BIODIVERSITAS
Volume 19, Number 3, May 2018
Pages: 1176-1180

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190343

Short Communication: Attractiveness of subterranean termite *Coptotermes formosanus* to plant leaf extracts

YULIATI INDRAYANI[1,♥], MUSRIZAL MUIN[2,♥♥], CYNTHIA ADILLA[3,♥♥♥], TSUYOSHI YOSHIMURA[4,♥♥♥♥]
[1]Faculty of Forestry, Tanjungpura University. Jl. Imam Bonjol, Pontianak 78124, West Kalimantan, Indonesia. Tel./fax.: +62-561-767673. ♥email: mandaupermai@yahoo.com
[2]Faculty of Forestry, Hasanuddin University, Jl. Perintis Kemerdekaan Km 10, Makasar 90245, South Sulawesi, Indonesia. ♥♥email: musrizal@unhas.ac.id
[3]Faculty of Mathematics and Natural Sciences, Tanjungpura University. Jl. Prof. Hadari Nawawi, Pontianak 78124, West Kalimantan, Indonesia. ♥♥♥email: cynthiaarief@gmail.com
[4]Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho-uji, Kyoto 611-0011, Japan. ♥♥♥♥email: tsuyoshi@rish.kyoto-u.ac.jp

Manuscript received: 2 May 2018. Revision accepted: 28 May 2018.

Abstract. *Indrayani Y, Muin M, Adilla C, Yoshimura T. 2018. Short Communication: Attractiveness of subterranean termite* Coptotermes formosanus *to plant leaf extracts. Biodiversitas 19: 1176-1180.* Plant-derived compounds have many potential uses. One such use is in agriculture for managing pests with less risk than with synthetic compounds that are toxicologically and environmentally undesirable. The current study focuses on the potential use of leaf extracts from five tropical plant species in attractant bait systems for controlling subterranean termites. Leaves from clove [*Syzygium aromaticum* (L.)], cajuput (*Melaleuca leucadendra* L.), cinnamon [*Cinnamomum burmannii* (Nees & T. Nees) Blume], basil (*Ocimum sanctum* L.) and bay (*Syzygium polyanthum* Wight) were extracted with 70% ethanol solution. Each extract was evaluated for its attractant properties for *Coptotermes formosanus* Shiraki using olfactory and Y-line trail following tests, respectively. Extract solutions were diluted to 1% concentration based on their eugenol content. For each test, which was replicated 15 times, a single worker was introduced into the test unit. The results showed that *M. leucadendra* crude extract was the most attractive among the five plant crude extracts, and the *S. aromaticum* crude extract was attractive especially in the trail-following test. The attraction rates were 40.00%, 80.00%, 53.33%, 60.00% and 46.67% for the *S. aromaticum*, *M. leucadendra*, *C. burmannii*, *O. sanctum* and *S. polyanthum* extracts, respectively. The trail-following rates were 86.67% for the *S. aromaticum* extract, 66.67% for the *M. leucadendra* extract, 53.33% for the *C. burmannii* extract, 20% for the *O. sanctum* extract as well as 26.67% for the *S. polyanthum* extract.

Keywords: Attractants, attractive properties, *Coptotermes formosanus*, leaf extracts, trail-following test

INTRODUCTION

Subterranean termites are well-known for causing damage to wooden structures, and considerable efforts have been made for controlling them. Subterranean termite treatment has changed dramatically in terms of systems, application techniques, and products during the last two decades. Currently, the use of bait systems with various methods of application has become a common commercially developed approach. Baiting systems for termite control have been intensely researched (Myles 1996; Getty et al. 2000; Thorne and Forschler 2000; Grace and Su 2001; Kubota 2011). The common goal in bait systems is to exploit the process of termite workers in foraging for food and bringing it back to the colony. In baiting technology, a food source as a part of the system should be viable and accepted by worker termites. They then feed other colony members with the bait, leading to the elimination of termite colony. Therefore, for a baiting system to be successful, the food sources must contain attractants for the bait matrix. Many toxicants have been considered as potential active ingredients in termite baits (Eger et al. 2012). However, for economic and environmental reasons, research has explored the potential of using bio-based materials for termite control (Sajap and Aloysius 2000; Sajap et al. 2006; Safian et al. 2011).

Plant extracts contain a variety of chemical compounds, some of which may have potential uses in agriculture for managing insect pests. These compounds may also be associated with less risk than synthetic compounds that are toxicologically and environmentally undesirable. The use of plant extracts for termite control has been conducted by many researchers. Singh et al. (2001) and Ding and Hu (2010) reported plant extracts having anti-termite properties and described termite-resistant formulations. Uses of plant extracts as bio-based attractant for termites bait formulation has not been reported so far.

Waller et al. (1999) reported the importance of chemical directional cues as one of the factors affecting termite recruitment to bait. Crude plant extracts contain a mixture of compounds that are likely to perform differently compared to a single compound. Consequently, in addition to their attractant effects, crude extracts may also have repellent effects for termites. Some studies have been conducted to explore the performance of crude plant extracts against insects in term of calling behavior, but was limited to *Sogatella furcifera* (Homoptera: Delphacidae) (Khan and Saxena 1986). This current study aimed at examining the direct response of subterranean termites *Coptotermes formosanus* Shiraki to crude extracts of leaves from five tropical plant species in terms of their attractiveness and repellency.

MATERIALS AND METHODS

Termites

Mature subterranean termite workers were obtained from the termitarium of the Deterioration Organisms Laboratory (DOL), Research Institute for Sustainable Humanosphere (RISH), Kyoto University, Japan. Only health workers were used in this study.

Extracts and treatments

Leaf extracts from the following five tropical plant species were prepared according to Indrayani et al. (2017): clove [*Syzygium aromaticum* (L.) Merr. & L.M. Perry], cajuput (*Melaleuca leucadendra* L.), cinnamon [*Cinnamomum burmannii* (Ness & T. Nees) Blume], basil (*Ocimum sanctum* L.), and bay (*Syzygium polyanthum* Wight). Prior to the extraction, the plant leaves were air-dried for 3 days and crosscut into small segments. The extraction was conducted with 70% ethanol solution for three 24-hours, and then rotary evaporated at 60°C for two 24-hour periods until the extract became a gel. For treatments, all leaf extracts were diluted with 70% ethanol solution to achieve concentration of eugenol (1%). This concentration was calculated based on the eugenol content of each extract revealed by GC-MS analyses in preliminary testing.

Olfactory response test

The response of termites to the extract was determined using an olfactometer test unit modified from Wang et al. (2012) as shown in Figure 1. The unit consisted of distilled water bottle, molecular sieve 3A bottle, molecular sieve 5A bottle, charcoal bottle, circulating air pump, air flow meters, and a Y-tube boom. The parts were connected in series with odorless silicon tubing (Sanplatec, Japan) and permitted the use of clean air to blow the odor source. The Y-tube boom was 7 cm and 3 cm for the main and side arms, respectively, with an inside diameter of 1 cm.

Twenty-five microliters of extract were pipetted onto a 1.3-mm paper disk (Advantec, Toyo Roshi Kaisha, Ltd.), air-dried, and put into one of the Y-tube side arms. Another paper disk with ethanol solvent alone was placed in the other Y-tube side arm as a control. The two Y-tube side arms were then ventilated with two lines of air pump at the same flow rate of 0.6 L min-1. One termite worker was introduced into the main arm and allowed to move towards either of the side arms; this test was replicated 15 times, with a different worker each time. The test unit was covered with a sheet of transparent red plastic wrap (Rengo, Osaka, Japan) to protect termites from direct light. The number of workers that went into either of the side arms and remained for more than 30 seconds was recorded. The Y-tube boom was cleaned with ethanol and dried. After each replicate, the two side arms were alternated for treatment and control.

Trail-following test

The trail-following test was performed according to the method developed by Hall and Traniello (1985) with a modification in the Y line measurement. A Y-shaped pencil line was drawn onto a 12.5-cm-diameter filter paper (Whatman No. 2, United Kingdom) with 3-cm-and 7-cm-lengths of main stem and arms, respectively. The stem and one arm of each Y were treated with 75 mm3 extracts of each plant species, while the other arms were treated with the ethanol solvent as a control. The extracts were dried for approximately 2 minutes, and one termite worker was then placed at the base of the Y stem, held there with a 1-cm-diameter plastic cylinder and for one minute prior to being released. The test unit was covered with a 12.5-cm-diameter Petri dish coated with transparent red plastic wrap (Rengo, Osaka, Japan) to protect the termite from the ambient environment. The test was replicated 15 times for each extract. Following the line treated with an extract was considered a positive response, and the number of individual termites with a positive response was recorded for each extract.

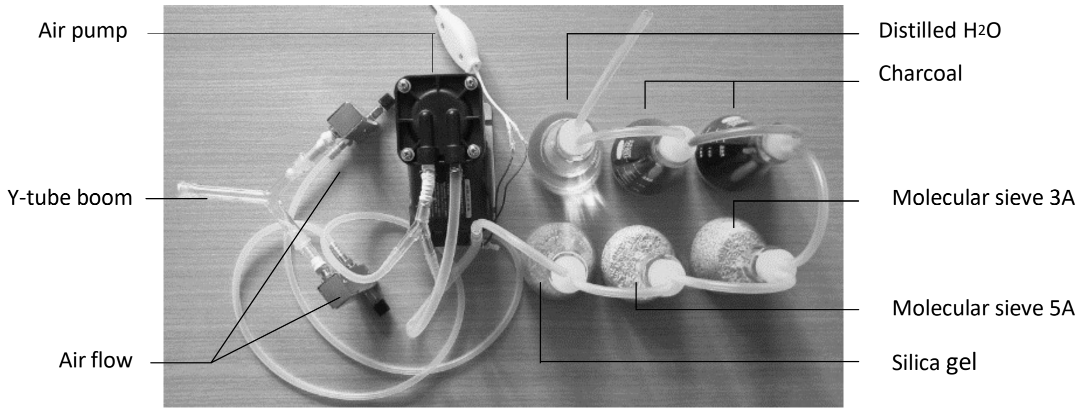

Figure 1. Experimental unit for the olfactory response test

Data analysis

The repellency rate of each of the crude extracts was calculated based on the number of termites that selected the control and the total number of tested termites. The positive response rate for each of the crude extracts was calculated based on the number of termites that followed the extract lines and the total amount of tested termites. All data were analyzed by using the Chi-Square Test to compare between treatment and control.

RESULTS AND DISCUSSION

Olfactory response

In the olfactory test, the termites responded differently to treated and untreated paper disks. Compared to untreated control, only the paper disk treated with *M. leucadendra* extract had significant attractive rates ($P < 0.001$). No significant response was found for the other tested extracts. The odour attractiveness rates of extracts were 40.00% for *S. aromaticum*, 80.00% for *M. leucadendra*, 53.33% for *C. burmannii*, 60.00% for *O. sanctum*, and 46.67% for *S. polyanthum*. The different responses of the tested plant extracts indicate that each of them has different chemotypes exerting different biological actions. In this study, *M. leucadendra* extract had a significant attractant effect for the termites, with the repellent rate being only 20%.

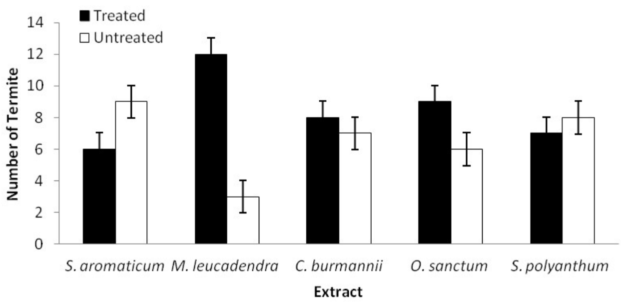

Figure 2. Responses of worker termites to leaf extracts in the olfactory test

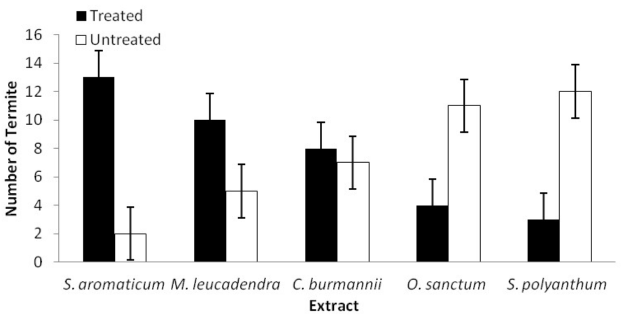

Figure 3. Responses of worker termites to leaf extracts in the following trial test

Trail following

In the trail-following test, positive responses were found for the *S. aromaticum* and *M. leucadendra* extracts as shown in Figure 2. The number of termites that followed the extracted line of *S. aromaticum* was significantly greater than the number other ways (significant at $P < 0.05$). A significant response by termites was also found for the *M. leucadendra* extract (significant at $P < 0.05$) but the difference for the extracted line of *C. burmannii* was not significant ($P > 0.05$). Negative responses were observed for the *O. sanctum* and *S. polyanthum* extracts. The number of termites that followed the extracted line of *O. sanctum* was significantly less than the number that went other ways (significant at $P < 0.05$). The same significantly negative response by termites was also found for the extracted line of *S. polyanthum* (significant at $P < 0.05$). Hence, the attractant rates for *O. sanctum* and *S. polyanthum* extracts were lower than those of *S. aromaticum* and *M. leucadendra* extracts. The trail-following rates were 86.67% for *S. aromaticum*, 66.67% for *M. leucadendra*, 53.33% for *C. burmannii*, 26.67% for *O. sanctum*, and 20.00% for *S. polyanthum*.

Our bioassay showed that among the five plant crude extracts, *M. leucadendra* crude extract was the most attractive to termites based on their olfactory behavior tested using the olfactory response test and trail-following test. *Syzygium aromaticum* crude extract could also be considered as attractive for termites, especially in the trail-following test. The remaining crude extracts, including *C. burmannii*, *O. sanctum*, and *S. polyanthum* were not attractive. Our results agree with previous studies reporting many plants as having anti-termitic activities, such as clove (Koul et al. 2008; Zhu et al. 2001a, b) and cinnamon (Koul et al. 2008; Sbeghen et al. 2002). Basil is also known for its varied pest control properties (Koul et al. 2008), and bay leaves possess repellent properties for controlling structural pests such as termite (Onyambu et al. 2014).

The attractiveness level of plant extracts is acknowledged to have the relation with the type and concentration of their chemical compounds. For *M. leucadendra*, one of the chemotypes associated with its attractiveness is the high content of cineole and low content of eugenol when compared to other plant extracts. Although the two chemical compounds have been known to have repellent and toxicant properties against insects, depending on their concentrations, they can be considered as essential oil with a capacity to attract termites. Besides, the chemical responses are believed to be the resultant effect from a complex interaction between its constituents, which produce both synergistic and antagonistic responses between the components. Therefore, understanding such interactions is important in comparing plant extracts on the basis of their chemical composition.

Earlier studies suggested that the anti-termitic activities of many plants were associated with the presence of eugenol in their tissues. Cornelius, Grace, and Yates (1997) reported that eugenol caused 100% subterranean termite *C. formosanus* mortality at a concentration as low as 0.06 μLg^{-1} of sand. Furthermore, previous data have shown that the crude extracts of *S. aromaticum, M. leucadendra*, and

C. burmannii contain eugenol. GCMS analysis showed eugenol in each crude extract used in this study as follows: *S. aromaticum* (7.92%), *C. burmannii* (4.64%), and *M. leucadendra*, (3.36%), while the crude extract of the other two plant species contained derivatives of eugenol such as methyl eugenol (2.24%) in *O. sanctum* and acetyl eugenol in *S. polyanthum* (6.54%) (Indrayani et al. 2017). The result of this study confirms that *M. leucadendra* crude extract, which is the most attractive to *C. formosanus*, has the lowest amount of eugenol.

The result also demonstrated the potential for *S. polyanthum* extracts to be either attractant or repellent substances for termites because repellent and trail-following rates were 60.00% and 86.67%, respectively. These effects should be carefully considered with respect to application of *S. polyanthum* extract as it was found to have a high eugenol content (Indrayani et al. 2017). A study using a 10% crude extract of *S. aromaticum* (equal to a eugenol content of 0.00792%) was found to attract termites (Indrayani et al. 2017). The reason for these different responses by termites is unclear and need to be further explored, particularly related to the appropriate concentration and synergic interaction of active compound present in crude extract of *S. aromaticum*. More studies are necessary to prove their mechanism of action.

The current result will be useful in determining the compounds in different crude extract that are attractive to subterranean termites. The data concerning chemotypes and biological properties of various plant extracts enable us to determine whether these crude extracts are compromised as part of a bait matrix formulation, particularly for developing bait system technologies for termite controls. In this case, the concentration of each active chemical compounds should be taken into consideration together with its synergistic or antagonistic properties to other compounds.

In conclusion, the tested leaf extracts performed differently in terms of their effects on subterranean termites (*C. formosanus*). Positive responses were found for *S. aromaticum* and *M. leucadendra* extracts as the termites prefer to move along the extract lines in the trail-following tests. However, *S. aromaticum* extract also demonstrated a negative reaction as the termites go toward the untreated control in the olfactory response test. No significant termite response was observed on the treatments using *C. burmannii*, *O. sanctum*, and *S. polyanthum* extracts. The *M. leucadendra* extract is found to have potential toward meeting the needs of termite control because of its high attractant and low repellent rates. To use *M. leucadendra* leaf extract as bio-attractants for termites, other related data on active chemical compounds of the extract are required since they are responsible for the termite attractiveness. Further study is indispensable for searching the most appropriate type of chemical compounds containing in *M. leucadendra* leaf extract as bio-attractants for termites.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the DOL/LSF Collaborative Research Program at Research Institute for Sustainable Humanosphere (RISH), Kyoto University and the Directorate General of Higher Education of Indonesia for financial support.

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