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Original article (Orijinal araştırma)

Constituents and termiticide potential of some wood extracts against *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae)

Bazı odun ekstraktlarının bileşenleri ve *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae)'ye karşı termitisit potansiyeli

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Summary

Wood extractives are one of the main reasons for the resistance of wood to termite attack. A study was carried out to determine the chemical constituents of wood extractives from *Pinus roxburghii* Sargent*, Morus alba* L. and *Eucalyptus camaldulensis* Dehnh against *Coptotermes heimi* (Wasmann) under laboratory conditions in Forman Christian College University (Lahor, Pakistan) in June 2015. Gas chromatography-mass spectrometry of wood extractives of *P. roxburghii* detected 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester and octadecanoic acid (methyl ester). Hexadecanoic acid (methyl ester) and 1-methyl-3-(1-methylethyl)-benzene were present in *M. alba* in addition to the compounds present in *P. roxburghii* and in *E. camaldulensis* 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester and β-phellandrene were also present. Based on the feeding activity, wood extracts were arranged in descending order of preference; *P. roxburghii* > *M. alba* > *E. camaldulensis*. Extracts of *P. roxburghii, M. alba* and *E. camaldulensis* proved repellent at higher concentrations, with tunneling activity almost fully inhibited. So these could prove useful in developing a soil barrier to block termite activity and serve as a replacement to synthetic chemicals.

Keywords: Barrier*, Eucalyptus camaldulensis,* extractives*, Morus alba, Pinus roxburghii,* wood

Özet

Odun özütleri, termit saldırılarına karşı odun dayanıklılığının ana nedenlerinden birisidir. Bu çalışma 2015 yılı haziran ayında, Forman Christian College Üniversitesi (Lahor, Pakistan)'nde laboratuvar koşullarında *Coptotermes heimi* (Wasmann)'ye karşı *Pinus roxburghii* Sargent*, Morus alba* L. ve *Eucalyptus camaldulensis* Dehnh'in odun özütlerinin kimyasal bileşenlerini belirlemek amacıyla yürütülmüştür. *P.* roxburghi'nin odun özütlerinin gaz kromatografisi-kütle spektrometresi sonucunda, 1,2-benzendikarboksilik asit, mono (2-etilheksil) ester ve oktadekanoik asit (metil ester) tespit edilmiştir. *M. alba*'da *P. roxburghii*'de mevcut bileşiklere ilave olarak heksadekanoik asit (metil ester) ve 1-metil-3-(1-metil-etil)-benzen bulunurken, *E. camaldulensis*'te 1,2-benzendikarboksilik asit, mono (2-etilheksil) ester ve β-fellandren de bulunmuştur. Odun özütleri beslenme aktivitesi tercih sırasına göre, *P. roxburghii* > *M. alba* > *E. camaldulensis* şeklinde olmuştur. *P. roxburghii*, *M. alba* ve *E. camaldulensis* özütlerinin daha yüksek konsantrasyonlarda itici olduğu tespit edilmiş, tünel aktivitesi ise neredeyse tamamen engellenmiştir. Böylece, bu özütler termit aktivitesini engellemek için bir toprak bariyer geliştirilmesinde yararlı olabilir ve sentetik kimyasallara bir alternatif olarak hizmet edebilir.

Anahtar sözcükler: Bariyer*, Eucalyptus camaldulensis,* özütler*, Morus alba, Pinus roxburghii,* odun

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Introduction

Termites remain the most dreaded insect pest of human dwellings and other structures because of their ability to destroy wood and wood products (Pedigo, 1986). Wood being a biological material is readily degraded by bacteria, fungi and termites (Walker, 1993; Schultz & Nicholas, 2002). Termites are responsible for much of the degradation of wood and other cellulose materials in the terrestrial environment mainly in the tropics and subtropics (Peralta et al., 2003, 2004). The injudicious use of pesticides for the control of termites has generated a number of biological and environmental hazards in air, water, soil and food. More than 1000 species of plants have been reported to have chemicals in leaves, stems, flowers, seeds and roots which have insecticidal properties, but only a few of them have been used for practical insect control on a commercial scale. The chemical poisons of plants are mostly alkaloids (Shahid, 2003). Termites are members of the insect order, Isoptera, and more than 2600 species of termites are found around the world (Kambhampati & Eggleton, 2000; Eggleton, 2001, Ohkuma et al., 2004). Ecologically, termites are classified under three main groups based on their feeding and nesting behavior: damp wood termites, dry wood termites and subterranean termites (Chung & Lee, 1999). Termites have economic importance because they damage a wide variety and quantity of wood work in buildings, crops, local plantation and forests. For the prevention of heavy timber damage, we need extensive knowledge of natural resistance of native plants to termites and their feeding preference (Rasib, 2008). The fauna of Pakistan includes a range of termites species having diverse feeding preferences in various ecological zones (Akhtar, 1974).

In the past, the control of termites was totally based on the synthetic insecticides especially the persistent organochlorine (Khan & Singh, 1985; Anonymous, 2000, Ahmed et al., 2006). Their maximum residual effects, as well as the development of resistance in target pests, are considered their main drawbacks. Also, adverse effects on human health and concerns about environmental deterioration (Potter & Hillery, 2001; Verkerk & Bravery, 2001) have resulted in the replacement of synthetic insecticides with biorational ones, and is now universally accepted and practiced worldwide. The pool of plants possessing insecticidal substances is enormous, with plant chemicals from roots, stems, leaves, flowers and seeds of more than 1000 species having been used for insect control. Like alkaloids, plant flavonoids are naturally occurring substances and play an important role in insect control. These compounds function as constitutive or inducible anti-insect compounds (Dixon, 1999), so they may serve as natural pesticides. Many of them are being tested for repellent and deterrent effects against the insects (Simmonds, 2003). Flavonoids can also modulate the feeding behavior of the insects (Nawrot et al., 1986).

***Eucalyptus camaldulensis* Dehn.**

Eucalyptus species have promising essential oils with repellent and pesticide effects (Nerio *et al.*, 2010). From an environmental and toxicological point of view, the use of eucalyptus essential oil as a natural pesticide has a better effect than irregular use of other pesticides, and thereby the problem of pest resistance can be overcome (Batish et al., 2008). Durable and termites-resistant *Eucalyptus camaldulensis* Dehn. wood is extensively used in some industrial processes like pulp, paper, chipboard and plywood manufacturing (Abbas et al., 2010).

***Morus alba* L.**

White mulberry heartwood (*Morus alba* L.) is the main timber used for crafting three traditional long necked lutes in Iran. The chemical structure of wood is composed not only of its primary constituents, but also of a relatively small fraction of extractable compounds. Extractives can include different categories like phenols, tannins and oils. The primary role of these compounds is to protect the tree and timber against decay agents; therefore it is expected that these would have termiticidal, fungicidal and even toxic properties (Hillis, 1962; Highley & Scheffer, 1970; Tsunoda, 1990; Schultz & Nicolas, 2000; Taylor et al., 2002).

***Pinus roxburghii* Sargent**

Pinus roxburghii Sargent is a large evergreen tree, which is sometimes deciduous in dry locations or dry seasons (Troup, 1921). *Pinus roxburghii* oil has been traditionally used to treat cuts, wounds, boils, and blisters (Gewali, 2008). *Pinus roxburghii* is known for its natural resistance to termites (Rasib, 2008).

This study was specifically designed to assess the effectiveness of wood extractives against *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae) by determining different compounds present in these three tree species using Soxhlet extraction and gas chromatography-mass spectrometry (GC-MS) to understand their efficacy against termites and then suggest their application for termite control as an alternative to synthetic insecticides.

Materials and Methods

Selection of plant species

Commercial timber species were selected for this study. Wood of *E. camaldulensis, M. alba* and *P. roxburghii* was cut from standing trees in the, Forman Christian College Botanical Garden, Lahore, Pakistan in June 2015

Extraction method

For crude extracts of the wood, a non-polar and polar solvents, n-hexane and methanol, were used by incubating sawdust of *E. camaldulensis, M. alba* and *P. roxburghii* at 70^{o}C for 72 h in order to remove moisture. The samples were then preserved in zip lock bags for future use and to avoid any contamination. Two hundred g of each species with solvent were placed separately in a Soxhlet extractor and extracted with 150 ml of hexane and methanol according to ASTM (2003) standards. After extraction, each extract was kept separately under refrigeration at 4°C and subsequently the bioactivity of the extracted compounds was checked against termites.

Gas chromatography-mass spectrometry

For the identification of components extracts from the Soxhlet extractor were processed through hydro distillation, for GC-MS analysis. Samples were distilled below 200°C and filtered through 0.20 µm pore size filter paper. The gas chromatography temperature ranged of 50 to 250 °C with 4°C/min, with a solvent delay of 5 min. The temperature of the injector was 250°C. The inert gas was helium at a flow rate of 1.0 mL/min, and 2 µL of sample was injected sample in the splitless mode. The percent composition of the samples was calculated. The quantitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each compound was compared with those of NIST 98 (Mass Spectral Library, National Institute of Standards and Technology, MD, USA) as modified by Jianu et al., 2013.

Collection of *Coptotermes heimi*

Coptotermes heimi were collected from different areas of the Lahore District including visits to Changa Manga Forest, Jallo Park, Wagha Border, Jinnah Garden, but mainly from Forman Christian College. Some were also collected through the baiting. Collection were made feasible using artificial baiting methods such as bucket traps, wetted toilet rolls, and cardboard in of plastic bottles with small holes in the base and sides to permit the entry of termites. The baits were buried in soil and termites were collected as and when required.

Feeding bioassays

Choice test and repellency bioassay

Repellent responses of wood extracts from *E. camaldulensis, M. alba* and *P. roxburghii* were observed against *C. heimi* by cutting Whatman filter paper No. 1 into two halves according to size of Petri dish (70 x 10 mm) and placed into the dish in such a way that there was space between two halves to allow separate treatment. One half was treated with specific amount of plant extracts 2, 5, 10, 20 or 30% by pipette while the other half was treated with water to serve as a control. Then filter papers were air dried for few minutes. Three replicates of each extract including control were prepared. Ten mature workers of *C. heimi* were released separately into each dish between treated and untreated zone and observations were made at 15-min intervals. After introduction of termites into the dishes, the number of termites oriented towards the control half were counted as repelled. A treatment concentration was considered repellent when 21 (sum of three replicates) of 30 termites were present on untreated area against respective percent concentration.

No choice test

Filter paper for each concentration *E. camaldulensis, M. alba* and *P. roxburghii* was treated separately as mentioned above to evaluate the mortality and feeding of *C. heimi.* Termite mortality was recorded periodically during the bioassays up to 7 days. Percentage mortality was calculated using the following formula:

$$Mc = (Mo - Me) / (100 - Me) \times 100$$

Where, Mo = mortality rate of treated termite (%); Me = mortality rate of control (%); Mc =corrected mortality rate (%).

Tunneling test

Tunneling apparatus

Tunneling apparatus consists of a transparent tube with two chambers i.e. nest/feeding chamber and treated chamber attached with a rectangular plate. Termites were released into the nest chamber which was treated with the distilled water. The chamber was cylindrical (53 mm high by 55 mm wide) with holes in the lid to provide a source of oxygen to the termites. The nest chamber was connected with treated chamber through a tube (71 mm long, 8 mm diameter). Termites could move from this tube to reach the treated chamber. The treated chamber was rectangular (253 mm long x 75 mm wide x 14 mm high) and treated with the different concentrations of the wood extracts.

Tunneling method

Tunneling apparatus was filled with soil in both chambers i.e. nest/feeding chamber and treated chamber. In the nest chamber, a layer of 10 g of soil was evenly spread and moistened with distilled water. In the treated chamber, 48.9 g of soil was evenly spread with filter paper in the center to provide a food source for the termites. This compartment was treated with five different concentrations (2, 5, 10, 20 and 30%) of each wood extract. A group of 60 workers were added to each nest container, with three replicates for each wood extract. Test set ups were examined at 24-h intervals for one week after establishment. At each examination, tunnel length was measured.

Statistical analysis

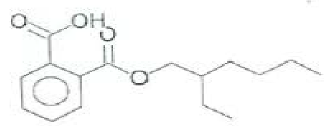
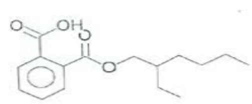
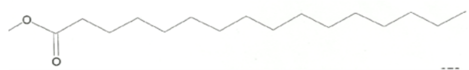
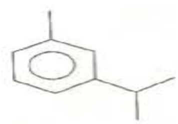
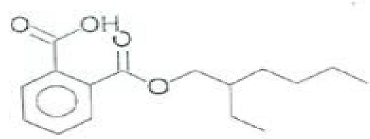
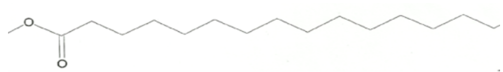
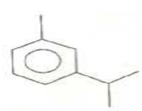
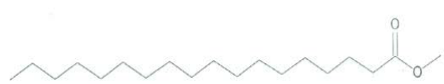
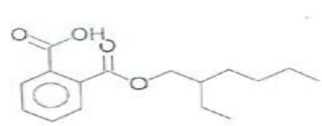
Data were analyzed statistically by using paired t-test and standard deviation to evaluate the consumption, mortality and the tunneling activity on the wooden extracts against *C. heimi* in MINITAB 15 software (Minitab Inc., State College, PA, USA). Results were statistically significant in all cases ($P < 0.05$).

Results

Compounds identified in extracts of *Eucalyptus cameldulensis, Morus alba* and *Pinus roxburghii*

Compounds identified in gas chromatography using methanol and hexane as extraction solvent are given in Table 1.

Table 1. Phytocompounds identified in chromatogram of *Eucalyptus cameldulensis, Morus alba* and *Pinus roxburghii* using hexane and methanol as extraction solvent

Tree species	Retention time (minutes)	Phytocompounds	Extraction solvent	Structural formulae
Eucaluyptus camaldulensis	21.264 and 21.695	1,2-Benzenedicarboxylic acid, Mono (2-ethylhexyl) ester	hexane	
Morus alba	18.080	Octadedanoic acid, methyl ester	methanol	
	21.633	1,2 Benzenedicarboxylic acid, mono(2-ethylhexyl) ester		
	16.185	Hexadecanoic acid, methyl ester		
	5.119	Benzene, 1- methyl-3-(1-methylethyl)-methanol		
	18.080	Octadedanoic acid, methyl ester		
	21.633	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester		
	16.185	Hexadecanoic acid, methyl ester		
	5.119	Benzene, 1- methyl-3-(1-methylethyl)-		
Pinus roxburghii	18.074	Octadedanoic acid, Methyl ester	hexane	
	21.639	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester		

Effect of wood extracts on *Coptotermes heimi* under laboratory conditions

No choice laboratory trials

Table 2 shows workers of *C. heimi* exposed to different *E. camaldulensis* extract concentrations. The highest mortality rate of 97.5% was observed at an extract concentration of 30% and the lowest mortality rate of 30.1% at an extract concentration of 2%. The table also shows the consumption of filter paper by *C. heimi* when treated with different concentrations of *E. camaldulensis* extract. The maximum consumption of 0.41 g was observed at an extract concentration of 2% and minimum consumption was at an extract concentration of 30%. There were statistically significant concentration effects on mortality (t = 7.22; P = 0.002; P > 0.05) and feeding (t = 2.53; P = 0.065; P > 0.05).

Table 2. Mortality of and filter paper consumption by *Coptotermes heimi* exposed for 7 days to varying concentrations of wood extracts of *Eucalyptus cameldulensis, Morus alba* and *Pinus roxburghii* using hexane as the solvent

Tree species	Extract concentration (%)	Mean mortality (%)	Filter paper consumed (g)	Solvent Used
Eucalyptus camaldulensis	Control	0.00	0.50	hexane
	2	30.1	0.41	
	5	50.2	0.35	
	10	70.8	0.24	
	20	84.7	0.18	
	30	97.5	0.13	
Morus alba	Control	0.00	0.49	methanol
	2	20.8	0.41	
	5	40.2	0.39	
	10	40.8	0.37	
	20	50.7	0.31	
	30	70.6	0.24	
Pinus roxburghii	Control	0.00	0.47	hexane
	2	20.5	0.42	
	5	30.8	0.38	
	10	70.9	0.25	
	20	80.2	0.18	
	30	90.6	0.14	

N = 100 (sum of three replicates).

Table 2 shows workers of *C. heimi* exposed to different concentrations of *M. alba* extract. The highest mortality rate of 70.6% was observed at an extract concentration of 30% and the mortality rate of 20.8% was at an extract concentration of 2%. The table also shows the consumption of filter paper by *C. heimi* when treated with different concentrations of *M. alba* extract. Maximum consumption of 0.41 g was observed at an extract concentration of 2% and minimum consumption of 0.24 g was at an extract concentration of 30%. There statistically significant concentration effects on mortality (t = 8.68; P =0.001; P > 0.05) and feeding (t = 2.52; P = 0.065; P > 0.05).

When workers of *C. heimi* were exposed to the different concentrations of *P. roxburghii* extract, the highest mortality rate of 90.6% was observed at an extract concentration of 30% and the lowest mortality rate of 20.5% was at an extract concentration of 2% (Table 2). The table also shows the consumption of filter paper by *C. heimi* when treated with different percentage concentrations of *P. roxburghii*. The maximum consumption of 0.42 g was observed at an extract concentration of 2% and minimum consumption of 0.14 g was at an extract concentration of 30%. There were statistically significant concentration effects on mortality ($t = 4.77$; $P = 0.009$; $P > 0.05$) and feeding ($t = 2.52$; $P = 0.065$; $P > 0.05$).

Choice test and repellency bioassay

Table 3 shows the results when workers of *C. heimi* were exposed to *E. camaldulensis* extracts to detect the repellent and attractive effects on treated and untreated filter paper in Petri dishes. There were repellent effects on termites, which moved from treated to untreated; at 2%, 18 termites were observed on untreated filter paper as the concentrations increased fewer termites were observed on treated filter paper. However, at 10-30%, concentration most of the termites were present on untreated filter paper indicating repellency.

Table 3. Repellency effects of wood extracts on *Coptotermes heimi* when exposed to filter paper with five different concentrations of *Eucalyptus camaldulensis, Morus alba* and *Pinus roxburghii* extracts for 60 mins

Tree species	Extract concentration (%)	Termites* on treated filter paper	Termites* on untreated filter paper
Eucalyptus camaldulensis	2	12	18
	5	15	15
	10	9	**21**
	20	6	**24**
	30	2	**28**
Morus alba	2	18	12
	5	12	18
	10	6	**24**
	20	6	**24**
	30	0	**30**
Pinus roxburghii	2	12	18
	5	15	15
	10	9	**21**
	20	6	**24**
	30	2	**28**

*N = 30 (sum of three replicates). Values in bold in columns indicate repellency.

Table 3 shows the results when workers of *C. heimi* were exposed to *M. alba* extracts to detect the repellent and attractive effects on treated and untreated filter paper in Petri dishes. There were repellent effects on termites, which moved from treated to untreated; at 2%, 12 termites were observed on untreated filter paper as the concentrations increased fewer termites were observed on treated filter paper. However at 10-30% concentration, most of the termites were present on untreated filter paper indicating repellency.

Table 3 shows the results when workers of *C. heimi* were exposed to *P. roxburghii* extracts at different concentrations to detect the repellent and attractive effects on treated and untreated filter paper in Petri dishes. There were repellent effects on the termites, which moved from treated to untreated; at 2%, 18 termites were observed on untreated filter paper as the concentrations increased fewer termites were observed on treated filter paper. However at 10-30% concentration, most of the termites were present on untreated filter paper indicating repellency.

Effect of wood extracts on tunneling activity of *Coptotermes heimi*

The tunnel length formed by *C. heimi* at 2, 5, 10, 20 and 30% concentrations of wood extracts of *E. camaldulensis*, *M. alba* and *P. roxburghii* in different solvents is given in Table 4.

Table 4. Effect of different wood extracts on tunneling activity of *Coptotermes heimi* at varying concentrations of wood extracts of *Eucalyptus camaldulensis, Morus alba* and *Pinus roxburghii* after 7 days exposure

Extract concentration (%)	Tunneling distance (mm, mean ± SD)		
	Eucalyptus camaldulensis	*Pinus roxburghii*	*Morus alba*
Control	294.2 ± 1.53	298.3 ± 1.32	295.4 ± 1.68
2	60.1 ± 0.98	72.0 ± 1.42	70.1 ± 1.95
5	46.1 ± 1.05	56.2 ± 1.47	55.1 ± 1.58
10	24.2 ± 0.95	34.2 ± 1.20	33.2 ± 1.205
20	20.4 ± 1.21	25.3 ± 0.86	24.3 ± 0.80
30	10.9 ± 1.00	12.7 ± 1.05	11.6 ± 1.2

Discussion

Compounds present in the extracts of *M. alba* wood have been previously reported (Venkataraman, 1972; Rowe & Conner, 1979; Se Golpayegani, 2007; Sadeghifar et al., 2011). Higher hydrocarbons, fatty acids, sterols and phenols constitute the majority of the compounds found in white mulberry. Extracts of *M. alba* were protective against decay caused by fungi and termites (Se Golpayegani et al., 2014). Siramon et al. (2007), investigated the essential oils from *E. camaldulensis* leaves and found both fumigants and contact toxicants against *Coptotermes formosanus*. The antitermite functions were dependent on the chemical composition of the oils. The various chemical constituents isolated from the bark of *P. roxburghii* include 3,4-dihydroxybenzoic acid, terpenoids, flavonoids, tannins, xanthones and some other compounds and all showed antitermite activity (Kaushik et al., 2013). In the present study, similar results were found using *E. camaldulensis, M. alba* and *P. roxburghii.* The preference of termites for a particular wood species could be altered by the wood combination offered (Smythe & Carter, 1970a; Morales-Ramos & Rojas, 2001). The choice feeding test was a more appropriate method for determining termite wood preference than no choice test (forced feeding), which is why it was adopted here because in the later test method, termites were forced to feed on whatever resource was available to survivor (Smythe & Carter, 1970b). The results of present study were consistent with above findings, as the workers of *C. heimi* more easily identified the palatable food source than the distasteful one when offered these in choice feeding experiments. In the laboratory trials more termites were observed feeding on palatable food source. *Eucalyptus camaldulensis, M. alba* and *P. roxburghii* sawdust with ethanol, methanol and hexane in the bioassay of *C. heimi* exhibited mortality at different concentrations of the extracts. In the feeding bioassays on *C. heimi*, the impact of different wooden extracts used against this termite indicated the following descending order of feeding preference: *P. roxburghii* > *M. alba* > *E, camaldulensis*. Resistance to these species to termites after preservation could be due to the repellent characteristics of the extractives. The repellent characteristics could be due to the toxic chemical composition of the various wood extractives and the durability of the heartwood of the tree species from which they were extracted.

Conclusions and recommendations

1) In the present study, the chemical constituents of wood extracts from *E. camaldulensis, M. alba* and *P. roxburghii* was studied using gas chromatographic analysis. Several compounds were identified. Fatty acids and waxes were the major groups of compounds detected and identified.

2) The main compounds detected in *P. roxburghii* were 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester and octadecanoic acid, methyl ester. The main compounds detected in *M. alba* were 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, octadecanoic acid (methyl ester), hexadecanoic acid (methyl ester) and 1-methyl-3-(1-methylethyl)-benzene. The main compounds detected in *E. camaldulensis* were 1, 2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester. Bioactivity bioassay revealed antitermite activity was common and were different as far as their feeding was concerned.

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