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The Effect of Ethanol Extracts and Essential Oils Obtained from Different Varieties of Mint on Wood Molding

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Abstract: This paper presents the results of research on the effect of essential oils and ethanol extracts on the growth of mold fungi on Scots pine (*Pinus sylvestris* L.) wood. The analysis of fungal growth on the microbial medium showed that the degree of inhibition of microorganism growth depends on the amount of the extract added to the medium. At the same time, it was found that the highest dose of the extract, amounting to 5.0 cm^3, almost completely inhibited the growth of fungi. In addition, it was found that mint ethanol extracts, the application of which in the wood is at least 40 g/m^2, have a fungistatic effect at the initial stage of fungal development. Solutions of essential oils turned out to be more active against fungi, although also in this case the desired biocidal effect was not achieved. Essential oils significantly slowed down the growth of the fungus *Ch. globosum*, with the strongest fungistatic effect found for 'Morocco' spearmint oil (*Mentha spicata* L.). Despite the fact that in tests on agar-maltose medium, the strongest biocidal activity against *Ch. globosum* was found for spearmint 'Crispa' (*Mentha spicata* L.) oil, the effect of growth inhibition was not so clearly visible in studies on wood. Essential oils applied to the surface of the wood slowed down the growth of *T. viride* fungus, but not to the extent that it was found in the case of *Ch. globosum*. The qualitative and quantitative composition of substances belonging to the group of terpenes and their derivatives was characterized using the GCMS technique. It was shown that the ethanol extracts of mints were dominated by substances belonging to the oxygen-containing monoterpenoid and monoterpene groups. In terms of quality, the composition of essential oils turned out to be richer.

Keywords: *Mentha* sp.; essential oil; ethanol extracts; wood; mold growth

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1. Introduction

Treating wood with preservatives is intended to increase the durability of the material. Extending the durability of wood in various working environments is possible only as a result of treating it with biocides containing active substances, permitted for use in European Union countries, in accordance with the guidelines of the Regulation of the European Parliament and of the Council No. 528/2012 on the provision and use of products biocides [1]. The regulation in question greatly limited the market of active substances permitted for use in biocidal products, which was related to the imposition of restrictions on EU Member States to ensure the maximum safety of biocides. Among the biocidal substances used to protect wood against biodegradation, arsenic and chromium compounds, fluorine compounds, phenols, naphthalenes, and numerous petroleum distillation products have been withdrawn from the market. There are only those substances for which it has

been shown in appropriate toxicological and ecotoxicological studies, as well as risk and exposure assessment, that they are safe for humans, animals, and the environment while treating or using treated wood [2]. Other active substances to be used in wood preservation are subject to a continuous process of review and testing, and when new information on possible harmfulness is obtained, confirmed by the latest state of knowledge, these substances are withdrawn from wood preservatives. Taking into account the safety of users of biocides and environmental protection considerations, more and more research is undertaken to assess the biocidal effectiveness of substances or chemical compounds of natural origin [3–5]. Compounds such as essential oils [6–9], alkaloids [10], and natural resins [11], as well as substances of animal origin or from microbial cultures [12,13], and even the microorganisms themselves [14] are evaluated. It should also be mentioned that Regulation No. 528/2012 does not limit the possibility of extending the list of active substances approved for use. Article 56 of this regulation guarantees the possibility of development and research into new substances and biocidal products [1]. Taking into account the legal provisions of Regulation No. 528/2012, it is worth looking for new, safe, and effective biocides naturally occurring in nature, which can be an alternative to a number of synthetic substances currently used in wood protection. Due to the increasing burden on the environment, decisive steps should be taken towards ecological wood treatment.

Plants are the source of many substances with fungicide activity; thus, they are of growing interest in the field of wood protection and preservation. The literature data indicate that various plant components have been tested in wood preservation against the harmful effects of fungi [15,16]. Essential oils in particular can constitute an important group of diverse substances that could be included in the formulation of traditional biocides, reducing their harmfulness to a minimum level. In the literature on the subject, the most frequently evaluated oils of plant origin in protecting wood against brown decay and mold were thyme [17,18], oregano [19–21], lavender [22], and clove [23–25]. The high fungicide potential of Lavandula angustifolia Mill oils and Lavandula latifolia Medik, as well as good wood preservation against termites, was shown by Šimůnková et al. [26]. The authors of the study showed the biocidal activity of lavender oils similar to the level of effectiveness of commercial biocides based on trivalent boron and quaternary ammonium salt. Xie et al. [27] found that Origanum vulgare L. oil is highly toxic to white and brown rot fungi. An interesting study of the literature on the effectiveness of essential oils as active ingredients in wood preservation was presented by Woźniak [28]. The author of the publication has compiled a set of results of the evaluation of the fungicide properties of various plant oils (lemongrass, neem, peppermint, rosemary, thyme), indicating very good results in protecting wood against fungi.

The biocidal potential of plant oils largely depends on the method and place of their extraction. Ludwiczuk et al. [29] report that the availability and quality of essential oils depend on the quality of the soil, the climate in which the plant grew, the amount of annual atmospheric precipitation, the time of harvesting, the method of drying, and the morphological part. The same authors indicate that the exact identification of the plant is of key importance in its further use. Studies using gas chromatography have shown that mint leaves may contain over 70 active substances belonging to the group of terpenes [30], while oregano leaves may contain up to 40 potential active substances [31]. The composition, and thus the biocidal potential of essential oils, is also determined by the genetic factors of plants, which is important in the case of successively emerging new cultivars, created by man in order to obtain the appropriate plant characteristics. An example of such diversity is the separation of different mint chemotypes even within the species [32].

The development of innovative, natural biocides as an alternative to synthetic fungicides should be one of the methods of modern development of wood preservatives. The fungicide potential of plant components could be used for research activities in the development of new, pro-ecological biocides. The interaction between natural ingredients and synthetic ingredients of wood preservative preparations is little studied. Obtaining the effect of enhancing the biocidal effect by introducing substances of natural origin

into the formulation of wood protection products, while reducing the content of active chemical substances, often harmful to the environment, would be a great prospect for the development of a new group of impregnations, safer and more environmentally friendly. Wood preservatives containing biocides of natural origin are undoubtedly important for the development of innovation and a knowledge-based economy. In addition, they clearly fit into the concepts of sustainable development, based on technological progress, but maintaining natural balance and the durability of basic natural processes. By creating new environmentally friendly biocides, we minimize problems related to the biodegradation of preparations and the residue of harmful waste in the environment.

While in the literature we can find a large group of studies describing the antifungal properties of various mint extracts, these studies mainly concern experiments conducted on agar media. The assessment of fungal growth on a porous material such as wood has not been the subject of deeper analyses so far. In addition, the possibility of evaluating the antifungal properties of different varieties of mint and identifying a variety with high fungicidal potential allows for a better characterization of the fungicide variability of different varieties of this plant and the indication of a variety that has significant indications for use in the production of new environmentally friendly biocides.

In this study, an attempt was made to determine the effect of ethanol extracts and essential oils obtained from various varieties of mint on the growth of mold fungi. An analysis of the content of ingredients that may have a biocidal effect was carried out. The intention of the authors was to determine the potential possibilities of using extracts from plant raw materials as ingredients in the development of environmentally friendly wood impregnation agents.

2. Materials and Methods

2.1. The Plant Material

Four varieties of mint were used in the research: peppermint 'Almira' (*Mentha piperita* L.) (No. 6), spearmint 'Crispa' (*Mentha spicata* L.) (No. 3) spearmint 'Morocco' (*Mentha spicata* L.) (No. 5), pineapple mint 'Variegata' (*Mentha suaveolens* Ehrh.) (No. 8) (Figure 1).

Figure 1. *Mentha* sp. used in the experiment: No. 3. *Mentha spicata* 'Morocco', No. 5. *M. spicata* 'Crispa', No. 6. *M. piperita* 'Almira', No. 8. *M. suaveolens* 'Variegata' (photographed by Anna and Marcin Dadasiewicz).

All mint varieties were cultivated on the experimental fields of the Research and Science Innovation Center in Wola Zadybska near Lublin (Poland) (51°44′49″ N 21°50′38″ E). The plants were cultivated on lessive soil, of light mineral agronomic category, with the granulometric composition of loamy sand. The soil was slightly acidic (pHKCl 6.1–6.2), with medium humus content, very high content of assimilable forms of phosphorus and

potassium, high content of magnesium, average content of manganese, zinc and iron, and low content of copper and boron. The plantations were located in the temperate climate zone, where the average daily air temperature in the growing season (May-September) was, respectively: 14.2; 17.2; 19.2; 18.1 and 12.9 °C, and the average monthly amount of precipitation ranged from 62.4 to 91.8 mm (determined on the basis of data from the Institute of Meteorology and Water Management—National Research Institute (IMiGW-PIB) from the measuring station located in Jarczew (51°48′52″ N 21°58′21″ E). Plants were harvested for testing just prior to flowering at BBCH 29 (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) growth stage. Immediately after cutting, the mint herb was dried in a laboratory drier with forced air circulation at a temperature of 32 °C. After drying the raw material, the leaves were separated from the stems. Only leaves were used for further analysis.

2.1.1. Preparation of Ethanol Extracts

A total of 10 g of mint leaves were flooded with 200 cm^3 of 60% ethanol (Polmos, Poland) and shaken for 72 h on a laboratory shaker (IKA KS 3000 icontrol, IKA-Werke GmbH & Co.KG, Staufen, Germany). The extracts were cleaned of leaves and then sterilized. Syringe filters were used for sterilization. The pore diameter of the filters was 0.22 µm.

2.1.2. The Method of Obtaining Essential Oils

The oils used in this study were obtained by distilling the dried leaves in a Deryng apparatus. After weighing 30 g (with an accuracy of 0.01 g) of the dried leaves of the tested mint cultivars, they were placed in turn in a 1000 cm^3 round-bottomed flask, covered with 300 cm^3 of distilled water and connected to the Deryng apparatus. The flask, heater, and condenser were connected and distilled for 3 h from the start of condensation. The operation was repeated for each variety of mint until obtaining samples with a volume of at least 2 cm^3.

2.2. Methods

2.2.1. Evaluation of Fungicide Properties on Agar Medium

The effect of essential oils and ethanol extracts on the growth of mold fungi was carried out on an agar medium. Two species of fungi, significant in wood preservatives testing, were used in the study: *Trichoderma viride* Pers., strain A-102, and *Chaetomium globosum* Kunze, strain A-141 (ATCC 6205), from the collection of pure cultures of the Institute of Wood Sciences and Furniture, at the Warsaw University of Life Sciences. Ethanol extracts of mint, in the amounts of 0.1, 0.5, 1.0, 2.5, and 5 cm^3, were added to a sterile Petri dish and then covered with 10 cm^3 of maltose-agar medium. Essential oils were diluted in ethyl alcohol (60 µL of oil per 10 cm^3 of 60% ethanol). Oil solutions were dosed onto the plate in the amount of 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0 cm^3. The inoculum of mold fungi, 5–6 mm in size, was inoculated centrally into Petri dishes. Cultures were performed in a Thermolyne Type 42,000 model thermal incubator (ThermoFisher Scientific, Waltham, MA, USA). The temperature of the culture was 26 ± 2 °C, and the relative air humidity was 63 ± 2%. At 48-h intervals, the growth diameter of mold fungi was measured. The diameter of mycelial growth was measured in two perpendicular directions. The tests were considered completed when the Petri dish was completely covered in the control samples. The analysis of variance using the Snedecor statistics was used to verify the statistical analysis. Statistical inference was carried out for the significance level $\alpha = 0.05$. In the case of rejection of the null hypothesis, Tukey's test was performed. The statistical hypothesis was as follows: H0: Ø 0.5 = Ø 5 = Ø 10 = Ø 15 = Ø 20 = Ø 40 = Ø 60 = ØK, H1: There are at least two means that differ significantly.

2.2.2. Wood Treatment and Assessment of Fungi Overgrowth

Samples of sapwood from pine wood (*Pinus sylvestris* L.) were selected for surface treatment. The dimensions of the wood samples were 40 × 40 × 4 mm. The density of the

wood at 12% humidity was 400 kg/m^3. Wood samples were sterilized in a steam autoclave (SMS, Warsaw, Poland). The impregnation of wood samples was carried out under sterile conditions in a laminar chamber. Using a sterile pipette, ethanol extracts of mints and ethanol solutions of essential oils were applied to one of the larger surfaces of the wood and then spread over this surface. Ethanol was applied to the control samples. Treatment was carried out by applying an appropriate amount of ethanol extract or essential oil to the largest surface of the wood samples. In the case of the ethanol extract, the spreads were 24.0, 32.0, and 40.0 g/m^2, in the case of the essential oil solution, the spreads were 140 i 180 g/m^2. The impregnated samples were left for 24 h in empty sterile vessels, and after that time they were transferred to Petri dishes with overgrown mycelium.

Treated wood samples were placed on a maltose-agar medium overgrown with mycelium, on glass spacers, avoiding the direct contact of wood with the substrate. The degree of overgrowth of the wood sample by fungi was determined on the basis of high-resolution photographic images taken periodically for each tested sample. The growth of fungi on the sample was determined as the percentage of the mycelium overgrown area of the sample to the total area of the sample tested. The percentage of overgrowth of the samples was determined with an accuracy of 5% with the help of ImageJ2 (Fiji v.1.52i) image analysis software [33]. The attack of mold fungi on wood is superficial, especially in the first period of this attack. The assumed duration of the test (14 days) corresponded to this first stage—the attack of mold on the wood surface [5].

2.2.3. GC-MS Analysis

The analysis of components in both types of samples (essential oils and ethanol extracts) was carried out on the GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) coupled with the GCMS-QP2010 mass spectrometer. An AQUATIC-2 capillary column (Phenomenex, Torrance, CA, USA) with a length of 60 m, diameter of 0.25 mm, and a film thickness of 0.25 µm was used. Samples were introduced to the column with the AOC-20i autosampler. GCMS software version 2.72 (Shimadzu, Kyoto, Japan) was used for sample analysis and the peaks were identified with the NIST11 and NIST11b mass spectrum libraries.

GC-MS separation conditions were as follows:

- Starting temperature: 75 °C, hold time 6 min, ramp rate 5 °C/min to 160 °C, hold time 4 min, ramp rate 5 °C/min to 180 °C, hold time 4 min; ramp rate 5 °C/min to 200 °C, hold time 4 min; ramp rate 5 °C/min to 220 °C, hold time 4 min; ramp rate 5 °C/min to 240 °C, hold time 4 min;
- Carrier gas: helium 5.0 (PGNiG, Warsaw, Poland);
- Carrier gas flow: 1.55 cm^3/min;
- Injection mode: split—1.8;
- Temperature of the injection: 250 °C;
- Detector voltage: 0.7 kV;
- Ion source temperature: 200 °C;
- Interface temperature: 250 °C.

3. Results and Discussion

3.1. Antifungal Properties

The analysis of fungal growth on the maltose-agar medium showed that the degree of growth inhibition depends on the amount of extract added to the medium (Tables 1 and 2). Ethanol extracts showed weak fungicidal activity. At the same time, it was found that the highest dose of the extract, amounting to 5.0 cm^3, either completely inhibited the growth of the fungi or significantly reduced the growth. Due to the fact that the solvent used also has a fungicidal effect [34], an analysis of the effect of ethanol on the growth of fungi was carried out. In order to confirm or exclude the biocidal effect of 60% ethanol on the growth of fungi, control tests were performed. It was not found that the ethanol used in amounts from 0.5 to 5 cm^3 had an inhibitory effect on the growth of the test fungi.

Table 1. Diameter of mycelium *T. viride* on a microbial medium with ethanol mint extracts.

Plant Materials	Concentration of Mint Extracts in Growth Medium (cm^3/100 cm^3)	Day of Observation			*p*-Value	α
		2	4	6		
		Growth (mm)			Tukey's Test	
(No. 3) *M. spicata* 'Morocco'	statistics F				2.02×10^{-24}	0.05
	0 (control)	65.4	90.0	-	a	
	0.5	31.8	86.7	-	a	
	1.0	29.0	74.8	-	b	
	0.1	32.0	72.0	-	b	
	2.5	17.8	55.8	-	c	
	5.0	0.0	0.0	-	d	
(No. 5) *M. spicata* 'Crispa'	statistics F				2.88×10^{-96}	0.05
	0 (control)	32.7	65.0	90.0	a	
	0.1	28.3	65.3	90.0	a	
	0.5	29.5	73.7	90.0	a	
	1.0	25.8	64.2	90.0	a	
	2.5	19.5	55.7	90.0	a	
	5.0	0.0	0.0	0.0	b	
(No. 6) *M. piperita* 'Almira'	statistics F				2.88×10^{-96}	0.05
	0 (control)	32.7	65.0	90.0	a	
	0.1	28.0	67.2	90.0	a	
	0.5	27.5	71.2	90.0	a	
	1.0	25.3	63.2	90.0	a	
	2.5	18.0	51.2	90.0	a	
	5.0	0.0	0.0	0.0	b	
(No. 8) *M. suaveolens* 'Variegata'	statistics F				2.34×10^{-31}	0.05
	0 (control)	32.7	65.0	90.0	a	
	0.1	25.7	60.0	90.0	a	
	0.5	28.3	69.2	90.0	a	
	1.0	28.2	74.2	90.0	a	
	2.5	18.3	56.5	90.0	a	
	5.0	0.0	0.0	7.2	b	

abcd is homogeneous groups by the Tukey test, *p*-value—significance of the F statistic, α—statistical significance level.

Table 2. Diameter of mycelium *Ch. globosum* on a microbial medium with ethanol mint extracts.

Plant Materials	Concentration of Mint Extracts in Growth Medium (cm^3/100 cm^3)	Day of Observation					*p*-Value	α
		2	4	6	8	10		
		Growth (mm)					Tukey's Test	
(No. 3) *M. spicata* 'Morocco'	statistics F						2.50×10^{-35}	0.05
	0 (control)	19.3	34.5	58.0	80.3	90.0	a	
	0.1	28.0	64.2	90.0	90.0	90.0	a	
	0.5	28.2	63.7	90.0	90.0	90.0	a	
	1.0	23.2	49.0	76.3	90.0	90.0	a	
	2.5	11.5	17.7	22.5	33.7	48.8	b	
	5.0	0.0	0.0	0.0	0.0	0.0	c	
(No. 5) *M. spicata* 'Crispa'	statistics F						1.49×10^{-26}	0.05
	0 (control)	20.8	51.3	90.0	-	-	a	
	0.1	24.2	54.3	88.5	-	-	ab	
	0.5	23.2	50.7	85.0	-	-	abc	

Table 2. *Cont.*

Plant Materials	Concentration of Mint Extracts in Growth Medium (cm^3/100 cm^3)	Day of Observation					*p*-Value	α
		2	4	6	8	10		
		Growth (mm)					Tukey's Test	
(No. 5) *M. spicata* 'Crispa'	1.0	22.2	48.8	79.7	-	-	abc	
	2.5	17.2	21.0	26.5	-	-	d	
	5.0	0.0	0.0	0.0	-	-	e	
	statistics F						1.44×10^{-27}	0.05
(No. 6) *M. piperita* 'Almira'	0 (control)	20.8	51.5	90.0	-	-	a	
	0.1	20.2	47.5	76.8	-	-	b	
	0.5	20.0	44.0	72.7	-	-	b	
	1.0	19.3	39.2	64.2	-	-	c	
	2.5	15.7	20.2	24.0	-	-	d	
	5.0	0.0	0.0	11.7	-	-	e	
	statistics F						6.40×10^{-31}	0.05
(No. 8) *M. suaveolens* 'Variegata'	0 (control)	20.8	51.3	90.0	-	-	a	
	0.5	21.5	52.2	90.0	-	-	a	
	0.1	22.7	54.5	82.7	-	-	b	
	1.0	19.8	44.5	73.2	-	-	c	
	2.5	16.2	20.0	24.8	-	-	d	
	5.0	0.0	0.0	12.5	-	-	e	

abcde is homogeneous groups by the Tukey test, *p*-value—significance of the F statistic, α—statistical significance level.

On the basis of the obtained results, different sensitivity of the fungi to the applied extracts was found. The growth rate of *Ch. globosum* on media with ethanol extracts was definitely lower than the growth rate of *T. viride* at the same time of the study (Tables 1 and 2). The dominant biocidal effect of a particular variety of mint on the growth of fungi was not demonstrated.

The *T. viride* fungus was insensitive to the addition of essential oil solutions. The doses used in the tests did not show fungicide activity against one of the most dangerous fungi—causing gray decay of wood. On the fourth day of culture, complete overgrowth of the microbial media was observed (Table 3). Different biocidal activity of the solutions of the tested essential oils was demonstrated against the fungus *Ch. globosum*. Growth of the fungus was completely inhibited in medium containing 2.0 cm^3 and 1 cm^3, respectively, of an essential oil solution obtained from *M. piperita* 'Almira' and *M. spicata* 'Moroc-co' (Table 4).

Table 3. Diameter of mycelium *T. viride* on a microbial medium with essential oils solution.

Plant Materials	Concentration of Mint Extracts in Growth Medium (cm^3/100 cm^3)	Day of Observation			*p*-Value	α
		2	4	6		
		Growth (mm)			Tukey's Test	
	statistics F				5.23×10^{-9}	0.05
(No. 3) *M. spicata* 'Morocco'	0 (control)	54.7	90.0	-	a	
	0.05	54.5	90.0	-	a	
	0.1	46.8	90.0	-	a	
	0.25	49.8	90.0	-	a	
	0.5	50.0	90.0	-	a	
	1.0	42.8	90.0	-	a	
	1.5	26.2	90.0	-	a	
	2.0	22.0	90.0	-	a	

Table 3. *Cont.*

Plant Materials	Concentration of Mint Extracts in Growth Medium (cm^3/100 cm^3)	Day of Observation			*p*-Value	α
		2	4	6		
		Growth (mm)			Tukey's Test	
(No. 5) *M. spicata* 'Crispa'		statistics F			1.00	0.05
	0 (control)	54.7	90.0	-	a	
	0.05	46.5	90.0	-	a	
	0.1	44.5	90.0	-	a	
	0.25	49.7	90.0	-	a	
	0.5	47.8	90.0	-	a	
	1.0	53.7	90.0	-	a	
	1.5	44.8	90.0	-	a	
	2.0	48.5	90.0	-	a	
(No. 6) *M. piperita* 'Almira'		statistics F			3.82×10^{-58}	0.05
	0 (control)	54.7	90.0	-	a	
	0.05	48.3	90.0	-	a	
	0.1	51.5	90.0	-	a	
	1.0	51.2	90.0	-	a	
	0.25	49.3	90.0	-	a	
	1.0	51.2	90.0	-	a	
	1.5	45.7	90.0	-	a	
	2.0	10.7	90.0	-	a	
(No. 8) *M. suaveolens* 'Variegata'		statistics F			0.45	0.05
	0 (control)	54.7	90.0	-	a	
	0.05	52.7	90.0	-	a	
	0.1	52.3	90.0	-	a	
	0.25	48.7	90.0	-	a	
	0.5	48.5	90.0	-	a	
	1.0	40.5	90.0	-	a	
	1.5	33.2	90.0	-	a	
	2.0	47.7	88.3	-	a	

a is homogeneous groups by the Tukey test, *p*-value—significance of the F statistic, α—statistical significance level.

Table 4. Diameter of mycelium *Ch. globosum* on a microbial medium with essential oils solution.

Plant Materials	Concentration of Mint Extracts in Growth Medium (cm^3/100 cm^3)	Day of Observation			*p*-Value	α
		2	4	6		
		Growth (mm)			Tukey's Test	
(No. 3) *M. spicata* 'Morocco'		statistics F			3.85×10^{-70}	0.05
	0 (control)	25.2	61.7	90.0	a	
	0.05	27.7	67.7	90.0	ab	
	0.1	25.7	64.3	90.0	ab	
	0.25	11.0	12.0	13.5	c	
	0.5	6.5	7.8	9.2	d	
	1.0	0.0	0.0	0.0	e	
	1.5	0.0	0.0	0.0	e	
	2.0	0.0	0.0	0.0	e	
(No. 5) *M. spicata* 'Crispa'		statistics F			2.88×10^{-96}	0.05
	0 (control)	25.2	61.7	90.0	a	
	0.05	30.0	69.8	90.0	a	
	0.1	27.2	68.3	90.0	a	

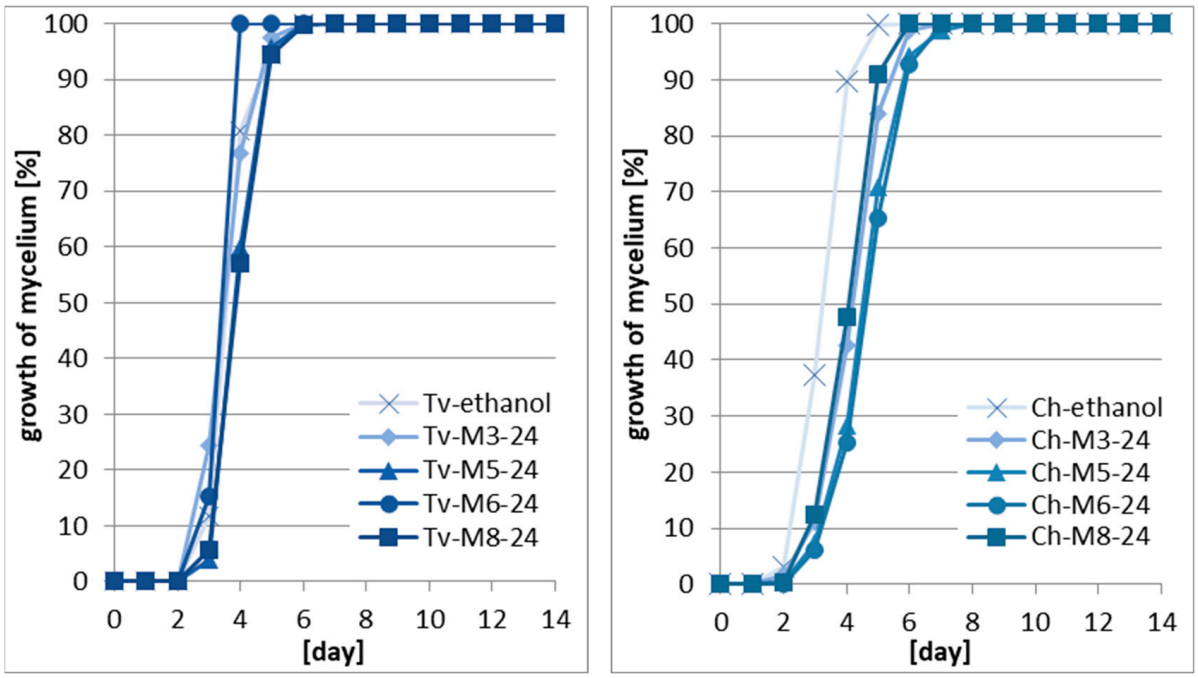
Table 4. *Cont.*

Plant Materials	Concentration of Mint Extracts in Growth Medium (cm^3/100 cm^3)	Day of Observation			*p*-Value	α
		2	4	6		
		Growth (mm)			Tukey's Test	
(No. 5) *M. spicata* 'Crispa'	0.25	21.3	50.3	77.6	ab	
	0.5	14.3	32.5	61.3	bc	
	1.0	15.5	27.8	50.2	c	
	1.5	9.5	12.8	17.2	d	
	2.0	0.0	2.5	3.7	d	
	statistics F				2.88×10^{-96}	0.05
(No. 6) *M. piperita* 'Almira'	0 (control)	25.2	61.7	90.0	a	
	0.05	25.7	62.5	90.0	a	
	0.1	25.5	56.5	90.0	a	
	0.25	27.0	65.7	88.3	ab	
	0.5	23.7	49.3	78.3	ab	
	1.0	14.8	19.3	57.0	c	
	1.5	14.2	30.3	25.2	d	
	2.0	0.0	0.0	0.0	e	
	statistics F				2.34×10^{-31}	0.05
(No. 8) *M. suaveolens* 'Variegata'	0 (control)	25.2	61.7	90.0	a	
	0.05	28.3	67.3	90.0	a	
	0.1	24.7	62.5	90.0	a	
	0.25	26.8	65.5	90.0	a	
	1.0	21.7	48.2	78.7	ab	
	0.5	21.2	44.7	71.5	b	
	1.5	13.2	28.8	48.7	c	
	2.0	13.0	20.3	32.2	d	

abcde is homogeneous groups by the Tukey test, *p*-value—significance of the F statistic, α—statistical significance level.

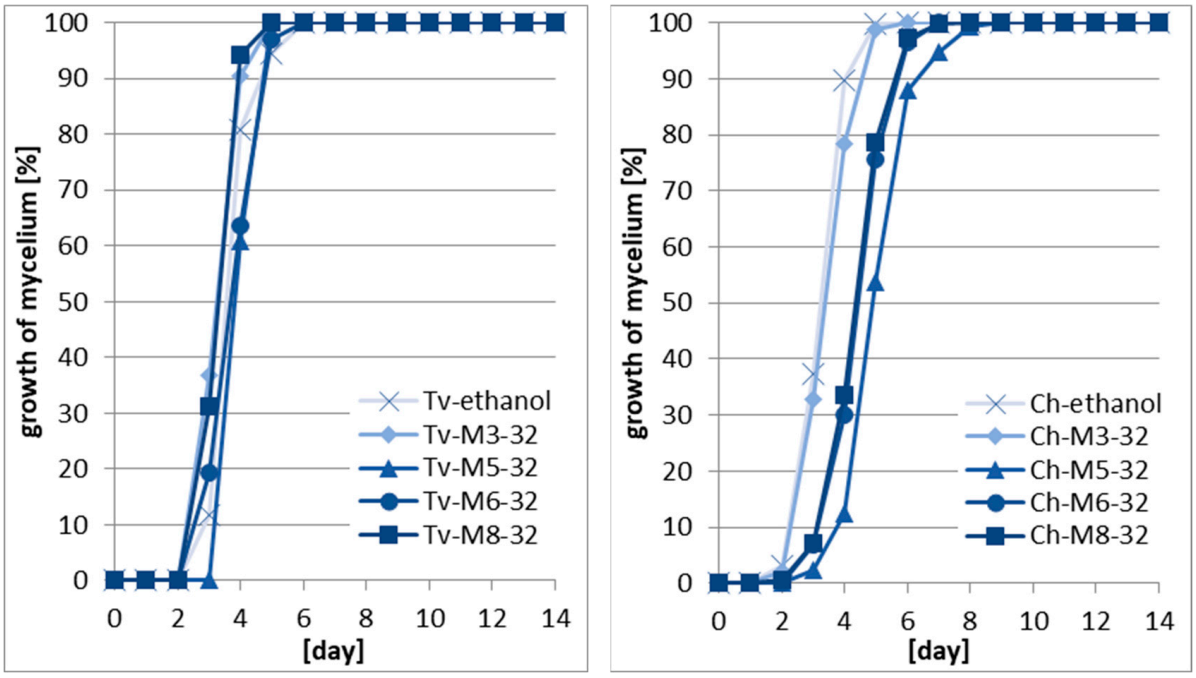
Figures 2–4 show the growth activity of mold fungi on the surface of wood samples treated with mint ethanol extracts. None of the extracts used was found to be effective in protecting wood against biodegradation. Despite the lack of fungicidal activity, it was noticed that some extracts clearly slow down the growth of the fungus *Ch. globosum*. This effect was particularly visible when the extract was applied to the wood surface in the amount of 40 g/m^2. With such application, the growth of the fungus *Ch. globosum* relative to the control was delayed by about 72 h (Figure 4b). It must therefore be concluded that mint ethanol extracts, the application of which in wood is at least 40 g/m^2, have a fungistatic effect in the initial stage of fungal development. Ethanol extracts in the doses used not only did not inhibit the growth of the *T. viride* fungus, but also stimulated the growth of the fungus, especially at lower doses (Figures 2a and 3a).

Solutions of essential oils turned out to be more active against fungi; although, in this case, the desired biocidal effect was not achieved. Essential oils significantly slowed down the growth of the fungus *Ch. globosum*, the strongest fungistatic effect was found for 'Marocco' spearmint oil (*Mentha spicata* L.) (No. 5) (Figures 5b and 6b). Although in tests on agar-maltose medium the strongest biocidal activity against *Ch. globosum* was found for oil from spearmint 'Crispa' (*Mentha spicata* L.) (No. 3), the growth inhibition effect was not so clearly visible in the studies on wood. Essential oils applied to the wood surface slowed down the growth of *T. viride* fungus, but not as markedly as was found in the case of *Ch. globosum*. In addition, it was noticed that a solution of pineapple mint 'Variegata' oil clearly stimulates the growth of the fungus (Figures 5a and 6a).



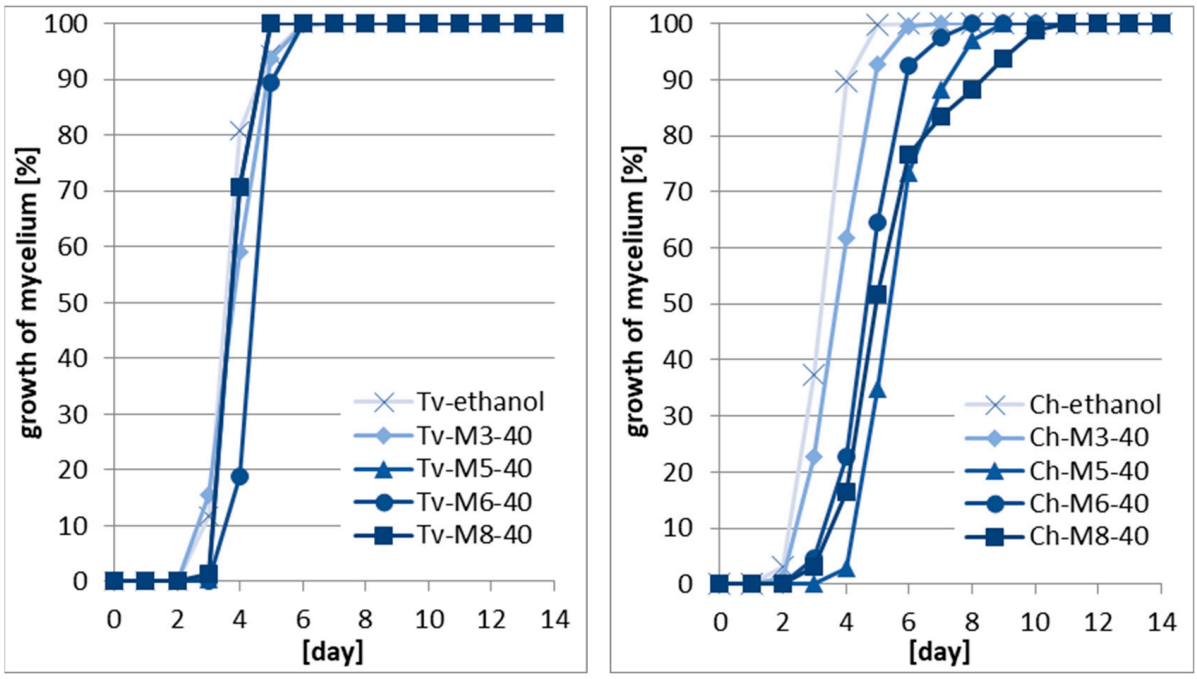
(**a**) (**b**)

Figure 2. Mold growth on surface of wood: (**a**) *T. viride*; (**b**) *Ch. globosum* (the legend of symbols: Tv-ethanol/Ch-ethanol—control, 24—sample code indicating the application of 24 g of extract per m^2, M3—*M. spicata* 'Morocco', M5—*M. spicata* 'Crispa', M6—*M. piperita* 'Almira', M8—*M. suaveolens* 'Variegata').



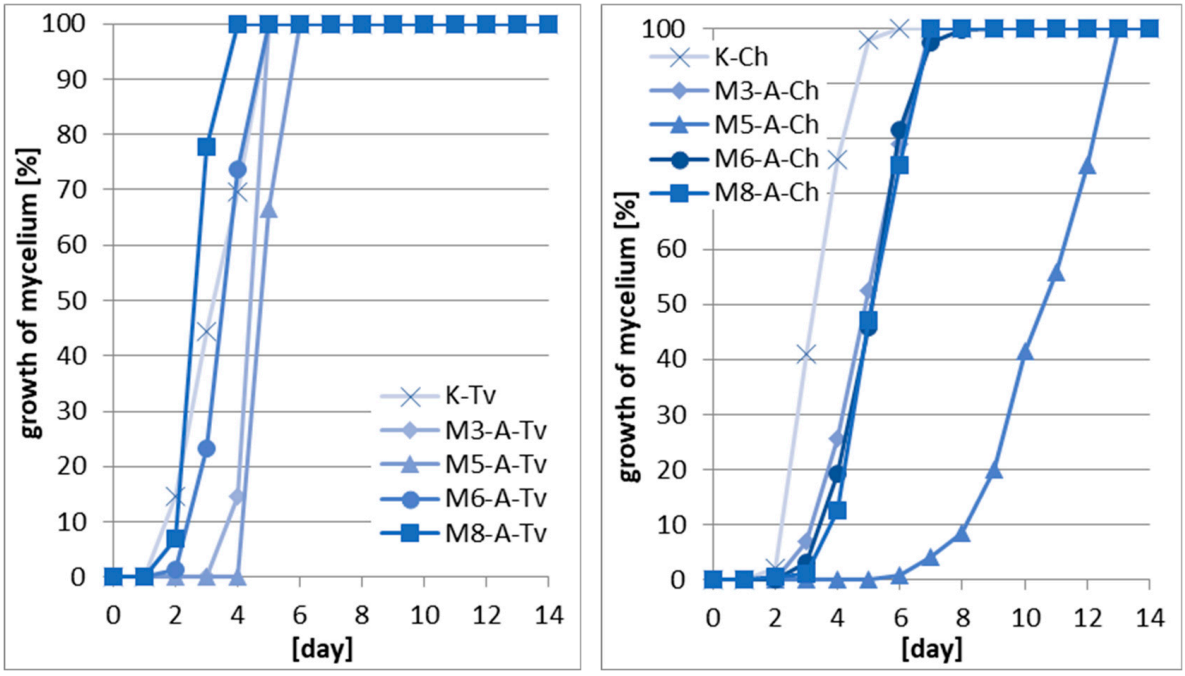
(**a**) (**b**)

Figure 3. Mold growth on surface of wood: (**a**) *T. viride*; (**b**) *Ch. globosum* the legend of symbols: Tv-ethanol/Ch-ethanol—control, 32—sample code indicating the application of 32 g of extract per m^2, M3—*M. spicata* 'Morocco', M5—*M. spicata* 'Crispa', M6—*M. piperita* 'Almira', M8—*M. suaveolens* 'Variegata').



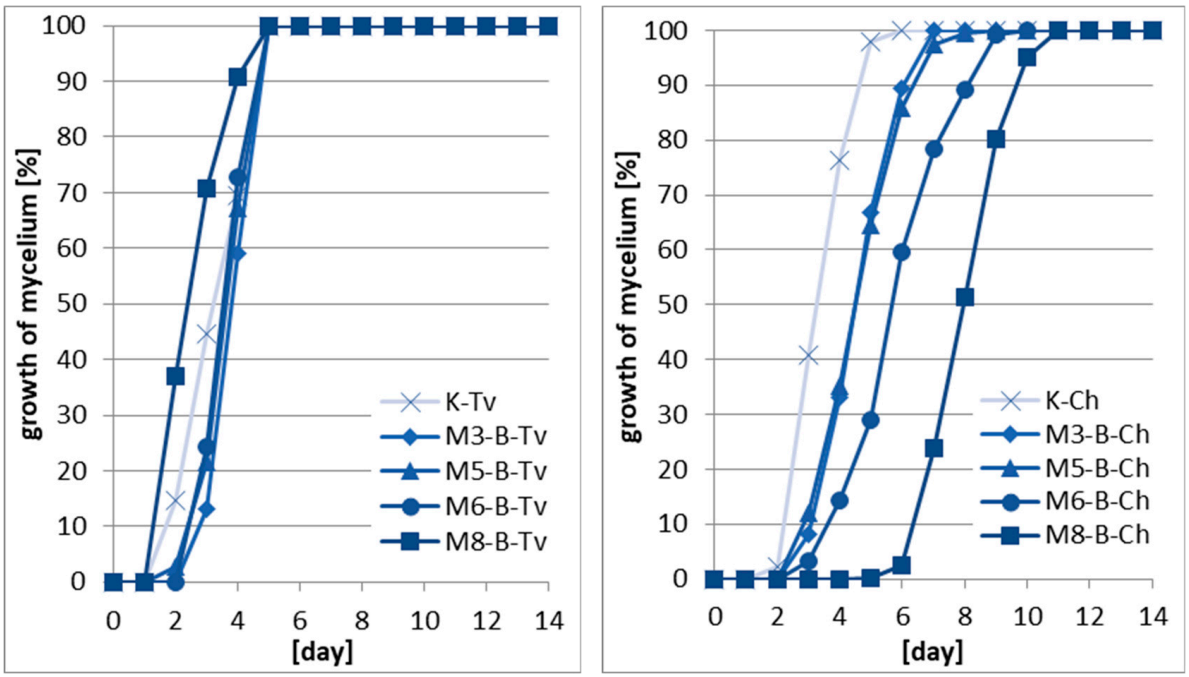
(**a**) (**b**)

Figure 4. Mold growth on surface of wood: (**a**) *T. viride*; (**b**) *Ch. globosum* (the legend of symbols: Tv-ethanol/Ch-ethanol—control, 40—sample code indicating the application of 40 g of extract per m^2, M3—*M. spicata* 'Morocco', M5—*M. spicata* 'Crispa', M6—*M. piperita* 'Almira', M8—*M. suaveolens* 'Variegata').



(**a**) (**b**)

Figure 5. Mold growth on surface of wood: (**a**) *T. viride*; (**b**) *Ch. globosum* (the legend of symbols: K-Tv/K-Ch—control, A—sample code indicating the application of 140 g of essential oils solution per m^2, M3—*M. spicata* 'Morocco', M5—*M. spicata* 'Crispa', M6—*M. piperita* 'Almira', M8—*M. suaveolens* 'Variegata').



(**a**) (**b**)

Figure 6. Mold growth on surface of wood: (**a**) *T. viride*; (**b**) *Ch. globosum* (the legend of symbols: K-Tv/K-Ch—ontrol, B—sample code indicating the application of 180 g of essential oils solution per m^2, M3—*M. spicata* 'Morocco', M5—*M. spicata* 'Crispa', M6—*M. piperita* 'Almira', M8—*M. suaveolens* 'Variegata').

The fungicide effectiveness of mint extracts largely depends on the dose of the substances used and the method of obtaining the active ingredients. Despite the failure to achieve high biocidal activity in the assumed experiment, there are examples in the scientific literature of research indicating that mint extracts can be used as products that completely inhibit the growth of mold fungi. Ali et al. [35] showed that oils obtained from *M. longifolia* cause 100% growth inhibition of *Aspergillus flavus* and *A. fumigatus*. The authors of the study, however, used much higher doses of extracts of 250–500 µL/mL. The fungicide properties of mint extracts are also confirmed by the studies of Singh et al. [36] and Desam et al. [37]. Džamić et al. [38] proved the fungicidal properties of mint essential oils against the fungi *Aspergillus flavus, A. fumigatus, Alternaria alternaria*, and *Fusarium oxyporum*. However, in this case, the authors of the study used much higher effective doses than in the presented studies. The research by Gulluce et al. [39] indicated that the method of obtaining active ingredients has a decisive influence on the effectiveness of plant extracts against mold fungi. The authors of the study found that the essential oil extracted from *M. longifolia* showed strong antimicrobial activity against the 30 microorganisms tested, while the methanol extract remained almost inactive. If mint extracts are to be a component of wood preservatives in the future, it should also be considered whether the application of such a product in doses at which full fungicidal effect was achieved would be acceptable to the producers of wood preservatives, taking into account the costs of obtaining, processing the raw material and proper wood preservation.

3.2. Chemical Composition

The qualitative and quantitative composition of substances belonging to the group of terpenes and their derivatives was characterized using the GCMS technique. These substances are known for their antimicrobial properties [40,41]. Studies have shown that mint ethanol extracts are dominated by substances belonging to the oxygen-containing monoterpenoid and monoterpene. Menthol dominated in extracts from spearmint 'Crispa'

(*Mentha spicata* L.) (No. 3) and spearmint 'Morocco' (*Mentha spicata* L.) (No. 5). On the other hand, in the ethanol extracts of peppermint 'Almira' (*Mentha piperita* L.) (No. 6) the most isomenthols were identified. Substances such as borneol, gamma-terpinene, or camphene were present only in *M. piperita* extracts. gamma-terpineol, 4-terpinenol, homocatechol, isophorone, and 3(10)-Caren-4-ol were identified only in *M. suaveolens* extracts (Table 5).

Table 5. Chemical composition of the mint ethanol extracts.

Compound	Class *	Concentration (%) Calculated Relative to the Area of Peaks Identified			
		(No. 3) *M. spicata* 'Morocco'	(No. 5) *M. spicata* 'Crispa'	(No. 6) *M. piperita* 'Almira'	(No. 8) *M. suaveolens* 'Variegata'
3-Carene	MH	0.29	-	0.51	-
Camphene	MH	-	-	2.16	-
alpha-Pinene	MH	0.92	-	1.54	5.95
beta-Pinene	MH	1.26	3.45	-	-
p-Cymene	NH	0.11	0.56	-	5.75
D-Limonene	MH	7.69	-	1.14	-
beta-Myrcene	MH	0.68	0.57	-	-
beta-Phellandrene	MH	0.67	-	1.92	2.41
Eucalyptol	OM	5.77	7.16	3.24	6.16
gamma-Terpinene	MH	-	-	1.87	-
Isopulegol	OM	0.17	-	-	-
Linalol	OM	0.6	1.6	3.34	-
Borneol	OM	-	-	0.62	-
Caproaldehyde	ON	-	1.97	-	-
Menthol	OM	69.39	58.41	-	16.97
Isomenthol	OM	-	1.97	8.66	-
alpha-Terpineol	OM	-	0.87	1.6	25.50
beta-Terpineol	OM	2.52	1.79	-	8.7
gamma-Terpineol	OM	-	-	-	7.01
4-Terpinenol	OM	-	-	-	2.55
Carveol	OM	4.93	15.67	2.15	-
Carvone	OM	1.05	28.31	-	5.46
Dihydrocarvone	OM	10.4	0.55	-	-
Pulegone	OM	0.13	3.01	1.93	5.85
Geraniol	OM	-	1.76	-	-
beta-Citral	OM	-	-	-	3.53
Piperitone	OM	0.94	0.98	-	-
Homocatechol	ON	-	-	-	15.34
Isophorone	ON	-	-	-	3.52
3(10)-Caren-4-ol	OM	-	-	-	5.46

* OM—oxygen-containing monoterpenoids, MH—monoterpenes, SH—sesquiterpenes, NH—non-terpene hydrocarbons, OS—oxygen-containing sesquiterpenoids, ON—oxygen-containing non-terpenoids.

In terms of quality, the composition of essential oils turned out to be richer, although oxygen-containing monoterpenoids also dominated (Table 6). Six substances belonging to sesquiterpenes were identified in essential oils, which were not found in ethanol extracts. In oils of spearmint 'Crispa' (*Mentha spicata* L.) (No. 3) and spearmint 'Morocco' (*Mentha spicata* L.) (No. 5). Large amounts of carvone have been identified, which is consistent with studies by other authors [42]. Chrysanthenone was identified only in 'Almira' peppermint oils (Table 6). The fungicidal activity of these substances was described in studies conducted by Reddy et al. [43].

Table 6. Chemical composition of the essential oils.

Compound	Class *	Concentration (%) Calculated Relative to the Area of Peaks Identified			
		(No. 3) *M. spicata* 'Morocco'	(No. 5) *M. spicata* 'Crispa'	(No. 6) *M. piperita* 'Almira'	(No. 8) *M. suaveolens* 'Variegata'
3-Carene	MH	0.78	0.57	-	-
alpha-Pinene	MH	-	-	1.54	5.95
beta-Pinene	MH	-	-	0.51	-
p-Cymene	NH	-	-	1.92	2.41
D-Limonene	MH	5.29	5.94	3.24	6.16
beta-Myrcene	MH	0.67	3.45	-	-
beta-Phellandrene	MH	-	-	-	-
Eucalyptol	OM	2.94	15.71	-	-
Linaol	OM	0.55	1.6	2.16	-
Borneol	OM	-	-	3.29	-
Caproaldehyde	ON	-	-	1.18	-
Ocimene	MH	-	2.22	-	-
Menthol	OM	-	2.11	-	-
Menthone	OM	-	13.56	-	-
Isomenthol	OM	-	1.97	-	-
Menthyl acetate	OM	-	1.76	-	-
alpha-Terpineol	OM	-	0.55	-	-
beta-Terpineol	OM	3.6	-	-	-
Carveol	OM	-	0.98	1.04	8.79
Carvone	OM	64.84	40.49	8.66	-
Dihydrocarvone	OM	7.24	-	-	-
p-Menth-1-en-9-al	OM	-	-	-	-
Valeraldehyde	ON	-	-	-	7.01
D-Verbenone	OM	-	-	-	8.7
Chrysanthenone	OM	-	-	67.73	-
Sabinene	MH	1.35	-	-	-
Piperitone	OM	-	1.38	-	-
Catechol	ON	0.46	-	-	2.55
Levulinic acid	ON	-	-	1.87	5.75
Coumarin	ON	1.65	0.87	1.6	16.71
p-Vinylguaiacol	ON	1.42	2.21	1.66	-
Hydroquinone	ON	-	-	1.05	5.46
Eugenol	ON	-	-	0.62	-
cis-Jasmone	ON	-	1.63	1.93	5.85
beta-Bourbonene	SH	3.36	1.79		-
Caryophyllene	SH	3.6	-	-	3.53
Humulene	SH	0.66	-	-	-
beta-Copaene	SH	1.59	-	-	15.34
beta-Elemene	SH	-	-	-	3.52
Farnesene	SH	-	-	-	5.46

* OM—oxygen-containing monoterpenoids, MH—monoterpenes, SH—sesquiterpenes, NH—non-terpene hydrocarbons, OS—oxygen-containing sesquiterpenoids.

4. Conclusions

The results obtained in the study indicate that with the applied doses of extracts and the method of wood impregnation, the full fungicidal effect was not achieved, which would indicate that mint ethanol extracts and essential oils completely protect wood against the development of mold fungi. Despite the lack of a fungicide effect on wood, several very important conclusions can be drawn:

1. The degree of fungi growth inhibition depends on the amount of extract added to the medium.
2. The highest dose of the extract, 5.0 cm^3, either completely inhibited or severely limited the growth of the fungi.

3. Ethanol extracts from the tested mints, the application of which in the wood is at least 40 g/m^2, have a fungistatic effect in the initial stage of fungal development.
4. Solutions of essential oils turned out to be more active against fungi; although, in this case, the desired biocidal effect was not achieved.
5. Essential oils significantly slow down the growth of the fungus *Ch. globosum*, with the strongest fungistatic effect found in 'Morocco' spearmint oil (*Mentha spicata* L.).
6. It was shown that ethanol extracts from mints were dominated by substances belonging to the oxygen-containing monoterpenoid and monoterpene groups. In terms of quality, the composition of essential oils is richer, although oxygen-containing monoterpenoids also dominated.
7. Six substances belonging to sesquiterpenes were identified in essential oils, which were not found in ethanol extracts.

At this stage of the findings, it is impossible to talk about the practical aspect of the obtained results. The extracts used in the proposed concentrations and the proposed application did not protect the wood against growth by mold fungi; however, the obtained results allow us to observe some differences in the biocidal activity that occurred for individual varieties of mint and individual types of extracts. This gives a starting point for further research and the search for ways to enhance the fungicide effect. If mint extracts were to be used in wood protection, further research should be undertaken to search for synergistic effects with other types of plant extracts or with synthetic fungicides. The latter research concept seems to be more promising and prospective.

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Conflicts of Interest: The authors declare no conflict of interest.

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