Annals of Warsaw University of Life Sciences – SGGW
Forestry and Wood Technology No. 110, 2020: 118-125
(Ann. WULS – SGGW, For. and Wood Technol. 110, 2020)

Evaluation of the fungicidal properties of essential oils from *Leptospermum scoparium* and *Azadirachta indica* in relation to selected fungi causing wood decay

JOANNA ŚWISTAK[1], IZABELA BETLEJ[2]
[1] Warsaw University of Life Sciences – SGGW, Faculty of Wood Technology
[2] Warsaw University of Life Sciences – SGGW, Institute of Wood Sciences and Furniture

Abstract: *Evaluation of the fungicidal properties of essential oils from Leptospermum scoparium and Azadirachta indica in relation to selected fungi causing wood decay*. The publication presents the results of evaluation of the fungicidal effect of two essential oils from *Leptospermum scoparium* (manuka) and *Azadirachta indica* (neem) on fungi that cause the decomposition of *Trametes versicolor* and *Coniophora puteana* wood. Research results indicate that the growth of the tested fungi is clearly inhibited with the participation of the highest concentrations of essential oils used. At the same time, it was found that the action of neem oil was stronger compared to the *Traetes versicolor* fungus. In contrast, manuka essential oil has a stronger inhibitory effect on the growth of *Coniophora puteana.*

Keywords: essential oils, fungicides, wood rotting fungi

INTRODUCTION

The durability of wood and wood-based materials depends primarily on the conditions of its exploitation. Under improper conditions of use, the service life of wood as a utility material drops sharply. One of the conditions for long-term use of wood is protection against biocorrosion by appropriate wood preservatives. Both the right choice of biocides and wood preservation methods determine the resistance of wood to the destructive effects of biotic agents. Wood preservatives are chemical impregnates that contain active substances that show biocidal activity against wood-destroying organisms (Krajewski and Witomski 2003, Piontek and Lechów, 2013). The use of chemicals to protect wood, protects the material from biocorrosion, but always remember that these substances can negatively affect the ecosystem. These are the issues of environmental, human and animal safety, contained in Directive 98/8/ EC of the European Parliament and of the Council concerning the placing of biocidal products on the market (Directive 98/8/EC, 1998), and then repeated in Regulation No 528/2012 of the European Parliament and of the Council on the provision and the use of biocidal products (Regulation (EU) No 528/2012, 2012) imposed tight restrictions on the marketing of wood preservative products. Those are commonly used and effective against fungi that cause deep wood decomposition, chemicals containing chromium, arsenic, or fluorine compounds, phenols, naphthalenes and numerous products of distillation of crude oil have been withdrawn from trading. The current state of active substances allowed for use in wood preservatives in the European Union is 45 active substances, including: propiconazole, boric acid, IPBC, permethrin, didecyl dimethylammonium chloride, copper oxide, copper (II) hydroxide copper (II) (1: 1), cypermethrin, K-HDO, Cu-HDO, fenoxycarb, sulfuryl fluoride (echa.europa.eu/pl/information-on-chemicals/biocidal-active-substances). Despite numerous restrictions, Regulation 528/2012 provides for the possibility of research into new chemicals. The formal requirement for the approval of such substances for use in the protection of wood, besides the guarantee of effectiveness against declared organisms, is to meet the criteria related to toxicological and ecotoxicological safety. That is, it should be demonstrated by appropriate tests that the new active substances proposed for use are safe for humans, animals and the environment, during the use of impregnating agents and when using impregnated wood. Issues related to new active substances, safe for the environment appeared in scientific discussions already in the 60s of the last century (Shelds and Atwell, 1963); however, detailed research and analyzes presented on the world stage have been intensified in the world of science after 2000. At the 2007 international conference of the International Research Group, scientific reports on the use of substances of natural origin in wood protection were presented. The authors of the presentation presented interesting results of the use of plant extracts such as essential oils in protecting wood against blue stain, as well as attempts to combine caffeine with propiconazole to strengthen the bio-protective effect against fungi causing wood decay (Kundzewicz 2007).

Plant extracts rich in various chemical components of antimicrobial importance can be used to protect numerous materials (Nuzhat and Vidyasagar 2013, Stupar et al. 2014, Kharbach et al. 2019). Attempts to use plant extracts to protect wood have been presented in numerous publications (Chittenden and Singh 2011, Mohareb et al. 2013, Pánek et al. 2014, Mansour and Salem 2015). Ahmed et al. (2020) assessed the effect of oils derived from linseed, eucalyptus, neem and jojoba on the effectiveness of protecting three species of wood against termites. In the presented research they showed that all oils, except eucalyptus, effectively protected the wood against decay caused by termites. In the research of Yildiz et al. (2020) were presented the biocidal properties of mistletoe leaf extracts and *Usnea flipendula* lichen on *Copniophora puteana*. An interesting issue was the assessment of the protection of Scots pine wood with a solution of propolis against decay caused by *Coniophora puteana* (2020). The authors of the study showed that the weight loss of wood impregnated with natural biocide was tewnty-one times lower than that of control wood. The fungistatic properties of propolis have been demonstrated even against such fungi species as *Penicillium italicum, Aspergillus niger, Trichoderma viride,* and *Colletotrichum gloeosporioides* (Garedew et al. 2004, Meneses et al. 2009, Peng et al. 2012). Essential oils are the most popular group of active compounds of natural origin with significant fungistatic or fungicidal properties. The strong fungicidal action of oils derived from distillation from the aerial parts of *Thymus bleicherianus* and *Thymus capitatus* was determined by El Ajjouri et al. (2008). The authors of the study have identified that phenolic and aldehyde compounds contained in oils have a strong fungicidal effect. Fungicidal properties of chemical compounds from the group of phenols (isoeugenol, eugenol, resorcinol, 2,6-dimethoxyphenol, pyrogalol) in relation to selected species of Basidiomycetes were the subject of studies by Zarzyński and Andres (2009, 2010a, 2010b). The effect of essential oils and plant extracts on the growth of *Trametes versicolor* and *Tyromyces palustris* was anazlialized by Kartal et al. (2006).

The purpose of the research presented in this article was to determine the effect of the addition of various concentrations of essential oils from the Indian Honey tree (*Azadirachta indica*) and the Manuka shrub (*Leptospermum scoparium*) on the growth of fungi causing wood decay: *Trametes versicolor* and *Coniophora puteana* on maltose-agar medium.

MATERIALS AND METHODS

Studies on the fungicidal action of essential oils in relation to fungi that cause deep wood decay were prepared based on the methodologies described by Gniewosz et al. (2012), Betlej and Andres (2019) and Nenoff et al. (1996).

Fungi-inhibiting factors were plant oils derived from *Azedirachta indica* A.Juss. (called neem) and *Leptospermum scoparium* J.R.Forst. & G.Forst. (called manuka), obtained in the form of essential oils (Pollena-Aroma, Nowy Dwór Mazowiecki, Poland). The assessment of fungicidal activity was carried out on two fungi causing deep wood decomposition - *Coniophora puteana* (Schumach) P. Karst., Strain EB97 and *Tramestes versicolor* (L) Lloyd, strain 30. The organisms tested came from the fungi collection of the Department of Wood Science and Wood Protection. The selection of the indicated species was dictated by the fact that, according to the EN 113: 2000 standard, these are the main species of test fungi, used in biological tests to determine the effectiveness of protecting wood against decay.

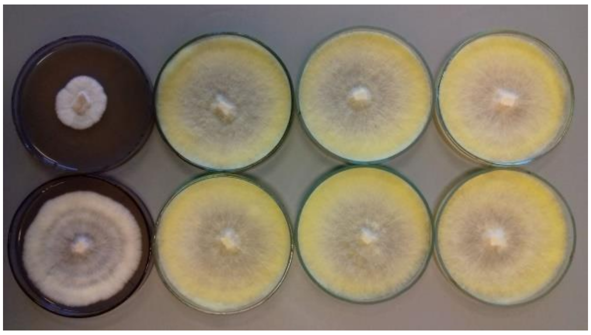
The evaluation of the fungicidal effect of selected essential oils was started by re-transferring fungi starting cultures from the collection in order to obtain microorganisms with high physiological activity. These microorganisms were grown in a medium containing maltose as a carbon source. On the 10th day fungi cultures were used for the analysis. A number of dilutions of essential oils in ethanol were prepared before starting the main tests. These oils in an amount of 1ml were added to a Petri dish (Ø90mm), on which 9ml sterile medium containing 2.5% maltose extract (Biomaxima, Lublin, Poland) and 2.5% agar (Difco, New Jersey, USA) was dosed. The final content of oils in the medium was 0.1, 0.5, 1.0 5.0, 10 and 50µl/l. At the same time, two control trials were prepared, defined as treatment and non-treatment control. The treatment control contained in its composition, apart from the medium, also ethanol, in the same amount that was added to the research tests. The preparation of such control was aimed at identifying whether the determined fungicidal effect was derived from plant extracts or was also the result of the action of ethanol. The non-treatment control contained only maltose medium.

The tested fungi were centrally inoculated onto the solidified medium. The inoculum size was about 5mm. The cultures prepared in this way were incubated in laboratory incubators at temperature and humidity conditions of 23 ± 2˚C and 66 ± 5%, respectively. The evaluation of the inhibitory effect on the growth of the studied fungi by the applied essential oils was made by measuring the diameter of the fungal colony in two opposite directions. The studies were conducted at 48h intervals until the medium in the non-treatment control was completely overgrown by test fungi. All tests were performed in triplicate.

RESULTS

Based on the obtained results, it was found that the addition of essential oils from *Leptospermum scoparium* and *Azedirachta indica* to the substrate affects the growth of test fungi. However, it should be noted that the degree of inhibition of fungal growth depends on the proportion of the substance in the culture medium. Comparing the fungicidal activity of the examined essential oils, it was found that the oil from *A. indica* has a stronger fungicidal effect in relation to the *T. versicolor* fungus than the oil from *L. scoparium*.

The growth of *T. versicolor* on a medium with neem essential oil on the 6th day of culture



The growth of *C. puteana* on a medium with manuka essential oil on the 8th day of culture

Figure 1. Visual growth of test fungi on the medium with the addition of the essential oils tested

The data presented in Figures 2 and 3 shows that the average percentage of overgrowth of *T. versicolor* mycelium on medium with manuka oil on the 6th day of culture, for most of the tested concentrations ranged from 85 to 100%. Only in the case of the addition of 50µl/l manuka oil to the medium, a strong inhibition of fungus growth was observed, whose percentage degree of growth on the medium, on the 6th day of culture was less than 13%. When assessing the effect of manuka essential oil on the growth of *C. puteana* on the 6th day of the experiment, it was found that the oil has a stronger effect on the growth of the tested fungus than the neem essential oil.

A visual assessment of the growth of *T. versicolor* and *C. puteana* fungi on the last day of culture on media containing the oils tested was presented in Figure 1.

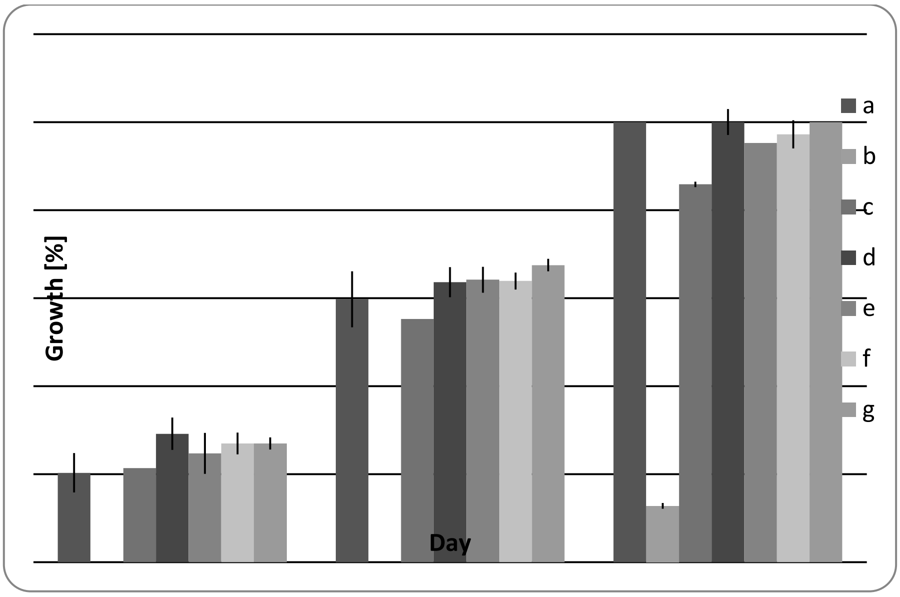


Figure 2. Percentage of growth of the medium with the addition of various concentrations of manuka essential oil by *T. versicolor*, at different breeding times (a-non treatment control, b-50, c-10, d-5.0, e-1.0, f-0.5, g- 0.1µl/l)

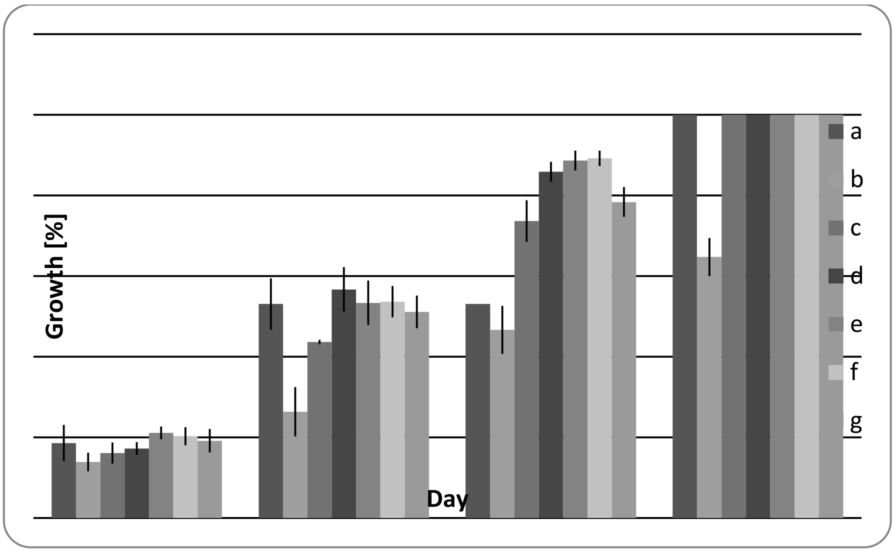


Figure 3. Percentage of growth of the medium with the addition of various concentrations of neem essential oil by *T. versicolor*, at different breeding times (a-non treatment control, b-50, c-10, d-5.0, e-1.0, f-0.5, g- 0.1µl/l)

When assessing the effect of neem essential oil on the growth of the fungus that causes white wood decay, it was noticed that this oil inhibits the growth of the species of fungus more strongly than manuka oil. On the 6th day of culture, no mycelial growth of the medium by 100% was achieved in any experimental variant. The most visible fungicidal effect was found for the highest concentration used. At the same time, it should be noted that when comparing the effects of the highest concentrations of manuka and neem oils on the growth of the test fungus, manuka oil showed a stronger fungicidal effect.

When assessing the effect of neem essential oil on the growth of *C. puteana* in the final stage of the experiment, it was found that only a concentration of 10 and 50µl/l in the culture medium inhibits the growth of the test fungus (Figure 4). The determined mycelial growth in the medium on the 6th day of culture was 47% and 92% with the addition of 50µl/l and 10µl/l, respectively. Manuka essential oil strongly influenced the growth rate of *C. puteana*. On the 6th day of the experiment, no full growth of the substrate was achieved in any of the tested samples (Figure 5). The smallest mycelium growth on the medium was found when the 50µl/l oil was added to the medium.

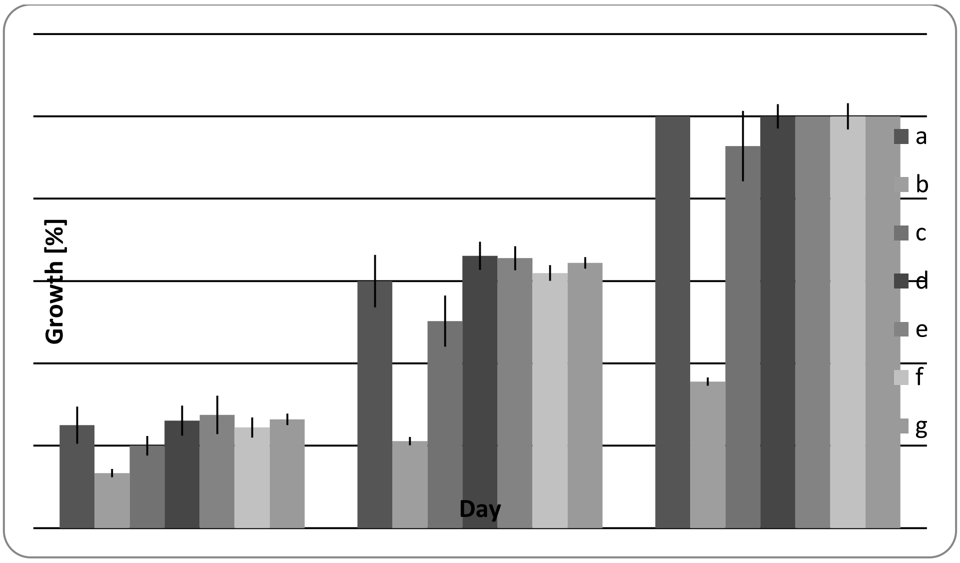


Figure 4. Percentage of growth of the medium with the addition of various concentrations of neem essential oil by *C. puteana*, at different breeding times (a-non treatment control, b-50, c-10, d-5.0, e-1.0, f-0.5, g- 0.1µl/l)

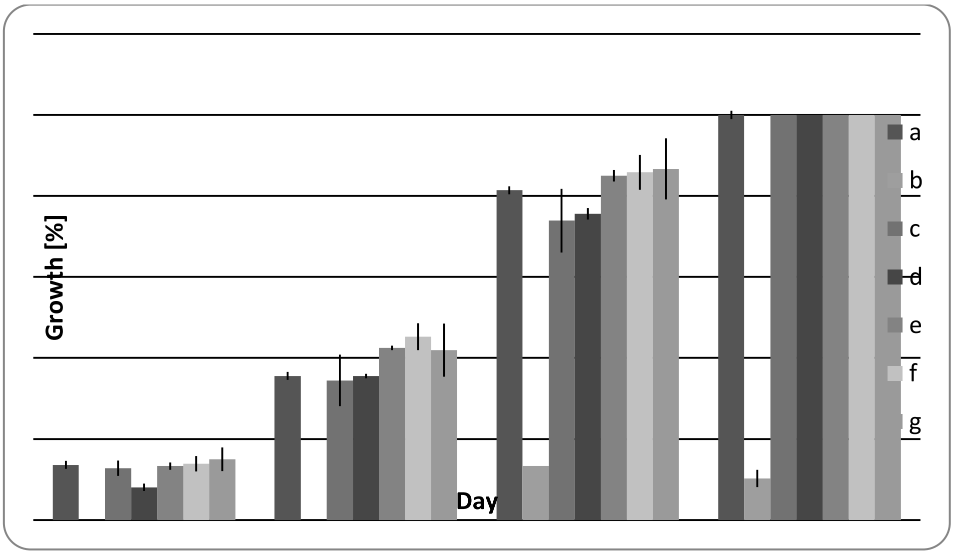


Figure 5. Percentage of growth of the medium with the addition of various concentrations of manuka essential oil by *C. puteana*, at different breeding times (a-non treatment control, b-50, c-10, d-5.0, e-1.0, f-0.5, g- 0.1µl/l)

Small differences in growth diameter were found on the treatment and non-treatment control samples. In the treatment control, the growth diameter of the test fungi was 3-6mm smaller than in the non-treatment control. This result led to the conclusion that the effect of ethanol on inhibiting the growth of fungi is minimal.

CONCLUSIONS

Analyzing the obtained test results, it was found that neem and manuka essential oils, at certain concentrations, have inhibitory effects on the growth of fungi causing wood decay. The tested fungi species react differently to the presence of essential oils in the culture medium. Manuka essential oil has a stronger fungicidal effect on the *C. puteana* fungus than neem essential oil. An inverse relationship was found in the test using the *T. versicolor* fungus.

REFERENCES

1. AHMED S., FATIMA R., HASSAN B., 2020: Evaluation of different plant derived oils as wood preservatives against subterranean termite Odontotermes obesus. Maderas. Ciencia y tecnología No. 22(1); 109-120
2. BETLEJ I., ANDRES B., 2019: Ocena właściwości biobójczych metabolitów grzybów, jako potencjalnych fungicydów w środkach do konserwacji drewna. Jakość produktów przemysłowych: wybrane aspekty /red. nauk. Jerzy Szakiel, Paweł Turek. om - Radom: Instytut Technologii Eksploatacji, (Towaroznawstwo w badaniach i praktyce); 163-173
3. CAFER YILDIZ U., KILIÇ C., GÜRGEN A., YILDIZ S., 2020: Possibility of using lichen and mistletoe extracts as potential natural wood preservative. Maderas: Ciencia y tecnología 22(2); 179-188
4. CHITTENDEN C., SINGH T., 2011: Antifungal activity of essential oils against wood degrading fungi and their applications as wood preservatives. International Wood Products Journal No. 2(1); 44-48
5. DIRECTIVE 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market
6. EL AJJOURI M., SATRANI B., GHANMI M., AAFI A., FARAH, A, RAHOUTI M., AMARTI F., ABERCHANE M., 2008: Antifungal activity of the Thymus bleicherianus Pomel and Thymus capitatus (l.) Hoffm. & Link essential oils against wood-decay fungi. Biotechnologie agronomie societe et environnement No. 12(4); 345-351
7. GAREDEW A., SCHMOLZ E., LAMPRECHT I. 2004: Microbiological and calorimetric investigations on the antimicrobial actions of different propolis extracts: an in vitro approach. Thermochimica Acta No. 422(1-2); 115–124
8. GNIEWOSZ M., STOBNICKA A., ADAMSKA E., 2012: Porównanie działania przeciwdrobnoustrojowego olejków wterycznych Manuka (Leptospermum scoparium) i kanuka (Kunzea ericoides). Bromatologia i Chemia Toksykologiczna 2012 No. 45 (3); 639-644
9. https://echa.europa.eu/pl/information-on-chemicals/biocidal-active-substances
10. KARTAL S. N., HWANG W.-J., IMAMURA Y., SEKINE Y., 2006: Effect of essential oil compounds and plant extracts on decay and termite resistance of wood. Holz Als Roh- und Werkstoff, 64(6); 455–461
11. KHARBACH M., MARMOUZI I., EL JEMLI M., BOUKLOUZE A., Heyden Y. V., 2019: Recent advances in untargeted and targeted approaches applied in herbal-extracts and essential-oils fingerprinting - A review. Journal of Pharmaceutical and Biomedical Analysis No. 177 (112849); 1-52
12. KRAJEWSKI A., WITOMSKI P., 2003: Ochrona Drewna, Warszawa: Wyd. SGGW
13. KUNDZEWICZ A., 2007: Kierunki rozwoju ochrony drewna w świetle 38 konferencji IRG. Ochrona drewna XXII Sympozjum Rogów; 29-36

14. MANSOUR, M. M. A., & SALEM, M. Z. M. (2015). Evaluation of wood treated with some natural extracts and Paraloid B-72 against the fungus Trichoderma harzianum: Wood elemental composition, in-vitro and application evidence. International Biodeterioration & Biodegradation No. 100; 62-69
15. MENESES E. A., DURANGO D. L., GARCÍA C. M., 2009: Antifungal activity against postharvest fungi by extracts from Colombian propolis. Química Nova No. 32(8); 2011–2017
16. MOHAREB A. S. O., BADAWY M. E. I., ABDELGALEIL S. A. M., 2013: Antifungal activity of essential oils isolated from Egyptian plants against wood decay fungi. Journal of Wood Science No. 59(6); 499–505
17. NENOFF P., HAUSTEIN U.-F., BRANDT W., 1996: Antifungal Activity of the essential oil of Melaleuca alternifolia (Tea Tree Oil) against pathogenic fungi in vitro. Skin Pharmacology and Physiology No..9(6); 388–394
18. NUZHAT T., VIDYASAGAR G.M., 2013: Antifungal investigations on plant essential oils - A review. International Journal of Pharmacy and Pharmaceutical Sciences No. 5(2); 19-28
19. PÁNEK M., REINPRECHT L., HULLA M., 2014: The essential oils for beech wood protection - efficacy against wood-destroying fungi and moulds, and effect on wood Discoloration. BioResources No. 9(3); 5588-5603
20. PENG L., YANG S., CHENG Y. J., CHEN F., PAN S., FAN G., 2012: Antifungal activity and action mode of pinocembrin from propolis against Penicillium italicum. Food Science and Biotechnology No. 21(6); 1533–1539
21. PIONTEK M., LECHÓW H., 2013: Produkty biobójcze stosowane w ochronie elewacji przed korozjąbiologiczną, Zeszyty Naukowe. Inżynieria Śrorodowiska/Uniwersytet Zielonogórski No. 151(31); 86 - 95
22. REGULATION (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products
23. SHELDS J.K. ATWELL E.A., 1963:Effect of a mold, Trichoderma viride, on decay of birch by four stoprage-rot fungi. Forest Product Journal 13(7), 262-265
24. STUPAR M., GRBIĆ M., DŽAMIĆ A., UNKOVIĆ N., RISTIĆ M., JELIKIĆ A., VUKOJEVIĆ J., 2014: Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. South African Journal of Botany No. 93; 118-124
25. WOŹNIAK M., KWAŚNIEWSKA-SIP P., WAŚKIEWICZ A., COFTA G., RATAJCZAK I., 2020: Thepossibility of propolis extract application in wood Protection. Forests, 11(4), 465; 1-11
26. ZARZYŃSKI P., ANDRES B., 2009: The connection between chosen phenolic compounds occurring in wood and the range of trophic abilities of quinine fungus (Laricifomes officinalis (Vill.) Kotl. Et Pouzar) Acta Scientarum Polonorum No. 8(3); 71-80
27. ZARZYŃSKI P., ANDRES B., 2010a: Laboratoryjna ocena możliwości wykorzystania wybranych związków fenolowych naturalnie występujących w drewnie do zabezpieczania drewna lipowego przed rozkładem przez grzyby. Sylwan No. 8; 515-523
28. ZARZYŃSKI P., ANDRES B., 2010b: The connection between chosen phenolic compounds occurring in wood and the range of trophic abilities of birch bracket (Piptoporus betulinus (Bull.) P. Karst.) Folia Forestalia Polonica, Seria B, No. 41; 13-21.

Streszczenie: *Ocena właściwosci grzybobójczych olejków z Leptospermum scoparium i Azadirachta indica w stosounku do wybranych grzybów powodujacych rozkład drewna*. W publikacji przedstawiono wyniki oceny działania fungicydowego dwóch olejków eterycznych z *L. scoparium* (manuka) i *A. indica* (neem) na grzyby powodujące rozkład drewna *Trametes versicolor* i *Coniophora puteana*. Rezultaty badań wskazują, że wzrost testowanych grzybów jest wyraźnie hamowany przy udziale największych z zastosowanych stężeń badanych substancji chemicznych. Jednocześnie stwierdzono, że działanie olejku neem było mocniejsze w stosounku do grzyba *T. versicolor*. Z kolei olejek manuka działał silniej inhibujaco na wzrost grzyba *C. puteana*.

Corresponding author:

Joanna Świstak
students of the Faculty of Wood Technology
Warsaw University of Life Sciences – SGGW
email: joannaswistak123@gmail.com