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# Evaluation of fungicidal properties of post-cultured liquid medium from the culture of Kombucha microorganisms against selected mold fungi

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**Abstract:** *Evaluation of fungicidal properties of post-cultured liqiud medium from the culture of Kombucha microorganisms against selected mold fungi*. The paper presents the results of the evaluation of fungicidal activity of post-cultured liquid medium from Kombucha microrganism on molds *Alternaria alternata* and *Trichoderma viride*. The obtained results confirmed that the medium on which Kombucha microorganisms grew, have fungicidal activity against mold fungi. The lowest concentration of post-cultured liqiud medium, inhibiting the growth of *A. alternata* and *T. viride*, on the 4th day of culture, was respectively: 5% and 15%. The total absence of fungal growth was observed on medium containing 20% medium from Kombucha culture.

*Keywords*: mold fungi, Kombucha, post-cultured liquid medium

## INTRODUCTION

Wood impregnation with preservatives is currently the only method that guarantees durability of wood in various classes of use. Wood protection products, due to their chemical nature, are subject to strict requirements of law, established in the Regulation of the European Parliament and the Council No. 528/2012 on the provision and use of biocidal products. This regulation imposes on the producers of preparations for wood impregnation ensuring safety for humans, animals and the environment, during the use of impregnants and during the use of impregnated wood. One of the requirements of guaranteed safety is a positive assessment of toxicological reports, ecotoxicological biocides and risk and exposure assessments, which are one of the basic documents guaranteeing obtaining a permit for trade in wood protection products. Due to the fact that many biocides, which until recently placed on the market, were not positively verified, the market for wood protection products narrowed to those where substances that are not potentially hazardous are predominant. With regard to the safety of users of biocides and the environment, more and more studies are undertaken to assess the biocidal effectiveness of substances or chemical compounds of natural origin. Both natural substances such as essential oils, alkaloids, natural resins, but also substances of animal origin or from cultures of microorganisms and even microorganisms are evaluated. Ziglio et al. [1] used an extract of fresh chili peppers to protect pine wood from decomposition caused by *Paecilomyces variotii* and *Pycnoporus sanguineus*, while Dasilveri et al. [2] used *Acacia mearnsii* extract containing tannins as a biocide against *Pycnoporus sanguineus*, causing white decomposition of wood. Applications of plant alkaloids as potential fungicides have been the subject of research by Cofta et al. [3], while teak extracts to protect pine wood from degradation caused by brown fungus and white decay fungi were subject to analyzes carried out by Brocco et al. [4]. Substances isolated from wood with potential biocidal properties were tested by Nakayama et al. [5], Kirker et al. [6] and Mohammed et al. [7].

The use of metabolites of microorganisms for the biological protection of wood was already undertaken in the 90s of the last century, by Kundzewicz and Ważny [8]. They determined the influence of metabolites synthesized by *Trichoderma viride* on the growth of selected fungi decomposing the wood. Betlej and Andres [9] evaluated the effect of *S. cerevisiae* and *L. brevis* strains as potential biological biocides on the growth of the white fungus *Trametes versicolor*. Similar studies using microorganisms as biocides were conducted by Bruce [10] and Horvath et al. [11].

*S. cerevisiae* and *L. brevis* strains belong to microorganisms belonging to the Kombucha biofilm microflora [12]. Kombucha are symbiotic bacteria and yeasts that carry out acetic fermentation. During the fermentation process, these organisms produce numerous metabolites, including polyphenols, organic acids and ethanol, which are characterized by antimicrobial activity. Pei et al. [13] showed that *Lactobacillus plantarum*, which is a component of Kombucha microflora, produces bacteriocin, which exerts a bactericidal effect on gram-positive and gram-negative bacteria. The biocidal effects of metabolites from Kombucha culture on selected *Candid*a yeast fungi have been observed by Battikh et al. [14]. The fungicidal effect of metabolites produced by Kombuch against mold fungi found on wood has not been studied so far.

This publication presents the results of screening test of influence a post-culture liquid medium from the Kombucha microorganisms on the growth of mold fungi: *Trichoderma viride* and *Alternaria alternata*.

## MATERIALS AND METHODS

Studies on the fungicide effect of post-culture liquid medium obtained from Kombucha biofilm cultures were carried out on selected mold fungus species from the collection of the Department of Wood Science and Wood Preservation: *Trichoderma viride*, strain A-102 and *Alternaria alternata*, strain A-166. Kombucha microorganisms (wild strain) also came from the collection of this Department. The microbiological characteristics of microorganisms that are part of the Kombucha biofilm were not analyzed at this stage of the experiment. Kombucha microorganisms were grown on a liquid medium containing the ingredients: mannitol, yeast extract and peptone. The composition of the medium was in accordance with the ATCC instructions for the *Gluconacetobacter xylinus* strain. The liquid medium from the Kombucha culture was sterilized by means of syringe filters, using filters with a diameter of 0.25μm. The prepared liquid was added to the maltose-agar medium at 0.5, 1, 5, 10, 15, 20 and 30ml, in such a way that the final volume of the medium was 100ml. After thorough mixing of the contents, 10ml of the medium was poured onto Petri dishes, on which mycelium of the molds were centrally grafted. At the same time, control samples were prepared that do not contain liquid medium from the Kombucha microorganism culture. The culture was carried out under temperature - humidity conditions of 22˚C and 65 ± 2%, respectively. The assessment of the influence of medium from Kombucha on the growth of selected mold fungi was based on the measurement of mycelium growth in two perpendicular directions. The colony growth was measured at 48h intervals. Tests were terminated on the day when the substrate was completely covered in the control. Each study was done in triplicate. The results obtained were used to determine the MIC value - the minimum inhibitory concentration inhibiting the growth of microorganisms.

To verify statistical hypotheses, analysis of variance for single classification was used, using Snedecor statistics. Statistical inference was carried out for the significance level $\alpha = 0.05$ and $\alpha = 0.01$. In the case of rejection of the null hypothesis, the next step was to compare the average test of multiple comparisons - Tukey.

Statistical hypothesis:

H0: Ø 0.5= Ø 1= Ø 5= Ø 10= Ø 15= Ø 20= Ø 30= Ø K

H1: There are at least two averages that differ significantly

## RESULTS

Analyzing the obtained results, it was found that post-culture medium from Kombucha microorganisms show fungicide properties in relation to the mold fungi used in the studies. It was found that the degree of inhibition of fungal growth was dependent on the proportion of liquid medium in the growth medium. Tables 1 and 2 present the results of the assessment of

fungal growth on medium with different content of post-culture medium from Kombucha culture.

Table 1. The diameter of mycelium (*A. alternata)* growth on medium containing various concentrations of post-culture medium from the Kombucha culture

| No. | Content of metabolites in the culture medium | Day | | | | | Variability: diameter of colony growth at a given concentration of metabolites | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | $F_{emp}$ | $F_{0,05}$ | Division by Tukey |
| | [ml/100ml] | Diameter of growth | | | | | 7099.3 | 2.41 | |
| | | [mm] | | | | | Average | | |
| 1. | Control | 13.0 | 37.5 | 56.5 | 79.8 | 90.0 | 90.0 | | a |
| 2. | 0.5 | 10.5 | 35.3 | 54.3 | 78.0 | 90.0 | 90.0 | | ab |
| 3. | 1 | 12.8 | 36.0 | 53.8 | 76.0 | 90.0 | 90.0 | | ab |
| 4. | 5 | 9.8 | 28.5 | 44.8 | 64.0 | 83.5 | 83.5 | | c |
| 5. | 10 | 0.0 | 16.8 | 26.0 | 39.7 | 65.3 | 65.3 | | d |
| 6. | 15 | 0.0 | 0.0 | 10.5 | 21.2 | 40.5 | 40.5 | | e |
| 7. | 20 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | f |

Source: own study.

Table 2. The diameter of mycelium (*T. viride)* growth on medium containing various concentrations of post-culture medium from the Kombucha culture

| L.p. | Content of metabolites in the culture medium | Day | | | | | Variability: diameter of colony growth at a given concentration of metabolites | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | $F_{emp}$ | $F_{0.05}$ | Division by Tukey |
| | [ml/100ml] | Diameter of growth | | | | | 839.07 | 2.39 | |
| | | [mm] | | | | | Average | | |
| 1. | Control | 43.3 | 90.0 | - | - | - | 90.0 | | a |
| 2. | 0.5 | 42.3 | 90.0 | - | - | - | 90.0 | | ab |
| 3. | 1 | 44.0 | 90.0 | - | - | - | 90.0 | | abc |
| 4. | 5 | 30.3 | 90.0 | - | - | - | 90.0 | | abcd |
| 5. | 10 | 15.7 | 88.3 | - | - | - | 88.3 | | abcd |
| 6. | 15 | 7.0 | 61.3 | - | - | - | 61.3 | | e |
| 7. | 20 | 0.0 | 0,0 | - | - | - | 0.0 | | f |

Source: own study.

The significantly stronger fungicidal activity of post-culture medium from Kombucha microorganisms was observed for the *A. alternata* fungus. The lowest concentration of medium, inhibiting fungal growth on the second day of culture, was 5%. Complete cultivation of the mycelium medium in the control culture took place on the eighth day of the culture. At the same time, in medium containing 5% of Kombucha culture medium, the diameter of the

fungus growth was 83,5mm. At the content of 15% liquid medium, the *A. alternata* growth diameter was halved compared to the growth in the control sample. At the concentration of 20% post-culture medium, the growth of the fungus was completely inhibited. The influence of liqid medium from Kombucha culture on the growth of *T. viride* was less intense. At the same time, it was noticed that the growth of the examined fungal species in the control sample was much faster than the growth of *A. alternata*. On the fourth day of cultivation, mycelium *T. viride* completely overgrew the control medium. Determined on the fourth day of culture, 10%was the lowest concentration of Kombucha metabolites which inhibited the growth of *T. viride*. At the content of 20% of liquid medium, the growth of the fungus was completely inhibited.

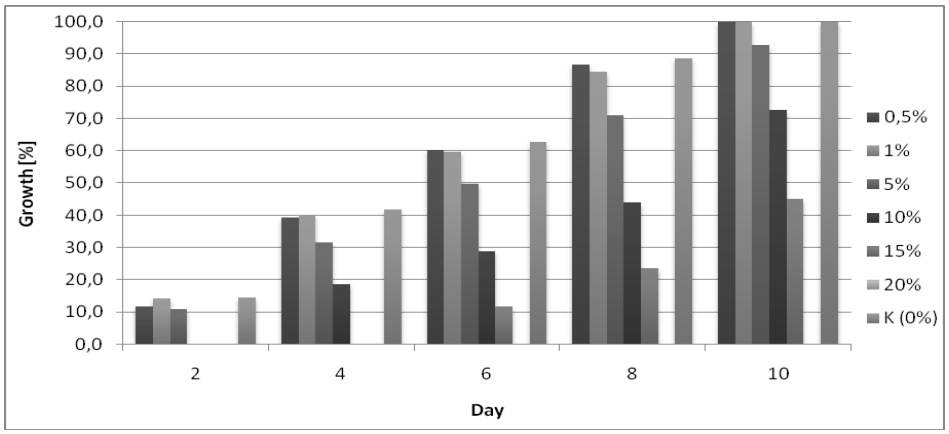


Figure 1. Percentage of overgrowth of agar-maltose medium with the addition of different concentrations of post-culture medium from Kombucha cultivation by *A. alternata* mycelium at different times of breeding
Source: own study.

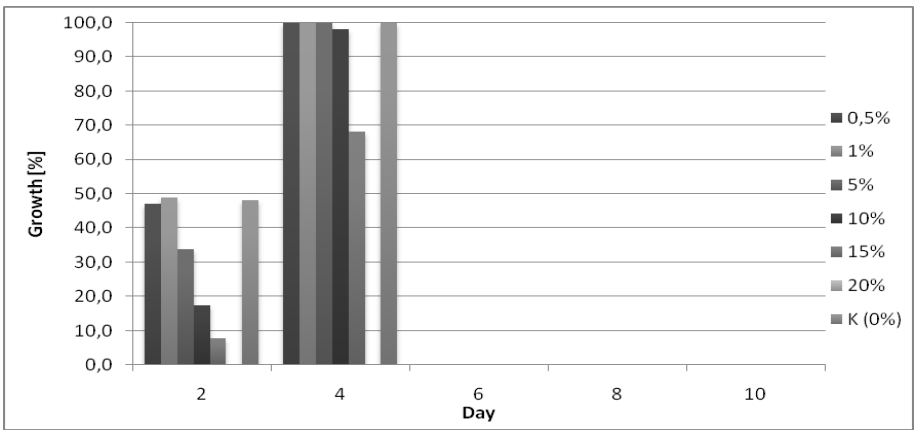


Figure 2. Percentage of overgrowth of agar-maltose medium with the addition of different concentrations of post-culture medium from Kombucha cultivation by *T. viride* mycelium at different times of breeding
Source: own study.

A graphical analysis of the percentage of growth of medium containing various concentrations of post-culture liuid medium from Kombucha microorganism's cultivation by mold fungi is presented in Figures 1 and 2.

In the assessment of the medium from Kombucha influence on growth of *A. alternata*, significant differences were found between the control growth and the test culture containing liquid medium from Kombucha in the amounts from 5 to 20%. However, no significant differences were observed in the growth of the fungus on media containing 0.5 and 1% of Kombucha culture fluid, compared to the control. In the case of the assessment of the increase of liquid medium from the Kombucha culture on the growth of the *T. viride* fungus,

significant differences were found between the control sample and the test sample only in two cases in which the content of post-culture medium in the growth medium was 15 and 20%.

## CONCLUSION

Analyzing the obtained results, it was found that the post-culture medium from Kombucha microorganisms, show a fungicidal effect on mold fungi used in the presented research. The obtained results are the basis for further analyzes, including: checking what metabolites are produced by Kombucha during their cultivation, what is the composition of microorganisms in the Kombucha biofilm and what will be the effect of this medium on fungi if it is impregnated with wood.

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**Streszczenie**: *Ocena właściwości fungicydowych podłoża pohodowlanego z hodowli mikroorganizmów Kombucha, w stosunku do wybranych grzybów pleśniowych.* W pracy przedstawiono wyniki oceny działania fungicydowego płynu pohodowlanego z hodowli mikroorganizmów Kombucha na grzyby pleśniowe *Alternaria alternata* i *Trichoderma viridie*. Uzyskane wyniki potwierdziły, że podłoże pochodzące z hodowli Kombucha wykazuje działanie fungicydowe wobec badanych grzybów pleśni. Najmniejsze stężenie podłoża, hamujące wzrost *A. alternata* i *T. viride*, w 4–tej dobie hodowli, wynosiło odpowiednio: 5% i 15%. Całkowity brak wzrostu grzybów zaobserwowano na pożywce, zawierającej w swym składzie 20% płynu pohodowlanego z hodowli Kombucha.

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