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ANTIFUNGAL AND LARVICIDAL EFFECTS OF WOOD VINEGAR ON WOOD-DESTROYING FUNGI AND INSECTS

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ABSTRACT

Wood vinegar is a natural organic pesticide that is effective against plant diseases and harmful insects and is used in agriculture in particular for the improvement of plant and soil quality.In different application areas, wood vinegar provides effective protection against various harmful bacteria, fungi, and insects. Based on its demonstrated protection as a pesticide and antifungal, this study aimed to use wood vinegar as an impregnation agent in wood materials. For this purpose, using the full-cell process, (*Pinus sylvestris* and *Fagus orientalis*) samples were impregnated with concentrations of 1 %, 5 %, 3 % and 6 % oak wood vinegar, obtained via the pyrolysis of sessile (*Quercus petraea*) wood at 350 °C.The samples were then subjected to tests for brown-rot (*Serpula lacrymans*) and white-rot (*Trametes versicolor*) fungi, for *Hylotrupes bajulus* (Coleoptera: Cerambycidae) larvae, and for three different mold fungi (*Aspergillus niger* Tiegh JAG-04-1003, *Penicillium brevicompactum* Dierckx FS-31, and *Trichoderma harzianum* Rifai FS-19). According to the results, the wood vinegar was found to exhibit antifungal, antimold, and larvicidal properties and consequently, could be used effectively in wood protection.

Keywords: Antifungal, pesticide, pyroligneous acids, wood preservation, wood vinegar.

INTRODUCTION

Wood vinegar (also called wood acid, liquid smoke, and pyroligneous acid) is obtained by condensing the smoke released during the process ofwood pyrolysis (Nakai *et al.* 2007, Zuraida and Budijanto 2011). Wood vinegar contains mostly acid, phenol, and carbonyl compounds. These compounds show antifungal, biopreservative, antioxidant, and insecticidal effects in addition to their use as flavoring agents (Faisal *et al.* 2019). Because of these properties, wood vinegar offers the opportunity for a wide variety of applications, includingas a sterilizing agent, deodorizer, fertilizer, antimicrobial and plant growth promoting agent, antioxidant, and wood preservative (Mathew and Zakaria 2015, Oramahi and Diba 2013, Toledo 2007, Yahayu *et al.* 2017, Zhai *et al.* 2015). Salim *et al.* (2021) reported that wood vinegar exhibited anti-mold properties in both sesenduk (*Endospermum* spp.) and jelutong (*Dyera costulata*) hardwood species as a result of treating the wood with volatile and non-volatile wood vinegar. The antifungal effects of wood vinegar obtained from various tree species and bamboo wood was investigated by Theapparat *et al.* (2015), who reported that all wood vinegars exhibited antifungal effects and that the wood vinegars from bamboo and rubberwood with higher total phenolic concentrations especially showed higher antifungal effects. As a result of the investigation of the antimicrobial effect of wood vinegar obtained from pear tree branches at different temperatures, Wei *et al.* (2010) stated that phenolic and organic acids played an important role in the antimicrobial activity. Yahayu *et al.* (2017) reported that wood vinegar obtained from pinewood residues exhibited anti-termite and anti-fungal properties, and that the 2,6-dimethoxyphenol, 3-hydroxy-4-methoxybenzoic acid and 2-methoxy-4-methylphenolcompounds in this wood vinegar made an important contribution to these properties. Effective data on anti-termite and anti-fungal properties could be obtainedfrom future applications using wood vinegar in wood protection. The

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acetic acid and phenols in wood vinegar from broad-leaved trees and bamboohave been reported to show effective antifungal properties in wood preservation (Sulaiman *et al.* 2005, Velmurugan *et al.* 2009). Oramahi and Yoshimura (2013) investigated the properties of wood vinegar obtained from the *Vitex pubescens* tree at different temperatures on a white-rot fungus (*Trametes versicolor*), a brown-rot fungus (*Fomitopsis palustris*), and termities.They reported that it had significantly repellent effect and mortality rate to termites even at low concentrations and had an antifungal effect on both fungal species. Adfa *et al.* (2017) stated that as a result of using *Toona sinensis* wood vinegar prepared at different concentrations against the *Coptotermes curvignathus* termite, termite mortality increased in parallel with the increase in concentration and after three days at the highest concentration of 8 %, all termites had died. As a result of the investigation of the antifungal effect of wood vinegar obtained from the woods of sugi (*Cryptomeria japonica*) and acacia (*Acacia mangium*) against brown-rot *Fomitopsis palustris* and white-rot *T. versicolor*, Kartal *et al.* (2004a) reported that the antifungal effect of wood vinegar increased with the increase of phenolic compounds.Wood vinegars produced from three different tree species at two different temperatures were used to impregnate beech and Scots pine samples using the full-cell process. These samples were subjected to white- and brown-rot tests before and after the leaching test. According to the results, the highest chemical retention rate was determined for wood vinegar obtained at 350 ℃ and the highest leaching rate was found for wood vinegar obtained at 280 ℃. It has been reported that wood vinegar obtained at 350 ℃ is much more effective against fungi and meets the mass loss values specified in the EN 113 (1996) (Firouzbehi *et al.* 2021).The effectiveness of different concentrations of wood vinegar obtained from oil palm empty fruit bunches and bengkirai wood at different temperatures against *Fomitopsis palustris* and *T.versicolor* fungi and the subterranean termite *Coptotermes formosanus*was investigated by (Oramahi *et al.* 2020*).* Wood vinegar obtained from both trees showed a strong antifungal effect and they reported thatwood vinegar obtained from oil palm empty fruit bunches at high temperatures (450 °C) showed anti-termite properties. Nakai *et al.* (2007) investigated the antifungal effect of wood vinegar obtained from wood-based composites produced with phenol or urea-type adhesive and meranti wood material. Theyreported that a high antifungal effect was exhibited bythe wood vinegar produced at high temperatures from the wood-based composites with phenol formaldehyde glue and by the vinegar produced at low temperatures from the composites with urea formaldehyde glue.In addition, they pointed out that wood vinegar resulting from the pyrolysis process of wood and wood-based composite materials contained many valuable chemicals and emphasized the importance of developing some of these chemicals for wood protection against fungal degradation.

The objective of this study was toevaluate the effects of wood vinegear derived from sessile oak on mould and decay fungi as well as old house boorer in laboratory scale.

MATERIALS AND METHODS

Wood samples

Scots pine (*Pinus sylvestris* L.), and Eastern beech (*Fagus orientalis* L.) sapwood was obtained from Düzce Province, Turkey. Wood specimens were cut 50 × 10 × 5 mm^3 (longitudinal × radial × tangential) for fungal tests, 50 × 25 × 15 mm^3 for powder post beetle, and 50 × 50 × 5 mm^3 for mold tests. The specimens to be exposed to the fungi were dried at 103 ºC ±2 °C overnight and weighted.

Wood vinegar

The wood vinegar obtained from Turkuaz Organic Gübre A.Ş. was produced by condensing the steam generated during the pyrolysis process applied at 350 °C to convert the oak wood to coal.The extract was reddish-brown, has a pH of 3,51 and a total acid ratio 114,1 g/100 mL (Table 1). pH values for the treatment solutions prepared were measured as 4,05; 3,80 and 3,69 for 1,5 %, 3 %, and 6 %, respectively.

Table 1: Physicochemical properties of the wood vinegar.

Analysis	Value
Ignition Residue	2,690%
Total Acidity Content	114,100 g/100 mL
Specific Gravity	1,008 g/cm^3
Tar Content	10,020 %
Color	Reddish-brown
pH	3,51

GS/MS analysis

The wood vinegar was on an Agilent 7890A GC System coupled to an Agilent 5975C inert MSD with Triple Axis Detector withAgilent HP5-MS (30 m × 0,25 mm × 0,25 µm). The oven temperature was held at 40 °C for 5 min, ramped at 5 °C / min to 100 °C for 5 min, then ramped at 20 °C / min to 225 °C and held at this temperature for 8 min. The total run time was 33,25 min. The injector temperature was fixed at 200 °C and the splitless mode was used with helium as the carrier gas. The ion source was electron ionization and the MS source temperature was set at 230 °C. The injection volume was 1,0 µL.

Impregnation of wood samples

The wood specimens were dried at 103 °C ± 2 °C for 24 h, and the dried weighted (M0) impregnation process with 1,5; 3 and 6 % concentrations of wood vinegar in water. The test specimens, except for the controls, were placed into the solution in the impregnation tank and kept under a vacuum of 600 mmHg and 3 atm pressure for 30 min. After impregnation, the remaining wood vinegar solution was rapidly wiped away using a paper towel. Next, the impregnated samples were weighed (M1). The retention amount of the samples was calculated using Equation 1. All samples were then conditioned at 20 ºC ± 2 °C and 65 % ± 2 % relative humidity (RH) for two weeks after treatment.

$$R_{(kg/m^3)} = \left(\frac{(M1-M0)\times C}{V}\right) \quad (1)$$

Where *M0* is the wood mass before treatment (kg), *M1* is the wood mass after treatment (kg), *C* is the concentration of the wood vinegar treatment solution (%), and *V* is the volume of the wood blocks (m^3).

Decay tests

The decay resistance tests of the wood vinegar-treated Scots pine and beech specimens against white-rot (*T. versicolor*) and brown-rot (*S. lacrymans*) fungi were conducted with minor modifications in the application according to the EN 113 (1996) standard. *T. versicolor* was obtained from RISH (Kyoto University, Japan), and *S. lacrymans* FD-143 was kindly provided by the Forest Products Laboratory (Madison, Wisconsin, USA). Malt extract agar (Merck, Darmstadt, Germany) medium (3,7 %) was used to grow the fungi cultures. The media and all wood samples were sterilized in an autoclave at 121 °C and 1,1 atm. pressure for 20 min. The media were then transferred to 90 mm x 15 mm Petri dishes. After inoculation (one-week alive fungal cultures were used for the test), the dishes were kept at 26 °C and 80 % RH. When the media surfaces were completely colonized by the test fungi, the treated and untreated wood samples were placed into the Petri dishes. Six replicates were used for each concentration level. The wood samples were incubatedat 26 °C and 80 % RH for 16 weeks. thenthe exposed wood samples were cleaned of the fungi mycelium and dried at 103 ºC ±2 °C overnight, then weighed again. The percent mass loss caused by the fungi was then calculated from the differences in the masses before and after the decay.

Larvae tests

The larvae tests were conducted according to EN 47 (2016) standard. Newly hatched *Hylotrupes bajulus* (Coleoptera: Cerambycidae) larvae were used for the larvae tests. Five replicates were used for each concentration level. Six larvae were inserted in a hole opened in the control and treated wood samples. A total of 120 newly hatched larvae were used, which included the control samples, according to the EN 47 (2016). The wood blocks were kept in a conditioner cabinet at 26 ºC ± 2 °C and 80% RH. All wood blocks were cut open after 16 weeks and the numbers of live and dead larvae were recorded. Larvae mortality rates were calculated by using Equation 2.

$$Mortality = \left(N_f / N_t\right) \times 100 \quad (2)$$

Here, N_t is the total larvae number before the larvae test and N_f is the number of dead larvae after the test.

Mold tests

Three mold fungi, *Aspergillus niger* Tiegh JAG-04-1003, *Penicillium brevicompactum* Dierckx FS-31, and *Trichoderma harzianum* Rifai FS-19 were grown and maintained on 3,7 % malt agar at 26 °C and 80

% RH. The *A. niger, T.harzianum,* and *P. brevicompactum* were provided by the Forest Products Laboratory (Madison, Wisconsin, USA). The suspensions of the three test mold fungi were prepared by washing the surface of the Petri dish cultures with 10-15 mL of sterile pure water. Washings were collected in three spray bottles. Three replicates were used for the each concentration level. Each of the wood specimens including controls were sprayed with 1 mL of mold spore suspension. After spraying, the wood samples were inserted into polyethylene bags and incubated at 26 °C and 80 % RH for six weeks,Specimens were visually rated on a scale of 0-5 according to ASTM 4445-10 (2019) standard in Table 2.

Table 2: Scale used to evaluate percent of wood surface covered by fungal growth a mould test (ASTM D 4445-10 2019).

Evaluation	Mold Growth %
5	>75
4	51-75
3	26-50
2	11-25
1	1-10
0	No Fungus

Statistical analyses

The SPSS software (2010) was utilized as the statistical tool.Multiple analyses of variance (ANOVA) were used to evaluate the wood species, fungal speciesi, and concentration levels and their interactions on mass losses and retention values. In addition, the Duncan's test was used to compare values.

RESULTS AND DISCUSSION

GS/MS results

The GS/MS chemical analysis of the wood vinegar obtained from oak wood at 350 °C are given in Table 3.

Results of the GC/MS analysis showed that the most abundant content was 2-methoxy-phenol (11,91 %), whereas heptane (0,52 %) was the lowest. Moreover,2-cresol (10,93 %), 4-methyl-phenol (10,86 %), 4-methoxy-3-methylbenzyl alcohol (9,7 %), 2-methoxy-4-methylphenol (9,26 %), 2,6-dimethoxy-phenol (7,38 %), 2,3-dimethylphenol (6,47 %), and phenol (5,03 %) were found in significantly higher amounts than the other compounds. Many studies have analysed pyroligneous acid with more than 200 components reported from different sources, included aldehydes, ketones, alcohols, organic acids, esters, furan and pyran derivatives, phenolics, hydrocarbons, and nitrogen compounds (Wei *et al.* 2010).

Table 3: Wood vinegar content analysis via GC/MS.

Compound Name	Percentage (%)
N-ethenyl-N-methyl-Acetamide	1,768
Heptane	0,522
3,5-Dimethylpyrazole	1,582
2-Methyl-2-cyclopentenone	1,866
2-Furyl methyl ketone	0,754
1-Methyl-1-cyclopenten-3-one	2,198
Phenol	5,032
Corylone	2,204
2,3-Dimethyl-2-cyclopenten-1-one	2,309
2-Cresol	10,935
4-methyl-Phenol	10,868
2-methoxy-Phenol	11,918
3-ethyl-2-hydroxy-2-Cyclopenten-1-one	1,155
2,3-Dimethylphenol	6,474
3,5-Dimethylphenol	4,906
2-Methoxy-4-methylphenol	9,267
4-Methoxy-3-methylbenzyl Alcohol	9,701
2,6-dimethoxy-Phenol	7,382
2-methoxy-4-propyl-phenol	2,018
2,6 - di - methoxy - 4 - methyl - phenol	4,045
7,8-dimethylbenzocyclooctene	2,419
2,3,5-Trimethoxytoluene	
2-(N-Methylamino)-4,5-dimethoxyanilinemonohydrochloride	
4-Hydroxy-3-methoxyphenylethyl alcohol	0,677

Retention decay results

Table 4 gives the retention and decay result values of the beech and Scots pine samples impregnated with oak wood vinegar at 1,5 %; 3 % and 6 % concentrations via the full-cell process and exposed to white-rot fungus (*T. versicolor*) and brown-rot fungus (*S. lacrymans*).

Scots pine and beech samples impregnated with 6 % wood vinegar exposed to white-rot fungus (*T. versicolor*) had the highest retention values (202,12 kg/m^3 and 136,58 kg/m^3), whereas the lowest values (44,24 kg/m^3 and 18,32 kg/m^3) were detected in samples impregnated at 1,5 % concentration. As with the white-rot, in Scots pine and beech samples exposed to brown-rot fungus (*S. lacrymans*), the highest retention values were found in samples impregnated at 6 % concentration (155,84 kg/m^3 and 166,06 kg/m^3) and the lowest in samples impregnated at 1,5 % concentration (46,34 kg/m^3 and 41,42 kg/m^3). Different values were obtained at the same concentration in the same tree species.This was attributed to factors such as the anatomical structure, heterogeneous structure, and density and porosity of the wood material that might have caused a difference in the retention values (Akinyele *et al.* 2015).

After 12 months, the weight loss from white-rot fungus *T. versicolor* and brown-rot fungus *S. lacrymans* was statistically significant in the samples impregnated with 1,5 %, 3 % and 6 % wood vinegar and the untreated (control group) Scots pine and Eastern beech samples. The results are given in Table 4.

Table 4: Weight loss and retention resultin Scots pine and Eastern beech for white-rot and brown-rot fungi.

Wood species	Fungal species	Concentration	Mass loss (%)	Retention results (kg/m^3)
Scots pine	TV	C	31,1 (1,29) a	
		1,5%	8,2 (0,69) b	44,24 (4,15) c
		3%	6,1 (0,23) b	102,12 (14,14) b
		6%	6,9 (0,19) b	202,12 (23,66) a
	SL	C	20,3 (1,73) a	
		1,5%	15,9 (1,21) b	46,34 (7,89) c
		3%	15,2 (1,41) b	82,83 (4,46) b
		6%	6,2 (1,10) c	155,84 (10,9) a
Beech	TV	C	53 (1,64) a	
		1,5%	26,2 (1,28) b	18,32 (3,82) c
		3%	22,5 (2,44) b	48,86 (12,25) b
		6%	11,3 (3,16) c	136,58 (12,59) a
	SL	C	30,7 (0,93) a	
		1,5%	14,4 (0,51) b	41,42 (8,89) c
		3%	13,3 (1,36) b	74,08 (21,18) b
		6%	7,98 (1,88) c	166,06 (17,74) a

TV: *Trametes versicolor*, SL: *Serpula lacrymans* Values in parentheses are the standard errors.
Different letters in the mass loss column indicate statistical differences at the 95 % confidence level.

In the Scots pine wood, *T. versicolor* white-rot fungi were found to cause the highest weight loss (31,1 %) in the control samples, where as the lowest weight loss (6,1 %) was in the samples treated with 3 % wood vinegar. No statistically significant difference was found among the weight loss rates of Scots pine samples impregnated with wood vinegar. In the Scots pine wood, *S. lacrymans* brown-rot fungi caused the highest weight loss (20,3 %) in thecontrol samples, whereas the lowest weight loss (6,2 %) was found in the samples treated with 6 % wood vinegar. No statistically significant difference was seen in the weight loss of the Scots pine samples treated with wood vinegar at 1,5 % and 3 % concentrations. In the beech wood, *T. versicolor* white-rot fungi caused the highest weight loss (53 %) in the control samples and the lowest (11,3 %) in the samples treated with 6 % wood vinegar. No statistically significant weight loss difference was found between the beech samples treated with wood vinegar at 1,5 % or 3 % concentrations.In the beech wood, *S. lacrymans* brown-rot fungus caused the highest weight loss (30,7 %), whereas the lowest weight loss (7,98 %) was obtained in the samples treated with 6 % wood vinegar. No statistically significant difference was found betweenthe weight loss of beech samplestreated with wood vinegar at 1,5 % and 3 % concentrations.

According to the results, the sessile oak wood vinegar stopped the destruction caused by both white-rot *T. versicolor* and brown-rot *S. Lacrymans*. In the literature, it has been reported that the wood vinegars of different woods or barks obtained via the pyrolysis process exhibit an antifungal effect (Adfa *et al.* 2020, Oramahi *et al.* 2018, Oramahi and Yoshimura 2013). Faisal *et al.* (2019) used copper to modify palm kernel shell wood vinegar at various concentrations (1 %, 2 % and 3 %). Even at the lowest concentration, they found

that the copper-modified wood vinegar significantly inhibited growth of the white-rot fungus *Schizophyllum commune* Fr. on Scots pine wood. The highest inhibition was found in the samples treated with the 3 % wood vinegar with added copper. Kartal *et al.* (2004b) impregnated wood samples with wood vinegar obtained via the hydrolysis of sugi (*Cryptomeria japonica* D.) and acacia (*Acacia mangium* Willd.) woods at 270 °C and 300 °C. They reported that the wood vinegar increased the resistance of the wood material against brown-rot and white-rot. The results of the GC/MS analysis of the wood vinegar obtained from oak wood (Table 3) showed that the 4-methyl-phenol, 2-methoxy-phenol, and 4-methoxy-3-methylbenzyl alcohol substances in its compound had played an active role in the antifungal effect of the wood vinegar. Adfa *et al.* (2020) used wood vinegar from *Cinnamomum parthenoxylon* (CP) wood against *Schizophyllum commune* white-rot fungus and *Fomitopsis palustris* brown-rot fungus and found that the wood vinegar had a more toxic effect against the white-rot fungus than againstthe brown-rot fungus, and that the wood vinegar at 0,9 % and 1 % concentrations inhibited the development of both fungal species by 100 %. They stated that the wood vinegar exhibited a good antifungal effect and that there were phenolic compounds released during the lignin degradation.It has been reported in the literaturethat the phenolic and acidic acid compounds in wood vinegar have a significant effect on its antifungal properties (Faisal *et al.* 2019, Sulaiman *et al.* 2005, Velmurugan *et al.* 2009, Yahayu *et al.* 2017).

Mold test results

Scots pine samples impregnated with 1,5 % and 3 % wood vinegar and untreated (control) samples were treated with Trichoderma harzianum (Th), Aspergillus niger Tiegh (An), Penicillium brevicompactum (P) and a mixture of all three fungi (M). The average growth values at the end of the 3rd and 6th weeks are given in Table 5.

Table 5: Mold growth in Scots pine samples impregnated with wood vinegar.

Concentration	Mold species	3rd week average	6th week average
1,5%	Th	0	0,5
	An	0,3	0,3
	P	0,3	0,3
	M(Th+An+P)	0	0
6%	Th	0	0
	An	0	0
	P	0	0,25
	M(Th+An+P)	0	0
Control	Th	4,67	5
	An	4,67	5
	P	5	5
	M(Th+An+P)	3,33	5

Th: Trichoderma harzianum, An: Aspergillus niger Tiegh, P: Penicillium brevicompactum, M: Combination of three mushrooms (Th+An+P).

At the end of three weeks, on Scots pine samples treated with wood vinegar at a concentration of 1,5 %, the average mold growth value of Th mold fungus was 0, whereasat the end of six weeks the average mold growth value was 0,5 and a negligible amount of mold growth was observed. At the same concentration, at the end of three and six weeks, An and P mold fungi were detected on Scots pine samples at an average rating of 0,3. It was determined that M (the mixture of the three mold fungi Th+An+P) was unable to grow on the same samples (0). At the end of three and six weeks, no growth (0) of the Th or An mold fungi or the mixture M was observed on the Scots pine samples impregnated with 6 % wood vinegar. Although the P mold did not develop at the end of three weeks, it developed only 0,25 on average at the end of six weeks. In the untreated Scots pine control group, the Th fungus showed an average growth rate of 4,67 at the end of three weeks, whereas an averagemold growth rate of 5 was detected at the end of six weeks.The An mold fungus, on the other hand, showed an average growth rate of 4,67 on the control samples at the end of three weeks, whereas an average mold growth rateof 5 was seen at the end of six weeks. The P mold fungus showed an average mold growth rate of 5 on the entire sample surface of the control group at the end of both three and six weeks. However, the average value of all three molds on the control samples was 3,33 at the end of three weeks and 5 at the end of six weeks. As a result, it was observed that although mold growth occurred at high rates on the untreated control samples, the mold fungi were unable to develop in the samples impregnated with wood vinegar. Salim *et al.* (2014) impregnated *Pinus densiflora* (Japanese red pine) samples with oak wood vinegar in different concentrations and reported that mold test results showed that the undiluted wood vinegar provided protection

against mold formationfor at least eight weeks, and wood vinegar diluted 1:1 with water protected for two weeks. Shen *et al.* (2010) stated that bamboo wood vinegar, even at 10-fold dilution, had a 99 % inhibitory rate against Aspergillus niger, Trichoderma viride, and Penicillium citrinum mold fungi. Yahayu *et al.* (2017) found that wood vinegar obtained from pineapple waste successfully inhibited the growth of Aspergillus niger (ATCC 9642) and Botryodiplodia theobromae (WML 007) mold fungi. They stated that the phenolic and acid concentrations in wood vinegar were very important for its antifungal effect. The high percentage of phenolic compounds contained in the oak wood vinegar, as shown by the GC/MS analysis (Table 3), was believed to beeffective in stopping mold fungus growth.

Larvae test results

Thirty *Hylotrupes bajulus* larvae were placed in each Scots pine sample of all groups (untreated control and those impregnated with 1,5 %; 3 % and 6 % concentrations of wood vinegar). The number of dead and live larvae and average mortality rates at the end of 16 weeks are given in Table 6.

Table 6: Larval mortality rates as a result of the larval test.

Wood vinegar concentration	Dead larvae	Live larvae	Mortality rate (%)	Average larval mortality rate (%)
Control	0	6	0	10
	1	5	16,6	
	1	5	16,6	
	1	5	16,6	
	0	6	0	
1,5%	5	1	83,3	93,4
	6	0	100	
	6	0	100	
	6	0	100	
	5	1	100	
3%	6	0	100	100
	6	0	100	
	6	0	100	
	6	0	100	
	6	0	100	
6%	6	0	100	100
	6	0	100	
	6	0	100	

Results of the larvae tests revealed that out of 30 live larvae, only three were found dead in the control samples and the average larval mortality rate was 10%. In the Scots pine samples treated with wood vinegar at a concentration of 1,5 %; 28 out of 30 larvae were found dead and the average mortality rate was 94,4 %. In the Scots pine samples impregnated with 3 % and 6 % wood vinegar, all 30 larvae died and the mortality rate was determined as 100 %. The type of wood used in the wood vinegar, the differences in the chemical structure of the wood vinegar, and the concentration levelapplied were thought to have affected the larval mortality. Lee *et al.* (2010) reported that as a termiticide, the use of wood vinegar obtained from different tree species at different temperatures accelerated the death rate of termites. Adfa *et al.* (2020) found that wood vinegar obtained from *Cinnamomum parthenoxylon* wood significantly increased the mortality rate of termites. Wititsiri (2011) stated that organic acids (acetic acid 42,91 %; 3-butenoic acid 6,89 %, butanoic acid, 2-propenyl ester 2,26 %), and ketones (1-hydroxy-2-propanone 5,14 %, 3-methylcyclopentane-1,2-dione 2,34 %) in wood vinegar played an important role in termite mortality.Termiticidal and pesticidal activities were investigated against *Odontotermes* sp. termites and striped mealy bugs (*Ferrisia virgata)* via direct contact application of wood vinegars produced from coconut shell, coir, and holy basil. The wood vinegar obtained from coconut shells resulted in the highest termite mortality (81,71 %), whereas a mixture of coconut shell and coir wood vinegars achieved insect a mortality rate of 95,12 %. They concluded that the active ingredients in the wood vinegar made an important contribution to the termiticide and pesticide properties.In this study, high amounts of 2-cresol, 4-methyl-phenol, 2-methoxy-phenol, 2-methoxy-4-methylphenol, 4-methoxy-3-methylbenzyl alcohol, and 2,6-dimethoxy-phenol were seen in the oak wood vinegar content analysis (Table 3). These components were thought to have had a significant effect on *Hylotrupes bajulus* larval mortality. Temiz *et al.* (2010) impregnated Scots pine wood vinegar obtained at different temperatures into the same wood species according

to the EN 47 via the full- and empty-cell processes. At the end of an average of 12 weeks, they achieved a mortality rate of 60% against *Hylotrupes bajulus* larvae. When we compared the Scots pine wood vinegar used in that literature study with the oak wood vinegar used in our study, we considered the wood or lignocellulosic type used in the production of the wood vinegar to have had a significant effect on its insecticidal property.

CONCLUSIONS

Scots pine and Eastern beech samples were impregnated with wood vinegar obtained from oak wood at concentrations of 1,5 %, 3 % and 6 % using the full-cell process. As a result of the examination of the weight losses from the white-rot fungus *T. versicolor* and the brown-rot fungus *S. lacrymans* at the end of 16 months, the wood vinegar was determined to have had an antifungal effect at all concentrations and in general, this antifungal effect increased in parallel with the increase in the concentration of the wood vinegar.

At the end of the 3rd and 6th weeks, the study evaluated the growth of *T. harzianum* (*Th*), *A. niger* Tiegh (*An*), *P. brevicompactum* (*P*), and a mixture of all three fungi (*M*) on Scots pine samples impregnated with 1,5 %, 3 % and 6 % wood vinegar. Nearly 100 % mold development was observed in almost all of the control (untreated) samples, whereas a negligible (0 - 0,5) mold growth was detected in the Scots pine samples impregnated with wood vinegar. The oak wood vinegar was thuseffective against mold growth in the impregnated Scots pine samples.

H. bajulus larvae were placed in the Scots pine samples impregnated with wood vinegar at 1,5 %, 3 % and 6 % concentrations, and at the end of 16 weeks, an examination of the average mortality rates revealed that the oak wood vinegar could be considered as an effective pesticide against *H. bajulus* larvae.

During the study, it was noted that the oak wood vinegar had a sharp odor. For this reason, we recommend that oak wood vinegar might be more suitable for outdoor use rather than for indoor fixtures.

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