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Preliminary evaluation of storax and its constituents: Fungal decay, mold and termite resistance

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ABSTRACT

Essential oils and their derivatives might be one of the promising preserving agents to prevent fungal decay and termite/insect attack in wood since such compounds have a long history of safe usage as antimicrobial agents in various industries. Considerable research has focused on utilizing bioactive essential oils and wood extractives based on green technologies to develop environmentally friendly wood preservatives. This study evaluated biological activities of storax from *Liquidambar orientalis* tree, some storax constituents and commercial styrax against wood-decaying fungi, mold fungi, and termites in laboratory conditions. Scots pine sapwood specimens were treated with various concentrations of styrax, storax and storax constituents i.e. cinnamyl acetate, cinnamyl alcohol and ethyl cinnamate. Treated specimens were subjected to leaching followed by soil block decay tests using two brown rot and two white rot fungi. Specimens were also subjected to two different laboratory termite resistance tests based on the standards methods by the American Society for Testing and Materials (ASTM) and Japanese Industrial Standards (JIS). Inhibitory effects of the compounds were evaluated *in vitro* against Basidiomycetes, mold, and staining fungi. Treated wood specimens were also tested for mold growth. The storax constituents, storax and styrax did not inhibit fungal decay by the brown rot fungi. The constituents and storax-treated specimens resulted in mostly "moderate resistance" to fungal decay by the white rot fungi based on the ASTM D2017 classification. Styrax only was effective against the Basidiomycetes fungi ***in vitro*** tests: however, the mold and staining fungi tested were not completely inhibited. Natural storax at the concentration level of 0.5% inhibited all fungi except *Trametes versicolor* and *Ceratocystis pilifera.* Cinnamyl alcohol inhibited fungal growth with the exception of *Aspergillus niger.* In mold tests, complete inhibition was not observed for any of the test fungi: however, cinnamyl alcohol reduced mold growth considerably on treated wood specimens. Cinnamyl alcohol was also effective against termites in unleached specimens: however, styrax and natural storax at the concentration level of 10% reduced wood consumption in treated specimens.

1. Introduction

Due to their toxic effects to the environment and mammals, certain commercial wood preservatives and broad spectrum biocides containing heavy metals are being restricted for wood protection purposes (Kartal et al., 2006). As a result, considerable amount of research has focused on developing new environmentally friendly wood preservatives to protect wood against biotic and abiotic factors (Kartal et al., 2004a, 2004b; Yang and Clausen, 2007; Clausen and Green, 2009). Essential oils and extractives from herbacious plants and trees, used most often in the food, cosmetics and pharmaceutical industries are now being evaluated as potential wood preserving compounds based on green technologies (Chang et al.. 1999, 2000; Mater et al., 2006; Yang and Clausen, 2007; Clausen et al., 2010; Du et al., 2011; Kawamura et al., 2011). Essential oils and heartwood and bark extractives of trees might be natural alternatives to traditional wood preservatives and would be

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considered safe and effective replacements since these components have both fungicidal and anti-oxidant properties (Schultz et al., 1995; Nicholas 2000; Schultz and Nicholas, 2000; Windeisen et al., 2002; Voda et al., 2003; Gerardin et al., 2004; Alfredsen et al., 2008; Yang and Clausen. 2008; Little et al., 2010; Wang et al., 2011).

Storax obtained by injuring sigla trees (*Liquidambar orientalis* Mill.) shows antiseptic properties and is used as a fixative agent in perfumery, whilst its anti-oxidant and organoleptic properties are important in the cosmetic and food industries for preservation and improvement of flavor. Sağdıç et al. (2005) have stated that the sigla tree as a member of *Hamamelidaceae* family and an endemic tree species in Turkey is locally distributed in the south-westem coastal area of the country. Major constituents of the essential oil of *L. orientalis* are terpinen-4-ol, α-terpinol, sabinene and y-terpinene along with cinnamyl cinnamete, phenylpropyl cinnamete cinnamaldehyde, cinnamyl alcohol, ethyl cinnamate, methyl cinnamate and cinnamyl acetate (Hafizoglu, 1982; Hafizoglu et al., 1996; Duru et al., 2002; Sağdıç et al., 2005; Fernandez et al., 2005; Hovaneissian et al., 2008).

We previously studied various formulations containing cinnamon bark oil. cinnamaldehyde, cinnamic acid, methyl and ethyl cinnamate, cinnamyl alcohol, cinnamyl acetate, 2-amylcinnamaldehyde and hexylcinnamaldehyde for their ability to inhibit wood decay and termite attack (Kartal et al., 2006). One test formulation with cinnamaldehyde was found to be effective against both brown rot and white rot test fungi selected for the study. Of the formulations with cinnamic acid derivatives, only two formulations showed inhibitory effects against the fungi tested. One formulation with cinnamic acid was effective against the white rot fungus. A series of formulations with cinnamon bark oil, cinnamaldehyde, methyl and ethyl cinnamate, cinnamyl alcohol, and cinnamyl acetates provided no protection against the decay fungi. The two formulations with cinnamaldehyde and cinnamic acid had strong activity against subterranean termites, *Coptotermes formosanus* Shiraki. Cinnamic acid and most of its derivatives are believed to be toxic to many microorganisms. Said et al. (2004) showed that cinnamic acid effectively inhibited *Neurospora crassa* in glucose and sucrose medium. Wang et al. (2005) found that cinnamaldehyde had strong antifungal activity against *Trametes versicolor* and *Laetiporus sulphureus* in agar tests. Chang and Cheng (2002) and Park et al. (2000) have stated that cinnamic acid and its derivatives are effective against termites and insects. Sağdıç et al. (2005) studied inhibitory effects of storax against 20 species of bacteria. Seven of these were not inhibited by any of the concentrations of storax; however, the results showed that storax had antibacterial activity against many bacteria at concentrations of 10% and against some bacteria at concentrations of 1.0, 0.4 and 0.2%.

Due to its potential anti-oxidant and biocidal properties, storax from *L. orientalis* tree bark, gum storax (commercial styrax) and some storax constituents (cinnamyl alcohol, ethyl cinnamate. and cinnamyl acetate) along with heartwood of *L. orientalis* were tested against various fungi and termites in laboratory conditions. Scots pine specimens were first treated with various concentrations of the above stated compounds and subjected to leaching before decay and termite resistance tests. In the study, the effects of the compounds on mold growth in wood specimens and inhibition of fungal growth in vitro were also evaluated.

2. Materials and methods

2.1. Wood specimens

A Scots pine (*Pinus sylvestris* L.) tree was harvested from Aladağ, Bolu, Turkey on February 10, 2009 for the research. All test specimens were prepared from Scots pine sapwood. The specimens were free of knots, and visible concentration of resins, and showed no visible evidence of infection by mold, stain, or wood-destroying fungi.

2.2. Preparation of constituents of storax wood for preservative treatments

Gum storax (commercial styrax), cinnamyl alcohol ($C_6H_5CH=CHCH_2OH$), ethyl cinnamate ($C_6H_5CH=CHCOOC_2H_5$) and cinnamyl acetate ($CH_3CO_2CH_2CH=CHC_6H_5$) as constituents of storax were tested in the study (Fig. 1). All storax constituents and commercial styrax were purchased from Sigma–Aldrich, Germany. Natural storax was obtained from *L. orientalis* trees grown in Mugla, Turkey and storax solutions were prepared by using ethyl alcohol. For preparations of working solutions of styrax and cinnamyl alcohol, ethyl alcohol only was used as a solvent. Cinnamyl acetate and ethyl cinnamate were first dissolved with ethyl alcohol and working solutions were then prepared with distilled water (DI).

2.3. Treatment of wood specimens

All specimens were pre-weighed and conditioned at 20 °C and 65%RH (relative humidity) for two weeks before treatment. Specimens were vacuum-treated (45-min vacuum at 550-mm Hg) with the solutions. Treated specimens were dried at 40 °C for 3 days, reconditioned at 20 °C and 65% RH for two weeks and reweighed.

2.4. Leaching procedure-I

This procedure was performed for decay and termite resistance tests based on the AWPA E10-09 (AWPA, 2010) and the ASTM D3345-08 (ASTM, 2010) test methods, respectively. The leaching procedure was similar to the American Wood Protection Association (AWPA) Standard Method E11-06 (AWPA, 2010) for one half of the specimens. After the conditioning period, specimens were placed into a 1000 ml bottle, submerged in deionized water, and subjected to a vacuum to impregnate the blocks with deionized water. The specimen bottles were subjected to mild agitation and the water was replaced after 6 h, 1 day, 2 days, and every 2 days thereafter for a total of 14 days.

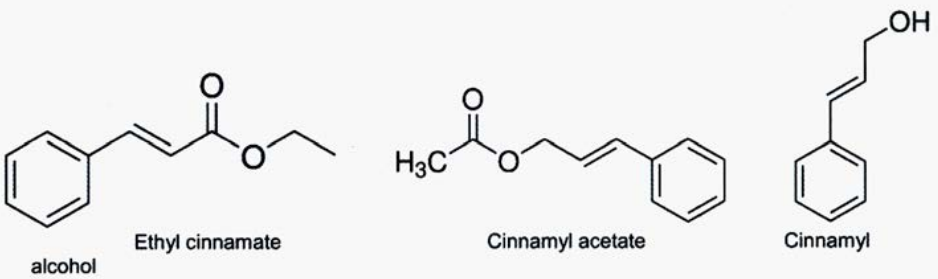

Fig. 1. Chemical formulas of ethyl cinnamate. cinnamyl acetate, and cinnamyl alcohol.

2.5. Leaching procedure-I1

A second leach test was carried out for termite resistance tests based on the JIS K 1571 (JIS, 2010) test method. The procedure was conducted according to the same standard test method. The JIS leaching process involved immersing wood blocks in distilled water, stirring with a magnetic stirrer (400–500rpm) at 27 °C for 8 h followed by drying at 60 °C for 16 h. This cycle was repeated 9 times. After each leaching cycle, water was replaced at a ratio of 10 volumes of water to 1 volume of wood.

2.6. Decay resistance rests

Treated and then leached wood specimens (19 by 19 by 19 mm) were tested against two brown rot and two white rot fungi in soil block tests based on the AWPA E10-09 standard method (AWPA. 2010). Wood specimens (20 by 20 by 10 mm) from heartwood portions of storax trees were also tested for natural durability by using the same method and fungi. In the tests, southern pine feeders were inoculated with the brown rot fungi *Postia placenta* (Fries) Lars. & Lomb. MAD 698, and Gloeophyllum trabeum (Pers:-Fries) Murrill, MAD 617, and maple feeders were inoculated with the white rot fungus, *Irpex lacteus* HHB 7328 and *T. versicolor* (L.: Fr.) Quel. MAD 697. The cultures were obtained from USDA Forest Service, Forest Products Laboratory, Madison, WI, USA. All cultures were maintained on malt-agar medium in Petri dishes at 27 ± 2 °C until needed. A piece of fungus inoculum equivalent to approximately 10 mm square from near the leading edge of mycelium in Petri dish cultures was cut. Sections of inoculum was placed on an edge of the feeder strip on the soil. The culture bottles with lids released one-fourth turn from a tightened position were incubated at 27 °C and 70% RH for 3 weeks until the fungus completely colonized each feeder. Following conditioning period, treated wood specimens were steam-sterilized and placed on actively growing feeders in soil bottle cultures. Soil bottles were incubated at 27 °C, 70% RH for 12 weeks. Following incubation, surface mycelium was brushed from each specimen before the specimens were oven-dried at 40 °C for 24 h, and reconditioned at 27 °C and 70% RH to a constant weight. Average percentage mass loss was calculated for specimens in each treatment group.

2.7. Assessment of inhibition of fungal growth

The compounds were also used to determine their ability to inhibit growth of various fungi by inclusion in 2%.w/v (malt extract agar) MEA dishes in vitro. The solutions of the constituents were first prepared at a concentration of 10%as stated before and then these solutions were mixed with liquid growth medium (2%MEA). The mutant strain brown rot fungi, *P. placenta* and *G. trabeum*, white rot fungi, *T. versicolor* and *I. lacteus*, sapstaining fungus, *Ceratocystis pilifera* (*Ophiostoma piliferum*) (Fr.:Fr.) Syd. & P. Syd. CPI 6702, and mold fungus *Aspergillus niger* 2.242 cultures (7 days old) that were incubated on 2% malt extract agar (MEA) plates at 27 °C were used for the preparation of individual inocula. The brown rot and white rot fungi were obtained from USDA Forest Service Forest Products Laboratory, Madison, WI, USA and the sapstaining and mold fungi were from RISH, Kyoto University, Kyoto, Japan. A piece of fungus inoculum equivalent to approximately 10 mm square from near the leading edge of mycelium in Petri dish cultures was cut. The pieces were then placed in the center of 2%agar plates incorporated with the test constituents. Growth inhibition of the fungi on the plates was tested by incorporating the constituents at varying dilutions prepared with liquid growth medium (2% MEA) (0.005%, 0.01%, 0.05%, 0.1%,0.5% v/v). The growth medium and solutions were autoclaved for 15 min at 121 °C and 103.4 kPa (15 psi). The medium was then poured into Petri dishes (40 mm diameter) and left in a conditioning room at 27 "C. After cooling, the Petri dishes were centrally inoculated with a single plug (5 mm diameter) of each fungus. Controls were set up using Petri dishes containing growth medium only. Three replicates were set up for all tests and controls. All cultures were incubated in a conditioning room at 27 °C until the growth of the fungi in the control plate had reached the edge of the Petri dishes. The colony diameter was measured daily and the percentage mycelia inhibition calculated according to the following equation:

$$I = [(C - T)/C] \times 100$$

where,

I = inhibition, %
C = colony diameter of mycelium from control Petri dishes, mm
T = colony diameter of mycelium from the Petri dishes containing the solutions, mm

If the inhibitory ratio was greater than 20%, the test fungus was considered inhibited and the minimal inhibitory concentration (MIC) for that fungus was then marked in bold in the result table.

2.8. Mold resistance tests

Treated wood specimens (7 mm tangential by 20 mm radial by 7 cm long) were evaluated for resistance to mold fungi according to the American Society for Testing and Material (ASTM) D4445-10 (ASTM, 2010). No leaching was performed before mold testing. Three mold fungi, A. niger 2.242. *Penicillium chrysogenum* PH02. and *Trichoderma viride* ATCC 20476 were grown and maintained on 2% malt-agar (Difco, Detroit, MI, USA) at 27 °C. and 80% RH. All fungi were obtained from the USDA Forest Service Forest Products Laboratory, Madison, WI. USA. A mixed spore suspension of the three test fungi were prepared by washing the surface of individual 2 week-old Petri plate cultures with 10–15 ml of sterile DI water. Washings were combined in a spray bottle and diluted to approximately 100 ml with DI water to yield approximately 3×10^7 spores ml-1. The spray bottle was adjusted to deliver 1 ml inoculum per spray. Wood specimens (5 specimens per treatment group) were sprayed with 1 ml of mixed mold spore suspension and incubated at 27 °C and 80% RH for 4 weeks. Following incubation, specimens were visually rated on a scale of 0–5 with 0 indicating the specimen is completely free of mold growth and 5 indicating the specimen was completely covered with mold growth (0: no growth, 1:20%, 2: 40%, 3: 60%, 4: 80%, 5: 100% coverage with mold fungi).

2.9. Termite resistance tests based on the ASIM D3345-08 test method

A no-choice termite resistance test with *Reticulitermes flavipes* Kollar (Eastern subterranean termites) was performed using five leached and unleached test specimens (25 by 25 by 5 mm) for each treatment group. Termites were collected from Janesville, WI, USA. One specimen was placed in the bottom of an acrylic cylindrical container (90 mm diameter and 60 mm height) with 1 g of *R. flavipes* and moist sand. The containers were maintained at 27 °C and 85% RH for 4 weeks based on the ASTM D3345-08 standard method (ASTM,2010). Tests were periodically checked for moisture and mortality. At the end of the test period of 4 weeks, wood specimens were taken out. oven-dried, reconditioned at 27 °C and 70%RH, and reweighed to calculate mass losses.

2.10. Termite resistance tests based on the JIS K 1571 test method

Untreated and treated specimens (20 × 20 × 10 mm) were exposed to the subterranean termites, *C. formosanus* Shiraki, according to the JIS K 1571 standard method (JIS, 2010) for three weeks. An acrylic cylinder (80 mm diameter, 60 mm height) whose lower end was sealed with a 5 mm thick hard plaster (GC New Plastone, Dental Stone, GC Dental Industrial Corp., Tokyo, Japan) was used as a container. A test specimen was placed at the center of the plaster bottom of the test container. A total of 150 worker termites collected from a laboratory colony of Research Institute for Sustainable Humanosphere (RISH), Kyoto University, Japan were introduced into each test container together with 15 termite soldiers. Five wood specimens per treatment were assayed against the termites. The assembled containers were set on damp cotton pads to supply water to the specimens and kept at 28 °C and >85% RH in darkness for three weeks. The mass losses of the specimens due to termite attack were calculated based on the differences in the initial and final oven-dry (60 °C, 3 days) weights of the specimens after cleaning off the debris from the termite attack. Five leached and unleached specimens were tested for each treatment group.

2.11. Statistical analysis

Statistical analysis was conducted using the SPSS program in conjunction with analysis of variance (ANOVA). Duncan s multiple range test (DMRT) was used to test statistical significance at $\alpha = 0.05$ level.

3 Results and discussion

Storax is obtained by injuring L orientalis trees and scraping their bark away. It is a semi-liquid, sticky, and transparent compound with bitter taste and odor. It has a characteristically cinnamic acid smell. Its originally brownish-yellow color turns to greyish when exposed to weather (Hafizoglu.1982).

The mass losses that occurred in treated specimens exposed to decay fungi for three months are shown in Table 1. None of the formulations containing commercial styrax, storax and its constituents showed complete inhibition of decay fungi. In the tests against the white rot fungus, *T. versicolor*, only commercial styrax and storax at 10%, and cinnamyl alcohol at 3 and 5% (unleached ,specimens) concentrations resulted in decreased mass losses when compared to control scots pine specimens. There was a mixed effect of the constituents on decay resistance of treated specimens with the solutions of commercial styrax and storax constituents. In most cases, mass losses in treated specimens were less than those found in untreated scots pine specimens; however, no complete protection by the constituents was provided. The constituents resulted in mostly moderate resistance to fungal decay when treated specimens were exposed to the white rot fungi tested based on the ASTM D2017 classification (Table 2) (ASTM, 2010). However, the brown rot fungi tested caused higher mass loss in the treated specimens compared to the white rot fungi and were all consequently classified as non-resistant based on the ASTM classification. Interestingly, mass losses in some leached specfimens were lower than those in unleached specimens. In this study, however, no chemical analyses were performed to determine the amount of the

Table 1
Mass losses (%) in the specimens in decay resistance tests.

Compounds/Specimens	Concentration	Leaching	Fungi							
			I. lacteus		*T. versicolor*		*G. trabeum*		*P. placenta*	
			Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
Styrax	1%	Leached	47.02c	4.26	35.66c	5.73	53.29cd	5.24	66.11a	4.00
	1%	Unleached	51.62b	5.54	40.07b	1.54	55.71c	8.16	64.28 ab	3.11
	3%	Leached	33.17f	5.66	33.93c	10.07	45.80ef	2.57	21.62g	3.16
	3%	Unleached	27.18h	4.82	34.66c	6.73	53.59cd	5.08	57.66c	6.80
	5%	**Leached**	28.53h	4.18	**21.62f**	3.16	25.56g	7.82	59.60b	3.41
	5%	**Unleached**	34.86f	6.97	**22.01f**	3.10	56.03c	1.68	59.11b	6.49
	10%	**Leached**	30.44g	3.42	**10.46gh**	**1.61**	61.57b	11.04	43.01d	5.10
	10%	**Unleached**	25.79hi	6.38	**12.77g**	**1.81**	51.81d	8.08	39.58e	8.08
Storax	1%	Leached	38.87e	3.12	41.02b	3.87	47.98e	3.76	54.21c	3.01
	1%	Unleached	45.98d	2.14	33.98c	4.12	43.21f	3.45	55.32c	3.63
	3%	Leached	30.32g	4.21	29.09d	2.90	38.98 fg	2.88	34.98f	2.91
	3%	Unleached	32.98g	7.32	34.76c	2.11	41.32f	3.54	53.21c	2.62
	5%	Leached	26.03h	2.71	25.98e	2.45	54.90cd	2.45	54.23c	3.40
	5%	**Unleached**	39.13e	2.09	**21.89f**	3.12	51.34d	2.90	56.89c	3.76
	10%	**Leached**	27.98h	3.29	**9.87h**	**1.09**	45.98ef	3.21	32.87f	2.78
	10%	**Unleached**	**23.01i**	2.05	**8.87h**	**1.00**	50.34d	5.67	39.45e	2.81
Cinnamyl acetate	3%	Leached	45.54d	6.05	32.02c	4.86	58.19c	5.42	67.81a	3.94
	3%	Unleached	44.52d	2.54	26.04e	5.66	57.45c	6.91	67.88a	1.15
	5%	Leached	37.81e	6.08	42.95b	10.18	51.95d	8.53	60.97b	3.63
	5%	Unleached	47.11c	1.71	40.42b	4.76	54.72cd	1.96	67.97a	4.24
Cinnamyl alcohol	3%	**Leached**	44.82d	2.85	**13.54g**	3.68	56.73c	9.75	61.45b	2.78
	3%	**Unleached**	45.51d	7.74	**8.14h**	**1.56**	49.90d	11.43	59.65b	5.85
	5%	**Leached**	48.31c	8.47	**11.85g**	4.16	51.92d	4.68	63.77 ab	3.10
	5%	**Unleached**	25.71hi	6.11	**8.96h**	**1.72**	37.55 fg	5.95	33.70f	8.86
Ethyl cinnamate	3%	Leached	39.07e	5.88	34.92c	5.78	70.03a	4.25	61.87b	3.80
	3%	Unleached	38.53e	4.43	42.42b	4.07	61.69b	2.25	65.21a	1.71
	5%	**Leached**	**18.47j**	5.84	**23.33f**	2.70	58.08c	4.22	66.85a	0.99
	5%	**Unleached**	**14.60k**	1.70	**21.56f**	3.22	46.35ef	2.75	55.65c	4.35
Storax heartwood	–	–	61.96a	1.94	62.53a	3.02	52.00d	2.40	67.46a	2.41
Pine sapwood	–	Alcohol extracted	51.48b	1.36	42.28b	4.28	61.46b	6.06	64.56 ab	2.31
		Unextracted	47.03c	3.39	40.95b	3.91	56.06c	2.36	66.28a	4.28

Each value is average of nine specimens for each fungus and treatment group. Alcohol extraction was carried out for 48 h in Soxhlet apparatus. The same letters in each column indicate that there is no statistical difference between the specimens according to Duncan's multiply range test. Bold figures state "highly resistant" and "resistant" specimens based on average mass losses occurred in the specimens (0–10%:highly resistant; 11–24%:resistant) according to the ASTM D2017 classification.

Table 2
Decay resistance expressed as either weight loss or residual weight according to ASIM D2017-05 (ASTM. 2010).

Average mass loss (%)	Average residual mass (%)	Indicated class of resistance to a specified test fungus
0 to 10	90 to 100	Highly resistant
11 to 24	76 to 89	Resistant
25 to 44	56 to 75	Moderately resistant
45 or above	55 or less	Slightly resistant or non-resistant

components remaining in the specimens after leaching in order to clarify the leaching effect on decreased mass losses.

Heartwood specimens of *L. orientalis* were also susceptible to fungal decay even though it has a dark color. The color of heartwood depends on the presence/absence. characteristics, and concentrations of extractives in the wood (Gierlinger et al., 2004). The heartwoods of some wood species have varying degrees of natural decay resistance, which is greatly affected by differences in the protective qualities of the wood extractives. However, Taylor et al. (2002) have reviewed that extractive content in heartwood does not necessarily correspond to natural durability and decay resistance is sometimes weakly correlated with variations in the heartwood compounds. Scheffer and Morrell (1998) also classified similar species *Liquidambar styraciflua* (styrax tree) heartwood as non-resistant or perishable based on laboratory tests. According to the ASTM classification, mass losses in control specimens prepared from pine sapwood classified the specimens as non-resistant to fungal decay by *I. lacreus,* and the two brown rot fungi tested; however, slightly lower mass losses occurred in the specimens exposed to *T. versicolor.*

Table 3 shows percentage inhibition obtained in antifungal activity tests *in vitro.* The storax constituents tested revealed different degrees of inhibition on the growth of the fungi. In the tests, when the inhibitory value was greater than 20%, the test fungus was considered inhibited. Based on this evaluation, concentrations of commercial styrax ranging from 0.1% to 0.5% were able to inhibit the growth of the white rot fungi, *I. lacteus, T. versicolor,* and the brown rot fungi, *P. placenta* and *G. trabeum;* however, the mold fungus, *A. niger* and the staining fungus, *C. pilifera* were not inhibited by styrax. Storax (0.5%) from *L. orientalis* bark inhibited *I. lacreus.* both brown rot fungi, and *A. niger.* Cinnamyl acetate only inhibited *I. lacreus, P. placenta* and *A. niger* only were inhibited by cinnamyl acetate. Only the highest concentration of cinnamyl alcohol was able to control all test fungi except *A. niger.* No inhibition for *C. pilifera* was seen for ethyl cinnamate; however, 0.5% of ethyl cinnamate controlled all other fungi in this test. *C. pilifera* was only inhibited by cinnamyl alcohol. As shown in the evaluation of inhibition values, the best results were attained at the concentration level of 0.5% and the concentrations lower than 0.5% was generally less effective against the fungi in the ***in vitro*** test.

Considering decay resistance tests, similar results were obtained with a number of formulations with cinnamaldehyde, methyl and ethyl cinnamate, cinnamyl alcohol, and cinnamyl acetate in decay resistance tests (Kartal et al.. 2006); even though cinnamic acid and most of its derivatives are known to be toxic to many microorganisms (Said et al., 2004). A study by Wang et al. (2005) showed that cinnamaldehyde had strong antifungal activities against white

Table 3
Percentage inhibition ratio values obtained in toxicity tests.[a]

Fungi	Concentration (%)	Compounds				
		Styrax	Cinnamyl acetate	Cinnamyl alcohol	Ethyl cinnamate	Storax
I. lacteus	0.005	0	0	0	0	0
	0.01	0	0	0	0	0
	0.05	0	0	0	0	0
	0.1	7.5	**100**	0	0	0
	0.5	**32.5**	**100**	**100**	**37.5**	**45**
T. versicolor	0.005	0	0	0	0	0
	0.01	0	0	0	0	0
	0.05	0	0	0	0	0
	0.1	**20**	0	0	0	0
	0.5	**50**	0	**100**	**37.5**	0
P. placenta	0.005	17.5	0	0	0	0
	0.01	12.5	0	0	0	0
	0.05	12.5	**100**	0	0	12.5
	0.1	**25.0**	**100**	0	0	12.5
	0.5	**57.5**	**100**	**100**	**62.5**	**57.5**
G. trabeum	0.005	15	0	0	0	0
	0.01	15	0	0	0	0
	0.05	**25**	0	0	0	0
	0.1	**25**	0	0	0	0
	0.5	**35**	0	**100**	**37.5**	**47.5**
A. niger	0.005	0	0	0	0	0
	0.01	0	0	0	0	0
	0.05	0	0	0	0	0
	0.1	0	0	0	0	0
	0.5	12.5	**25.0**	12.5	**37.5**	**47.5**
C. pilifera	0.005	0	0	0	0	0
	0.01	0	0	0	0	0
	0.05	0	0	0	0	0
	0.1	0	0	0	0	0
	0.5	7.5	7.5	**100**	7.5	0

[a] If the inhibitory ratio was greater than 20%, the test fungus was considered inhibited and the minimal inhibitory concentration (MIC) for that fungus was then marked in bold. Each value is average of two replicates Petri dishes.

Table 4
Mold resistance of specimens.[a]

Compounds/Specimens	Concentration	Week 4	Week 8	Week 12
Styrax	1%	3.2	2.9	3.4
	3%	3.2	3.8	3.8
	5%	3.5	3.7	3.7
	10%	3.1	3.3	3.7
Storax	1%	2.2	3.0	3.2
	3%	2.2	3.0	3.2
	5%	2.0	3.0	3.0
	10%	2.0	2.1	2.8
Cinnamyl acetate	3%	2.2	1.5	2.6
	5%	0.9	2.1	2.6
Cinnamyl alcohol	3%	0.2	2.5	2.5
	5%	0.5	1.0	1.5
Ethyl cinnamate	3%	1.9	1.9	2.3
	5%	0.9	2.2	2
Storax heartwood	–	0.2	0.8	1.2
Pine sapwood (extracted)	–	3.5	3.5	4.5
Pine sapwood (unextracted)	–	3.4	3.6	4.4

[a] Specimens were visually rated on a scale of 0–5with 0 indicating the specimen is completely free of mold growth and 5 indicating the specimen was completely covered with mold growth (0: no growth, 1: 20%, 2: 40%. 3: 60%, 4: 80%. 5: 100% coverage with mold fungi). Each value is average of ten specimens. Alcohol extraction was carried out for 48 h in Soxhlet apparatus using ethyl alcohol.

rot fungus, *T. versicolor* and brown rot fungus, *L. sulphureus* in agar tests. Said et al. (2004) found that cinnamic acid was effective in inhibition of *N. crassa* in glucose and sucrose medium.

Cinnamyl acetate, cinnamyl alcohol and ethyl cinnamate at the 5.0%concentration level demonstrated decreased development of mold fungi on Scots pine specimens after 4 weeks (Table 4). Naturally obtained storax from L ***orientalis*** at 10%concentration caused moderate inhibition of mold growth when compared to untreated control specimens; however, commercial styrax failed to inhibit mold growth on the wood specimens. The best results were seen in storax heartwood specimens at the end of the test period of 12 weeks. None of the compounds provided complete inhibition of mold growth on the wood specimens but 10% cinnamyl alcohol and storax heartwood showed sustained inhibition of mold growth for at least 12 weeks.

Table 5 and Table 6 show mass losses in the specimens after 4 week and 3 week termite resistance tests based on the ASTM D3345-08 (ASTM, 2010) and the JIS K 1517 (JIS, 2010) standard test methods, respectively. Of the constituents tested, only cinnamyl alcohol caused considerably lower mass losses in unleached specimens in comparison with leached specimens suggesting that leaching resulted in loss of components that might be effective against the termites. On the other hand, cinnamyl alcohol might have demonstrated synergism with Scots pine wood specimens when mass losses in the pine specimens are considered. Commercial styrax and storax at the concentration level of 10%only were effective against termite attack in both leached and unleached specimens. Mass loss in storax heartwood specimens was less than that in control Scots pine specimens in the ASTM test method; however, in the JIS K test method, the differences between storax heartwood and Scots pine sapwood were statistically important for alcohol extracted pine specimens only. In most cases, *R. flavipes* termites resulted in greater mass losses when compared to *C. formosanus* termites. The possible reasons for this phenomenon might be longer test duration (4 weeks vs. 3 weeks) and higher termite number (≈300 vs. 165) of the ASTM test method in comparison with the JIS test method. In a study by Kartal et al. (2006). two treatment solutions with cinnamaldehyde and cinnamic acid showed strong activity against the subterranean

Table 5
Mass losses in the specimens exposed to termite resistance tests based on the ASTM D3345-08 test method.

Compounds/Specimens	Concentration	Leaching	Mass loss (g)	Mass loss (%)
Styrax	1%	Yes	0.77 (0.11)	40.10 (4.88)bc
	1%	No	0.63 (0.06)	31.08 (0.99)de
	3%	Yes	0.54 (0.11)	25.46 (3.73)fg
	3%	No	0.42 (0.12)	19.13 (4.76)h
	5%	Yes	0.24 (0.03)	12.30 (2.03)m
	5%	No	0.22 (0.04)	10.69 (1.43)k
	10%	Yes	0.14 (0.05)	6.41 (2.40)bc
	10%	No	0.21 (0.02)	9.22 (0.26)f
Storax	1%	Yes	0.73 (0.10)	39.11 (3.12)bc
	1%	No	0.59 (0.04)	28.45 (1.23)f
	3%	Yes	0.55 (0.09)	26.34 (2.34)fg
	3%	No	0.41 (0.03)	18.34 (3.21)h
	5%	Yes	0.25 (0.02)	13.45 (0.54)j
	5%	No	0.21 (0.03)	10.45 (0.89)k
	10%	Yes	0.11 (0.04)	5.67 (2.11)m
	10%	No	0.19 (0.04)	8.95 (2.03)l
Cinnamyl acetate	3%	Yes	0.72 (0.04)	36.36 (1.71)d
	3%	No	0.42 (0.07)	21.49 (3.12)g
	5%	Yes	0.67 (0.03)	33.08 (1.23)e
	5%	No	0.60 (0.12)	29.70 (5.96)f
Cinnamyl alcohol	3%	Yes	0.78 (0.11)	46.03 (7.35)a
	3%	No	0.08 (0.02)	3.92 (1.40)n
	5%	Yes	0.63 (0.38)	32.38 (19.86)e
	5%	No	0.07 (0.01)	3.75 (0.28)n
Ethyl cinnamate	3%	Yes	0.26 (0.29)	13.79 (15.62)j
	3%	No	0.81 (0.11)	39.38 (7.11)bc
	5%	Yes	0.43 (0.36)	20.11 (16.73)g
	5%	No	0.52 (0.07)	24.68 (4.87)fg
Pine sapwood		Alcohol extracted	0.84 (0.11)	43.71 (13.53)b
		No extraction	0.52 (0.11)	25.87 (5.41)fg
Storax heartwood		–	0.40 (0.05)	16.04 (2.27)i

Each value is average of five specimens for each fungus and treatment group. Values in parentheses are standard deviations. Alcohol extraction was carried out for 48 h in Soxhlet apparatus using ethyl alcohol. The same letters in each column indicate that there is no statistical difference between the specimens according to Duncan's multiply range test.

Table 6
Mass losses in the specimens exposed to termite resistance tests based on the JIS K 1571 test methods.

Compounds/Specimens	Concentration	Leaching	Mass loss(%)
Styrax	1%	Yes	37.78 (2.90)a
	1%	No	35.10 (0.78)ab
	3%	Yes	31.09 (2.10)b
	3%	No	15.23 (1.98)e
	5%	Yes	16.10 (0.45)e
	5%	No	13.23 (0.97)f
	10%	Yes	5.23 (0.12)ij
	10%	No	8.18 (0.13)h
Storax	1%	Yes	34.23 (1.98)ab
	1%	No	29.12 (0.34)b
	3%	Yes	27.23 (2.12)bc
	3%	No	20.34 (1.98)d
	5%	Yes	10.23 (0.98)g
	5%	No	7.45 (0.34)i
	10%	Yes	6.54 (1.11)i
	10%	No	5.78 (0.45)ij
Cinnamyl acetate	3%	Yes	27.19 (2.03)bc
	3%	No	16.87 (1.67)c
	5%	Yes	37.67 (3.78)a
	5%	No	30.89 (2.17)b
Cinnamyl alcohol	3%	Yes	29.12 (1.11)b
	3%	No	3.88 (1.92)j
	5%	Yes	29.09 (3.24)b
	5%	No	2.14 (3.42)j
Ethyl cinnamate	3%	Yes	9.98 (2.45)g
	3%	No	21.09 (3.89)d
	5%	Yes	11.98 (2.43)f
	5%	No	19.23 (3.21)d
Pine sapwood	–	Alcohol extracted	29.45 (2.09)b
	–	No extraction	23.67 (3.21)c
Storax heartwood	–	–	24.56 (2.22)c

Each value is average of five specimens for each fungus and treatment group. Values in parentheses are standard deviations. Alcohol extraction was carried out for 48 h in Soxhlet apparatus using ethyl alcohol. The same letters in each column indicate that there is no statistical difference between the specimens according to Duncan's multiply range test.

termites, *C. formosanus* in the JIS standard tests. Chang and Cheng (2002) and Park et al. (2000) have also stated that cinnamic acid and its derivatives are effective against termites and insects.

4. Conclusion

Essential oils and extracts from plants and trees contain chemical substances with low molecular weight. Such components may have applications as a preservative or flavoring ingredient in food industry or pharmaceutics. With regard to developing environmentally benign wood preservatives, the activities of various storax constituents, commercial styrax and storax from *L. orientalis* tree were evaluated. The storax constituents, storax and commercial styrax were not able to inhibit the white and brown rot fungi completely in the soil block tests. Of the components tested, styrax was effective against the Basidiomycetes fungi: however, the mold and staining fungi were not inhibited in *in vitro*. Natural storax at the concentration level of 0.5% only inhibited all test fungi except for *T. versicolor* and *C. pilifera*. On the other hand, cinnamyl alcohol inhibited all fungi with the exception of *A. niger*. In mold resistance tests, cinnamyl alcohol only was the best constituent tested in terms of inhibition of mold growth on Scots pine specimens even though no complete inhibition was seen in the tests. Cinnamy alcohol was also effective against *R. flavipes* and *C. formosanus* termites in unleached specimens only. Commercial styrax and storax at 10% concentration resulted in reduced mass losses in both leached and unleached specimens. We suggest that storax wood might be highly susceptible to decay fungi and termites when used in outdoor applications. Most constituents of storax tested were not effective as potential wood preservatives. Using higher concentration levels of the constituents (more than 10%) might be beneficial to increase resistance of treated specimens against fungi and termites.

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