

ANTIFUNGAL ACTIVITY OF ORGANIC ACIDS AND THEIR IMPACT ON WOOD DECAY RESISTANCE¹

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Abstract. Organic acids are present in many bio-based chemicals suggested as active ingredients for wood preservative formulations, but their effects in wood have not been studied. However, an understanding of their effect in wood is necessary. The aim of this study was to determine the antifungal and wood-preserving activities in vitro of acetic, formic, and propionic acids against the wood-decaying fungi *Coniophora puteana*, *Rhodonia (Poria) placenta*, *Gloeophyllum trabeum*, and *Trametes versicolor*. Mixes of these three acids were also tested for possible synergetic effects. All the acids and mixtures showed antifungal activity against the pure cultures of wood-decaying fungi. Propionic acid by itself had the best performance, inhibiting at 1 g/L concentration the growth of all the decay fungi by 99-100%. Despite the antifungal activity of the organic acids, the pine sapwood specimens treated with 3% and 6% acid solutions and exposed to decay by *C. puteana* and *G. trabeum* did not differ significantly from the untreated pine. Leaching of the sapwood specimens caused an incremental mass loss of the sapwood specimens. In addition, the leached specimens had a lower mass than the same specimens before the acid impregnation, indicating that they were damaged by the acidic chemicals. The presence and acidity of organic acids in wood-derived bio-based chemicals need to be assessed before they are used as wood preservatives.

Keywords: Wood degradation, wood preservation, biorefining, fungistatic, organic acid.

INTRODUCTION

Transferable durability is based on the transfer of extracts from durable materials to wood to increase the wood's durability (Kirker et al 2013; Eller et al 2020). This technique's relevance has increased in recent years, as new preservation applications have gradually replaced many traditional wood preservatives whose use has become limited, such as chromated copper arsenate (Liu et al 2018) and, more

recently, boron-based compounds (Hu et al 2017). However, as wood treated with these chemicals is still in use, hazards to public health will remain (eg Augustsson et al 2017) unless these structures are rebuilt or their materials are replaced with more benignly treated ones. Thus, finding nontoxic substitutes for traditional wood preservatives that can be used in wooden structures is of high importance and can lead to the safer use of wood in the long term.

Bio-based chemicals are commonly studied as alternatives to creosote and metal salts in the field of wood preservation. The antifungal activity of many bio-based chemicals, such as tannins (Lomelí Ramírez et al 2012; Anttila et al 2013),

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stilbenes (Lu *et al* 2016), essential oils (Chittenden and Singh 2011; Cai *et al* 2020), bark extractives (Harun and Labosky Jr. 1985), and the derivatives of organic acids (Barbero-López *et al* 2018), makes them promising chemicals for transferable durability. These antifungals can be extracted from forestry and agricultural side streams, such as bark, via conventional as well as newly emerging methods (Zwingelstein *et al* 2020), resulting in chemical mixtures that are able to inhibit the harmful effects of wood-decaying fungi. Some known antifungals can be found in wood and its chemically refined products, such as wound-wood extracts (Vek *et al* 2013) and pyrolysis distillates (Barbero-López *et al* 2019). Nevertheless, most bio-based chemicals, including caffeine (Kwaśniewska-Sip *et al* 2019), silverskin extracts (Barbero-López *et al* 2020), and tannins (Tomak and Gonultas 2018), can easily leach out from wood because of their water-soluble nature, which drastically reduces their efficiency as wood preservatives.

Pyrolysis distillates represent tar-like chemical mixtures that result from biomass pyrolysis at conditions exceeding 350°C under inert environments. They have antifungal properties that combat wood-decaying fungi, and they are rich in many different chemicals (Mourant *et al* 2007). Tar and similar chemical mixes are used in traditional wood preservation, from wooden boats to roof tiles (González-Laredo *et al* 2015; Bailly *et al* 2016). Pyrolysis distillates, which contain organic acids, have been tested by several research groups as wood preservatives with successful results (Lourençon *et al* 2016; de Souza Araújo *et al* 2018; Barbero-López *et al* 2019). Similar chemicals, such as wood vinegars, are known to have antifungal properties against wood-decaying fungi and also contain organic acids (Oramahi and Yoshimura 2013; Oramahi *et al* 2018). Organic acids are present in many bio-based chemicals and have been identified by previous studies as potential fungal inhibitors against wood-decaying fungi (Oasmaa and Czernik 1999; Bahmani *et al* 2016).

The effect of organic acids in wood demands more attention, as these acids are present in many

of the bio-based chemicals suggested for wood preservation, and they may also play a role in wood decay. In a study conducted by Barbero-López *et al* (2019), propionic acid was found to be highly antifungal against several decay fungi, and propionic acid and acetic acid were also found to protect against date and oil palm decay (Bahmani *et al* 2016). Formic acid has not been reported as a successful wood preservative; however, it is used by ants to disinfect their nests, and it produces strong antimicrobials (Brütsch *et al* 2017). The presence of organic acids in pyrolysis distillates has been shown to be related to their antifungal activity (Oramahi and Yoshimura 2013; Barbero-López *et al* 2019) as well as in other bio-based chemicals (Barbero-López 2020). The study performed by Oramahi *et al* (2018), additionally, found that the only pyrolysis vinegar performed well as antifungal agent contained propionic acid and had the highest acetic acid concentration of the ones tested. Other research groups have found that phenolics are responsible for the antifungal activity of both pyrolysis distillates and wood vinegars (Mourant *et al* 2005; Baimark and Niamsa 2009), whereas Mattos *et al* (2019) highlighted that the antimicrobial activity of pyrolysis distillates is related to the presence of organic acids—acetic acid specifically—phenolic derivatives and carbonyl compounds and their synergies, although chemical analyses of distillates are often not performed. Thus, there is not enough knowledge of the role played by acids and their synergies in wood preservation, as the impact that acids have on wood decay is often not isolated from the other constituents of bio-based chemicals.

The aim of this study was to assess the antifungal performance of acetic, formic, and propionic acids on wood-decaying fungi *in vitro* and to determine how the wood decay process is affected once these acids are impregnated into a wood substrate. Wood decay tests were performed with Scots pine sapwood impregnated with the organic acids and their mixtures to examine the effect of these acids on mass loss and to determine whether we can derive conclusions

regarding the chemicals' performance in anti-fungal assays from their activity in wood. The results of these experiments demonstrate the potential of organic acids found in thermal distillates of wood as active components of bio-based wood preservatives against decay fungi.

MATERIALS AND METHODS

Fungal Culture Preparation

Brown rot fungi *Coniophora puteana* (strain BAM 112), *Rhodonía (Poria) placenta* (strain BAM 113), and *Gloeophyllum trabeum* (strain BAM 115), as well as white-rot fungus *Trametes versicolor* (strain BAM 116), were purchased from the Federal Institute for Materials Research and Testing (BAM, Berlin, Germany). Before any tests were performed, the fungal cultures were prepared to have actively growing fungi for the antifungal test. The fungi were inoculated under sterile conditions in petri dishes (Ø 90 mm) with 15 mL of 4% malt powder and 2% agar growth media and one fungus per dish. Afterward, the petri dishes were sealed with parafilm and kept in a growing chamber ($22 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH). Once the mycelium covered the entire surface of each dish, the dishes were moved to a fridge (10°C) and taken back to the growing chamber 2 d before the antifungal test.

In the cases of *C. puteana* and *G. trabeum*, the same procedure was used to prepare the platforms for the wood decay test. Brown rot fungi were chosen for this test as they usually decay softwoods (Martínez et al 2005) which are the most common wood species for outdoor uses. Petri dishes (Ø 90 mm and 15 mm height) with 4% malt powder and 2% agar were prepared and

inoculated with either *C. puteana* or *G. trabeum* and sealed with parafilm under sterile conditions. The dishes were kept in a growing chamber ($22 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH) until the mycelia covered their entire surface.

Antifungal Test

Acetic acid (100%), propionic acid (99%), and formic acid (85%) were purchased in Merck KGaA, Darmstadt, Germany. Growth media amended with acetic, propionic, and formic acids and their mixtures were prepared in petri dishes (Ø 90 mm). To allow the agar to set, pH of the acids and acid mixtures was adjusted to 4 with 0.1 M NaOH. The growth media for the antifungal test were prepared in Milli-Q water, with 4% malt powder, 2% agar, and one of the organic acids or their mixtures, as presented in Table 1. For example, the 1 g/L acetic acid and propionic acid mixture included 0.5 g/L each of propionic acid and acetic acid. The same mixture—4% malt powder and 2% agar in Milli-Q water without any acid—was included in the controls. Each growth solution was autoclaved (120°C , 15 min), and 15 mL was cast in each petri dish under sterile conditions.

Using a plug (Ø 5.5 mm), a spherical inoculum from the previously prepared fungal colonies was placed in the center of each petri dish under sterile conditions. The dishes were then sealed with parafilm and placed in a growing chamber ($22 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH), and the growth of the fungal colonies was checked daily until the mycelia of the controls reached the edges of the dishes (between 11 and 14 d). Fungal growth

Table 1. Total concentration (g/L) of the individual organic acids and their mixtures (w/w) tested in the antifungal test.

Organic acid and its mixture	Tested concentrations (g/L)			
Acetic acid	0.1	0.25	0.5	1
Propionic acid	0.1	0.25	0.5	1
Formic acid	0.1	0.25	0.5	1
Acetic acid + propionic acid (1:1)	0.2		0.5	1
Propionic acid + formic acid (1:1)	0.2		0.5	1
Acetic acid + formic acid (1:1)	0.2		0.5	1
Acetic acid + propionic acid + formic acid (1:1:1)	0.3		0.75	1

inhibition was measured by modifying the following formula proposed by Chang *et al.* (1999):

$$\text{Inhibition (\%)} = \left(1 - \frac{[\text{AT} - \text{IA}]}{[\text{AC} - \text{IA}]}\right) \times 100.$$

Here, AT is the area of the experimental plate, AC is the area of the control plate, and IA is the surface area (mm^2) of the inoculated plug. For each organic acid or mixture, concentration, and fungus, eight replicates were prepared and measured, and their mean inhibitions were calculated.

Wood Retention and Decay Test

Dried Scots pine (*Pinus sylvestris*) sapwood pieces of $5 \times 40 \times 10 \text{ mm}^3$ (radial \times longitudinal \times tangential) bought from a sawmill in Kerimäki, Finland, were used as a wood substrate. These pieces were tagged and oven-dried at 50°C until a constant mass was reached, and their masses were recorded.

A modified version of the European Standard EN 113 (CEN 1996) test described by Lu *et al.* (2016) was used to perform the wood decay test. The sapwood specimens were pressure-impregnated with one of the following acids or acid mixture solutions in Milli-Q water: propionic, acetic, or formic acid at 3% or 6%; a formic and propionic acid mixture (1:1) at 3% or 6%; and an acetic, formic, and propionic acid mixture (1:1:1) at total acid concentrations of 3% and 6%. At least 16 sapwood specimens were impregnated for each treatment and concentration. First, the wood pieces were exposed to partial vacuum at 0.15 bar for 20 min. The pressure was then slowly increased until 10 bar was reached, held for 60 min, and then slowly released. No final vacuum typical of the Bethel process was applied. The wet masses of the impregnated wood specimens were then measured to assess the liquid penetration. To calculate the chemical retention, the wood pieces were again oven-dried at 50°C until a constant mass was reached, and their masses were recorded.

Eight randomly chosen sapwood specimens for each treatment were then exposed to leaching following European Standard EN 84. Afterward, the specimens were oven-dried at 50°C until a constant mass was reached, and their masses were recorded. Then, all the specimens were sterilized using gamma radiation (31.7–32.3 kGy) at Scandinavian Clinics Estonia OÜ (Alliku, Estonia).

Under sterile conditions, four wood pieces were placed in each fungal mycelium-covered petri dish, each over a plastic mesh of $\sim 50 \times 15 \text{ mm}^2$ to avoid direct contact with the growth media. The wood pieces in each dish included leached and unleached specimens of different concentrations (3% and 6%) of the same acid or acid mixtures and were placed consistently in the order shown in Fig 1. In the case of the controls, two wood specimens were placed in each petri dish, and two extra wood specimens—which were not part of the experiment—were added to each of these dishes for a total of four specimens per dish. Eight replicates per treatment were used in this experiment. Then, the dishes were sealed with parafilm and kept in a growing chamber at $22 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH. After 16 wk, the wood specimens were removed from the dishes, the fungal mycelia were smoothly removed with the help of a brush, and the specimens were oven-dried at 50°C until a constant mass was reached. The masses of the specimens were measured to calculate the decay rate (mass loss) caused by *C. puteana*.

RESULTS

Antifungal Test

The inhibition caused by the different acids and their mixtures varied significantly depending on the fungus (Table 2). Despite this fact, propionic acid at 1 g/L significantly inhibited the growth of *G. trabeum* by about 99% and fully inhibited the other fungi. The lowest propionic acid concentration was also able to cause some inhibition of all the (wood-decaying) fungi, except *G. trabeum*. Acetic acid and formic acid inhibited *C. puteana* at 1 g/L. *G. trabeum* and *T. versicolor*

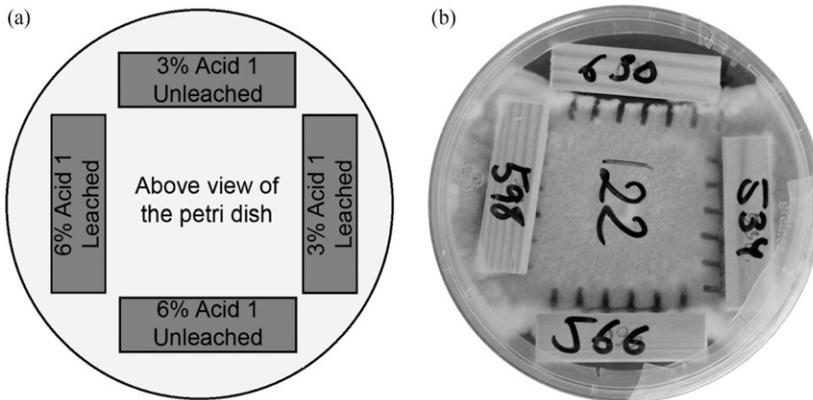


Figure 1. (a) Schematic of the setup used in the wood decay experiment with *Coniophora puteana* and *Gloeophyllum trabeum*. The sapwood specimens were placed in the petri dishes according to this scheme. (b) One of the petri dishes 1 wk after having had the sapwood specimens were placed in it.

were also significantly, albeit not fully, inhibited by these acids at 1 g/L. The effect of the three acids on the growth inhibition of the fungi decreased as the concentrations of the acids decreased.

In the case of *R. placenta*, the inhibition achieved by acetic acid and formic acid at 1 g/L was 15% and 5%, respectively, and lower concentrations of these acids were noninhibitive.

Table 2. Fungal growth inhibition (%) caused by the different acids and their mixtures. Results are presented as mean \pm SE.

Acid	Concentration (g/L)	<i>Coniophora puteana</i>	<i>Gloeophyllum trabeum</i>	<i>Rhodonia (Porcia) placenta</i>	<i>Trametes versicolor</i>
Acetic acid	0.1	42 \pm 4*	8 \pm 1	-7 \pm 5	7 \pm 2
	0.25	71 \pm 2*	12 \pm 2*	-5 \pm 6	-4 \pm 3
	0.5	94 \pm 1*	16 \pm 3*	-2 \pm 4	20 \pm 4*
	1	100 \pm 0*	66 \pm 1*	15 \pm 4	43 \pm 1*
Formic acid	0.1	71 \pm 3*	17 \pm 2*	-11 \pm 5	11 \pm 3
	0.25	79 \pm 4*	31 \pm 2*	-12 \pm 6	29 \pm 2*
	0.5	87 \pm 2*	60 \pm 1*	-1 \pm 4	52 \pm 3*
	1	100 \pm 0*	79 \pm 1*	5 \pm 4	85 \pm 5*
Propionic acid	0.1	39 \pm 4 *	2 \pm 2	17 \pm 5*	16 \pm 4*
	0.25	98 \pm 0*	28 \pm 1*	60 \pm 3*	60 \pm 2*
	0.5	100 \pm 0*	59 \pm 1*	89 \pm 1*	92 \pm 1*
	1	100 \pm 0*	99 \pm 0*	100 \pm 0*	100 \pm 0*
Acetic acid + formic acid	0.2	25 \pm 7*	-21 \pm 4*	18 \pm 1*	26 \pm 6*
	0.5	24 \pm 3*	33 \pm 2*	23 \pm 2*	63 \pm 3*
	1	100 \pm 0*	75 \pm 2*	52 \pm 2*	96 \pm 0*
Acetic acid + propionic acid	0.2	35 \pm 5*	-7 \pm 3	30 \pm 1*	42 \pm 4*
	0.5	92 \pm 1*	13 \pm 3*	23 \pm 2*	67 \pm 2*
	1	100 \pm 0*	40 \pm 3*	52 \pm 2*	83 \pm 2*
Propionic acid + formic acid	0.2	-9 \pm 3	11 \pm 3	31 \pm 1*	47 \pm 3*
	0.5	76 \pm 3*	27 \pm 2*	57 \pm 1*	74 \pm 2*
	1	100 \pm 0*	94 \pm 1*	83 \pm 1*	100 \pm 0*
Acetic acid + formic acid + propionic acid	0.3	77 \pm 3*	-11 \pm 3	25 \pm 2*	24 \pm 4*
	0.75	100 \pm 0*	31 \pm 2*	39 \pm 1*	66 \pm 2*
	1	100 \pm 0*	48 \pm 2*	53 \pm 1*	82 \pm 2*
Control	0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0

*Acid concentrations that caused a significant growth inhibition—or promotion—compared with the controls of the same fungal species according to Tukey's test, which was performed as a post hoc for the analysis of variance (ANOVA) test. Inhibition values over 95% are highlighted in bold, as they were considered the best-performing acids or mixtures.

The two acid mixtures were able to fully inhibit *C. puteana* at 1 g/L; however, neither of them completely inhibited *G. trabeum* or *R. placenta*. The white-rot fungus *T. versicolor* was inhibited by between 80% and 100% by these mixtures at 1 g/L. The mixture with all the acids fully inhibited *C. puteana* but did not significantly inhibit other fungi. The inhibition caused by the acid mixtures in all the fungi decreased as the concentration decreased, and in some cases, the lowest concentration even promoted the growth of some fungi; for instance, the acetic acid and formic acid mixture at 0.2 g/L promoted approximately 21% growth of *G. trabeum*.

Chemical Retention in Wood

The mass of the sapwood specimens increased between 9 and 17.5 kg/m³ when treated with the acids and their mixtures at 3%, and from 9 to 24 kg/m³ when treated with the acids and their mixtures at 6% (Table 3). All the leached specimens had negative retention values compared with their masses before impregnation. The mass loss varied for all the acids and mixtures between 6 and 1.5 kg/m³.

Wood Decay Test

The acid and acid mixture impregnation in the wood did not cause significant reductions in the masses of the sapwood specimens (Table 4). However, the leaching treatment led to a

significant difference between the leached and unleached specimens.

In the sapwood specimens exposed to *C. puteana*, the mass loss of the control specimens was about 30%. None of the unleached specimens treated with the acids or acid mixtures had a mass loss of lower than 20%. The leached specimens had a higher mean mass loss than the respective unleached acid and acid mixture-treated specimens. Although these differences are not significant, some of the leached specimens had a higher mean mass loss than the controls.

The mass loss caused by *G. trabeum* was lower than that caused by *C. puteana*. The controls had a mass loss of about 10%, whereas all the unleached specimens treated with the acids and acid mixtures had higher mass losses of between 14% and 20%. The leached specimens lost about 5% more mass than the unleached specimens, except the acetic acid at both concentrations and the mixture of the three acids at 3% concentration, which showed almost the same mass loss in the leached and unleached specimens.

DISCUSSION

The organic acids that were studied showed antifungal activity against the wood-decaying fungi. Propionic acid was the most effective acid, as it inhibited all the fungi used in the study. The results of this study support previous findings and highlight the potential of propionic acid as an antifungal; our previous work (Barbero-López et al 2019) determined that propionic acid performed even better than the copper-based commercial

Table 3. Chemical retention (kg/m³) of the different acids in the leached and unleached Scots pine sapwood specimens, measured by comparing the dry mass before and after impregnation with the acids. Results are presented as mean ± SE.

Acid	Concentration (%)	Unleached (kg/m ³)	Leached (kg/m ³)
Acetic acid	3	9.1 ± 1.2	-5.2 ± 0.2
	6	12.2 ± 0.3	-4.4 ± 0.2
Formic acid	3	8.2 ± 1.9	-5.4 ± 0.3
	6	9.7 ± 0.5	-6.3 ± 0.5
Propionic acid	3	12.2 ± 0.4	-4.9 ± 0.2
	6	13.9 ± 0.5	-3.2 ± 0.2
Formic acid + propionic acid	3	15.7 ± 0.4	-2.5 ± 0.8
	6	21.4 ± 0.7	-3.0 ± 0.4
Acetic acid + formic acid + propionic acid	3	17.5 ± 0.5	-4.5 ± 0.5
	6	23.3 ± 1.0	-1.6 ± 0.2

Table 4. Dry mass loss (%) caused by *C. puteana* and *G. trabeum* in acid-treated leached and unleached Scots pine sapwood specimens. Results are presented as mean \pm SE.

Acid or mixture	Total acid concentration (g/L)	<i>C. puteana</i> (%)		<i>G. trabeum</i> (%)	
		Unleached	Leached	Unleached	Leached
Acetic acid	3	21.3 \pm 2.3	34.2 \pm 4.2	16.9 \pm 1.6	17.3 \pm 1.1
	6	21.6 \pm 3.7	33.6 \pm 3.7	19.2 \pm 1.8	17.0 \pm 2.2
Formic acid	3	19.3 \pm 2.7	27.2 \pm 5.0	17.2 \pm 1.9	21.0 \pm 1.4
	6	19.9 \pm 4.5	28.6 \pm 4.1	14.7 \pm 2.4	20.5 \pm 1.7
Propionic acid	3	24.3 \pm 5.1	32.1 \pm 5.6	15.8 \pm 2.6	20.6 \pm 1.4
	6	21.0 \pm 3.5	25.5 \pm 3.0	14.4 \pm 2.6	23.5 \pm 1.5
Formic acid + propionic acid	3	29.1 \pm 3.6	27.2 \pm 3.1	17.6 \pm 2.4	22.2 \pm 1.2
	6	26.6 \pm 3.7	28.9 \pm 5.1	18.4 \pm 2.3	22.2 \pm 1.1
Acetic acid + formic acid + propionic acid	3	23.3 \pm 4.7	27.3 \pm 6.2	20.3 \pm 2.0	20.6 \pm 1.7
	6	25.1 \pm 4.7	33.7 \pm 6.7	15.6 \pm 1.8	21.3 \pm 0.7
Control	0	29.5 \pm 3.5	—	10.2 \pm 2.8	—

C. puteana, *Coniophora puteana*; *G. trabeum*, *Gloeophyllum trabeum*.

wood preservative used in the same study against *C. puteana*. Bahmani et al (2016) found that propionic and acetic acids reduced the mass loss caused by several decaying fungi in palm wood as well as inhibited mold growth. Our previous studies found also that other organic acids from different bio-based chemicals, such as coffee silverskin extracts (Barbero-López et al 2020) and fruit peel extracts (Barbero-López 2020), can inhibit wood-decaying fungi. The study at hand demonstrates that organic acids inhibit decay fungi and support the previous findings in this field.

All our fungi were inhibited at a certain point by acetic acid, except *R. placenta*, the least sensitive fungus to acids used in our study. These species were not included in Bahmani et al's (2016) study, which is why our study was different. These differences highlight the relevance of testing possible antifungals against multiple wood-decaying fungi, as different fungi—and strains—can vary significantly in their responses.

In this experiment, the mixtures of the acids did not present synergies within them, except in the combination between acetic acid and formic acid, where their effect combined at 1 g/L was much higher than the effect of each of these acids independently at 0.5 and 1 g/L. The rest of the mixtures, all of which included propionic acid, caused a lower inhibition of the wood-decaying fungi than propionic acid at the same concentration. Oramahi and Yoshimura (2013) found that the antifungal nature of pyrolysis distillates is related to

their acid content. Barbero-López et al (2019) concluded that the antifungal nature of pyrolysis distillates comes from the synergy between organic acids and phenolics. Mattos et al (2019) highlighted that based on the literature, the antimicrobial activity of these distillates comes from their phenolics, fatty acids, and acetic acid. Although organic acids play an antifungal role, our study suggests that the synergies between them alone are not responsible for the antifungal nature of pyrolysis distillates. Based on our work and the literature, the antifungal activity of pyrolysis distillates comes from the combined effect of organic acids and other constituents, or from the independent activity of some of these constituents, such as phenolics, which are often related to the antifungal activity of distillates (Mourant et al 2005; Baimark and Niamsa 2009; Temiz et al 2010).

The sapwood specimens were all successfully impregnated with the acids and their mixtures, as all of them increased in mass. The acid mixture specimens gained more mass than the acid specimens individually, indicating that a better impregnation was achieved when the acids were not alone. However, all the acids and mixtures leached out from the specimens during the leaching test. In addition, the mass of the specimens after leaching was lower than that before the impregnation, indicating a possible chemical degradation of the specimens due to the acids, as wood is widely known to be degraded by strong acidic solutions (Browning 1963; Kass et al 1970). These results highlight the relevance of

neutralizing pyrolysis distillates and acidic solutions for wood preservation applications.

The acids and acid mixtures were not effective at preventing wood decay; thus, they cannot be considered functional antifungal constituents for future wood preservatives. For both fungi species, the mass loss was greater for leached specimens than that for unleached specimens. Our wood decay results contrast with the findings of Bahmani *et al.* (2016), which showed that acetic and propionic acids protected palm wood. Propionic acid was also found to protect wood chips from decay (Esllyn 1973). Our results may be closely linked to the results found in the antifungal activity and the wood retention tests we discussed. Organic acids initiate wood degradation, as acids can break down hemicelluloses and thus increase the biodegradability of wood (Li *et al.* 2000; Keskin *et al.* 2019) but still retain some of their antifungal activity. However, when organic acids leach out from wood, the decay process caused by fungi is strong because of the lack of antifungal activity slowing down the process.

The results of this study highlight the high antifungal activity of some organic acids; however, they also show that because of their acidity, other acids have no—or even a negative—effect on wood and its decay. The results of this study present the *in vitro* performance of acetic, propionic, and formic acids against wood-decaying fungi but do not present the synergic effect of the acids with other constituents, such as phenolics. Thus, the synergies of organic acids with other constituents of pyrolysis distillates, such as phenolics, should be studied. Further tests should be performed *in vivo* to understand the effects of the acids in a real environment; tests to determine the effect of pH on wood and its degradation should also be carried out, as bio-based wood preservatives are often suggested by researchers as being acidic and rich in organic acids, such as pyrolysis distillates.

CONCLUSIONS

Acetic, formic, and propionic acids exhibit antifungal effects against wood-decaying fungi in pure cultures. Propionic acid was the only acid out of the three that presented better fungal inhibition,

as it was able to inhibit the fungal growth of the four species by over 99% when it was present at 1 g/L in the growth media. When applied to wood, the acids leached out from the wood, did not prevent wood decay, and caused the sapwood to lose mass possibly because of the chemical wood degradation caused by the acids' acidity. Thus, the presence of organic acids in wood-derived bio-based chemicals and their acidity needs to be considered before their use as wood preservatives.

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