission of the State of Michoacán (cofom.michoacan.gob.mx) (IPLAEM 2020; COFOM 2022). Particularly for fungal organisms, their pleomorphic capacity and phenotypic variability make descriptive studies necessary to continue correctly assessing their biodiversity (Bass and Richards 2011).

In this work, most of the fungi found belong to the Dikarya family, but the presence of different fungal genera reveals an interesting diversity. Furthermore, stress-strain graphs allow for the evaluation of the behavior of a material's elastic zone. Specifically, in wood, they provide useful information about the load-bearing capacity it supports before reaching a yield point (where the slope changes) and a failure point (where the plastic zone begins), which translates to a loss of microstructure within the material (Ansell 2015).

The mechanical behavior of wood has shown a pattern where the material treated with CCA and H_3BO_3 preservatives increases its density, which, in turn, modifies the material's mechanical strength. CCA salts can bond with lignin, ensuring reduced leaching by occupying available spaces in the wood's porosity. Boric acid also occupies available spaces within the wood matrix, but its chemical interactions are weaker (higher leaching rates), making it less durable than CCA. While both preservatives increase wood density, the molecular weight varies between formulations. CCA salts have a higher mass, and the metallic compounds are more crystalline. This factor influences the higher mechanical strength of the treated specimens, coinciding with their increased stiffness. It is important to mention that some interaction between the wood and the polyphenol extract appears to occur regardless of the presence of an organism, reducing the material's resistance to mechanical stress.

It is relevant to mention that the fungi's nutritional tropism helps to better understand the changes observed in the elasticity of the uninoculated wood specimens treated with preservatives after the metabolic action of the fungi. Changes of physical and mechanical properties are a useful tool to infer the repercussions of the enzymatic or physicochemical mechanisms that generate the biotransformation of this highly complex substrate (wood) by microorganisms (Ansell 2015; Vasconez 2021).

In the case of wood exposed to the *Trametes* isolate, the material becomes more deformable regardless of the preservative used, which is consistent with its nutritional preference and its ability to modify the mechanical properties of *Pinus* wood (Witomski et al. 2016), due to the role that lignin plays in the wood, cementing and strengthening the cellulose and

hemicellulose fibers in the cell wall, limiting excess flexibility and softening the wood tissue. It should also be noted that the mechanical plasticity of the wood specimens treated with CCA is modified to a lesser extent due to the interaction of salts with lignin, while the wood specimens treated with boric acid (higher leaching rate) are more exposed to the fungus.

The ExPol extract does not show a notable difference in mechanical strength before failure between inoculated and non-inoculated specimens. In the extract, color diminished with the fungus, which might be due to the substrate's chemical similarity to the phenylpropanoid compounds. As a white-rot fungus, *Trametes* can generate nonspecific and efficient enzymes for degrading lignin: lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), and laccases (EC 1.10.3.2). The enzymes of the peroxidase system contain a heme group rich in glycine, requiring the presence of hydrogen peroxide to perform Fenton reaction and degrade the aromatic structures present in the polymer. Laccases, in addition to catabolizing phenolic and non-phenolic compounds by electronic oxidation through the copper in their active center, are not only capable of degrading the substrate but are also used in the paper industry to bleach the material without affecting fiber strength (Rameshaiah and Jagadish 2015).

Meanwhile, in wood exposed to *Paecilomyces* and *Gloeophyllum*, fungi with distinct nutritional characteristics, an increase in the material's stiffness was observed, because of cellulose and hemicellulose fibrils depolymerization, where lignin remnants in the wood are susceptible to degradation. Once the material becomes more rigid, with the lignin remaining predominantly, the material becomes markedly brittle. The preserved, uninoculated specimens also show higher mechanical strength, explained by the interweaving of the different polymer fibers that were not compromised prior to inoculation. This phenomenon is also explained by the fact that they are also structurally responsible for distributing the mechanical forces exerted on the material before failure due to mechanical stress.

Paecilomyces and *Gloeophyllum* are fungi categorized as causal agents of brown rot (Rostami et al. 2023), degrading the structural components of cellulose and hemicelluloses, giving a characteristic geometric pattern that seriously compromises the wood's structure, decreasing its mechanical properties (Amaresan et al. 2020). These fungi mainly secrete a triad of enzymes: endoglucanases (EC 3.2.1.4) that are responsible for reducing the size of units joined in the polymer into smaller fragments, these changes also being useful to be enzymatically catalyzed by cellobiohydrolases (EC 3.2.1.91), responsible for causing changes in the crystalline regions of cellulose thus facilitating its degradation into increasingly smaller constituents (Mansfield et al. 1998).

In this work, the enzymatic profile secreted by fungal isolates shows that they are related to damage and mechanical behavior, for example, the genus *Aspergillus* is not usually attributed as a causal organism of white, soft or brown rot. However, it has demonstrated its ability to use wood as a substrate, being classified mostly as chromogenic, that is, it can pigment the surface where it develops, the existence of pathogenic strains of plants, humans and even causing problems for the food industry, capable of biosynthesizing aflatoxins is also known (Baker and Bennett 2007). In *Aspergillus*, the activity of carboxymethyl cellulase (EC: 3.2.1.203), beta glucosidase (EC: 3.2.1.21) and cellobiohydrolase (EC. 3.2.1.91) has been characterized (Díaz et al. 2021). In our study, the *Aspergillus* isolate exhibited a similar phenomenon as the *Gloeophyllum* isolate, obtaining higher maximum load values than the control treatment,

but lower deformation values. This is explained by the presence of carboxymethyl cellulase, beta-glucosidase, and cellobiohydrolase, which are believed to be degrading and altering the proportions of cellulose and hemicellulose in the wood, thus changing its mechanical behavior (Díaz et al. 2021).

The wood protection industry using compounds that are toxic to the environment is a common practice. The organism to be controlled is not considered, nor has the concentration necessary to control the damage been regulated. In this study, the results contribute to the understanding of which preservative compound is appropriate for the fungus to be controlled. For example, one of the most common problems in the forestry industry involved in wood processing is the presence of chromogenic fungi. Our results have shown that, based on the weight loss in the test probes for *Aspergillus* control, the use of ExPol is a better option than CCA and H_3BO_3, primarily due to its low toxicity and lower weight loss. Another example is the control of *Gloeophyllum* growth using H_3BO_3. Since the weight loss in test probes treated with CCA and H_3BO_3 is similar, it is preferable not to use CCA (Zabel and Morrell 2020).

It is important to note that none of the preservatives currently on the market are categorized as environmentally friendly. They all have pros and cons, *v.g.* CCA is perhaps the most effective preservative that exists but also one of the most toxic and prohibited, with greater residuality in the environment, attributable to the fact that the metallic elements of CCA have different uses in preservation, with Copper and Arsenic being elements capable of altering fungal metabolism, decreasing the levels of electronic energy in cellular respiration, while Chromium forms complexes with lignin preventing its leaching (Caldeira 2010; Zabel and Morrell 2020).

The boric acid and other borates have been shown to dehydrate organisms and generate alkaline environments that hinder their development. In some organisms, high exposure to boron can disintegrate cell membranes (Caldeira 2010; Zabel and Morrell 2020). There are also reports showing that aqueous wood preservatives can modify mechanical properties, as this is directly related to the changes generated within the material matrix (Winandy 1995).

The residuality and leaching of preservatives is another aspect to consider since CCA has greater residuality in the environment but less leaching, because the metallic atoms in the formulation generate electrostatic interactions, while the copper maintains a bond thanks to the chromium on the lignin. Boric acid doesn't bond with wood, and it easily leaches out, particularly when exposed to rain outdoors. The ExPol preservative compound showed a high content of phenols in its composition, reaching 4.577 mE gallic acid. However, one proposed mechanism of action on fungi could be explained by the ability to generate reactive oxygen species, which can have harmful effects on cellular organelles and disrupt signal transduction. The knowledge generated in this work transcends the point in that there are proposals for their use in relation to the preservative, condition, and organism to be controlled.

CONCLUSIONS

Xylophagous fungi isolation from collection sites corroborates the presence of etiological entities that affect the economic use of wood at different processing points and derived products. Additionally, it proves they are in the area researched where these organisms are a high phytosanitary risk. Changes in weight, density, and mechanical strength of the wood

specimens subjected to the different treatments were evident. The addition of substances such as CCA and H_3BO_3 salts modified the density of the material, reflecting a difference in mechanical strength. On the other hand, the polyphenolic extract decreased the strength of the material, contrary to expectations. Additionally, the finished material has an unsightly appearance. The metabolic tropism of fungi and their response to preservatives helps to understand the changes in the elastic modulus, stiffness, and strength of the material. It also indicates the optimal preservative compound for each organism, taking ecological factors and resistance into account.

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