Environmental Science and Pollution Research (2020) 27:3076–3085
https://doi.org/10.1007/s11356-019-07202-7

RESEARCH ARTICLE

Synergistic effect of heartwood extracts in combination with linseed oil as wood preservatives against subterranean termite *Heterotermes indicola* (Blattodea: Rhinotermitidae)

Babar Hassan[1] · Sohail Ahmed[2] · Grant Kirker[3] · Mark E. Mankowski[3,4] · Muhammad Misbah ul Haq[5]

Received: 20 June 2019 / Accepted: 28 November 2019 / Published online: 14 December 2019

Abstract
This study evaluated the effect of wood extracts from *Tectona grandis*, *Dalbergia sissoo*, *Cedrus deodara*, and *Pinus roxburghii* combined with linseed oil as protectants of two non-durable wood species against the termite, *Heterotermes indicola*. Heartwood blocks (19 × 19 × 19 mm) and wood shavings were extracted using an ethanol/toluene (2:1) solvent system. Results of choice and no-choice tests with solvent-extracted and non-extracted heartwood blocks showed greater wood mass loss from termite feeding on solvent-extracted blocks compared with non-extracted blocks for all wood species. Significantly higher termite mortality was observed after termite exposure to non-extracted blocks compared with extracted blocks for all durable species. Sapwood blocks of two non-durable wood species (southern pine and cottonwood) were vacuum/pressure impregnated separately with each of the four types of extract at a concentration of 7.5 mg ml^{-1}, linseed oil (20%) and a mixture of oil (20%) and extracts (4.25 mg ml^{-1}) for the laboratory and field tests. Results showed that extract-oil mixture imparted significantly higher termite resistance compared with linseed or extracts alone under laboratory conditions. This apparent synergistic effect was clearly noted when linseed oil was combined with extracts from *T. grandis* or *D. sissoo* followed by an extract-oil mixture using *C. deodara*. These extract oil mixtures showed significantly less weight loss for the treated non-durable wood species and higher termite mortality (83–100%) compared with the control treatments and other extract-linseed oil mixtures tested. Treatment of both non-durable wood species with *T. grandis* + oil and *D. sissoo* + oil prevented termite damage compared with other treatments when blocks and stakes were exposed in the field for a period of 2 years. Results of the current study indicated that a mixture of a particular heartwood extract with linseed oil has potential to be used as environmentally friendly wood protectants.

Keywords Termite · Wood protection · Natural products · Synergism · Environmentally friendly · Oil · Field tests

Introduction

Extending the service life of wood and wood products employing traditional chemical wood preservatives is being more strictly regulated due to concerns over human chemical toxicity and environmental effects (Hwang et al. 2007; Singh and Singh 2012). This has led to the increasing removal of treated wood and wood products in service each year (Coudert et al. 2013). Different counteractive actions that can limit and/or remove the synthetic wood protection chemicals have necessitated investigations of suitable alternative, less toxic natural sources to treat wood in service against biodeterioration (Singh and Singh 2012). One approach for the development of new wood protectant systems is use of plant oils and heartwood extracts of durable wood species that have insecticidal, fungicidal, and antioxidant properties (Schultz and Nicholas 2000; Hwang et al. 2007; Terziev and Panov 2011; Hassan

Responsible editor: Giovanni Benelli

✉ Babar Hassan
sialuaf@gmail.com

1 South China Agricultural University, Guangzhou 510642, People's Republic of China

2 University of Agriculture Faisalabad, University Main Rd., Faisalabad, Punjab 38000, Pakistan

3 USDA-FS, Forest Products Laboratory, 1 Gifford Pinchot Dr, Madison, WI 53726, USA

4 USDA-FS, Forest Products Laboratory, 201 Lincoln Green, Starkville, MS 39759, USA

5 Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Peshawar, Khyber-Pakhtunkhwa Province, Pakistan

et al. 2017a; Eller et al. 2018; Hassan et al. 2018a, 2018b ; Lee et al. 2018; Hassan et al. 2019a). However, the potential of these natural compounds to be as effective as, for example, chromated copper arsenate (CCA) in protecting wood is questionable. The combination of one or more organic biocides can be one method to increase efficacy of natural wood protectants. The benefits of combining biocides have long been known, and in synergy with an additive formulation can reduce the cost of production and increase in effectiveness against wood-degrading organisms (Leightley 2003; Green and Schultz 2003; Hwang et al. 2007).

Certain wood species contain specific non-structural chemical compounds (wood extracts) in their heartwood that generally constitutes a small part of wood microstructure (Hinterstoisser et al. 2000). These compounds protect wood against certain wood-degrading organisms and are a major contributor to wood durability (Chang et al. 1999; Morimoto et al. 2006). Wood extracts of naturally durable wood species have been shown to have toxic, repellant, and antifeedant properties against termites. Specifically, these extracts are detrimental to symbiotic gut protozoan within the termite gut (Hassan et al. 2017a). Application of extracted wood compounds can protect non-durable wood species such as southern pine and cottonwood from biological attack. Previous studies showed that transferring durability of resistant wood species using heartwood components provides protection to non-durable wood species against fungi and termite attack (Chang et al. 1999; Kirker et al. 2013; Mankowski et al. 2016a, 2016b; Hassan et al. 2018a; Ahmed et al. 2018; Hassan et al. 2019a, 2019b; Afzal et al. 2019).

Oils extracted from the foliage and seeds of several plant species have similar modes of action on insects and other organisms as exhibited by heartwood extracts. These compounds are antimicrobial, antioxidant, antifeedant, and repellent. A number of vegetable oils have also been tested against termites (Hammer et al. 1999; Siger et al. 2008; Kaithwas et al. 2011; Fatima and Morrell 2015). One property of oils, besides being toxic, is their ability to transfer a toxicant into wood for protection against termites and fungi (Hwang et al. 2007; Ahmed et al. 2014; Fatima and Morrell 2015). Several reports illustrate their efficacy against decay and termites. Linseed oil–treated non-durable wood at a high retention performed well against decay in both ground proximity and field stake tests (Edlund and Jermer 2007; Panov and Terziev 2015). Beech wood and Norway spruce treated with tung and linseed oils showed increased protection against brown and white rot fungi (Humar and Lesar 2013). Linseed oil was also used to increase the retention of boron in wood and increased the resistance of wood against termites (Lyon et al. 2007). Moreover, linseed oil is a good solvent for pentachlorophenol as it has excellent drying properties and provides adequate penetration to the wood (Fatima and Morrell 2015). Epoxidized linseed oil treatments significantly reduced water absorption and improved the durability of Scots pine treated with bio-oil against *Reticulitermes flavipes* (Kollar) (Temiz et al. 2013). It is generally assumed that wood extract and biological oil systems when used separately might be useful to control wood deteriorating organisms such as fungi and termites. In this study, we combined two natural biocides to improve the delivery of wood extracts and observe potentially synergistic effects of linseed oil combined with wood extracts to protect non-durable wood against *Heterotermes indicola* (Wasmann) for the development of novel wood preservative systems.

Materials and methods

Termite collection and maintenance

Methods described by Misbah-Ul-Haq et al. (2015) were used to collect termites. *H. indicola* foraging locations were identified by randomly installing untreated cottonwood (*Populus* sp.) stakes (4 cm × 2.5 cm × 28 cm) at a distance of 10 m with each other in the ground. Spots were checked every 2 weeks, and infested stakes were switched with a concealed monitoring station by excavating a hole in soil in a way that top edge of station just touching surface of soil. The monitoring station included five wooden poplar slices (15 cm high × 8 cm wide × 1 cm thick) wrapped with blotting paper, held together with a rubber band, and enclosed by a 2-mm-thick plastic collar (17 cm diameter × 22 cm high). The space between slices in each bundle and plastic collar was packed with soil, and the upper end of the plastic collar was enclosed with a poly bag. Stations were examined every 2 weeks, and infested bundles were replaced with new ones. Infested bundles were carried to the laboratory, and collected termites were kept in glass Petri dishes (14-cm dia.) containing two pieces of moistened blotting paper (14-cm dia.). Petri dishes with termites were kept in an incubator at 27 ± 2 °C and 75 ± 1% RH for at least 2 weeks prior to use in the experiments.

Procurement of wood and sample preparation

Extracts were prepared from the four durable wood species listed in Table 1. Two non-durable wood species, southern pine (*Pinus* sp.) and cottonwood (*Populus* sp.), were selected to test the effectiveness of heartwood extracts on non-durable species. Defect free logs of heartwood from the four test species except *Tectona grandis* L.f. were purchased from a timber market located at Jhang Road Faisalabad, Pakistan, and shipped to the Forest Products Laboratory in Starkville, MS. Marine grade *T. grandis* was acquired from a supplier in the USA (McIlvain, Pittsburg, PA) and shipped to the Starkville laboratory. After air drying for 1 week, wooden logs of heartwood and non-durable wood were cut into 19 × 19 × 19 mm

Table 1 Properties of selected wood species (Hassan 2017)

Wood species	Class	Family	Wood density (kg m^{-3})	Type	Information
Tectona grandis L.f. (Teak)	Dicotyledonae	Verbenaceae	550	Hardwood	Durable reference material. Class 1 (very durable). Contain high levels of quinones
Dalbergia sissoo Roxb. (Shisham)	Dicotyledonae	Fabaceae	809	Hardwood	Commercial species in Pakistan, heartwood contains stilbenes/polyphenols, Class 1 (very durable)
Cedrus deodara (D. Don) (Deodar)	Conifers	Pinaceae	515	Soft wood	Widely available softwood in Pakistan, medicinal properties. Class 3 (moderately durable)
Pinus roxburghii Sargent (Chir Pine)	Conifers	Pinaceae	497	Soft wood	Widely grown and utilized in Pakistan, building, medicinal, stilbenes. Class 4 (non-resistant)

blocks for the laboratory tests. For the field tests, the size of test block specimens was 12.5 × 3.75 × 2.5 cm for the ground proximity test (AWPA E26) and stake size was 45.7 × 1.9 × 1.9 cm for the field stake test (AWPA E7).

For the preparation of extracts, a part of wood from durable species was converted into shavings using an electric planer (Hassan et al. 2018a).

Preparation of extracts and solvent-extracted wood

Heartwood extracts were prepared using the method described by Hassan et al. (2017a). Air-dried heartwood shavings were extracted using 300 ml of an ethanol/toluene (2:1) solvent system according to ASTM D1105-96 "Standard Test Method for Preparation of Extractive-Free Wood" (ASTM 2014). Shavings (12–15 g) were added to 20 Soxhlet apparatuses with a small pad of cotton below and above shavings in each, and the extraction process was run for 6 h. The resulting aliquot was evaporated at reduced pressure by using rotary evaporator, and extraction yield was calculated per gram of wood shavings (Ordonez et al. 2006). Extracts were stored at 4 °C in airtight bottles, and these were further diluted using ethanol/toluene (2:1) to prepare concentrations for treatment of non-durable wood.

For preparation of solvent-extracted wood, blocks measuring 19 × 19 × 19 mm were extracted according to ASTM D1105-96 with the following modifications. Conditioned blocks (33 °C, 62 ± 3%) were numbered and weighed prior to being placed in Soxhlet apparatus and extracted for 6 h using 300 ml of ethanol/toluene (2:1). Blocks were then washed by dipping in alcohol extracted again for 6 h in ethanol (95%) alone. Extracted blocks were air dried overnight and then boiled for 6 h in 3-l deionized water with water changes every hour. Blocks were re-conditioned before the termite tests.

Boiled linseed (*Linum usitatissimum* L.) oil was purchased from a commercial source (Menards, Eu Claire, WI). Oil was diluted (20% concentration) using ethanol/toluene (2:1) as solvent for treatment of non-durable wood.

Wood treatment process

Non-durable wood blocks for laboratory tests were treated using the method described by Hassan et al. (2018a). Briefly, weighed and conditioned (33 °C, 62 ± 3% RH) blocks of southern pine and cottonwood (19 × 19 × 19 mm) were vacuum pressure treated with extracts (7.5 mg ml^{-1}) from each durable wood species, mixture of linseed oil and extracts (4.25 mg ml^{-1} + 20% oil), and linseed oil (20%) alone of each heartwood separately in a vacuum pressure chamber. A subset of pine or cottonwood blocks was treated with solvent (ethanol/toluene) or water as control treatments. For vacuum pressure treatment, five blocks were placed in a beaker (300 ml) containing 250 ml of the treatment solution and placed in a vacuum pressure chamber. A vacuum (91.4 kPa) was held for 30 min, and then, pressure was applied (275.8 kPa) for 60 min. Treated blocks of both woods were blotted dry using paper towels, weighed, reconditioned at 33 °C and 62 ± 3% RH, and re-weighed.

Non-durable wood blocks for field tests were treated using the method described by Hassan et al. (2017a). Briefly, all test samples were treated using full cell vacuum pressure with initial vacuum (91 kPa) for 30 min, and then, the pressure (1034 kPa) was applied for 60 min. The blocks and stakes were treated using same treatment solution as described above for the laboratory tests specimens. Additionally, stakes of both non-durable wood species were treated with oil-based copper naphthenate (Cu-NAP), and blocks were treated with a borate solution (disodium octaborate tetrahydrate (DOT); as Tim-Bor) for the positive chemical control in both tests.

Schematic representation of laboratory and field tests is given in Fig. 1.

Choice and no-choice tests on solvent-extracted and non-extracted durable wood

Solvent-extracted and non-extracted heartwood blocks of each species were tested against termites in choice and no-choice feeding tests according to the American Wood

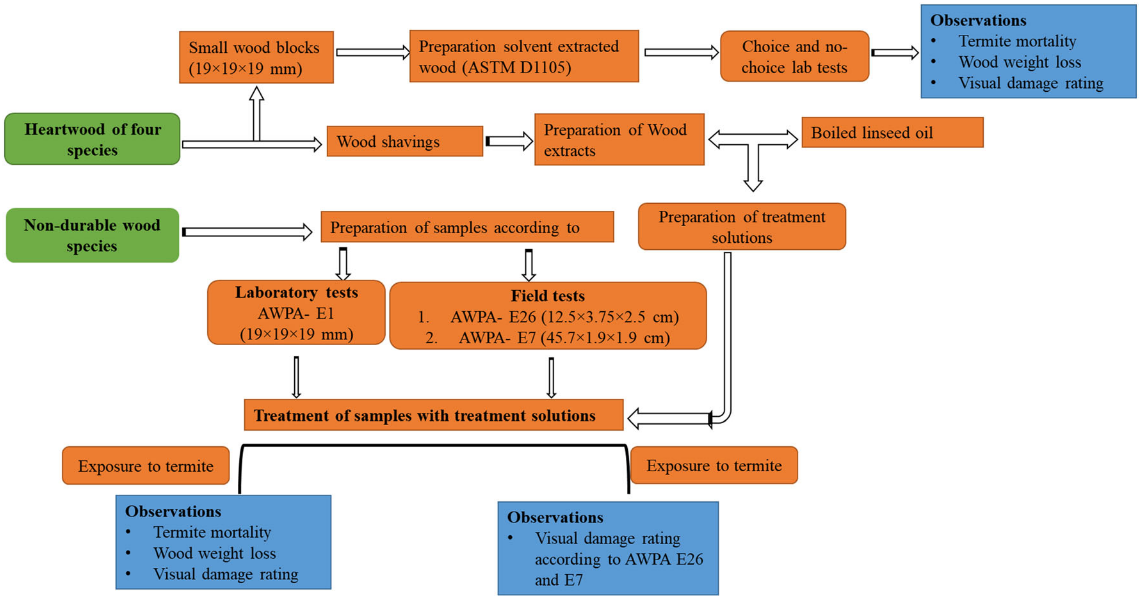

Fig. 1 Schematic representation of experimental methods

Protection Association E1-17 standard test in the laboratory (AWPA 2017). Screw top jars were filled with 150-g sand along with 27 ml distilled water and held for 2 h to equilibrate moisture. For the no-choice test, solvent-extracted and non-extracted blocks were weighed after conditioning and placed on top of the dampened sand with one block in each jar. For the choice test, each jar received one solvent-extracted and one non-extracted block. Both experiments were replicated five times. A total of 400 termites (396 workers and 4 soldiers) were released in each jar. Non-extracted blocks of cotton wood and southern pine were also fed to termite in a similar way for the comparison. Jars were kept in an incubator at 27 ± 2 °C and 75 ± 1% RH for 28 days. At the end of 28 days, the number of live termites was counted. Blocks were brushed to remove sand, conditioned for 1 week, and re-weighed to determine weight loss. All blocks were rated visually using 0–10 scale as described in the AWPA E1-17 standard (Table 2).

Table 2 AWPA E1-17 visual termite damage rating scheme

10	Sound
9.5	Trace, surface nibbles permitted
9	Slight attack, up to 3% of cross-sectional area affected
8	Moderate attack, 3–10% of cross-sectional area affected
7	Moderate/severe attack, penetration, 10–30% of cross-sectional area affected
6	Severe attack, 30–50% of cross-sectional area affected
4	Very severe attack, 50–75% of cross-sectional area affected
0	Failure

Termite bioassay with non-durable wood pressure treated with heartwood extracts and linseed oil

Laboratory tests

The termite bioassay was conducted according American Wood Protection Association E1-17 standard with some modifications according to Hassan et al. (2018a) and as described in choice and no-choice tests. At the end of test, the numbers of live termites were counted. Blocks were brushed to remove sand, conditioned for 1 week, and re-weighed to determine weight loss. All blocks were rated visually using 0–10 scale as described in the AWPA standard (Table 2).

Field tests

Two field tests (ground proximity test and ground contact field stake test) were conducted to test preservative potential of heartwood extracts when combined with linseed oil. Ground proximity test was initiated following AWPA E26 standard. Similarly, ground contact field stake test was initiated according to the AWPA E7. Each treatment was replicated five times for both tests. After conditioning at 33 °C, 62 ± 3% RH, all blocks and stakes were labeled with durable, inert, and corrosion-resistant tags and were placed at field sites (Botanical Garden of Forman Christian College University) in Lahore Pakistan (AWPA 2014a, b; Hassan et al. 2017b). All blocks and stakes were rated visually using 0–10 scale as described in the AWPA standard after every 12th month of installation.

Statistical analysis

Weight loss and mortality in the no-choice test where extracted and non-extracted wood was fed to termites were analyzed using two-way ANOVA with wood type and extraction as factors, assuming all blocks as independent. Similarly, data from the transferable durability test was analyzed using a one-way ANOVA. However, weight loss from the choice test was analyzed using a split-plot design with block pairs within each wood type. Termite mortality in the choice test was analyzed using exact Wilcoxon two-sample test. Means of treatments were separated using Tukey's HSD test at 5% level of significance.

Results

Choice and no-choice bioassays for weight loss showed *H. indicola* ignored non-extracted heartwood blocks of all durable wood species, consuming more of the solvent-extracted blocks and non-extracted control blocks (southern pine and cottonwood) (Fig. 2a). Significant wood type (p = 0.05) and extraction effects (p = 0.00) on wood weight loss for the four wood species tested were observed. In both tests, termites consumed more of the extracted *C. deodara* and *P. roxburghii* blocks compared with extracted blocks of *D. sissoo* and *T. grandis*. However, no significant difference in wood consumption in the non-extracted blocks of all tested wood species was observed. Weight loss of extracted *C. deodara* and *P. roxburghii* was significantly higher compared with non-treated southern pine and cotton wood controls. Significantly higher mortality (> 86%) was observed in *H. indicola* after feeding on solvent-extracted *T. grandis*, followed by solvent-extracted *D. sissoo* (> 74%). Mortality after feeding on extracted blocks of *C. deodara* (52%) or *P. roxburghii* (45.7%) differed non-significantly in no-choice tests. Similarly, in the choice test, mortality of termites after feeding on extracted wood of all species did not differ significantly, except *T. grandis*. Mortality was > 92% when termites were fed on non-extracted wood of all species, and it was > 25% after feeding on non-extracted southern pine and cottonwood (Fig. 2b).

Results of the bioassay with non-durable wood blocks treated with extracts and linseed oil showed that solvent and water-treated southern pine controls lost 22.5 and 22.4% (Fig. 3b) mass with average damage ratings of 4.4 and 4.8 (Fig. 4a), respectively. Solvent and water-treated cottonwood controls lost 25.6 and 25.2% mass, respectively, with the average damage rating of 4.4 and 6.4. Weight losses of both non-durable wood species treated with water and solvent did not differ significantly. The extract and mixture of extract and oil-treated non-durable blocks had a similar retention, and lowest retentions were observed after treatment with linseed oil (20%) (Table 3). However, treatment of non-durable wood blocks with linseed oil when combined with each separate heartwood extract of *T. grandis* and *C. deodara* showed synergistic effects and significantly reduced the weight losses compared with linseed oil, linseed when combined with other heartwood extracts, and extracts alone. Non-durable wood treated with *T. grandis* heartwood extracts + linseed oil and *D. sissoo* + linseed oil showed minimum weight loss (< 3%) with average termite damage ratings of 9.5 and 9 for southern pine and cottonwood, respectively. Weight losses for non-durable wood treated with *P. roxburghii* + linseed oil were non-significantly different from wood loses after treatment with linseed oil alone after 28-day exposure to termites (Figs. 3 and 4a, b). Mortality of *H. indicola* was observed after feeding on non-durable wood blocks treated with extracts from all heartwood species, linseed oil, and all extracts combined separately with linseed oil. Complete mortality (100%) of *H. indicola* was observed after feeding on southern pine treated with extracts of *T. grandis* + oil, *D. sissoo* + oil, and *C. deodara* + oil. While mortality of termites after feeding on cottonwood treated with extracts of *T. grandis* + oil, *D. sissoo* + oil, and *C. deodara* + oil was > 87%. Significantly, lower mortality of termite was observed after feeding on southern

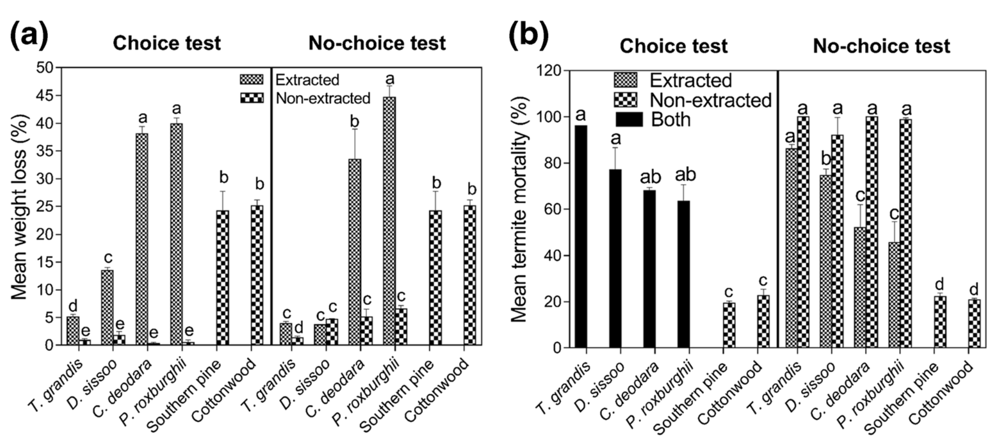

Fig. 2 Mean weight loss (**a**) for solvent-extracted and non-extracted blocks of four durable wood species and mortality (**b**) of *H. indicola* under choice and no-choice tests. (Bars with the same letter are not significantly different from each other at $p > 0.05$)

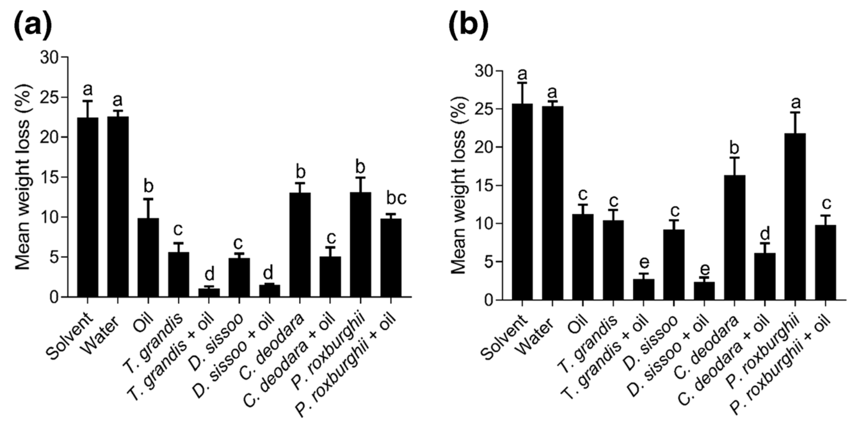

Fig. 3 Mean weight loss of southern pine (**a**) and cottonwood (**b**) treated with four-heartwood extracts and linseed oil after 28-day exposure to *H. indicola*. (Bars with the same letter are not significantly different from each other at $p > 0.05$)

pine and cottonwood treated with all extracts, linseed oil, and *P. roxburghii* extracts when combined with linseed oil (Fig. 5).

The average termite damage ratings for the southern pine and cottonwood test specimens in the ground proximity (AWPA E26) exposure test for 2 years is shown in Table 4. During first year of study, southern pine and cottonwood blocks treated with solvent were severely attacked by termites with an average rating of 3.8 and 1.2, respectively. While blocks treated with linseed oil only showed moderate termite attack (8.4, 6.4). *Pinus roxburghii* extractive–treated blocks of both non-durable wood species had some termite attack, and termite mud tubes were found extensively on the array of blocks after 12 months. During the second year, all blocks of both species were severely damaged by the termite. Highest average rating was observed for the borate-treated (positive control) blocks that differ non-significantly with that of *D. sissoo* + oil, *T. grandis* + oil, and *T. grandis* treatments.

Results of field stake tests presented in Table 5 showed that after 1 year of exposure, heavy termite attack occurred on cottonwood stakes treated with solvent, linseed oil, *C. deodara* extracts, *P. roxburghii* extracts, and *P. roxburghii* + oil. While after 24 months, all cottonwood stakes were severely damaged by the termites and average rating was < 7 except stakes treated with Cu-NAP that showed significantly less damage (7.2). Southern pine stakes treated with solvent, linseed oil, and *P. roxburghii* + oil were severely damaged by the termite, and stakes treated with other treatments showed slight to moderate attack. However after 24 months of exposure, stakes treated with Cu-NAP, *T. grandis* + oil, *D. sissoo* + oil, and *C. deodara* + oil showed better resistance against termite attack and heavy termite attack occurred on all other treated stakes (Table 5).

Discussion

Increasing environmental regulation and public concern regarding the toxicity of synthetic wood preservatives has resulted efforts to minimize the amount of biocides and/or development of new less toxic wood preservative systems using natural compounds. In this study, we tested the combined preservative potential of extracts and linseed oil against *H. indicola*. Heartwood components from four wood species were removed as extracts, and termites were exposed to extracted and non-extracted durable wood in choice and no choice tests. There was significantly less mortality in *H. indicola* and high wood weight loss in all solvent-extracted blocks compared with non-extracted blocks in the

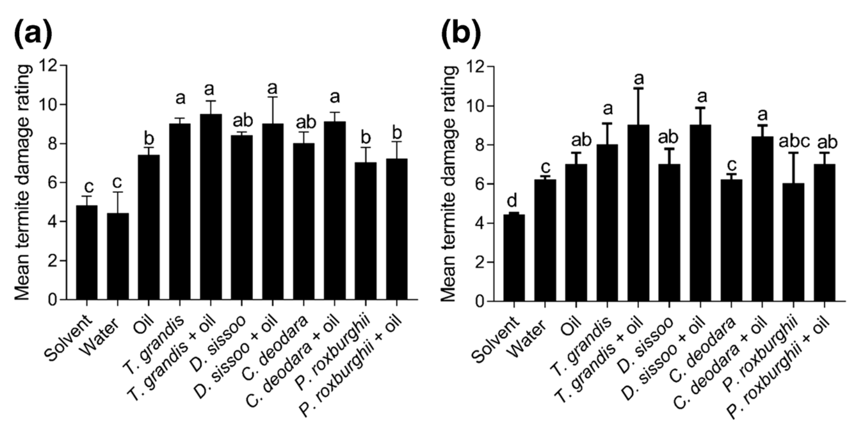

Fig. 4 Average rating of southern pine (**a**) and cottonwood (**b**) treated with four-heartwood extracts and linseed oil after 28-day exposure to *H. indicola*. (Bars with the same letter are not significantly different from each other at $p > 0.05$)

Table 3 Retention of treatment solutions in southern pine and cottonwood

Treatment solution	Retentions (kg m^{-3})	
	Southern pine	Cottonwood
Control	–	–
Oil (linseed)	99.23 ± 11.1	101.47 ± 2.06
T. grandis	79.05 ± 0.63	91.31 ± 1.55
T. grandis + oil	115.32 ± 6.86	120.84 ± 2.21
D. sissoo	86.05 ± 0.52	96.60 ± 0.76
D. sissoo + oil	123.96 ± 8.70	136.51 ± 3.40
C. deodara	80.61 ± 0.60	89.42 ± 1.66
C. deodara + oil	114.60 ± 11	122.04 ± 1.55
P. roxburghii	81.20 ± 0.78	88.13 ± 0.73
P. roxburghii + oil	118.26 ± 6.18	121.02 ± 11.60

no-choice test. However, in the choice test, there was no-significant difference in wood weight loss for *T. grandis* and *D. sissoo* before and after extraction. In both tests, *T. grandis* and *D. sissoo* showed comparatively more resistance to termites even after solvent/water extraction compared with the other two heartwood species. Continued durability of the blocks after solvent extraction indicates that not all of the toxic heartwood components in these wood species were removed using this particular solvent system and process. This durability could also be attributed to higher wood density and hardness (Peralta et al. 2004; Arango et al. 2006). Several studies showed that extracts are the main reason for termite and decay resistance in heartwood and removal of extracts from heartwood wood decreased termite resistance (Syofuna et al. 2012; Kibet et al. 2013; Zaharin 2013; Hassan et al. 2016a; Mankowski et al. 2016b; Hassan et al. 2018a, 2018c). The reduced resistance of all the heartwood species tested here after extraction indicates that some of the heartwood components responsible for termite resistance were removed.

Non-durable southern pine and cottonwood showed increased resistance to termites after treatment with extracts, linseed oil, and with mixtures of linseed oil + extracts, compared with non-extract–treated wood of the same species. The extract-treated non-durable samples had a similar retention, and weight losses of either non-durable wood were significantly reduced after treatment with mixture of extracts + linseed oil with an average rating > 8 except with *P. roxburghii* extracts + linseed oil mixture. However, during field test, stakes or wood blocks treated with *T. grandis* + oil and *D. sissoo* + oil showed better resistance against termites after exposure for the period of 2 years. Due to heavy infestation of termites, stakes or blocks treated with Cu-NAP or borate were also severely damaged by the termite.

Previously, we tested a series of extract concentrations from these wood species against *H. indicola* and *R. flavipes*. Results showed that 10 mg ml^{-1} extract concentration from each heartwood was effective against both of these termite species. In the current study, we used comparatively lower concentration (7.5 mg ml^{-1}) of each extract. These extracts were found to be repellant, antifeedant, free radical scavengers, and toxic to symbiotic protozoa of these two termite species in laboratory tests (Hassan et al. 2016a, 2016b; Mankowski et al. 2016a, 2016b; Hassan et al. 2017a; Hassan et al. 2018a, 2018b, 2018c). In addition to our studies, several other studies found these extracts effective against termites (Akhtar and Jabeen 1981; Lukmandaru and Takahashi 2008; Qureshi et al. 2012; Brocco et al. 2017). We have chemically analyzed these extracts and found high concentrations of anthraquinone and squalene in *T. grandis*; trimethoxyresveratrol in *D. sissoo*; terpenes, cuprenene, himachalene, and cedrene in *C. deodara*; and benzopyran from *P. roxburghii* (Mankowski et al. 2016a; Hassan et al. 2017a). These compounds have been observed to have strong biological activity against insects and other organisms (Reyes-Chilpa et al. 1995; Schultz and Nicholas 2000; Duchowicz et al. 2009; Xie et al. 2012; Nascimento et al. 2013; Xie et al. 2014; Hassan et al. 2017a).

Previous studies showed that addition of other chemicals might aid the action of heartwood extracts (Hwang et al. 2007) and further increase toxicity of extracts against termite. When

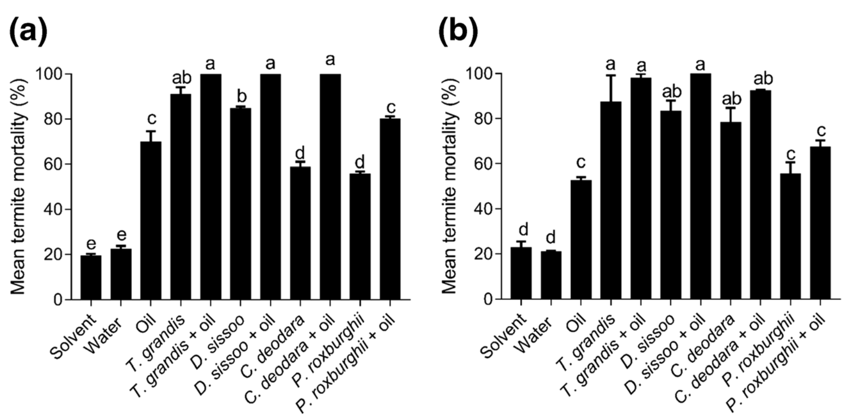

Fig. 5 Mean mortality of *H. indicola* after feeding on southern pine (**a**) and cottonwood (**b**) treated with four-heartwood extracts and linseed oil for 28 days. (Bars with the same letter are not significantly different from each other at $p > 0.05$)

Table 4 Average retention of treatment solutions and termite damage ratings of southern pine and cottonwood in ground proximity test (AWPA E26)

Treatments	Southern pine			Cottonwood		
	Retention (kg m^{-3})	Average rating		Retention (kg m^{-3})	Average rating	
		Year I	Year II		Year I	Year II
Solvent	–	3.8b	3.8bc	–	1.2c	0.0d
Linseed oil	497.84 ± 14.40	8.4a	3.6bc	570.64 ± 16.65	6.4ab	2.4cd
T. grandis	404.08 ± 13.22	9.6a	5.8a	461.62 ± 18.43	9.5a	3.6bc
T. grandis + oil	506.84 ± 8.79	9.9a	6.4a	582.31 ± 11.48	8.3a	6.4a
D. sissoo	484.41 ± 19.01	9.4a	4.4b	453.80 ± 12.50	8.2a	1.6d
D. sissoo + oil	526.40 ± 13.29	10a	5.4a	581.85 ± 14.18	9.3a	5.4ab
C. deodara	471.04 ± 11.81	9.8a	4.0b	454.07 ± 12.79	6.4ab	3.2c
C. deodara + oil	510.11 ± 19.00	9.9a	4.4b	527.57 ± 16.08	8.6a	5.2b
P. roxburghii	406.67 ± 14.13	8.6a	2.4c	476.07 ± 12.38	5.3b	1.6d
P. roxburghii + oil	521.31 ± 8.20	9.7a	4.6b	520.53 ± 14.11	8.3a	1.6d
Borate (DOT)	4.50	9.6a	7.2a	4.50	9.2a	7.4a

Means sharing same letter are not significantly different from each other at $p > 0.05$

linseed oil (20%) was added in extracts, it increased the efficacy of the extract synergistically as a wood protectant. Oils can have similar modes of action as some of the compounds described, and a number of vegetable oils have been tested against termites (Lyon et al. 2007; Ahmed et al. 2014). Previous studies showed that oils besides being lethal to termite due to suffocation have the ability to transfer a toxicant deep into wood for protection against termites and fungi (Ahmed et al. 2014; Fatima and Morrell 2015). Linseed oil also ensures water repellence and dimensional stability of the treated wood (Terziev and Panov 2011), and in combination with boron, it was used to develop low toxicity wood treatment against *Coptotermes formosanus* Shiraki and *R. santonensis* De Feytaud (Lyon et al. 2007). Several researchers have exploited the hydrophobicity of linseed and other plant oils in combination with other biocides. Results of the current study indicated that a mixture of a particular heartwood extract with linseed oil increased termite resistance of the treated non-durable wood. Moreover, current field study or field study previously reported indicated that *T. grandis* and *D. sissoo* extracts when separately combined with linseed oil protected southern pine and cottonwood from *R. flavipes*, *H. indicola*, and decay fungi in ground proximity and field stake tests (Hassan et al. 2017b). A literature search found that the wood preservative based on plant seed oils or wood extractives confers protection to non-durable wood. Most of

Table 5 Average retention of treatment solutions and termite damage ratings of southern pine and cottonwood in ground contact field stake test (AWPA E7)

Treatments	Southern pine			Cottonwood		
	Retention (kg m^{-3})	Average rating		Retention (kg m^{-3})	Average rating	
		Year I	Year II		Year I	Year II
Solvent	–	2.2d	0.0d	–	0.0c	0.0d
Linseed oil	396.50 ± 11.07	4.0c	1.6d	439.59 ± 21.95	7.4ab	4.4bc
T. grandis	305.42 ± 4.94	8.7ab	5.6ab	336.25 ± 21.43	8.0ab	4.4bc
T. grandis + oil	380.59 ± 6.56	10a	5.8a	464.62 ± 21.35	9.7a	5.8ab
D. sissoo	299.22 ± 5.45	7.6b	3.6c	353.25 ± 19.60	6.6ab	2.4cd
D. sissoo + oil	380.61 ± 10.20	10a	6.2a	479.31 ± 22.90	9.9a	6.6ab
C. deodara	288.23 ± 9.57	9.2a	3.0c	329.97 ± 15.40	4.4bc	3.8c
C. deodara + oil	391.29 ± 10.35	10a	5.8a	411.89 ± 12.44	9.5a	5.6b
P. roxburghii	295.68 ± 12.34	7.3b	4.4bc	371.47 ± 17.62	7.5ab	2.0cd
P. roxburghii + oil	373.62 ± 9.30	4.3c	4.8bc	467.15 ± 13.98	8.7ab	3.4c
Cu-NAP	1.20	9.9a	7.8a	1.20	9.8a	7.2a

Means sharing same letter are not significantly different from each other at $p > 0.05$

them were used in crude or semicrude form, and formulation based on these products have not been commercialized because of the cost and the un-stability of the extractives. Therefore, future studies will examine single-extract component isolates combined with oils to determine how these components act synergistically with particular heartwood components and linseed oil that will lead to new chemistries for industrialized wood preservative development.

Conclusions

Results indicated that extracts from the heartwood of *T. grandis*, *D. sissoo*, and *C. deodara* when combined with linseed oil provided protection of non-durable wood against *Heterotermes indicola*. Heartwood extracts in combination with linseed oil had not been tested against termites in laboratory or field conditions until this study. Field tests are still ongoing in Lahore (Pakistan) and Mississippi (USA).

Acknowledgments This manuscript is part of B. Hassan's PhD research (Hassan 2017). The authors wish to thank Craig Bell for preparation, handling, and processing the wood samples And Dr. Hamid Borazjani (Mississippi State University) for the use of his laboratory and Soxhlet apparatus. The authors gratefully acknowledge the USDA-FS International Program who granted fellowship to Babar Hassan to conduct research at USDA-FS Forest Products Laboratory and the Nuclear Institute for Food and Agriculture (NIFA) Peshawar. We would also like to thanks Dr. Khalid Zamir Raisb (FC-College Lahore) for his help during field experiments.

Funding information The study is financially supported by Higher Education Commission of Pakistan (HEC)

Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

References

Afzal M, Farman M, Rasib KZ, Qureshi NA (2019) Biocidal action of silver oak (*Grevillea robusta*) leaf extract on the termite *Heterotermes indicola* Wasmann (Blattodea: Rhinotermitidae). Int Biodeterior Biodegradation 139:1–10

Ahmed S, Fatima R, Nisar M, Hassan B (2014) Evaluation of castor bean oil on *Acacia nilotica* as wood preservative against *Odontotermes obesus* (Ramb.) (Termitidae: Isoptera). Int Wood Prod J 5:5–10

Ahmed S, Hassan B, Saleem MU, Riaz MA, Nisar MS (2018) Efficacy of heartwood extractives of *Albizia lebbeck* (L.) Benth against subterranean termites. Int Wood Prod J 9:194–199 1–6

Akhtar M, Jabeen M (1981) Responses of *Coptotermes heimi* (Wasmann) (Isoptera) to woods, wood extracts and essential oils of timbers. Mater und Org (Germany, FR) 163:199–206

American Society for Testing Materials (ASTM) (2014) D1105-96—standard test method for preparation of extractive-free wood. ASTM International, West Conshocken, pp 147–148

American Wood Protection Association (2017) AWPA E1-17 standard method for laboratory evaluation to determine resistance to subterranean termites. AWPA book of standards pp. 379–383. American Wood Protection Association, Birmingham

American Wood Protection Association Book of Standards (2014a) AWPA E7, standard method of evaluating wood preservatives by field tests with stakes. AWPA Book of Standards. American Wood Protection Association, Birmingham, pp 390–397

American Wood Protection Association Book of Standards (2014b) AWPA E26, Standard test method for the preservative treatments for lumber and timbers against subterranean termites in above ground protected applications (UC1 and UC2). In: AWPA Book of Standards. American Wood Protection Association, Birmingham, pp 476–482

Arango RA, Green F III, Hintz K, Lebow PK, Miller RB (2006) Natural durability of tropical and native woods against termite damage by *Reticulitermes flavipes* (Kollar). Int Biodeterior Biodegradation 57: 146–150

Brocco VF, Paes JB, da Costa LG, Brazolin S, Arantes MDC (2017) Potential of teak heartwood extracts as a natural wood preservative. J Clean Prod 142:2093–2099

Chang S-T, Wang S-Y, Wu C-L, Su Y-C, Kuo Y-H (1999) Antifungal compounds in the ethyl acetate soluble fraction of the extractives of Taiwania (*Taiwania cryptomerioides* Hayata) heartwood. Holzforschung 53:487–490

Coudert L, Blais J-F, Mercier G, Cooper P, Janin A (2013) Remediation processes for wood treated with organic and/or inorganic preservatives. In: Handbook of Recycled Concrete and Demolition Waste. Elsevier, pp 526–554

Duchowicz PR, Goodarzi M, Ocsachoque MA, Romanelli GP, Ortiz Edel V, Autino JC, Bennardi DO, Ruiz DM, Castro EA (2009) QSAR analysis on *Spodoptera litura* antifeedant activities for flavone derivatives. Sci Total Environ 408:277–285

Edlund M, Jermer J (2007) Durability of some alternatives to preservative-treated wood. Progress report 2: results from field tests after 5 years' exposure. In: Proceedings of the IRG-IUFRO Regional Research Symposium. International Union of Forest Research Organizations all Division 5 Conference, Taipei

Eller FJ, Hay WT, Kirker GT, Mankowski ME, Selling GW (2018) Hexadecyl ammonium chloride amylose inclusion complex to emulsify cedarwood oil and treat wood against termites and wood-decay fungi. Int Biodeterior Biodegradation 129:95–101

Fatima R, Morrell JJ (2015) Ability of plant-derived oils to inhibit damp wood termite (*Zootermopsis augusticollis*) activity. Maderas Ciencia y tecnología 17:685–690

Green F, Schultz TP (2003) New environmentally-benign concepts in wood protection: the combination of organic biocides and non-biocidal additives. In: ACS Symposium Series. Washington, American Chemical Society; 1999, pp 378–389

Hammer KA, Carson CF, Riley TV (1999) Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 86:985–990

Hassan B (2017) Studies on the effect of wood extractives in combination with plant oil on subterranean termites. Doctoral Thesis. University of agriculture, Faisalabad

Hassan B, Mankowski ME, Kirker GT, Ahmed S, ul Haq MM (2016a) Antitermitic activities of Shisham (*Dalbergia sissoo* Roxb.) heartwood extractives against two termite species. In: Proceedings IRG Annual Meeting (ISSN 2000–8953), IRG/WP 16–10856, the International Research Group on Wood Protection. pp 1–16

Hassan B, Ahmed S, Misbah-ul-Haq M, Mankowski ME, Nasir M (2016b) Antitermitic activities of *Pinus roxburghii* wood extractives against *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae). In: VII International Scientific Agriculture Symposium, Agrosym 2016, 6-9 October 2016, Jahorina, Bosnia and Herzegovina. Proceedings, University of East Sarajevo, Faculty of Agriculture, pp 1567–1575

Hassan B, Mankowski ME, Kirker G, Ahmed S (2017a) Effects of heartwood extractives on symbiotic protozoan communities and mortality in two termite species. Int Biodeterior Biodegradation 123:27–36

Hassan B, Ahmed S, Mankowski ME, Kirker G (2017b) Comparison of performance of wood extractives as preservatives in field tests against termites and decay in the USA and in Pakistan. In: In: Proceeding of the 113th AWPA Annual Meeting. American Wood Protection Association, Birmingham

Hassan B, Mankowski ME, Kirker G, Clausen C, Ahmed S (2018a) Effects of white mulberry (*Morus alba*) heartwood extract against *Reticulitermes flavipes* (Blattodea: Rhinotermitidae). J Econ Entomol 111:1337–1345

Hassan B, Ahmed S, Kirker G, Mankowski ME, Misbah-ul-Haq M (2018b) Antioxidant effects of four heartwood extractives on midgut enzyme activity in *Heterotermes indicola* (Blattodea: Rhinotermitidae). Environ Entomol 47:741–748

Hassan B, Ahmed S, Mankowski ME, Kirker G, Ibach R, Ul Haq MM (2018c) Effects of teak, *Tectona grandis* Linn, heartwood extractives against *Heterotermes indicola* (Isoptera: Rhinotermitidae). In: Proceedings, IRG annual meeting. The International Research Group on Wood Protection. pp 1–16

Hassan B, Ahmed S, Mehmood N, Mankowski ME, Misbah-ul-Haq M (2019a) Toxicity potential of heartwood extractives from two mulberry species against *Heterotermes indicola*. Maderas Ciencia y Tecnología 21:153–162

Hassan B, Mankowski ME, Kirker G, Ahmed S, Bishell A (2019b) Ex-situ performance of extracts from naturally durable heartwood species and their potential as wood preservatives. Eur J Wood Wood Prod 77:1–10

Hinterstoisser B, Stefke B, Schwanninger M (2000) Wood: raw material-material-source of energy for the future. Lignovisionen 2:29–36

Humar M, Lesar B (2013) Efficacy of linseed-and tung-oil-treated wood against wood-decay fungi and water uptake. Int Biodeterior Biodegradation 85:223–227

Hwang WJ, Kartal SN, Yoshimura T, Imamura Y (2007) Synergistic effect of heartwood extractives and quaternary ammonium compounds on termite resistance of treated wood Pest. Manag Sci: Formerly Pesticide Science 63:90–95

Kaithwas G, Mukerjee A, Kumar P, Majumdar DK (2011) *Linum usitatissimum* (linseed/flaxseed) fixed oil: antimicrobial activity and efficacy in bovine mastitis. Inflammopharmacology 19:45–52

Kibet S, Sirmah P, Mburu F, Muisu F (2013) Wood dimensional stability and extractives as reasons for termite and fungal resistance of the lesser known *Albizia malacophylla* Kenyan wood species. J Indian Acad Wood Sci 10:48–54

Kirker G, Blodgett A, Arango R, Lebow P, Clausen C (2013) The role of extractives in naturally durable wood species. Int Biodeterior Biodegradation 82:53–58

Lee SH et al (2018) Thermal treatment of wood using vegetable oils: a review. Constr Build Mater 181:408–419

Leightley LE (2003) Protection of wood using combinations of biocides. Wood Deterioration Preserv 845:390–398

Lukmandaru G, Takahashi K (2008) Variation in the natural termite resistance of teak (*Tectona grandis* Linn. fil.) wood as a function of tree age. Ann For Sci 65:1–8

Lyon F, Thevenon M-F, Hwang W-J, Imamura Y, Gril J, Pizzi A (2007) Effect of an oil heat treatment on the leachability and biological resistance of boric acid impregnated wood. Ann For Sci 64:673–678

Mankowski ME, Boyd B, Hassan B, Kirker GT GC-MS (2016a) Characterizations of termiticidal heartwood extractives from wood species utilized in Pakistan. In: IRG Annual Meeting (ISSN 2000-8953) IRG/WP 16-10857, The International Research Group On Wood Protection, Section 1 Biology. pp 1-16

Mankowski ME, Hassan B, Blodgett A, Kirker GT (2016b) Laboratory evaluations of woods from Pakistan and their extractives against *Postia placenta* and *Trametes versicolor*. In: Proceedings IRG Annual Meeting (ISSN 2000-8953), IRG/WP 16-10860 The International Research Group On Wood Protection, Section 1 Biology. pp 1-10

Misbah-ul-Haq M, Khan IA, Farid A, Ullah M (2015) Dose response relationship of subterranean termite, *Heterotermes indicola* (Wasmann) and two insect growth regulators, Hexaflumuron and Lufenuron. J Entomol Zool Stud 3:86–90

Morimoto M, Fukumoto H, Hiratani M, Chavasiri W, Komai K (2006) Insect antifeedants, pterocarpans and pterocarpol, in heartwood of *Pterocarpus macrocarpus* Kruz. Biosci Biotechnol Biochem 70: 1864–1868

Nascimento M, Santana A, Maranhão C, Oliveira L, Bieber L (2013) Phenolic extractives and natural resistance of wood. In: Biodegradation-Life of Science. InTech Open,

Ordonez AA, Gomez J, Vattuone M, Isla M (2006) Antioxidant activities of *Sechium edule* (Jacq) Swart extracts. Food Chem 97:452–458

Panov D, Terziev N (2015) Durability of epoxi-oil modified and alkoxysilane treated wood in field-testing. BioResources 10:2479–2491

Peralta RCG, Menezes EB, Carvalho AG, Aguiar-Menezes EL (2004) Wood consumption rates of forest species by subterranean termites (Isoptera) under field conditions. Revista Árvore 28:283–289

Qureshi NA, Qureshi MZ, Ali N, Athar M, Ullah A (2012) Protozoidal activities of *Eucalyptus cammeldulensis*, *Dalbergia sissoo* and *Acacia arabica* woods and their different parts on the entozoic flagellates of *Heterotermes indicola* and *Coptotermes heimi*. Afr J Biotechnol 11:12094–12102

Reyes-Chilpa R, Viveros-Rodriguez N, Gomez-Garibay F, Alavez-Solano D (1995) Antitermitic activity of *Lonchocarpus castilloi* flavonoids and heartwood extracts. J Chem Ecol 21:455–463

Schultz TP, Nicholas DD (2000) Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. Phytochemistry 54:47–52

Siger A, Nogala-Kalucka M, Lampart-Szczapa E (2008) The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. J Food Lipids 15:137–149

Singh T, Singh AP (2012) A review on natural products as wood protectant. Wood Sci Technol 46:851–870

Syofuna A, Banana A, Nakabonge G (2012) Efficiency of natural wood extractives as wood preservatives against termite attack. Maderas Ciencia y Tecnología 14:155–163

Temiz A, Kose G, Panov D, Terziev N, Alma MH, Palanti S, Akbas S (2013) Effect of bio-oil and epoxidized linseed oil on physical, mechanical, and biological properties of treated wood. J Appl Polym Sci 130:1562–1569

Terziev N, Panov D (2011) Plant oils as “green” substances for wood protection minimizing the environmental impact of the forest products. Industries:143–149

Xie Y, Huang Q, Yang F, Lei C (2012) Chemical variation in essential oil of *Cryptomeria fortunei* from various areas of China. Ind Crop Prod 36:308–312

Xie Y, Wang K, Huang Q, Lei C (2014) Evaluation toxicity of monoterpenes to subterranean termite, *Reticulitermes chinensis* Snyder. Ind Crop Prod 53:163–166

Zaharin FBA (2013) Effect of extractives on wood density and natural durability of *Tristaniopsis Whiteana* and *T. beccaril* (Myrtaceae). Master thesis. Universiti Malaysia Sarawak, Malaysia