Wood Science and Technology (2025) 59:12
https://doi.org/10.1007/s00226-024-01614-6

ORIGINAL

Evaluation of the antifungal efficiency of coatings on wood

Olena Myronycheva[1] · Injeong Kim[1] · Olov Karlsson[1] · Liudmyla Kiurcheva[2] · Peter Jacobsson[3] · Dick Sandberg[4,5]

Received: 16 May 2024 / Accepted: 30 September 2024

Abstract

Wood is an important construction material, but a significant problem hindering its widespread use is susceptibility to biodeterioration and biodegradation. To protect wood against degradation, a surface coating can be used, and it is important to be able to predict the ability of the coating to prevent fungal growth. The currently available standard method to determine the antifungal efficiency of a coating has two weaknesses, viz. no evaluation of the moisture content in the wood material, and no possibility to study antifungal effect of the coating towards an individual fungus. A new quantitative method of determining the antifungal efficiency of coatings is therefore proposed, where a coating is applied to wood and exposed to an individual fungus in a Petri dish. Six commercial water-based coatings containing synthetic biocides were studied on filter paper (EN 15457) and with the new test method on wood blocks. The results show the importance of studying the antifungal efficiency of a coating using individual fungi instead of a mixture of fungi, since individual fungi interact differently with a given biocide in the coating. The moisture content of the wood substrate during the test was affected by how the fungus was established on the coating. This new test approach shows promise in screening the antifungal efficiency of wood coatings containing preservative substances applied to wood material surfaces.

✉ Olena Myronycheva
olena.myronycheva@ltu.se

[1] Wood Science and Engineering, Luleå University of Technology, Forskargatan 1, 931 87 Skellefteå, Sweden

[2] Tavria State Agrotechnological University, Zhukovsky Street 66, Zaporizhzhia 69600, Ukraine

[3] Holmen AB, Martinsons Byggsystem AB, Strandgatan 48A, 931 34 Skellefteå, Sweden

[4] Wood modification, InnoRenew CoE, Livade 6a, 6310 Izola, Slovenia

[5] Department of Manufacturing and Civil Engineering, Norwegian University of Science and Technology (NTNU) , Gjøvik, Norway

Published online: 15 November 2024

Introduction

The United Nations initiative, "Sustainable Wood for a Sustainable World", advocates monitoring the life cycle of harvested wood, especially taking into consideration the role of wood-based building materials in the transition to a circular economy (FAO 2020). New testing methods and holistic approaches are required to study biodeterioration and biodegradation (Kutnik et al. 2014).

Wood-based building materials are construction materials with moderate carbon dioxide footprints (Hammond and Jones 2008), but untreated wood is susceptible to biodeterioration and biodegradation, particularly in environments that lead to elevated moisture contents in the wood material (Niemz et al. 2023). Mould and sporulating fungi in timber constructions can lead to respiratory diseases and detoxification of treated wood and this risk is exacerbated by high relative humidity, which can lead not only to moist wood-based materials but their further degradation by rot fungi and a reduction in the mechanical strength of the material (Rowell 2005; Zabel and Morrell 2020).

The prohibition of toxic chemicals and other biocidal products means that the wood industry must search for active substances with no or low environmental impact and a high efficiency against airborne fungi in above-ground constructions (US EPA 2015). One way to protect wood against degradation is by applying a protective surface coating (Sandberg et al. 2021). The moisture-barrier properties of the coating are, however, crucial, and these depend on the composition and thickness of the coating layer, as well as on the age and general condition of the coating (Copak et al. 2023). The ability of the coating to resist moisture and water may also influence its performance against microbial and fungal attack (Karlsson et al. 2022, Buck et al. 2023).

There are different standard methods to test the susceptibility of material to mold fungi. The EN 113 is used for testing preservatives in wooden blocks' efficiency against rot/basidiomycetous fungi, and mass loss is used for assessment (SS-EN 113 1996). Some tests already exist for assessing contaminated material, such as ISO 16000-21:2015 (SS-ISO 16000-21:2015 2015), or for generally describing procedures for artificially produced material and their resistance to mould fungi during environmental testing (SS-EN ISO 846:2019 2019; SIST EN 60068-2-10:2005/A1: 2018). The main standards used to evaluate mould growth include the British standard for paint resistance BS 3900 (BS 3900-G6:1989 1989), the American standard for unseasoned wood ASTM D4445 (ASTM D4445-10 2019) and an international standard for plastics ISO 16869 (SS-ISO 16869:2011 2011), where spore suspensions of different fungi are used as an inoculation. Both the British and ISO standards apply the adhesion methods and mixtures of fungi, but vary by using a box or agar plate as the test environment. Imken (Imken et al. 2020) studied both the British and ISO methods and found a poor correlation between them. As stated, this might be due to nutrients available in agar, or that it is difficult to evaluate the effect of one fungus since those fungi that use dry adhesion methods do not have a chance to propagate (Myronycheva et al. 2019).

The wet adhesion method is also used in the standard EN 15457 (CEN 2014a) standard method for evaluating the antifungal efficiency of a coating, which involves applying a coating to filter paper and exposing it to a mixture of different fungal spores. American and European standard methods assess the efficiency of active (biocidal or biostatic) substances applied to wood with the aid of a stereomicroscope as is the case with ASTM D3273 (ASTM 2021) or by rating the quantity and size of defects (CEN 2014b, 2016). The ASTM D7855 standard (ASTM 2013) employs the dry adhesion of several fungi, as shown in Fig. 1, whilst EN 152 (CEN 2011) employs the wet adhesion of several fungi. These two methods are often difficult to compare due to physiological differences in fungal spore germination in the absence or presence of moisture (Myronycheva et al. 2019). The fungal growth is quantified by counting colony-forming units (CFU) per gram of material in the ASTM D6329 standard (ASTM 2015) but this requires a special microbiological experience and access to a microbiological laboratory, and the moisture content of the wood material is determined only at the specimen-preparation stage, although the moisture content of the wood substrate varies depending on the type of coating and on the method of fungal inoculation (Myronycheva et al. 2019). Another factor affecting the evaluation of antifungal efficiency is the significant differences in fungal species on wooden surfaces in different geographical regions (Poohphajai et al. 2023). Additional complexity is that in microbial collections, fungal cultures stored for many years may lack information about their geographical origin (Gu 2016), whilst most published antifungal efficiency data relate to a mixture of fungi, with little information about the dominant isolates or strain physiology and little information about what kind of material and the geographical regions in which products were in service

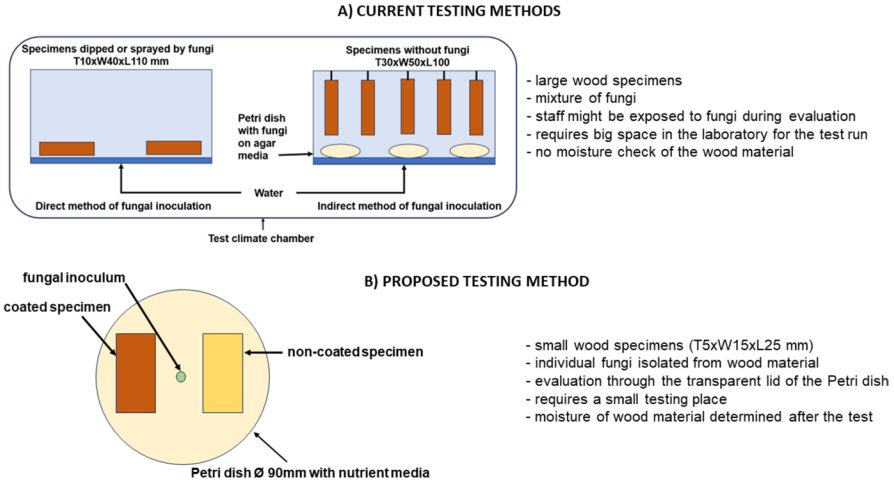

Fig. 1 Methods for the evaluation of antifungal efficiency of coatings applied on wood blocks: **a** the EN 152 (CEN 2011) standard test method for wet adhesion (direct method of inoculation) and the ASTM D7855 (ASTM 2013) standard test method for dry adhesion (indirect method of inoculation) where several fungi are applied simultaneously on the specimens, and **b** the suggested new method

(Gu 2016). Furthermore, the tests do not usually provide an in-depth understanding of fungal reactions in the tested substances (Eriksson et al. 1990).

To overcome the shortcomings of existing standardised methods for determining the antifungal efficiency of coatings, a new quantitative approach is proposed and evaluated in this study. The proposed testing procedure is shown in Fig. 1.

The proposed new method seeks to evaluate the antifungal efficiency of a coating applied on a wooden surface and to quantify the antifungal efficiency of the coating, providing information (1) on the geographical origin of the fungus, (2) on the inhibition effect of the coating with any synergistic fungicidal effects, and (3) on eventual changes in the moisture content of the specimen at the end of the test.

The suggested method quantifies how the surface coating responds to individual fungi, assesses the effect of the coating on fungal proliferation, and extends the understanding of the protecting properties of the coating on wood-based materials.

Materials and methods

Two different tests were performed: (1) an antifungal-efficiency test with a coating applied on filter paper and (2) an antifungal-efficiency test with a coating applied on wooden blocks.

Antifungal-efficiency test on filter paper

The test was carried out according to the EN 15457 standard (CEN 2014a) by applying the coating to a filter paper with a brush, allowing it to dry in air ambient conditions (approx. 20 °C, 50% RH) and placing it on a sterile nutrient agar medium in a Petri dish. About 1 ml of a suspension of fungi was added to each coated paper and spread over the paper with a Drigalski spatula. The Petri dish was incubated for two months at a temperature of 24 °C and 90% relative humidity (RH).

The EN 15457 test was modified using natural fungal isolates from wooden material tested in indoor and outdoor conditions in the region of Västerbotten, Sweden (Sehlstedt-Persson et al. 2011; Ahmed et al. 2013a, 2013c, 2013b). The fungi are stored in malt-agar stocks at + 4°C in the fungal collection at the Wood Science and Engineering Division, Luleå University of Technology, Campus Skellefteå, Sweden. The fungi were identified using an internal transcribed spacer (ITS) of ribosomal RNA (Schoch et al. 2012). The fungal species and their abbreviations are listed in Table 1

The selection of fungi was done as recommended in the EN 15457 standard: two fungi more likely to grow in an exterior environment—*Sydowia* sp. and *Cladosporium* sp.; two fungi more likely to grow in an interior environment—*Aspergillus* sp. and *Talaromyces* sp.; and soft rot—*Trichoderma* sp. (Lundell et al. 2014).

The fungal growth on the coated filter-paper surface was examined with an optical microscope and the growth was classified as: (1) no growth, (2) intermediate growth, i.e. 10 – 50% of the paper surface area covered by fungus, and (3) strong

Table 1 Fungi used in the study

Name of the fungus	Abbreviation
Sydowia polyspora (Bref. & Tavel) E. Müll. 1953	*Sydowia* sp.
Aspergillus niger van Tieghem 1867	*Aspergillus* sp.
Talaromyces radicus (A.D. Hocking & Whitelaw) Samson, Yilmaz, Frisvad & Seifert 2011	*Talaromyces* sp.
Cladosporium funiculosum W. Yamamoto, 1959	*Cladosporium* sp.
Trichoderma viridescens (A.S. Horn & H.S. Williamson) Jaklitsch & Samuels, 2006	*Trichoderma* sp.

growth, more than 50% covered. The active chemical substances in the coatings tested and their code letters are listed in Table 2. All the coatings were water-borne.

Antifungal efficiency test with a coating applied on wooden blocks

Nine pieces of industrially kiln-dried sawn timber of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) were randomly selected from a sawmill in Northern Sweden (slow-grown forest material). In the case of the pine, sapwood and heartwood were separated, and specimens 25 mm (radial) × 15 mm (longitudinal) × 5 mm (tangential) in size were prepared with a sawn surface. In the case of spruce, it was not possible to prepare specimens of sufficient size consisting only of sapwood and only heartwood specimens. The specimens were without defects and natural features such as knots. In total, 450 specimens were prepared.

From each of the nine pieces of sawn timber, ten samples were cut to determine the moisture content gravimetrically for heartwood and sapwood separately. The moisture contents of the specimens are presented in assumed to be an approximation of the specimens' initial moisture content when the test was started. The division for sapwood and heartwood was done to Scots pine due to the specificity of the mold fungi that attack wet sapwood (Zabel and Morrell 2020). The term "spruce mixed" was used for the specimens due to difficulties distinguishing sapwood from heartwood. After that, for all further procedures, the specimens were conditioned at 20 °C and a relative humidity of 65% (Table 3).

Table 2 Active chemical substances in the water/borne coatings

Coating code	Active substances
H	Tetramethylol acetylenediurea **(TA)** and iodopropynyl butyl carbamate **(IPBC)**
P	Iodopropynyl butyl carbamate (IPBC), 1,2-benzisotiazol-3(2H)-one (BIT), 2-methyl-2H-isothiazol-3-one (MIT) and 5-chloro-2-methyl-1,2-thiazol-3-one **(CMIT)** mixture
Si	No biocides, only potassium silicate as active substance against fungi
Sy	4,5-dichloro-2-n-octyl-3(2H)-isothiazolone, 1,2-benzisothiazol-3(2H)-one **(BIT)**, 2-Octyl-2H-isothiazol-3-one **(OIT)**
T	Iodopropynyl butyl carbamate **(IPBC)**

Table 3 Mean moisture contents (MC) with standard deviations (SD) of the sawn timber used for the specimens used in this study

Sawn timber	MC ± SD (%)	Sawn timber	MC ± SD (%)	Sawn timber	MC ± SD (%)
Pine sapwood 1	7.6 ± 0.1	Pine heartwood 1	7.0 ± 0.2	Spruce mixed 1	6.6 ± 0.1
Pine sapwood 2	7.7 ± 0.1	Pine heartwood 2	7.5 ± 0.1	Spruce mixed 2	6.3 ± 0.1
Pine sapwood 3	7.2 ± 0.2	Pine heartwood 3	7.1 ± 0.1	Spruce mixed 3	6.7 ± 0.1
Mean and SD of whole series:	7.5 ± 0.6		7.2 ± 0.6		6.5 ± 0.4

The coatings were deposited on the specimens with a brush. The average amount of coating per treatment is presented in Table 4. The specimens were first painted on five sides of the wooden block. After drying at ca. 20 °C and 50% RH, for 24 h, the remaining sixth surface (25 × 15 mm) was coated and dried in the same way.

Petri dishes were prepared aseptically with malt-extract agar (Merck KGaA, Germany). A sterilised plastic net was placed on the bottom of each Petri dish to prevent diffusion of coating to the nutrition agar. Two coated and uncoated specimens were placed in each Petri dish (Fig. 1). The specimens were attached to the net with a small piece of double-sized sterile tape to fix the specimen's position to fix the same distance in all replicates. Fungal inoculum with a diameter of 5 mm was placed in the middle of the dish on the nutrient agar.

The "inhibition effect of the coating" (the *Inhibition index*) was determined as the size of the inhibition zone measured from the nearest border of the wooden specimen to the edge of the fungal colony (see red lines in Fig. 2). Four such measurements were made and the average value of these distances was calculated. An *inhibition index* value of zero means that the coating has no effect on fungal growth, whereas values greater than zero are evidence that the coating had an effect.

The inhibition of fungal growth by the wood/coating system can be characterised by the fungal expansion. If the fungal growth extended beneath the specimen, i.e. between the specimen and the net, the *Beneath index* was defined as the ratio of the area covered by fungus and the total area of the surface (the surface directed towards the net). A *Beneath index* equal to 100% thus indicates that the coating had no effect and lower values means that the coating had an inhibiting effect. The tests were carried out in a laboratory climate chamber at 24 °C and an RH of 90% for one month.

After the test, the fungal mycelium was removed from the specimen's surface, the specimens were dried, and the moisture content of the specimens was

Table 4 Mean amount ± SD of coating applied to the wood surface (abbreviations see Table 2)

Code	H	P	Si	Sy	T
Amount (g/g oven dry wood)	0.20 ± 0.03	0.15 ± 0.04	0.06 ± 0.02	0.14 ± 0.03	0.01 ± 0.01

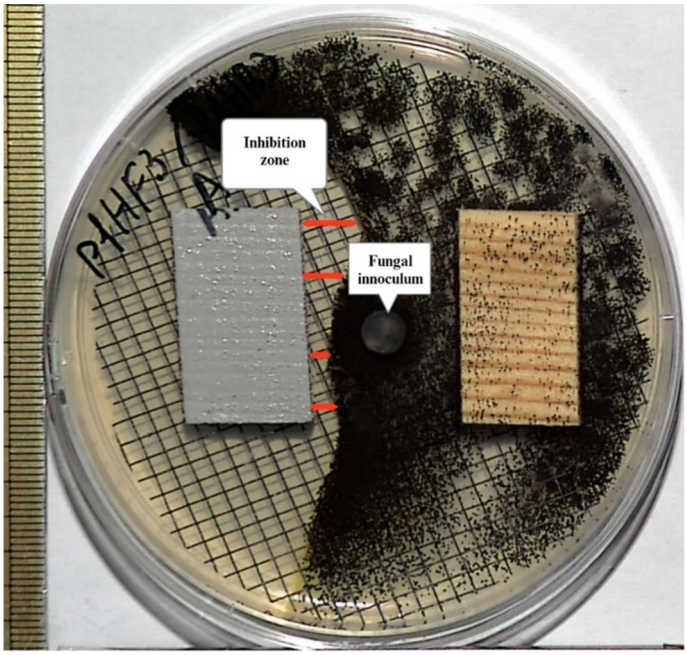

Fig. 2 Example of the inhibition zone where the distance between the border of the fungal growth and the edge of the specimen was measured four times (red lines) for calculating of an average "Inhibition index"

determined at the end of the test. The moisture content for sterile specimens without fungus was $53.17 \pm 10.24\%$. The results were subjected to a statistical analysis using SPSS Statistics software (IBM, USA and SIMCA, Sartorius, Umeå, Sweden).

Results

Antifungal efficiency test on filter paper

Table 5 shows how well the various coatings inhibited fungal growth without affecting the wood material to which they would normally be applied. The colours in the table show how much the coatings inhibited fungal growth.

With the P coating, no fungi grew after one month. The H coating positively reduced fungal growth in 14 specimens but failed in one specimen exposed to the fungus *Aspergillus* sp. The T coating did not stop the germination of spores in specimens contaminated with *Sydowia* sp. and *Aspergillus* sp., where single conidiophores were observed after 40 days of the test. The *Taloromyces* sp. developed heavy growth in only one of the 15 specimens.

The Sy coating had a more porous and glossier surface than the other coatings, but no biocidal effect was detected, even on the best coating with fewer pores.

Table 5 Fungal growth inhibition on the filter paper coated with the coatings containg the substances listed in Table 2

		Fungi				
Coating	*No. of replicate*	*Sydowia* sp.	*Cladosporium* sp.	*Trichoderma* sp.	*Aspergillus* sp.	*Talaromyces* sp.
H	1					
	2					
	3					
P	1					
	2					
	3					
Si (only K_2O_3Si)	1					
	2					
	3					
Sy	1					
	2					
	3					
T	1					
	2					
	3					

The colours describing the fungal growth on the filter paper and petri dish surface:

	Strong inhibition with no growth on the surface		Medium inhibition, where 10 to 50% of the surface was covered with fungal hyphae		Weak or no inhibition where > 50% of the surface covered

Sydowia sp. showed spots with colonies, *Cladosporium* sp. patches of conidiophores, and *Trichoderma* sp. showed a massive growth on the surface of the coating. *Aspergillus* and *Talaromyces* sp. were also observed in the pores of the filter paper. Signs of fungal growth on SG and Sy coatings were observed as early as day 6.

No growth of *Sydowia* sp. was observed on filter paper treated with Si, where contamination by *Aspergillus* sp., *Talaromyces* sp., and *Cladosporium* sp. was observed. The signs of fungal growth on the Si coating were observed already on day 8.

Control plates with no coating were overgrown by fungi from the first day of the experiment.

Antifungal efficiency test with a coating applied on wooden blocks

The coatings were tested using wood blocks and inoculum (Fig. 1), and the calculated Inhibition and Beneath indices are presented in Tables 6 and 7, respectively. Supplement 1 presents an overview of replicates and visual efficiency for all test specimens. A statistical analysis and general linear model showed a significant difference between the coatings for all the fungi but no difference between the types of wood based on the indices (Supplement 2).

Table 6 Average value of the inhibition index for the coatings on the coated wood blocks

	Inhibition index (mm)				
Coating:	H	P	Si	Sy	T
Type of wood	*Sydowia* sp.				
Pine sapwood	7.2	4.2	0.1	0	7.3
Pine heartwood	11.1	4.6	0.4	0	8.3
Spruce mixed	8.4	5.3	0	0	8.1
	Cladosporium sp.				
Pine sapwood	7.6	1.3	0	0	2.6
Pine heartwood	7.5	1.3	0	0	1.7
Spruce mixed	7.5	1.3	0	0	4.4
	Trichoderma sp.				
Pine sapwood	0	0	0	0	0
Pine heartwood	0	0	0	0	0
Spruce mixed	0	0	0	0	0
	Aspergillus sp.				
Pine sapwood	6.2	3.3	0	0	6.3
Pine heartwood	6.9	2.8	0	0	5.1
Spruce mixed	6.2	3.2	0	0	8.6
	Talaromyces sp.				
Pine sapwood	0.8	1.3	0	0	1.8
Pine heartwood	0.8	0.5	0	0	0.1
Spruce mixed	1.8	0	0	0	0.4

It is evident in Tables 6 and 7 that the best fungal inhibition was provided by coating H, as was also indicated by the filter-paper test. Specimens of pine heartwood coated with H inhibited the growth of four fungi, especially the fungus *Sydowia* sp. which is commonly found on wood from outdoor environment. However, no efficient coating was found against *Trichoderma* sp. which is characterised as a softrot fungus (Zabel and Morrell 2020).

The coatings Si and Sy had a low effect on fungus growth, no inhibition zones being found in the test.

A similar inhibition of fungal growth by the coatings was observed when the beneath indices were compared; H, P and T performed better than Si and Sy. Despite the inhibition of *Sydowia* sp. and *Aspergillus* sp. by the P coating, the fungi were still able to grow beneath the specimens. Similarly, in the case of *Cladosporium* sp., the inhibition was not complete, and some fungal hyphae managed to penetrate under the treated specimens. Similar, a tendency to partly inhibit growth of *Talaromyces* sp. was detected for P, H and T coatings.

The filter-paper test method may not provide results that are directly comparable to those obtained by the wood-block test method, particularly when assessing the fungal resistance of surface coatings. This is evident from the wood-block tests, where the presence of wood material influenced the outcomes—exposure to *Sydowia* sp. yielded notable effects, while exposure to *Cladosporium* sp. showed no

Table 7 Average values for the zone of fungus that went below the specimen (Beneath index), (% of total bottom specimen surface)

Coating:	Beneath index (%)				
	H	P	Si	Sy	T
Type of wood	*Sydowia* sp.				
Pine sapwood	0	0	56.7	100	0
Pine heartwood	0	0	51.7	100	0
Spruce heartwood	0	0	63.3	100	0
Cladosporium sp.					
Pine sapwood	0	1.7	91.7	96.7	1.7
Pine heartwood	0	9.3	83.3	71.7	0
Spruce heartwood	0	0.3	70.0	80.0	0
Trichoderma sp.					
Pine sapwood	100	100	100	100	100
Pine heartwood	100	100	100	100	100
Spruce heartwood	100	100	100	100	100
Aspergillus sp.					
Pine sapwood	0	0	76.7	100	0
Pine heartwood	0	0	46.7	100	0
Spruce heartwood	0	0	100	76.7	0
Talaromyces sp.					
Pine sapwood	3.0	5.0	26.7	23.3	5.0
Pine heartwood	4.3	13.3	21.7	16.7	10.3
Spruce heartwood	5.0	26.7	60.0	27.0	4.3

impact. Furthermore, coatings tested with *Trichoderma* sp. exhibited no significant effects. These observations (Tables 5, 6, and 7) suggest that while the filter-paper test method has its utility, it may exhibit limited validity when used to evaluate fungal resistance in surface coatings. Therefore, incorporating methods that involve wood substrates seems essential to achieve more accurate and representative results in such studies.

Moisture content in wood specimens after the test

Figures 3 and 4 present the moisture content after the antifungal efficiency test on wood blocks of treated and non-treated specimens paired in the Petri dishes during the fungal test. The initial moisture content before the test was approximately 7.0% for all specimens and 12.0% after conditioning (Table 3).

The moisture content increased considerably during the test for all specimens; however, the increase was generally lower and less variable (lower SD) for the coated specimens compared to uncoated ones. The change in moisture content was clearly influenced by the specific fungus tested, except in the cases involving *Trichoderma* sp. The specimens treated with Sy and exposed to *Trichoderma* sp. showed the highest reduction in moisture content (up to 70%) due to the applied coating.

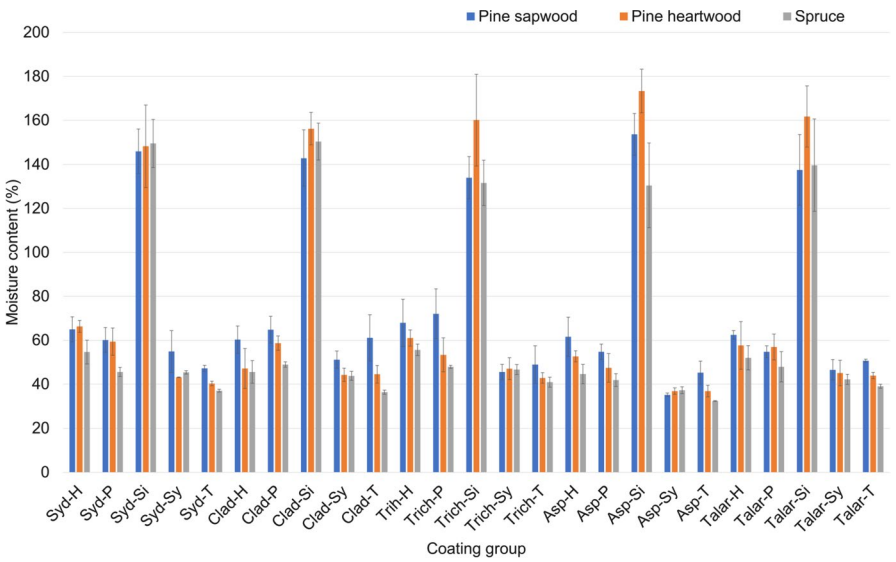

Fig. 3 Moisture content of the specimens from the antifungal-efficiency test on wood blocks, treated with coatings according to Table 1. Coding for the coating groups follows "fungus -coating"; Syd: Sydowia sp., Clad: Cladosporium sp., Trich: Trichoderma sp., Asp: Aspergillus sp., and Talar: Talaromyces sp. Bars represent standard deviation

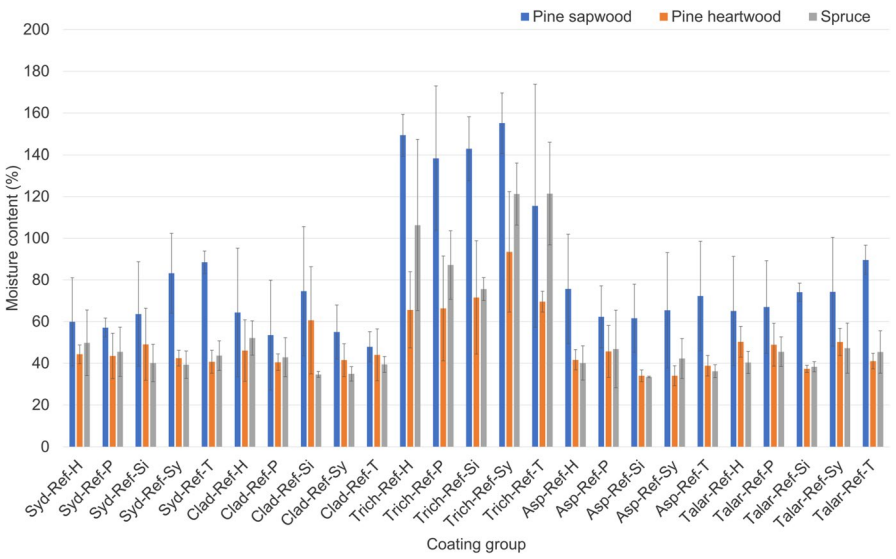

Fig. 4 Moisture content of the non-treated reference (Ref) specimens exposed to fungi according to Table 1. N.B. the coding for type of coating is given only to indicate to which type of coated specimen reference specimen were paired with in the Petri dish. Coding for the coating groups follows "fungus-Ref-coating"; Syd: Sydowia sp., Clad: Cladosporium sp., Trich: Trichoderma sp., Asp: Aspergillus sp., and Talar: Talaromyces sp. Bars reprsent standard deviation

The biocide-free coating (Si) showed a very high moisture-content increase during the test, more than three times for heartwood coated with potassium-silicate and exposed to *Aspergillus* sp. compared to the reference specimens that were in the same Petri dish (Figs. 2 and 3). This confirms the toxic-free and hydrophilic nature of potassium silicate, which has also been reported by Sonderegger et al. (2015) and its inefficiency as a water barrier (Sonderegger and Niemz 2009, Sonderegger et al. 2015).

Lower moisture content values were observed in spruce coated with T when applied to spruce heartwood exposed to *Aspergillus* sp. This suggests that reduced moisture content might contribute to the inhibition of fungal growth. However, coatings H and P generally showed higher moisture content in tests with *Aspergillus* sp. compared to the wood specimens with T coating. Additionally, the P coating demonstrated a weaker synergistic effect of its active substances across all wood types.

All non-treated reference specimens in *Trichoderma* sp. paired tests resulted in extremely high moisture content (values) in comparison to coated specimens (Figs. 2 and 3). In general, pine sapwood non-treated reference specimens had a significantly higher moisture content compared to pine and spruce heartwood. Non-coated reference spruce specimens had a lower moisture content (around 50%) compared to coated specimens with coating H (around 55%) when exposed to *Sydowia* sp. The hydrophilic properties of the coatings likely play a role in this difference. The moisture content of non-treated reference specimens of pine heartwood and spruce exposed to *Trichoderma* sp. was significantly lower than those treated with Si and exposed on the same plate. Heartwood contains more non-polar extractives like fatty and resin acids, waxes, and tannins (Fengel and Wegener 1989) and is more hydrophobic than sapwood and possesses water-resistance properties.

The correlation coefficients in Table 8 demonstrate how the type of coating applied on the wood substrate interacts with different fungi. None of the coatings have a strong protection against *Trichoderma* sp., and the protection against fungal growth of the other species depends considerable between type of coating.

These findings indicate the importance of studying the moisture-transfer properties of both the wood and coatings to find the most effective combination for protection against fungi. The study also suggests that the effect of coatings on moisture levels can vary depending on the type of fungus. Adding coating can increase moisture absorption for some fungi like *Sydowia* sp. and *Cladosporium* sp., while for others like *Talaromyces* sp. and *Aspergillus* sp., it might reduce moisture absorption.

The weak correlation between moisture content and the type of coating with *Trichoderma* sp. could be due to the fungus's own hydrophilic nature, as well as its ability to alter water content through the release of hygroscopic sugars (Luke et al. 2015, Hassett et al. 2015). Some specific correlations stood out:

- Coating P showed a strong positive correlation with *Sydowia* sp. and *Cladosporium* sp., while coating T had a strong positive correlation with *Sydowia* sp. However, coating H showed a notably negative correlation with *Talaromyces* sp. and less intense but still notable with *Aspergillus* sp. fungi tests.

Table 8 The average value for the zone of fungus that went below the specimen (% of total bottom sample surface)

	Beneath index (%)				
Coating:	H	P	Si	Sy	T
Type of wood	*Sydowia* sp.				
Pine sapwood	0	0	56.7	100	0
Pine heartwood	0	0	51.7	100	0
Spruce heartwood	0	0	63.3	100	0
	Cladosporium sp.				
Pine sapwood	0	1.7	91.7	96.7	1.7
Pine heartwood	0	9.3	83.3	71.7	0
Spruce heartwood	0	0.3	70.0	80.0	0
	Trichoderma sp.				
Pine sapwood	100	100	100	100	100
Pine heartwood	100	100	100	100	100
Spruce heartwood	100	100	100	100	100
	Aspergillus sp.				
Pine sapwood	0	0	76.7	100	0
Pine heartwood	0	0	46.7	100	0
Spruce heartwood	0	0	100	76.7	0
	Talaromyces sp.				
Pine sapwood	3.0	5.0	26.7	23.3	5.0
Pine heartwood	4.3	13.3	21.7	16.7	10.3
Spruce heartwood	5.0	26.7	60.0	27.0	4.3

- A moderately negative correlation was observed for coatings P and Sy when exposed to *Trichoderma* sp., possibly due to the coating's hydrophilic nature and variations in coating amounts.

Discussion

The differences in fungal phenotypic response in the study depend on the biocidal nature of tested coating. The most effective coatings contained active substances classified as synthetic preservatives and biocides, subject to specific EU regulations and widely approved for wood protection use. When such substances were applied to products and environmentally exposed, the effect of wind, rain, solar radiation, and coating efficiency must be considered, and coatings should be tested for the durability at those conditions. The fungicidal synergistic effect of coatings (largest inhibition zone) for coatings H and T containing IPBC, might be due to toxicity against *Aspergillus* sp. is showed in our study and agrees with the findings of other studies (Cook et al. 2002). However, the species diversity of the fungi found in both indoor and outdoor tests revealed some resistance to iodopropynyl butyl carbamate (IPBC), and the use of this substance in wood protection might be questionable due to environmental harm caused by photo-reactivity (Lanigan 1998). Both the H and T coatings contain IPBC in composition, but high antifungal efficiency of the coating H might be due to second compound tetramethylol acetylenediurea that is cancerogenic and toxic contributing to an overall synergistic effect (ECHA 2023) or lower applied amount of T (Table 4). Coating P contains a mixture of isothiazolinone

biocides and shows toxicity (Taubert et al. 2002) and showed antifungal efficiency for all fungi except for *Trichoderma* sp.

In this study, the effectiveness of active substances is attributed to synthetic biocides, particularly the resistance of *Trichoderma* sp. The variability in phenotypic responses of other fungi to the coatings highlights the need for testing approaches that focus on wood protection tailored to each fungus's specific physiology and ecology. Some species of *Sydowia* sp. are considered emerging tree pathogens with fungicidal resistance (CABI 2021). Introducing additional resistance may further promote the emergence of difficult-to-manage pathogens, especially in the context of modern biodiversity decline and climate change challenges.

The confirmation of the proposed indices for screening antifungal efficiency is crucial for future research. Further testing of the most effective coatings, H and P, in exterior or outdoor applications, where more extreme environmental conditions prevail, is needed. Additionally, the degradation rate of the active substances must be assessed. In this study, all active substances were adopted from the cosmetic industry for use in wood protection. However, our findings raise important concerns about the sustainability of using synthetic biocides in wood protection systems, particularly regarding their toxicological effects on the environment and the discovered fungal resistance. More detailed discussion about existing wood preservatives has been published (Niemz et al. 2023).

Therefore, the shift towards biocontrol strategies might be considered like in agricultural initiatives finding non-harmful but effective and efficient agents that will inhibit fungal growth via sensing mechanisms touched in the current study.

Conclusion

The present study was designed to determine the quantitative effect of wooden coatings containing preservatives with biocidal action on the market. The study has shown that the proposed methodology and indices can measure the inhibition or lack of effect of wood coatings on individual fungi under laboratory conditions. The interactions between fungi, wood, and coatings examined in this study will serve as a basis for future research on the antifungal effects of wood coatings with biocides on individual fungi. Despite its exploratory nature, this study provides some insights into the physiology of wood-inhabiting fungi and their influence on the moisture content of coated and non-coated wooden materials. An important addition to standard methods is the measurement of moisture-content changes in the specimens during the fungal test, as moisture can influence fungal physiology on wooden material.

The new wood-block methodology and proposed indices (inhibition zone, fungus under the specimen, and moisture content of coated and non-coated specimens) are proposed for evaluating and quantifying the antifungal effect using the wood-coating-fungi system under laboratory conditions. These indices, such as the inhibition zone and moisture content of coated versus non-coated specimens, could indicate the efficiency of coatings against different fungi. The study confirmed that the phenotypic response of individual fungi to coatings needs to be studied, and the proposed indices can characterize this response and serve as

predictors of resilience against fungal attacks. The study is limited by the lack of information about hardwood species and solvent-based coatings, which will be the focus of future research for method validation. This study has important implications for the future screening of antifungal substances for use in wooden coatings.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00226-024-01614-6.

Acknowledgements The authors gratefully acknowledge the considerable support of the CT WOOD—a centre of excellence at Luleå University of Technology. Financial support from the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), project "Fungal growth on modified wood-based products under subarctic conditions FORMAS 2017-00419", is also gratefully acknowledged.

Author contributions O.M, I.K., O.K, P.J., D.S. planned together experimental design and data collection. O.M. and I.K. conducted the experimental part. OM, LK conducted data analysis and wrote first draft of manuscript. All authors reviewed the manuscript and contributed to final version.

Funding Open access funding provided by Lulea University of Technology.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

References

Ahmed SA, Sehlstedt-Persson M, Morén T (2013a) Development of a new rapid method for mould testing in a climate chamber: preliminary tests. Eur J Wood Wood Prod 71(4):451–461. https://doi.org/10.1007/s00107-013-0697-0

Ahmed SA, Sehlstedt-Persson M, Morén T (2013b) Mould susceptibility of Scots pine (*Pinus sylvestris* L.) sapwood: impact of drying, thermal modification, and copper-based preservative. Int Biodeterior Biodegrad 85:284–288. https://doi.org/10.1016/j.ibiod.2013.06.031

Ahmed SA, Yang Q, Sehlstedt-Persson M, Morén T (2013c) Accelerated mold test on dried pine sapwood boards: impact of contact heat treatment. J Wood Chem Technol 33(3):174–187. https://doi.org/10.1080/02773813.2013.773041

ASTM D3273–21 (2021) Standard test method for resistance to growth of mold on the surface of interior coatings in an environmental chamber

ASTM D4445 - 10 (2019) Test method for fungicides for controlling sapstain and mold on unseasoned lumber (laboratory method)

ASTM D6329–98(2015) (2015) Standard guide for developing methodology for evaluating the ability of indoor materials to support microbial growth using static environmental chambers
ASTM D7855/D7855M - 13 (2013) Standard test method for determination of mold growth on coated building products designed for interior applications using an environmental chamber and indirect inoculation.
BS 3900-G6:1989 (1989) Methods of test for paints Assessment of resistance to fungal growth
Buck D, Wallentén P, Sehlstedt-Persson M, Öhman M (2023) Moisture- and mould-resistance: modelling of edge-sealed cross-laminated timber using multi-modal assessment leveraged by X-ray computed tomography. Mater Design. https://doi.org/10.1016/j.matdes.2023.111967
CABI (2021) Sydowia polyspora (dieback of pine). CABI Compendium, p 52193. https://doi.org/10.1079/cabicompendium.52193
CEN (2011) EN 152 Wood preservatives - Determination of the protective effectiveness of a preservative treatment against blue stain in wood in service - Laboratory method. European Committee for Standardization (CEN), Brussels, Belgium
CEN (2014a) EN 15457 Paints and varnishes - Laboratory method for testing the efficacy of film preservatives in a coating against fungi. European Committee for Standardization (CEN), Brussels, Belgium
CEN (2014b) EN 16492 Paints and varnishes - Evaluation of the surface disfigurement caused by fungi and algae on coatings. European Committee for Standardization (CEN), Brussels, Belgium
CEN (2016) Paints and varnishes - Evaluation of degradation of coatings - Designation of quantity and size of defects, and of intensity of uniform changes in appearance - Part 1: General introduction and designation system (ISO 4628-1:2016). European Committee for Standardization (CEN), Brussels, Belgium
Cook SR, Sullivan J, Dickinson DJ (2002) IRG/WP 02-10437. The bacterial biotransformation of IPBC. In: IRG/WP 02-10437. IRGWP
Copak A, Jirouš-Rajković V, Živković V, Miklečić J (2023) Water vapour transmission properties of uncoated and coated wood-based panels. Wood Mat Sci Eng 18(3):1086–1093. https://doi.org/10.1080/17480272.2022.2106582
ECHA (2023) Substance information - ECHA. Tetrahydro-1,3,4,6-tetrakis(hydroxymethyl)imidazo[4,5-d]imidazole-2,5(1H,3H)-dione. https://echa.europa.eu/substance-information/-/substanceinfo/100.024.007. Accessed 20 Dec 2023
Eriksson K-E, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer, Berlin, Heidelberg
FAO (2020) Sustainable Wood for a Sustainable World (SW4SW). In: The Food and Agriculture Organization of the United Nations. http://www.fao.org/forestry/sustainable-wood/en/. Accessed 6 Dec 2020
Fengel D, Wegener G (1989) Wood: chemistry, ultrastructure, reactions. de Gruyter, Berlin
Gu J-D (2016) Biodegradation testing: so many tests but very little new innovation. Appl Environ Biotechnol 1(1):92–95. https://doi.org/10.26789/AEB.2016.01.007
Hammond GP, Jones CI (2008) Embodied energy and carbon in construction materials. Proc Inst Civil Eng–energy 161(2):87–98. https://doi.org/10.1680/ener.2008.161.2.87
Hassett MO, Fischer MWF, Money NP (2015) Mushrooms as rainmakers: how spores act as nuclei for raindrops. PLoS ONE 10(10):e0140407. https://doi.org/10.1371/journal.pone.0140407
Imken AAP, Brischke C, Kögel S, Krause KC, Mai C (2020) Resistance of different wood-based materials against mould fungi: a comparison of methods. Eur J Wood Prod 78(4):661–671. https://doi.org/10.1007/s00107-020-01554-5
Karlsson O, Jones D, Sandberg D (2022) Phenol-formaldehyde-resin treatment of Scots pine sapwood for the reduction of resin exudation through coatings. Wood Mater Sci Eng 17(2):144–146
Kutnik M, Suttie E, Brischke C (2014) European standards on durability and performance of wood and wood-based products – trends and challenges. Wood Mat Sci Eng 9(3):122–133. https://doi.org/10.1080/17480272.2014.894574
Lanigan RS (1998) Final report on the safety assessment of iodopropynyl butylcarbamate (IPBC). Int J Toxicol 17(5_suppl):1–37. https://doi.org/10.1177/109158189801700503
Luke B, Faull J, Bateman R (2015) Using particle size analysis to determine the hydrophobicity and suspension of fungal conidia with particular relevance to formulation of biopesticide. Biocontrol Sci Technol 25(4):383–398. https://doi.org/10.1080/09583157.2014.979396

Lundell TK, Mäkelä MR, de Vries RP, Hildén KS (2014) Chapter eleven - genomics, lifestyles and future prospects of wood-decay and litter-decomposing basidiomycota. In: Martin FM (ed) Advances in botanical research. Academic Press, pp 329–370

Myronycheva O, Poohphajai F, Sehlstedt-Persson M, Vikberg T, Karlsson O, Junge H, Sandberg D (2019) Application of GRAS compounds for the control of mould growth on scots pine sapwood surfaces: multivariate modelling of mould grade. Forests 10(9):714. https://doi.org/10.3390/f10090714

Niemz P, Teischinger A, Sandberg D (eds) (2023) Springer handbook of wood science and technology, 2023rd edn. Springer International Publishing, Cham

Poohphajai F, Myronycheva O, Karlsson O, Belt T, Rautkari L, Sandak J, Gubenšek A, Zalar P, Gunde-Cimerman N, Sandak A (2023) Fungal colonisation on wood surfaces weathered at diverse climatic conditions. Heliyon 9(6):e17355. https://doi.org/10.1016/j.heliyon.2023.e17355

Rowell RM (ed) (2005) Handbook of wood chemistry and wood composites. CRC Press, Boca Raton, Fla

Schoch CL, Seifert KA, Huhndorf S et al (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci 109(16):6241–6246. https://doi.org/10.1073/pnas.1117018109

Sehlstedt-Persson M, Karlsson O, Wamming T, Morén T (2011) Mold growth on sapwood boards exposed outdoors : the impact of wood drying. For Prod J 61(2):170–179

SIST EN 60068-2-10:2005/A1:2018 Environmental testing–part 2–10: tests - test j and guidance: mould growth. In: iTeh standards. https://standards.iteh.ai/catalog/standards/sist/4b676fe4-6a2c-4d23-b051-4a396691e0e9/sist-en-60068-2-10-2005-a1-2018. Accessed 16 Aug 2024

Sandberg D, Kutnar A, Karlsson O, Jones D (2021) Wood modification technologies. Principles, sustainability, and the need for innovation. CRC Press, p 432

Sonderegger W, Niemz P (2009) Thermal conductivity and water vapour transmission properties of wood-based materials. Eur J Wood Prod 67(3):313–321. https://doi.org/10.1007/s00107-008-0304-y

Sonderegger W, Glaunsinger M, Mannes D, Volkmer T, Niemz P (2015) Investigations into the influence of two different wood coatings on water diffusion determined by means of neutron imaging. Eur J Wood Prod 73(6):793–799. https://doi.org/10.1007/s00107-015-0951-8

SS-EN 113 (1996) Wood preservatives – test method for determining the protective effectiveness against wood destroying basiomycetes – Determination of the toxic values

SS-EN 15457:2014 (2014) Paints and varnishes - laboratory method for testing the efficacy of film preservatives in a coating against fungi.

SS-EN 16492:2014 (2014) Paints and varnishes - evaluation of the surface disfigurement caused by fungi and algae on coatings

SS-EN ISO 846:2019 (2019) Standard - plastics - Evaluation of the action of microorganisms.

SS-EN ISO 4628-1:2016 (2016) Paints and varnishes -- Evaluation of degradation of coatings -- designation of quantity and size of defects, and of intensity of uniform changes in appearance -- part 1: general introduction and designation system

SS-ISO 16000–21:2015 (2015) Indoor air - part 21: Detection and enumeration of moulds – Sampling from materials

SS-ISO 16869:2011 (2011) Standard - plast - bedömning av effektiviteten av fungistatiska föreningar i plastmaterial

Taubert K, Kraus S, Schulze B (2002) Isothiazol-3(2H)-ones, part I: synthesis. React Biol Act Sulfur Rep 23(1):79–121. https://doi.org/10.1080/01961770208047968

US EPA O (2015) Overview of wood preservative chemicals. U.S. Environmental Protection Agency

Zabel RA, Morrell JJ (2020) Wood microbiology: decay and its prevention, 2nd edn. Elsevier Science Publishing Co. Inc, San Diego