Bacterial and Mold Resistance of Selected Tropical Wood Species

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The biological resistance of 21 tropical wood species against bacteria and molds was studied. The gram-positive bacterium *Staphylococcus aureus* and the gram-negative bacterium *Escherichia coli* had none or negligible activity on surfaces of zebrano and maçaranduba (up to 1 $\times 10^6$ CFU/mL), and they had negligible or medium activity on surfaces of macassar ebony, ovengol, santos rosewood, and iroko (from 1 $\times 10^6$ up to 1 $\times 10^7$ CFU/mL). These bacteria had high activity on surfaces of okoumé, tineo, doussié, makoré, and both reference woods, beech and Scots pine (usually over 1 $\times 10^7$ CFU/mL). The mold growth activity (MGA) of *Aspergillus niger* and *Penicillium brevicompactum* was minimal on surfaces of ipé, yellow balau, macassar ebony, doussié, bubinga, and merbau, but it was strong on surfaces of okoumé, cerejeira, ovengol, wengé, sapelli, and both reference woods. When comparing individual biological tests of (a) bacterial and mold but also (b) bacterial, mold and rot with decaying fungi *C. puteana* and *T. versicolor*, no significant relationships were found. These results confirm that the bio-durability of woods is influenced not only by their molecular structure, but also by the attacking biological pest group.

Keywords: Tropical woods; Biological resistance; Staphylococcus aureus; Escherichia coli; Aspergillus niger; Penicillium brevicompactum

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INTRODUCTION

Microbial colonization of wooden materials is connected with health hazards due to the presence of pathogenic bacteria, molds, and their associated toxins (Gerullis *et al.* 2018; Munir *et al.* 2019). Bacteria and molds live in domestic, restaurant, medical, and industrial settings in association with surfaces made from natural and synthetic materials. In many parts of the world, infectious diseases are controlled by improving hygienic standards. Thus, the hygienic properties of material surfaces, *e.g.*, their antibacterial and antimold properties, are very important (Kandelbauer and Widsten 2009; Nosáľ and Reinprecht 2019). Surfaces of less durable and chemically unprotected woods and wooden composites are accessible to inhabitation by various bacteria and molds, mainly in wet environments, where there may be parallel contamination with food or other organic residues (Vidholdová *et al.* 2015; Nosáľ and Reinprecht 2017). At present, environmentally suitable protection of less durable woods and wood composites can be performed with silver, zinc or copper based nanocompounds (Mantanis *et al.* 2014; Reinprecht *et al.* 2018). However, the use of the naturally bio-durable woods without content of synthetic chemicals is generally more acceptable. Several durable tropical woods, for example, maçaranduba, merbau, iroko or ipé, are often used in exteriors as terrace boards, in bathrooms and other demanding exposures.

The porous nature of wooden products, especially compared with the smoother surfaces of glass, stainless steel, or laminates, is not responsible for their limited hygiene parameters (Vainio-Kaila *et al.* 2011; Aviat *et al.* 2016; Munir *et al.* 2019). The higher porosity may even be an advantage for their microbiological status. The rough and porous surface of wooden materials often generates unfavourable conditions for microorganisms; for example, wood surfaces dry faster. In addition, some wood species contain extracts, including flavonoids, tannins, terpenoids, phenolic acids, quinones, stilbenes, and alkaloids (Reinprecht *et al.* 2010; Laireiter *et al.* 2013; Vainio-Kaila *et al.* 2017; Valette *et al.* 2017) that protect wood against the bacterial and fungal degradation (Wong *et al.* 2005; Reinprecht 2010; Munir *et al.* 2019).

The antibacterial effects of extracts isolated from the xylem, bark, and leaf parts of various tree species have been studied. Rovira *et al.* (1999) tested the antimicrobial activity of 203 extracts obtained from xylem and bark parts of trees growing in neotropical lowland rainforest in French Guiana against two well-known human pathogens, *Staphylococcus aureus* and *Escherichia coli*. Milling *et al.* (2005) and Mulyaningsih *et al*. (2011) found that commercial essential oils from leaves of three *Eucalyptus* tree species have a good antimicrobial effect against several bacteria, including *E. coli.* Patra (2012) examined the antibacterial properties of different classes of phytochemicals, flavonoids, organosulfur compounds, and essential oils obtained from natural products, *e.g.*, from different species and varieties of eucalyptus tree. Elsiddig *et al.* (2015) determined good antibacterial properties of alcoholic extracts from root, leaf, and stem parts of the *Anogeissus leiocarpous* tree, including against bacteria *S. aureus* and *E. coli*. Abdulah *et al.* (2017) studied the antibacterial properties of leaf extracts obtained from 34 primate-consumed plants growing in Indonesia against *E. coli* and *Bacillus subtilis.* Vainio-Kaila *et al.* (2017) tested the efficiency of lignin and extracts from Scots pine and Norway spruce woods against the bacteria *S. aureus* and *E. coli*; the strongest antibacterial effect was observed in extracts isolated from pine heartwood. Rauf *et al.* (2017) showed that *S. aureus* is significantly inhibited by the ether, ethyl acetate, and methanol extracts of the *Diospyros ebenum* tree, which is rich in α-cadinol, τ-cadinol, and τ-muurolol. Tasneem and Narsegowda (2018) demonstrated that the methanol extracts of two leaf varieties (yellow and red) of the *Terminalia catappa* tree had a different inhibitory effect against bacteria, including *S. aureus* and *E. coli*, and also against molds, including *Aspergillus niger*.

The antimold effect of wood extracts has been the focus of additional research. According to Feng *et al.* (2018), the natural resistance of woods against mold attacks is significantly affected by the chemical composition and amount of their extracts. For example, β- and γ-sitosterol, cedrol, α- and β-cedrene, and vanillin, all present in spruce (*Picea asperata*) wood, or vanillin, 4-hydroxy-3-menthoplcinnamaldehyde, benzyl alcohol, and benzotiazole, all present in Mangolian pine (*Pinus sylvestris* var. *mongolica*), have a key role in the mold resistance of these wood species. Philp *et al.* (1995) showed that extracts of Scots pine heartwood have a strong effect against six selected isolates of the *Trichoderma* mold. Salem *et al.* (2016) reported that extracts from pine (*Pinus rigida*) heartwood inhibit the growth of the molds *Alternaria alternata*, *Fusarium subglutinans, Chaetomium globosum, A. niger*, and *Trichoderma viride*. There were different inhibitory effect against the molds *Penicillium selerotigenum*, *Paecilomyces variotii,* and *A. niger* by extracts from the xylem, bark, and leaves of the *Brachychiton diversifolius* wood (Salem *et al*. 2014a), from xylem and bark of the *Delonix regia* wood (Salem *et al*. 2014b), or from xylem of *Morus alba* and *Citharexylum spinosum* woods (Mansour *et al.* 2015). Various extracts of tropical woods—*Lophopetalum wightianum*, *Citharexylum spinosum*, and

Argyreia cuneata—are effective against *Penicillium* species; the leaf extract has a higher inhibitory effect than the bark extract (Sahana *et al.* 2018). Extracts from camphor (*Cinnamomum camphora*), toon (*Toona ciliata*), teak (*Tectona grandis*), eucalyptus (*Eucalyptus urophylla*), and sweetgum (*Liquidambar formosana*) tropical woods exhibit remarkable inhibition effect against growth of molds; these results correlate well with the total content of antifungal compounds in wood flour extracts (Feng *et al.* 2018). Salem *et al.* (2016) found that concentrated extract from *Eucalyptus camaldulensis* leaves inhibits the growth of *A. niger* but not the molds *A. alternata*, *F. subglutinans*, *Ch. globosum*, and *T. viride*.

Gradeci *et al.* (2017) showed that the growth of molds on wood surfaces is linked to the extracts and other factors, such as (a) the moisture content of wood and its variation due to the relative humidity of air; (b) the temperature; (c) the roughness of wood surfaces; and (d) the used sawing and drying patterns. There are other mold growth criteria and design avoidance approaches in wood-based materials under a wide range of environmental conditions.

This work compared the bacterial and mold resistances of selected tropical wood species, and following compared these resistances with their resistance to rot caused by wood decaying fungi (Reinprecht and Vidholdová 2019).

EXPERIMENTAL

Tropical and Reference Woods

The experiment was performed with heart-zone of 21 tropical wood species: ipé (*Handroanthus serratifolius* [Vahl] S. O. Grose), okoumé (*Aucoumea klaineana* Pierre), tineo (*Weinmannia trichosperma* Cav.), dark red meranti (*Shorea curtisii* Dyer ex. King), yellow balau (*Shorea laevis* Ridl.), macassar ebony (*Diospyros celebica* Bakh.), doussié (*Afzelia bipindensis* Harms), cerejeira (*Amburana cearensis* A. C. Sm.), bubinga (*Guibourtia demeusei* J. Léonard), ovengol (*Guibourtia ehie* J. Léonard), merbau (*Intsia bijuga* O. Ktze.), santos rosewood (*Machaerium scleroxylon* Tul.), zebrano (*Microberlinia brazzavillensis* Chev.), wengé (*Millettia laurentii* De Wild.), padouk (*Pterocarpus soyauxii* Taub.), sapelli (*Entandrophragma cylindricum* Sprague), iroko (*Milicia excels* C. C. Berg), karri (*Eucalyptus diversicolor* F. Muell.), blue gum (*Eucalyptus globulus* Labill.), maçaranduba (*Manilkara bidentata* A. Chev.), and makoré (*Tieghemella heckelii* Pierre), and also with sap-zone of two reference European wood species (European beech, *Fagus sylvatica* L., and Scots pine, *Pinus sylvestris* L.). These tropical woods, having a different natural durability relative to rot action of wood-decaying fungi (EN 350: 2016; Reinprecht and Vidholdová 2019), were obtained from the trading company JAF Holz, Ltd. (Špačince, Slovak Republic) in the form of naturally dried and conditioned boards with a moisture content of 13 ± 2.5%.

Two types of specimens, *i.e.*, with dimensions of 50 mm × 50 mm × 5 mm (longitudinal × radial × tangential) for bacterial activity tests and with dimensions of 50 mm × 20 mm × 5 mm (longitudinal × radial × tangential) for mold growth activity tests, were prepared on a circular saw from conditioned boards. The specimens did not contain biological damage, knots, or other inhomogeneity, and their surfaces were not ground.

Bacterial Activity

The bacterial activity (BA) test was performed with the gram-positive bacterium

Staphylococcus aureus ATCC-25923 and the gram-negative bacterium *Escherichia coli* ATCC-25922. Wood specimens were cleaned and sterilized with alcohol solution (8.8 : 1.2 mixture of ethanol and 2-propanol). They were subsequently placed in Petri dishes with a diameter of 120 mm and inoculated with 0.1 mL of bacterial suspension with density of 0.5* according to the McFarland scale (1.5×10^8 CFU/mL). Incubation of bacteria on the top surfaces of wood specimens lasted 48 h at 37 ± 2 °C. The bacteria were removed from wood surfaces using a sterile swab and transferred to liquid culture medium for 48 h. Finally, the bacteria were pre-inoculated from the liquid cultural medium into the sodium chloride diagnostic soil present in glass containers. The bacterial resistance of individual wood specimens was assessed by determining the BA values (0 to 2), which were translated from values measured in CFU/mL using these criteria: 0, up to 1×10^6 CFU/mL represents none or minimal BA; 1, from 1×10^6 up to 1×10^7 CFU/mL represents medium BA; and 2, over 1×10^7 CFU/mL represents strong BA.

Mold Growth Activity

The mold growth activity (MGA) test was performed with the microscopic fungi *Aspergillus niger* Tiegh. and *Penicillium brevicompactum* Dierck by a modified Standard EN 15457 (2014); wood specimens with other shape and other mode of sterilization were used. Firstly, all surfaces of wood specimens were sterilized with the 30 W germicidal lamp (Chirana Medical a.s., Stará Turá, Slovak Republic) from a distance of 1 m at 22 °C ± 2 °C for 0.5 h. The sterilized specimens were placed into Petri dishes with a diameter of 120 mm, which previously were filled with a 3- to 4-mm thick layer of 4.9% Czapek-Dox agar medium (HiMedia Ltd., Mumbai, India). Specimens were inoculated with a mold spore suspension prepared in sterile water (10^6 to 10^7 spores/mL). The inoculated specimens were incubated 21 days at 24 ± 2 °C and a relative air humidity of 90 to 95%. The mold resistance of wood specimens was evaluated by the MGA values (0 to 4), using these criteria: 0, no mold growth on the top surfaces; 1, ≤ 10%; 2, > 10% but ≤ 30%; 3, > 30% but ≤ 50%; 4, > 50%.

RESULTS AND DISCUSSION

Resistance to Bacteria

The activity of *S. aureus* and *E. coli* on the top surfaces of tropical wood species was usually weaker than on the top surfaces of reference European woods (Fig. 1). Excellent bacterial resistance was shown by the surfaces of zebrano and maçaranduba (BA equal to 0). Lower bacterial resistance was observed for the surfaces of macassar ebony, ovengol, santos rosewood, and iroko (BA from 0 to 1). The lowest resistance against bacteria was observed on doussié, okoumé, tineo, and makoré (BA of 2, or BA equal 1 and 2, *i.e.* when both bacteria, or only one bacterium had activity over 1×10^7 CFU/mL). In sum, the tropical woods had comparable bacterial resistance to the gram-positive bacterium *S. aureus* with $BA_{S.aureus\text{-}Average}$ = 0.857 and the gram-negative bacterium *E. coli* with $BA_{E.coli\text{-}Average}$ = 1.000 (Fig. 1).

Generally, products made from a native wood have better resistance to bacteria than products made from other natural or synthetic materials. Milling *et al.* (2005) tested the bacterial activity of *E. coli* and *E. faecium* on pine and oak woods, which had better hygienic performance than plastics. This result was likely due to the hygroscopic properties of wood components and the antibacterial effect of wood extracts. These researchers determined a reduction in bacterial numbers also for other wood species— spruce, larch, poplar and beech—compared with plastics. Hedge (2015) confirmed and extended this knowledge, showing that the native beech wood has better resistance to *E. coli* than varnished beech wood, testing also different surface pre-treatments of native wood, *i.e.*, uncleaned, cleaned, or cleaned and dried.

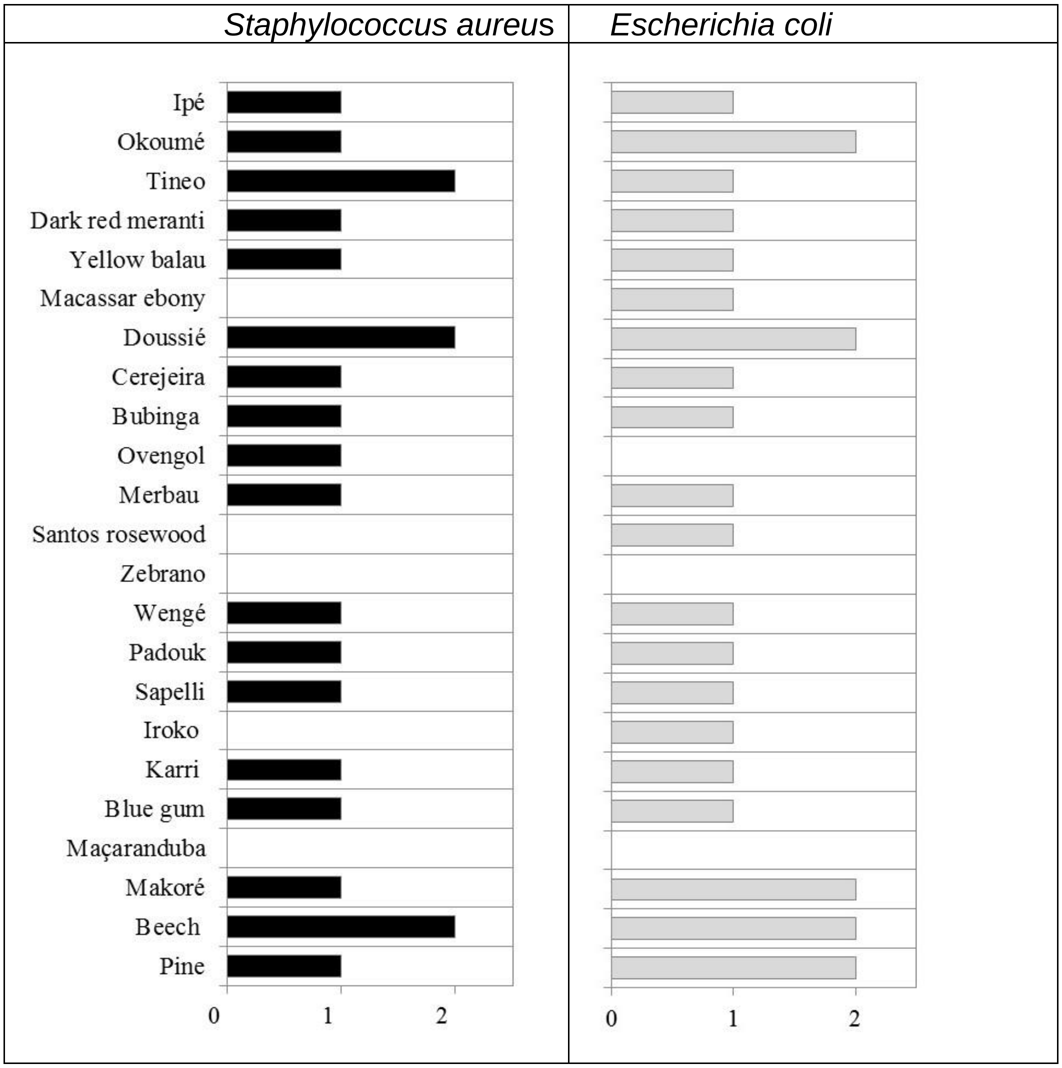


Fig. 1. Bacterial activity (BA) of *S. aureus* and *E. coli* on the top surfaces of 21 tropical woods and two reference European woods. Note: Mean values of BA (0 – 2) are from 4 replicates.

Resistance to Molds

The mold growth activity (MGA) of the microscopic fungi *Aspergillus niger* and *Penicillium brevicompactum* on the top surfaces of tropical wood species was weaker than on the reference European woods (Fig. 2). The lowest mold growth occurred on the top surfaces of ipé, yellow balau, macassar ebony, doussié, bubinga, and merbau (MGA from 0 to 2). Okoumé, cerejeira, ovengol, wengé, sapelli, and the reference European woods had the weakest resistance to molds (MGA from 3 to 4). The tropical woods had comparable mold resistance against both fungi, the *A. niger* with $MGA_{A.niger\text{-Average}} = 2.319$ and the *P. brevicompactum* with $MGA_{P.brevicompactum\text{-Average}} = 2.498$ (Fig. 2). Thus, both microscopic fungi are convenient for testing the antimold resistance of woods. However, results from the laboratory mold screening tests do not always correlate with results from the outdoor mould tests (Lie *et al.* 2019).

The high mold resistance of individual wood species is attributable largely to their molecular structure, mainly the type and amount of extractives having a higher or lower toxicity to microscopic fungi, *i.e.*, tannins, flavonoids, terpenoids, fatty acids, aldehydes, and ketones (Umezawa 2001; Nascimento *et al.* 2013; Vallete *et al.* 2017; Munir *et al.* 2019; Reinprecht and Vidholdová 2019). Feng *et al.* (2018) confirmed that a higher total content of antifungal compounds in wood extractives resulted in higher mold resistance.

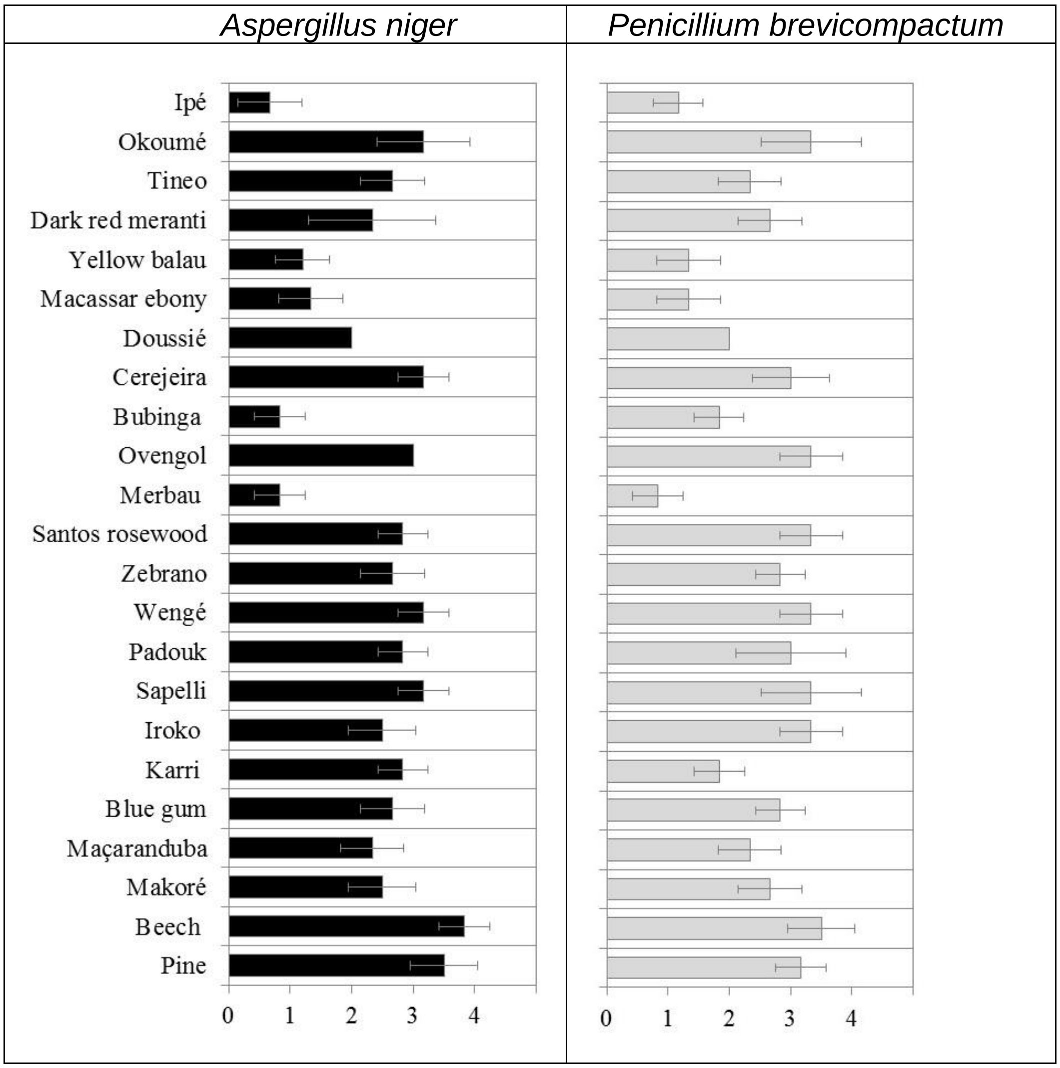


Fig. 2. Mold growth activity (MGA) of *A. niger* and *P. brevicompactum* on the top surfaces of 21 tropical woods and two reference European woods. Note: Mean values of MGA (0 – 4) are from 6 replicates.

Comparison of the Resistances to Bacteria, Molds, and Decaying Fungi

Of the tropical woods, only some species had a similar resistance to the bacterial attacks and the mold attacks. For example, macassar ebony was resistant to both bio-attacks (Figs. 1 and 2). However, for all tested wood species there was not a universal relationship between these two bio-attacks, probably due to specific biodegradation mechanisms of individual pests. Zebrano exhibited low BA and higher MGA; doussié demonstrated high BA and lower MGA (Figs. 1 and 2). Consequently, there was a very weak linear correlation between the bacterial resistance and the mold resistance of all tropical woods (Fig. 3). Hence, the biological durability of woods is influenced by the wood species determined by its molecular-chemical and anatomical structure, and also by the biological pest group attacking wood structure (Mansour *et al*. 2015).

Other studies have confirmed the high antibacterial and antifungal potential of phytochemicals, *i.e.*, naphthoquinone, pterocarane, deoxysanthalene, sandaline, stigmasterol, β-sitosterol, campesterol, spinoside, tunispinosides, verbascoside, and many others from various wood species (Surowiec *et al*. 2004; Romagnoli *et al*. 2013; Feng *et al*. 2018; Saidi *et al* 2018; Munir *et al*. 2019; Sharma 2019). As shown in Fig. 3, the biological activity of individual extracts can be more specific as universal.

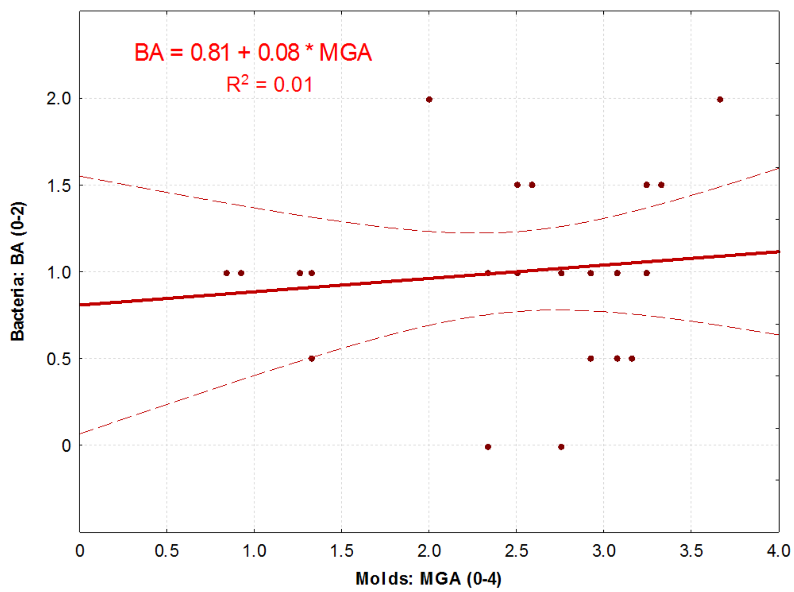
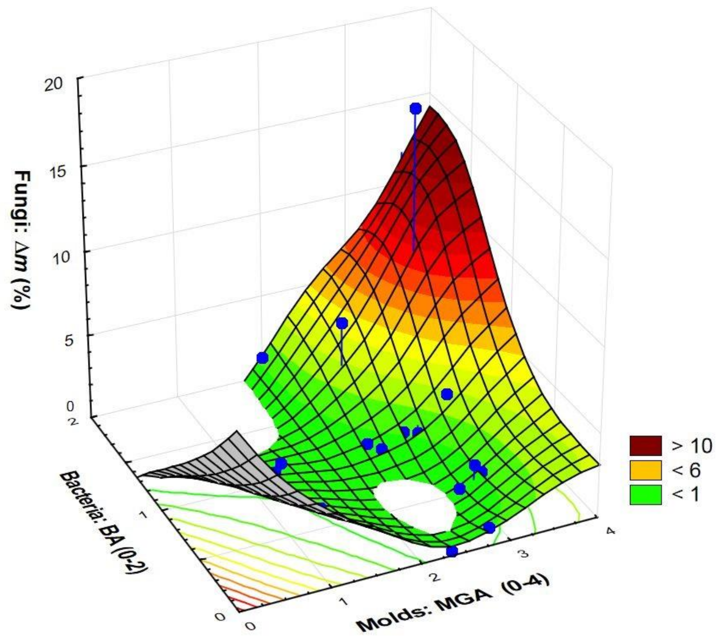


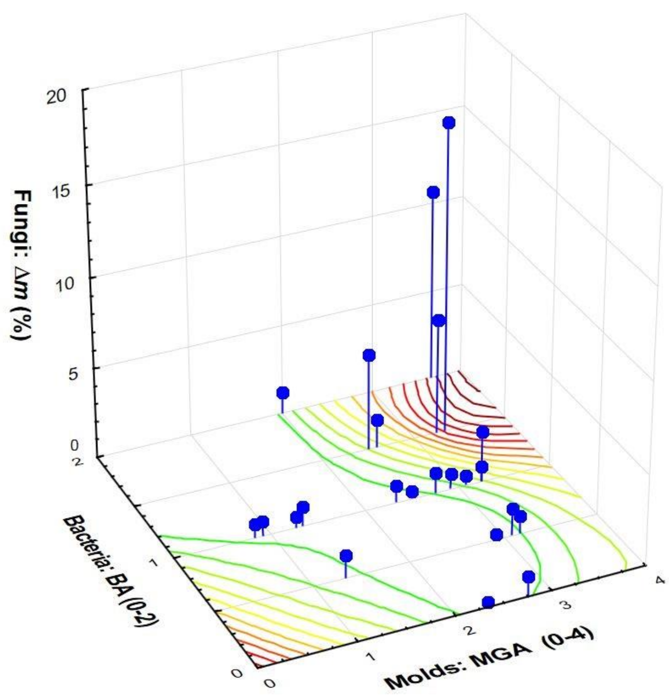
Fig. 3. A very weak linear correlation between the bacterial activity (BA – average of *S. aureus* and *E. coli*, by Fig. 1) and the mold growth activity (MGA – average of *A. niger* and *P. brevicompactum*, by Fig. 2) determined for 21 tropical wood species – as the slope is slight (0.08) and the coefficient of determination R^2 is very small (0.01).

The biological resistance of 21 tropical woods (heart-zones) and two reference European woods (sap-zones of beech and Scots pine) was analyzed more completely, as well, *i.e.*, against selected three biological pest groups: (I. → BA) average attack by two bacteria *S. aureus* and *E. coli*; (II. → MGA) average attack by two molds *A. niger* and *P. brevicompactum*; (III. → Δm) average weight loss at 6-weeks rot action of two wood-decaying fungi *Coniophora puteana* and *Trametes versicolor* – determined by Reinprecht and Vidholdová (2019). The complex analysis was performed by the 3D-response surface plots (Fig. 4a) and contour plots (Fig. 4b).

The reference woods had the lowest rot resistance, with Δ*m* equal to 10.64% for European beech and 17.01% for Scots pine, which also had low resistance to bacteria (average BA equal to 2 for beech and 1.5 for pine) and molds (average MGA equal to 3.67 for beech and 3.33 for pine). In contrast, 11 tropical woods – ipé, dark red meranti, yellow balau, cerejeira, bubinga, merbau, wengé, padouk, sapelli, karri, and blue gum – with a medium resistance to bacteria with BA always 1 (Figs. 1 and 4) had a relatively high rot resistance with Δ*m* from 0.41% to 2.84% (Fig. 4, and also Reinprecht and Vidholdová (2019)); however, there was a very different mold resistance with average MGA from 0.83 to 3.25 (Figs. 2 and 4).



(a)



(b)

Fig. 4. 3D-response surface plots (a) and contour plots (b) related to biological resistance of selected 21 tropical woods and two reference European woods – *i.e.* the average bacterial activity (BA; see Figs. 1 and 3) and the average growth mold resistance (MGA; see Figs. 2 and 3), analyzed together with the average rot resistance against the wood-decaying fungi *Coniophora puteana* and *Trametes versicolor* (Δm; average weight loss in percentage for two wood-decaying fungi – by Reinprecht and Vidholdová 2019).

Generally, some tropical wood species manifested as more resistant to bacteria and other ones to molds and decaying fungi. Such differences could be caused by different bio-effect of individual extractives on bacterial and fungal organisms – however this hypothesis has to be analysed in more detailed experiments.

CONCLUSIONS

1. The bacterial activity (BA) of *S. aureus* and *E. coli* and the mold growth activity (MGA) of *A. niger* and *P. brevicompactum* were evaluated on the top surfaces of 21 tropical wood species and two reference woods (sap-zones of European beech and Scots pine). The best bacterial resistance was exhibited by zebrano and maçaranduba. Macassar ebony, ovengol, santos rosewood, and iroko showed a very good resistance. The poorest bacterial resistance was shown by doussié, okoumé, tineo, makoré, and both reference species.
2. The best mold resistance was typical for ipé, yellow balau, macassar ebony, doussié, bubinga, and merbau. The poorest mold resistance was exhibited by okoumé, cerejeira, ovengol, wengé, sapelli, and both reference species.
3. In summary, tropical woods had a very similar average resistance against both bacteria, *i.e*. the gram-positive bacterium *S. aureus* with $BA_{Average} = 0.857$ and the gram-negative bacterium *E. coli* with $BA_{Average} = 1.0$, and also against both microscopic fungi, *i.e*., the mold *A. niger* with $MGA_{Average} = 2.319$ and the mold *P. brevicompactum* with $MGA_{Average} = 2.498$.
4. Only some of tropical woods had a similar level of resistance to the bacterial and mold attacks; for example, macassar ebony was resistant to both groups of pests. However, between 21 tropical woods there was not a universal relationship related to their biological resistance, probably due to specific biodegradation mechanisms of individual pests. For example, zebrano was characterized by low BA and higher MGA, and doussié showed the opposite effects of high BA and lower MGA.

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