



Antifungal Activity of Natural Products Derived from Plants and Fungi with The Potential to Control Wood-destroying Fungi



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Abstract

Wood-decaying fungi pose a considerable challenge to the timber industry, particularly in countries like Egypt that rely heavily on imported softwood. The growing interest in environmentally sustainable alternatives to synthetic wood preservatives has directed attention toward naturally derived antifungal agents. In this study, six bioactive natural compounds brefeldin A (compound A), anthracobic acid A (compound B), phomaxanthone A (compound C), leucodin (compound D), parthenolide (compound E), and vasicine (compound F) were isolated and assessed for their antifungal activity against two major wood-decay fungi: the brown-rot fungus *Tyromyces palustris* and the white-rot fungus *Irpex lacteus*. Miniature blocks of Scots pine (*Pinus sylvestris*) were treated with different concentrations (125–1000 ppm) of each compound and then exposed to fungal attack for eight weeks. The treated samples showed a significant reduction in mass loss, falling below 5% at the highest concentrations, compared to nearly 30% in untreated controls. These results confirm the strong antifungal performance of the tested compounds, suggesting their potential as green wood preservatives for use in above-ground applications.

Keywords: Wood-decaying, Brown-rot fungus, White-rot fungus, Brefeldin A, Anthracobic acid A, Phomaxanthone A.

Introduction

Pinus sylvestris (commonly known as Scots pine) is a commercially important softwood species widely used across the timber and furniture industries in the Northern Hemisphere (Krakau et al., 2013). In Egypt, where the local forestry sector is not sufficiently developed to supply the national demand for timber, softwood requirements are largely fulfilled through imports. In 2011, the country received about 1.1 million cubic meters of softwood from Sweden, ranking third among Sweden's export destinations after the United Kingdom and Germany (Edgren, 2012). These shipments represented roughly 9% of Sweden's total softwood exports, composed predominantly of redwood (*Pinus sylvestris* L., about 90%) and a smaller portion of whitewood (*Picea*

abies L., about 10%) (Edgren, 2012). However, imported softwoods are vulnerable to biological degradation, especially by wood-decay fungi. Among these, white-rot and brown-rot basidiomycetes are of particular concern, with an estimated 1,600 to 1,700 species identified. These fungi compromise wood quality and structural integrity by breaking down key components like lignin and cellulose. White-rot fungi typically utilize oxidative enzymatic pathways for lignin degradation, whereas brown-rot fungi rely on non-enzymatic mechanisms to decompose cellulose and hemicellulose (Witomski et al., 2016; Mäkelä et al., 2021). Two species frequently associated with the decay of coniferous wood, including Scots pine, are *Tyromyces palustris* (TYP) and *Irpex lacteus* (IRL) (Mäkelä et al., 2021).

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Conventional approaches to wood preservation predominantly employ synthetic chemical agents, which, despite their proven efficacy, present considerable environmental and health-related challenges. The escalating demand for sustainable and less hazardous alternatives have consequently fostered increasing interest in the application of natural products for wood protection (Mohareb *et al.*, 2013). A wide range of chemical preservatives both organic and inorganic are currently used in the wood industry, but there is a shift toward biologically based options with minimal ecological impact.

Natural bioactive compounds, particularly those obtained from plants and fungi, are well known for their wide-ranging antimicrobial and pharmaceutical activities. However, numerous secondary metabolites produced by fungi remain insufficiently investigated, especially regarding their potential role in preventing wood decay. This study aims to bridge that gap by evaluating six bioactive compounds of three plant-derived (leucodin, parthenolide, and vasicine) and three fungal-derived (brefeldin A, anthracobic acid A, and phomaxanthone A) for their effectiveness in protecting *Pinus sylvestris* wood from decay caused by *T. palustris* and *I. lacteus*.

Materials and Methods

Isolation and identification of compound A, compound B, and compound C

Compound A, compound B, and compound C were isolated from endophytic fungi *Penicillium brefeldianum*, *Anthracobia* sp. and *Phomopsis* sp., respectively. The fungal strains were identified by BEX Co. Ltd., Japan, using a DNA analysis of the 18S rDNA regions. After fermentation, the extracts of fungal media were subjected to chromatographic separation and purification. The chemical structure of the pure compounds was elucidated based on spectral analysis of UV, IR, HRMS and NMR. Details on isolation and identification were previously described by us (Saad *et al.*, 2021).

Isolation and identification of compound D, compound E, and compound F

Compound D, compound E, and compound F have been isolated from Egyptian plants *Achillea santolina* L., *Magnolia grandiflora* L. and *Adhatoda vasica* L., respectively. Isolation and identification of compounds were performed as described by Ahmed and Abdelgaleil (2005) and Abdelgaleil (2010). The chemical structures of tested compounds are shown in Figure 1.

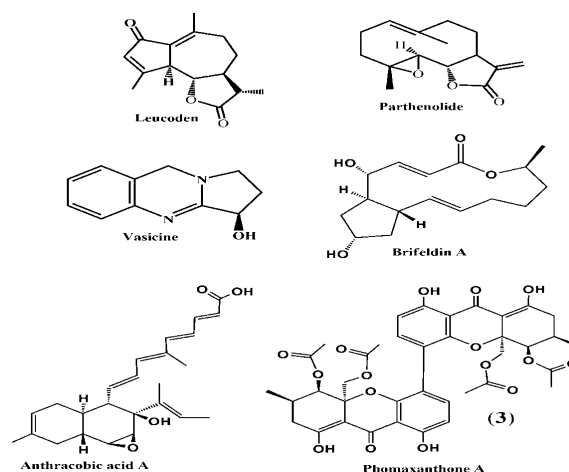


Fig. 1. The chemical structures of tested compounds brefeldin A (compound A), anthracobic acid A (compound B), phomaxanthone A (compound C), leucodin (compound D), parthenolide (compound E), and vasicine (compound F)

Dip treatment of wood by the tested natural compounds

Mini-block specimens (30 mm (L) x 10 mm (W) x 5 mm (T)) from Scots pine sapwood (*Pinus sylvestris* L.) are used. Stock solutions of the tested secondary metabolites were prepared in acetone. The codes of the tested compounds were presented in table 1 (alphabetically order from A to F). A series of at least four concentrations 125, 250, 500, and 1000 ppm were prepared and were coded from 1 to 4 in numeric order. Three replicates for each concentration were tested against control specimens. Sterilized Scots pine sapwood samples were dipped in each concentration for 15 seconds, following American Society for Testing and Materials (ASTM) test method D4445-91 (American Society for Testing and Materials (ASTM) 1998). The treated wood samples were kept in closed petri dishes overnight at room temperature.

Fungal biodegradation

The previously treated mini-blocks Scots pine sapwood were undertaken to assess biological resistance of the isolated natural products. Sterile culture medium (20 ml), prepared from malt 40 grams and agar 20 grams in distilled water (1 L), was placed in 90 mm diameter Petri dish with a small piece of mycelium of a freshly grown culture of *Irpex lacteus* HHB 7328 as a white rot fungus and *Tyromyces palustris* (Berk. et Curt) Murr. as a brown rot fungus. The culture was incubated for 2 weeks at 27°C and 70% HR to allow full colonization of the medium by the mycelium. Wood specimens were supported on sterile plastic mesh to prevent contact with culture medium (Mohareb *et al.*, 2010). All samples, previously oven dried at 60°C to constant

weight, were sterilized with radiation. Four blocks (three treated and one control) were placed in each Petri dish under sterile conditions, and all treatments were duplicated. Incubation was carried out for 8 weeks at 27°C under controlled humidity conditions of 70% RH in a climatic chamber WTB BINDER TYP KBF 240. At the end of the test period, 8 weeks, mycelia were removed, and all specimens were oven-dried to constant mass at 60°C and weighed. Mass loss (ML) was calculated as a percentage of the initial oven-dried weight of the samples according to equation 1:

$$ML (\%) = (M_0 - M_f) / M_0 \times 100 \quad (\text{Eq. 1})$$

Where M_0 is the mass of the oven-dried sample before the test, and M_f is the mass of the oven-dried sample after the test.

Results and Discussion

Characterization of natural compounds

A total of six natural compounds that were used in the present study have been isolated and identified in the previous studies as shown in Table 1. The chemical nature and origin of each natural compound.

In this investigation, six eco-friendly natural substances three derived from fungi and three from plants were utilized for the first time in Egypt as potential antifungal agents against wood-degrading fungi. The fungal metabolites (compound A, compound B, and compound C) were isolated from *Penicillium brefeldianum*, *Anthracoeba* sp., and *Phomopsis* sp., respectively. Meanwhile, the plant-based compounds (compound D, compound E, and compound F) were extracted from *Achillea santolina* L., *Magnolia grandiflora* L., and *Adhatoda vasica* L., respectively.

Structural analysis of these compounds revealed distinct chemical features influencing their bioactivity. Brefeldin A is a macrocyclic lactone belonging to the macrolide class (Dinos, 2017), while Anthracobic acid A features polyketide backbones enriched with tetrahydropyrans and polyhydroxyl chains (Zhang et al., 2019). Phomaxanthone A, a xanthone dimer, comprises two aromatic systems connected through a carbonyl-oxygen bridge (Wezeman et al., 2015).

Plant-derived constituents also exhibited structural diversity. Leucodin, a sesquiterpene lactone, contains a methylated methylene unit (Schafer, 2022). Parthenolide's notable functional groups include epoxide and α -methylene- γ -lactone moieties, contributing to its bioactivity (Srakaew & Tachaboonyakiat, 2017). Vasicine is a quinazoline alkaloid characterized by ketones, esters, and heterocyclic structures (Cheke et al., 2022).

These molecules are documented in the literature for various pharmacological effects, including antifungal, anti-inflammatory, and antioxidant properties (Paek, 2018; Abdelgaleil et al., 2024). Their known bioactivity supports their potential as wood preservatives.

Evaluation of Antifungal Efficacy

The antifungal activity of the tested compounds was assessed by measuring the mass loss of *Pinus sylvestris* mini-blocks after eight weeks of fungal exposure. The specimens were challenged with two representative wood-decaying fungi: the brown-rot *Tyromyces palustris* and the white-rot *Irpex lacteus*. The percentage of mass loss served as an indicator of the degree of fungal degradation and, consequently, the protective efficiency of the treatments.

As illustrated in Figures 2 and 3, increasing the compound concentration markedly reduced wood deterioration. At the maximum concentration of 1000 ppm, all treated samples exhibited strong resistance, with recorded mass losses below 5%, whereas untreated controls suffered nearly 30% loss due to fungal decay.

A clear dose-dependent trend was observed, confirming that higher concentrations of these natural agents enhanced antifungal protection. Figures 4 and 5 further illustrate the pronounced resistance of the treated mini-blocks compared to the controls. The antifungal mechanism is presumably associated with the bioactive components interfering with fungal enzymatic systems involved in lignocellulosic degradation.

These findings are consistent with the observations of Mohareb et al. (2013), who reported increased decay resistance in wood impregnated with essential oils derived from Egyptian plants. Similarly, Bari et al. (2018) demonstrated that fiber composites treated with plant-based extracts exhibited superior resistance against fungal colonization and structural deterioration. Likewise, Yang and Clausen (2007) demonstrated that essential oils applied to Southern Yellow Pine effectively inhibited fungal colonization for extended periods, suggesting that terpenoid and phenolic components are primarily responsible for antifungal protection.

Similar observations were reported by Chittenden and Singh (2011), who found that eugenol and cinnamaldehyde significantly reduced mass loss in *Pinus radiata* blocks, particularly under dry conditions. This is consistent with the strong resistance seen here, especially at higher concentrations where fungal enzymatic activity responsible for lignocellulose degradation may have been suppressed.

Recent studies have also emphasized the environmental importance of using plant-derived substances for wood preservation. Bahmani and Schmidt (2018) examined essential oils from several aromatic plants and confirmed their potential as eco-friendly antifungal agents, achieving comparable protection to conventional preservatives. Similarly, Pop *et al.* (2022) showed that clove (*Eugenia caryophyllata*) essential oil could serve as a sustainable antifungal treatment for cultural heritage wood, highlighting the potential of such natural products in long-term protection strategies.

The present findings are therefore in full agreement with the growing body of evidence that essential oils and their active compounds, particularly oxygenated monoterpenes, sesquiterpenes, and phenylpropanoids can effectively inhibit wood-decay fungi through disruption of fungal cell membranes and interference with ligninolytic enzyme systems. The strong dose response relationship observed here reinforces the hypothesis that increasing bioactive compound concentration enhances wood resistance, providing an environmentally responsible approach to wood preservation.

Implications and Application

The observed antifungal behavior of these compounds suggests their suitability for use in non-ground-contact wood applications, such as siding, decking, and window frames. Their natural origin and low toxicity make them favorable alternatives to synthetic preservatives. By improving the fungal resistance of wood while reducing environmental impact, these compounds offer a sustainable approach to timber protection.

Conclusions

The current research demonstrates the effectiveness of six natural compounds, obtained

from fungal and plant sources, in protecting wood from fungal degradation. Compounds such as brefeldin A, anthracobic acid A, phomoxanthone A, leucodin, parthenolide, and vasicine were shown to markedly reduce decay caused by *Tyromyces palustris* and *Irpex lacteus*, with treated wood samples exhibiting significantly lower mass losses. These outcomes support the role of natural products as viable substitutes for conventional chemical preservatives, especially in above-ground applications. Owing to their eco-friendly nature and low toxicity, these compounds could contribute to safer, more sustainable wood preservation strategies. Future research should aim to assess their durability over time, their ecological safety profile, and the potential for enhanced activity through formulation or synergistic blending.

Author Contributions

M.Z.Z. conceived and designed the study. Methodology was developed by M.Z.Z., A.S.M., and M.A.A. Software and computational analysis were performed by M.Z.Z. Validation and formal analysis were conducted collaboratively by M.Z.Z., S.A.A., M.A.A., and M.M.S. M.Z.Z. carried out the investigation and provided the necessary resources. Data curation was completed by M.Z.Z., S.A.A., M.A.A., and M.M.S. The original draft of the manuscript was written by M.Z.Z., and it was critically reviewed and edited by S.A.A., M.A.A., and M.M.S.

Conflicts of interest

All the authors declared that they have no competing interests.

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Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

TABLE 1. Name of compound, code for biological test, chemical class and source of fungal and plant secondary metabolites.

Name	Code for biological test	Chemical class	Source	Reference
1- Brefeldin A	A	Macrolide	Penicillium brefeldianum,	Saad et al., 2021 and Abdelgaleil et al, 2022
2-Anthractic Acids A	B	Polyketide	Anthracoia sp.	
3- Phomaxanthone A	C	Dimeric xanthone	Phomopsis sp	Ahmed and Abdelgaleil (2005) and Abdelgaleil (2010)
4- Leucodin	D	Tetracycline	Achillea santolina L.	
5- Parthenolide	E	Germacranolide	Magnolia grandiflora L.	
6- Vasicine	F	Quinazoline alkaloid	Adhatoda vasica L.	

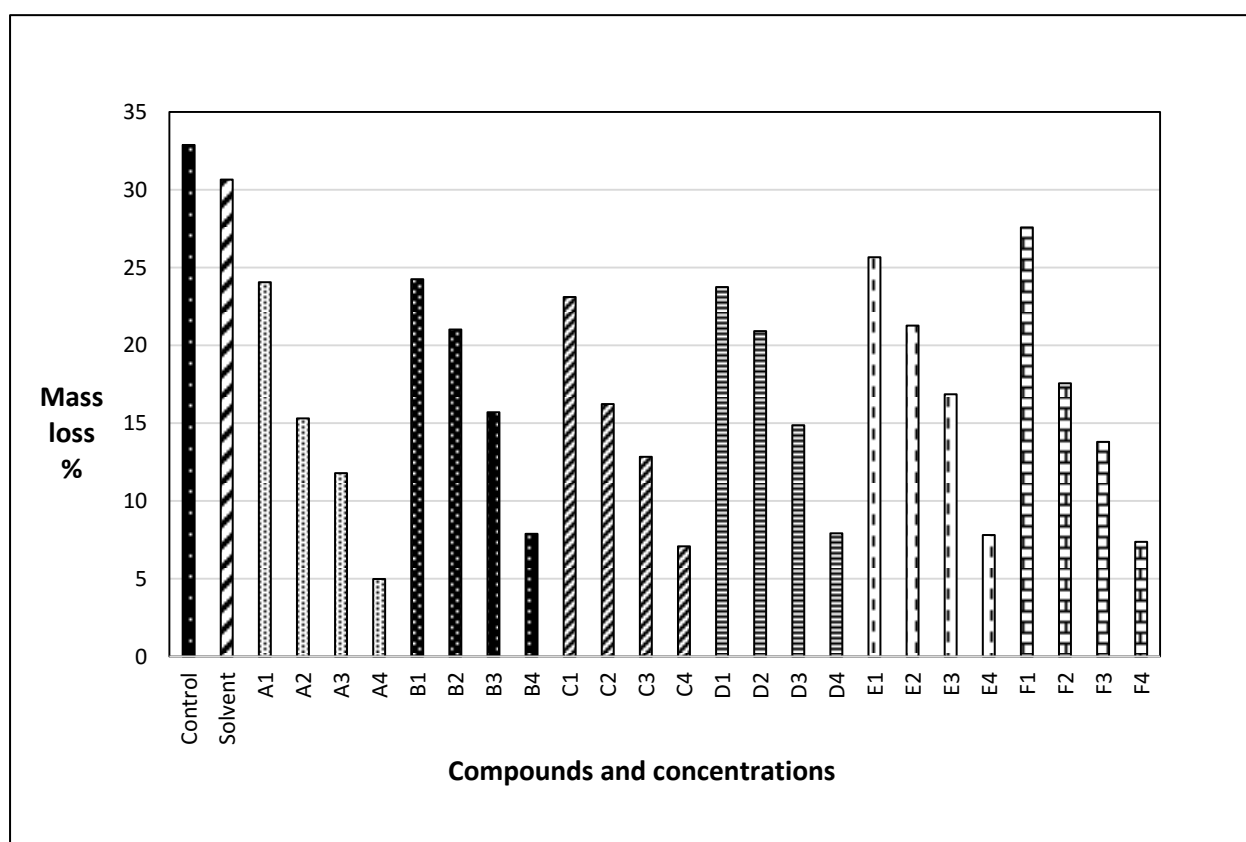


Fig. 2. Mean of mass losses (%) of Scots pine sapwood specimens treated with the tested natural compounds coded in table 1 with four concentrations. Treatments included: brefeldin A (Compound A: A1 = 125 ppm, A2 = 250 ppm, A3 = 500 ppm, A4 = 1000 ppm), anthracobic acid A (Compound B: B1 = 125 ppm, B2 = 250 ppm, B3 = 500 ppm, B4 = 1000 ppm), phomaxanthone A (Compound C: C1 = 125 ppm, C2 = 250 ppm, C3 = 500 ppm, C4 = 1000 ppm), leucodin (Compound D: D1 = 125 ppm, D2 = 250 ppm, D3 = 500 ppm, D4 = 1000 ppm), parthenolide (Compound E: E1 = 125 ppm, E2 = 250 ppm, E3 = 500 ppm, E4 = 1000 ppm), and vasicine (Compound F: F1 = 125 ppm, F2 = 250 ppm, F3 = 500 ppm, F4 = 1000 ppm). Measurements were taken after 8 weeks of exposure to the white rot fungus *Irpex lacteus*.

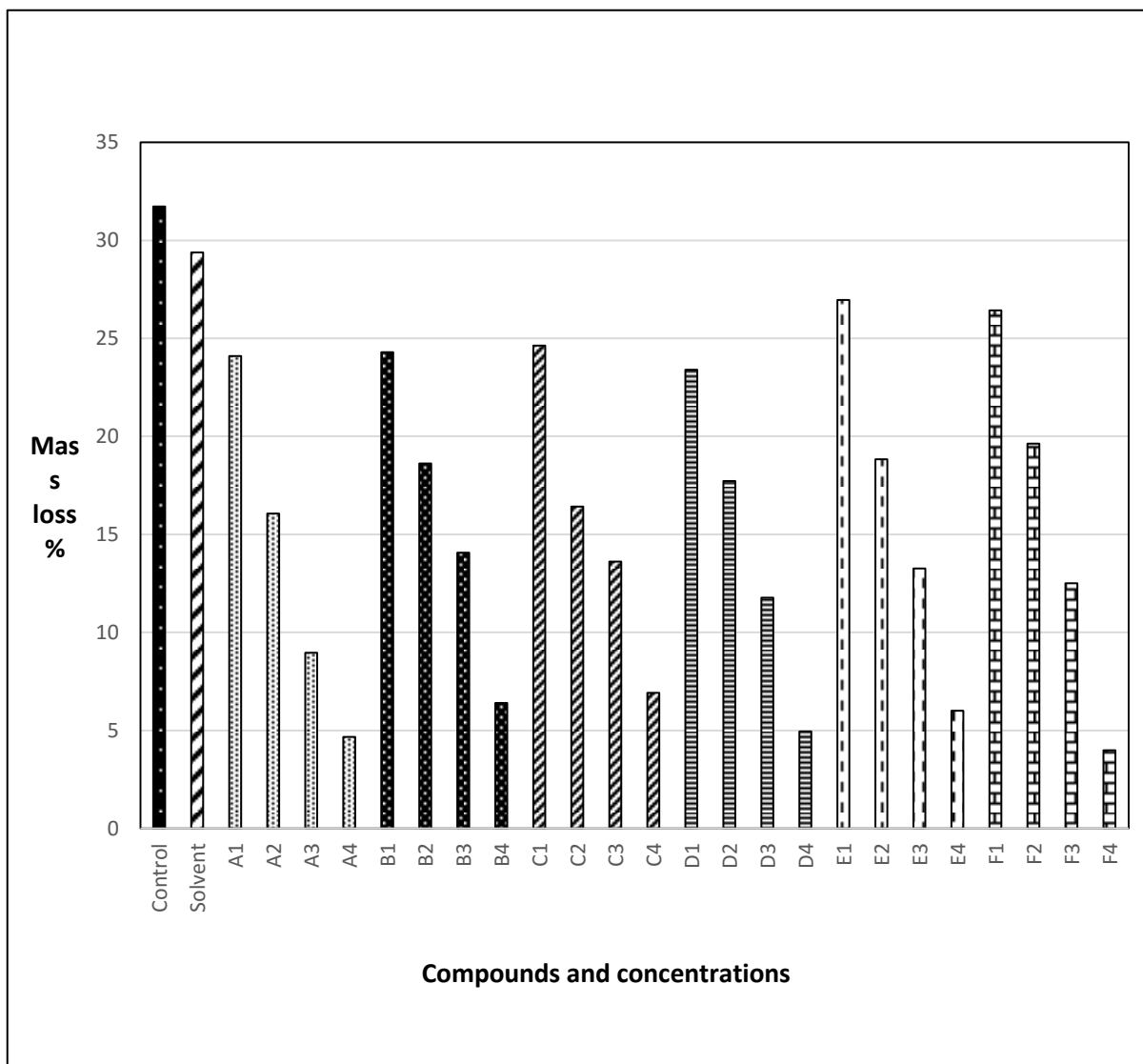
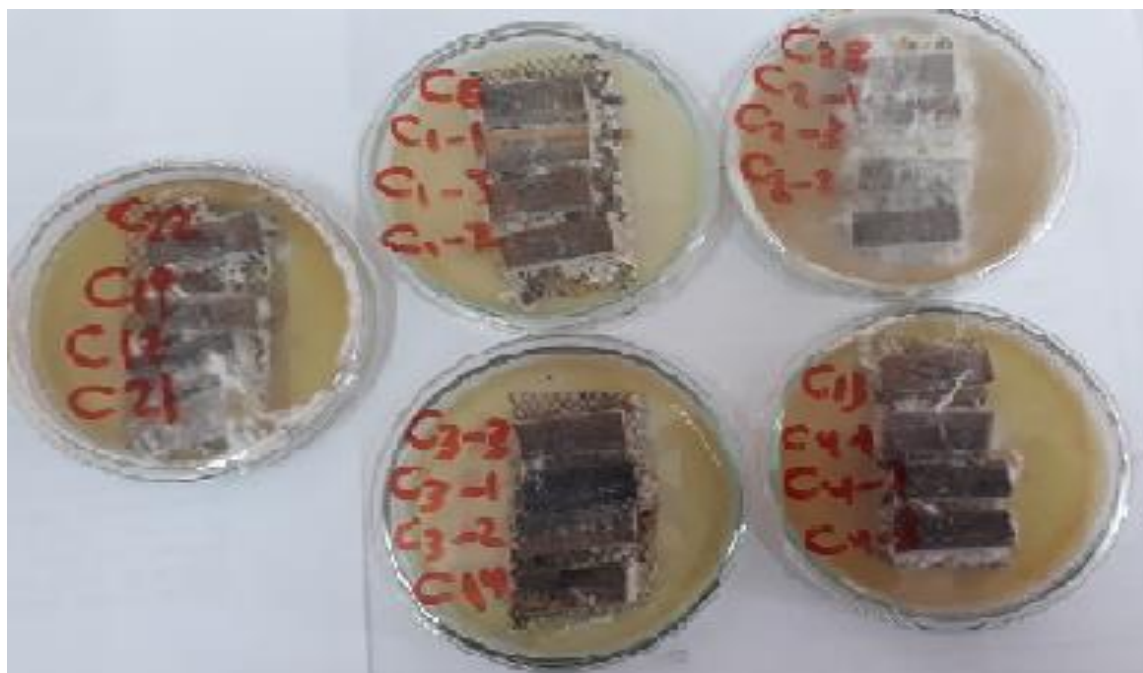
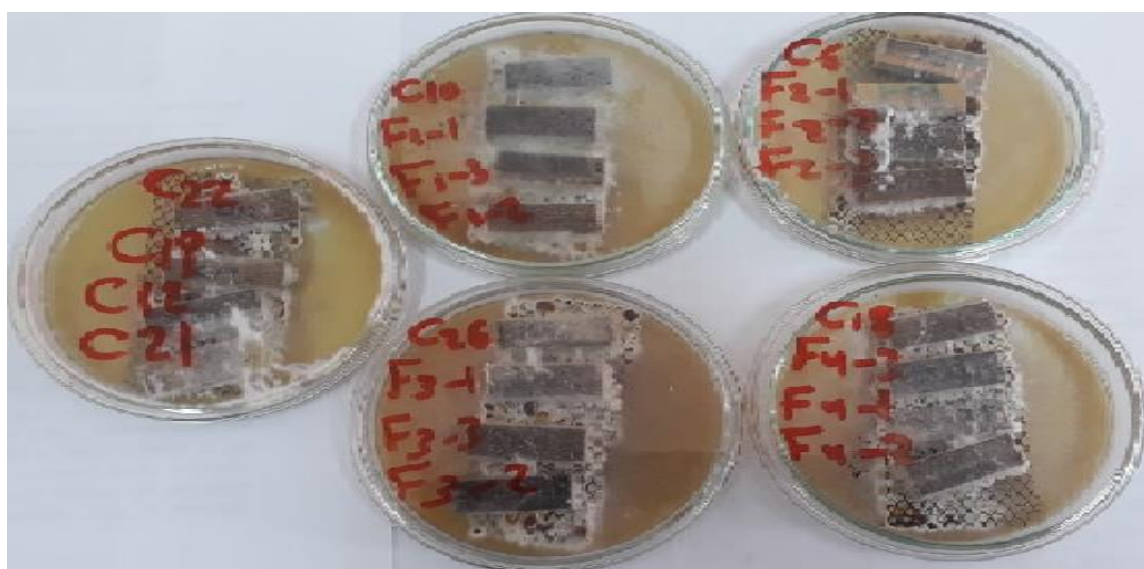


Fig. 3. Mean percentage mass loss (%) of *Scots pine* sapwood specimens treated with the tested natural compounds listed in Table 1 at four concentrations. Treatments included: brefeldin A (Compound A: A1 = 125 ppm, A2 = 250 ppm, A3 = 500 ppm, A4 = 1000 ppm), anthracobic acid A (Compound B: B1 = 125 ppm, B2 = 250 ppm, B3 = 500 ppm, B4 = 1000 ppm), phomaxanthone A (Compound C: C1 = 125 ppm, C2 = 250 ppm, C3 = 500 ppm, C4 = 1000 ppm), leucodin (Compound D: D1 = 125 ppm, D2 = 250 ppm, D3 = 500 ppm, D4 = 1000 ppm), parthenolide (Compound E: E1 = 125 ppm, E2 = 250 ppm, E3 = 500 ppm, E4 = 1000 ppm), and vasicine (Compound F: F1 = 125 ppm, F2 = 250 ppm, F3 = 500 ppm, F4 = 1000 ppm). Measurements were taken after 8 weeks of exposure to the brown-rot fungus *Tyromyces palustris*.

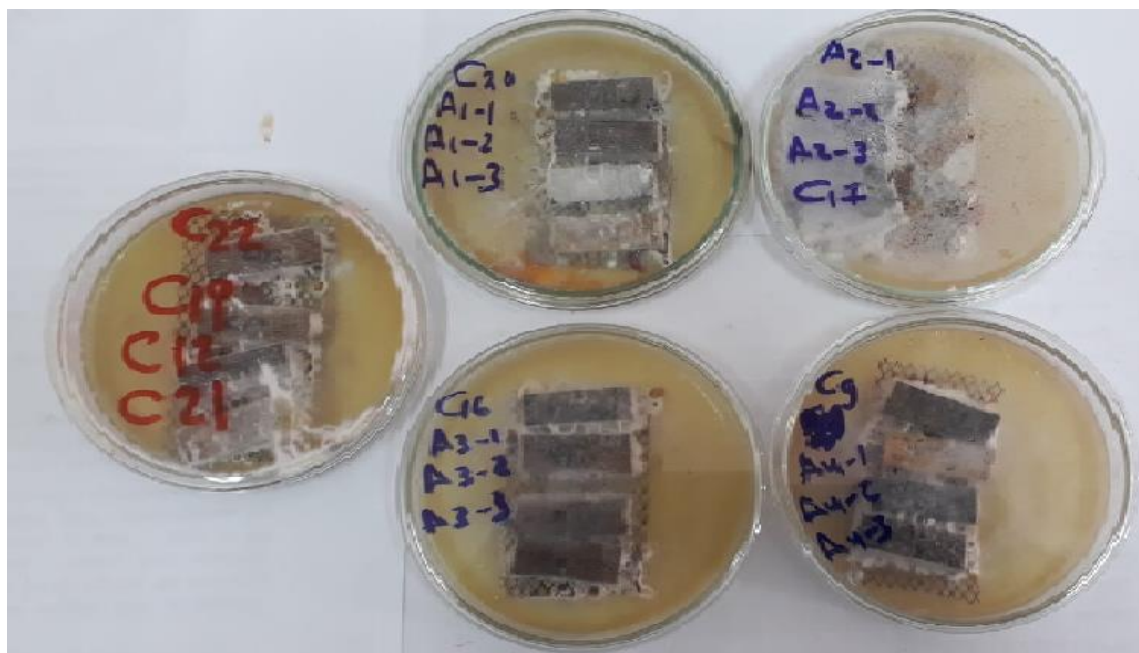


(a)

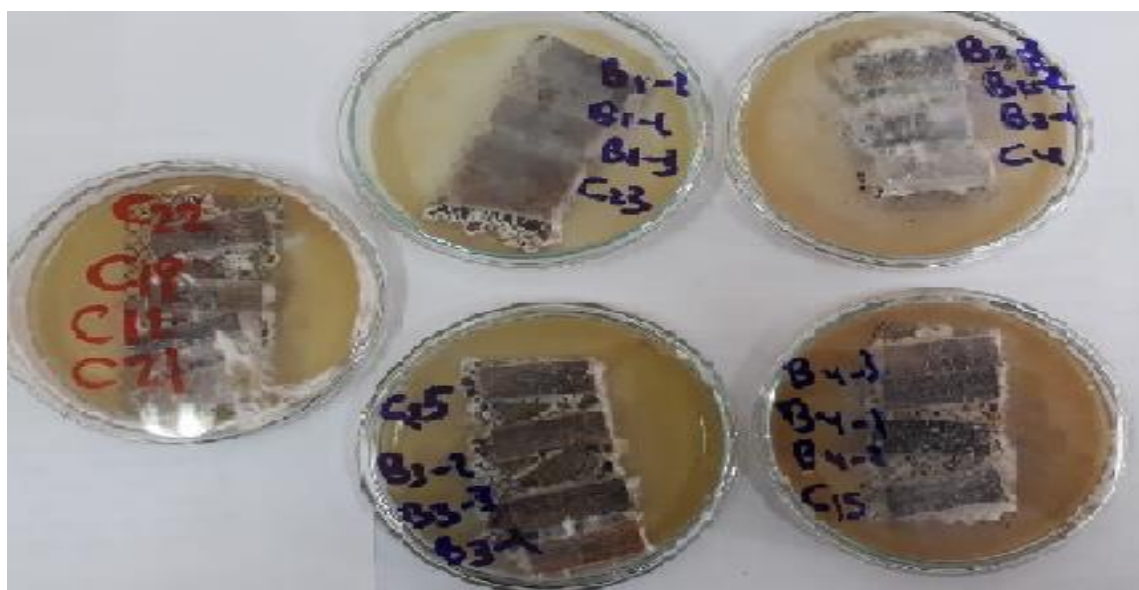


(b)

Fig. 4. Decay resistance of wood samples (*Pinus sylvestris*) treated with different natural products, (a) Phomaxanthone A (compound C: C1 (125 ppm) (C1-1, C1-2, C1-3), C2 (250 ppm) (C2-1, C2-2, C2-3), C3 (500 ppm) (C3-1, C3-2, C3-3) and C4 (1000 ppm) (C4-1, C4-2, C4-3)), (b) Vasicine (Compound F: F1 (125 ppm) (F1-1, F1-2, F1-3), F2 (250) (F2-1, F2-2, F2-3), F3 (500 ppm) (F3-1, F3-2, F3-3), F4 (1000 ppm) (F4-1, F4-2, F4-3)) against brown rot fungus *Tyromyces palustris* (TYP).



(a)



(b)

Fig. 5. Decay resistance of wood samples (*Pinus sylvestris*) treated with different natural products, (a) Brefeldin A (Compound A: A1 (125 ppm) (A1-1, A1-2, A1-3), A2 (250 ppm) (A2-1, A2-2, A2-3), A3 (500 ppm) (A3-1, A3-2, A3-3) and A4 (1000 ppm) (A4-1, A4-2, A4-3)), (b) Anthracobic acid A (compound B: B1(125 ppm) (B1-1, B1-2, B1-3), B2 (250 ppm) (B2-1, B2-2, B2-3), B3 (500 ppm) (B3-1, B3-2, B3-3) and B4 (1000 ppm) (B4-1, B4-2, B4-3)) against white rot fungus *Irpex lacteus* (IRL).

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الفعالية المضادة للفطريات للمنتجات الطبيعية المستخلصة من النباتات والفطريات ودورها المحتمل في السيطرة على الفطريات المدمرة للخشب

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الملخص

تُعَدّ الفطريات المسببة لتعفن الخشب تحديًا كبيرًا لصناعة الأخشاب، وخاصة في الدول التي تعتمد بشكل كبير على استيراد الأخشاب اللينة مثل مصر. وقد أدى الاهتمام المتزايد بالبدائل الصديقة للبيئة للمواد الحافظة الاصطناعية إلى توجيه الأنظار نحو العوامل الطبيعية ذات النشاط المضاد للفطريات. في هذه الدراسة، تم عزل وتقييم ستة مركبات طبيعية فعالة حيويًا هي: بريفيلدين A (المركب A)، حمض الأنثراكوبيك A (المركب B)، فوماتسانثون A (المركب C)، ليوكودين (المركب D)، بارثينولايد (المركب E)، وفاسيزين (المركب F)، وذلك من مصادر فطرية ونباتية، لدراسة فعاليتها المضادة للفطريات المسببة لتعفن الخشب، وهما الفطر تايروميسيس بالستريس (*Tyromyces palustris*) المسبب للتعفن البني، والفطر إريكس لكتيوس (*Irpex lacteus*) المسبب للتعفن الأبيض. تمت معالجة كتل صغيرة من خشب الصنوبر الاسكتلندي (*Pinus sylvestris*) بتركيزات مختلفة من كل مركب (125–1000 جزء في المليون)، ثم تعريضها للإصابة الفطرية لمدة ثمانية أسابيع. أظهرت العينات المعالجة انخفاضًا ملحوظًا في فقدان الكتلة، إذ لم تتجاوز نسبة الفقد 5% عند أعلى التركيزات، مقارنةً بنحو 30% في العينات غير المعالجة. تؤكد هذه النتائج الكفاءة العالية للمركبات الطبيعية المختبرة في مقاومة الفطريات، مما يشير إلى إمكانية استخدامها كمركبات حافظة خضراء وصديقة للبيئة مناسبة لتطبيقات حماية الأخشاب فوق سطح الأرض.

الكلمات الدالة: الفطريات المسببة لتعفن الخشب، التعفن البني، التعفن الأبيض، بريفيلدين A، حمض الأنثراكوبيك A، فوماتسانثون A.