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Effects of heartwood extractives on symbiotic protozoan communities and mortality in two termite species

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A B S T R A C T

Lower termites (Isoptera: Rhinotermitidae) are considered severe pests of wood in service, crops and plantation forests. Termites mechanically remove and digest lignocellulosic material as a food source. The ability to digest lignocellulose not only depends on their digestive tract physiology, but also on the symbiotic relationship between termites and their intestinal microbiota. The current study was designed to test the possible effects of four heartwood extractives (*Tectona grandis*, *Dalbergia sissoo*, *Cedrus deodara* and *Pinus roxburghii*) on the mortality, feeding rate and protozoan population in two lower termites, *Reticulitermes flavipes* and *Heterotermes indicola*. All wood extractives tested rapidly lowered protozoan numbers in the hindgut of termite workers, which was closely correlated with worker mortality. The average population of protozoans in both termite species was diminished in a dose-dependent manner after fifteen days feeding on treated filter paper. Mortality of termites increased when fed on filter paper treated with *T. grandis* or *D. sissoo* heartwood extractives with minimum feeding rate at the maximum concentration (10 mg ml^{-1}). Protozoan number and termite survival was also compared with starved termites and results showed that protozoan populations ware reduced up to 99.60 and 65.71% in *R. flavipes* and *H. indicola*, respectively as compared to untreated filter paper controls with ≅ 80% survival for both termite species. Characterizations of heartwood extractives were performed using Gas Chromatography-Mass spectrometry (GC-MS) and chemical profiles were obtained for extractives from each wood species. The largest chemical components, based on percentage of sample, identified from *T. grandis* were Squalene, 2-methyl-9, 10-Anthracenedione and 1-Methyl-3,4-dihydroisoquinoline. Tris-methoxyresveratrol, 1, 3-Diamino-8-n-butyl-5, 6 dihydrobenzoquinazoline and 6, 8-dimethyl-Benzanthracene were the largest components from *D. sissoo*. The largest percentage components from *C. deodara* were (E) − Atlantone, Di-epi-alpha-cedrene and alpha-Cuprenene. The main components of *P. roxburghii* were identified as 1, 2, 3, 4-tetrahydro-5, 8-dimethyl-Acridin-9-amine, 2, 3-dihydro-5, 7-dihydroxy-2phenyl-4H-1-Benzopyran-4-one and 5-hydroxy-7-methoxy-2-phenyl4H-1-Benzopyran-4-one. These are discussed in terms of their termiticidal and protozoicidal properties.

1. Introduction

Reticulitermes flavipes (Kollar) and *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae) are major pests of wood and wood products. *H. indicola* is a common wood destroying termite, predominantly in areas north of 20°N latitude which includes Pakistan, India, and Afghanistan (Maiti, 2006). Besides causing general damage to wooden structures, this species has also been observed to destroy paper, clothing and other stored products with cellulosic components. *R. flavipes* is found in the eastern North America as far north as Ontario, Canada, and south to Key Largo, Florida and it is considered to be one of the most destructive termite pest species in this region (Su et al., 2001; Hassan et al., 2016). To prevent termite damage, non-durable wood is often treated with chemical preservatives, some of which have been shown to be environmentally toxic to both humans and animals. Hence, some chemical treatments are not approved for indoor applications (Gautam and Henderson, 2008; Ward et al., 2009; Tascioglu et al., 2013). Due to their less toxic nature, other

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alternative methods have been developed such as use plant/wood oils, tannins and other naturally occurring plant extracts (Fatima and Morrell, 2015; González-Laredo et al., 2015) to make the cellulosic material inaccessible or repellent to termites as a food source.

Naturally durable woods with antitermitic properties, such as Alaskan yellow cedar (*Chamaecyparis nootkatensis*) (*D. Don*), Western red cedar (*Thuja plicata*) D. Don, Tung tree (*Aleurites fordii*), Red wood (*Sequoia sempervirens*) (D.Don), Western juniper (*Juniperus occidentalis*) Hook, and Teak (*Tectona grandis*) L. f, are considered very resistant to termites and are good alternatives to commonly used non-durable commercial timbers like southern yellow pine (*Pinus* spp.) and cotton wood (*Populus* spp.) (Beal et al., 1974; Bultman et al., 1979; Carter and Huffman, 1982; Carter et al., 1983; Grace and Yamamoto, 1994; Hutchins, 1997; Maistrello et al., 2003). In addition, they represent a valuable source of natural products (wood extractives) that could potentially be applied to more susceptible woods to control termite infestation (Adedeji et al., 2017). Wood extractives are substances present in the wood that, unlike lignin and cellulose, do not contribute to the structural integrity of wood. They are typically concentrated in the heartwood and are important in conferring resistance to attack by xylophagous organisms (Verma et al., 2009; Kirker et al., 2013). These compounds generally act as toxins, repellants or antifeedants to termites. For example, chamaecynone, an acetylenic sesquiterpenoid from *Chamaecyparis pisifera* (Siebold & Zucc.) Endl (Saeki et al., 1973), 7-methyljuglone, the naphthoquinone from *Diospyros virginiana* L (Carter et al., 1978), loganin, the iridoid glycoside from *Guettarda speciosa* L (Yaga and Kinjo, 1985) and the sesquiterpenoid alcohols, cedrol and widdol, from *Juniperus* spp. (Adams et al., 1988). have been reported to be toxic to the termites. Patchouli alcohol from *Pogostemon cablin* and nootkatone and its derivatives from *Vetiveria zizanioides* were very toxic and repellent to *Coptotermes formosanus* Shiraki (Maistrello et al., 2001a,b; Zhu et al., 2001a,b; Zhu et al., 2003; Ibrahim et al., 2004). Similarly, 2-furfuraldeyde from *Pinus sylvestris* L and other compounds were found to be repellent to termites (Becker et al., 1971). Compounds such as carboxylic acid from *Pinus lambertiana* Douglas (Scheffrahn and Rust, 1983), limonoids from *Phellodendron amurense* (Hayata & Kaneh.) C.E. Chang (Kawaguchi et al., 1989), and *Azadirachta indica* A. Jussieu (Ishida et al., 1992) and the diterpenes, ferruginol, manool and nezukol from *Taxodium distichum* (L.) Rich were feeding deterrents to termites (Scheffrahn et al., 1988). Solvent and aqueous extract of woods such as *Diospyros sylvatica* Roxb, *Eucalyptus camaldulensis* Dehnh, *Dalbergia sissoo* Roxb, *Morus alba* L, *Cedrus deodara* (Lamb.) G. Don, *Pinus roxburghii* Sarg and *Azadirachta indica* Adr. Juss, have also showed termiticidal activities (Hassan et al., 2016; Mankowski et al., 2016).

R. flavipes and *H. indicola* are lower termites (Family: Rhinotermitidae) that possess a variety of symbiotic microorganisms, including, protists, bacteria and archaea (Ikeda-Ohtsubo et al., 2007; Duarte et al., 2016), which are harbored in the distended portion of the hindgut known as the paunch. In this symbiotic system, termites contribute endogenous cellulases and mechanical maceration of wood fibers, while flagellate protists phagocytose the wood particles and digest them (Brune, 2014). Prokaryotes in the hindgut play a significant role in maintaining the physicochemical equilibrium within the termite hindgut (Brune and Ohkuma, 2010; Xie et al., 2012).

Increasing interest in the ligno-cellulolytic degradation process performed by termites and their symbiotic fauna, has led to a number of metagenomic and metatranscriptomic studies examining the effects of different diets on these holobionts (Scharf, 2015; Duarte et al., 2016). Current research suggests that changes in protozoan species composition and community structure in the termite gut appear to be influenced by wood extractives of different woods as well as by high or low weight carbon sources of diet (Mannesmann, 1972; Cook and Gold, 2000; Tanaka et al., 2006). Chemical defensive compounds present in wood (i.e. extractives) are introduced in the gut system by feeding, which has the potential to negatively affect hindgut protozoa. This influences the feeding rate of the host on different nutritive sources and can lead to a complete loss of symbionts, ultimately causing termite death.

It is apparent that the toxicity of various woods and their extractives to lower termites is related to their deleterious effect on hindgut protozoa (Mannesmann, 1972; Mauldin et al., 1981; Breznak, 1982). Limiting termite development through the destruction of the gut microbes is a control method that has shown some success (Adams, 2004; Doolittle et al., 2007). In this perspective, the current investigation was intended to examine the effects of extractives from the heartwood of durable wood species on protozoan populations within the termite hindgut. Extractives from heartwoods with protozoicidal properties may be potential substitutes for current synthetic chemicals used in termite treatment. Thus, these natural extracts are a unique approach for developing new wood preservatives (Salem et al., 2016).

2. Material and methods

2.1. Wood species and preparation of extractives

Four economically important timber species used in Pakistan were selected for this experiment. Raw lumber of *Dalbergia sissoo* Roxb, *Cedrus deodara* (Lamb.) G. Don and *Pinus roxburghii* Sarg were purchased from a timber market located on Jhang Road in Faisalabad (Pakistan). While marine grade *Tectona grandis* L. f was acquired from a supplier in the United States (McIlvain, Pittsburg, PA). These woods were shipped to the Forest Products Laboratory in Starkville, Mississippi (USA) where they were air dried and made into wood shavings using a planer. Air-dried wood shavings (12 g) were Soxhlet extracted using 300 ml of ethanol: toluene (2:1) according to ASTM D1105-96 (ASTM, 2014). Shavings were added to Soxhlets with a small amount of cotton placed below and above to contain the shavings and extracted for a total of six hours. The solvent containing extractive solutions were placed in a pre-weighed and tared round bottom flask and then evaporated to dryness at reduced pressure by using a rotary evaporator (BUCHI, Rotavapor R-114). Extraction yield was calculated per gram of wood shavings (Ordonez et al., 2006) for all wood species extracted, and the resulting dried extractive residue was re-weighed to identify the amount of dried extractives. The dried residue was re-dissolved with solvent (ethanol: toluene) for a final concentration of 100 mg ml^{-1} based on the final weight of the dried residue from the tared flask. This was used as a stock solution for production of a series of concentrations ranging from 10 mg ml^{-1} to 1.25 mg ml^{-1}. The stock solutions were stored in 1 L screw cap jars sealed with parafilm and kept in darkness at 4 °C.

2.2. Termite procurement and maintenance

Workers and soldiers of *R. flavipes* were collected from fallen logs and dead trees at Sam D. Hamilton Noxubee National Wildlife Refuge (Mississippi) and maintained in the laboratory at 25 °C in the dark. For the collection of *H. indicola*, foraging points were identified at Peshawar, Pakistan by installing untreated poplar (*Populus* sp.) stakes (4 cm wide x 2.5 cm thick x 28 cm high), which were examined every two weeks for the presence of termites. The infested stakes were exchanged with underground monitoring stations by digging a hole in the soil so that the upper edge of the station just touched the soil surface. The monitoring station was