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Method for controlling termite pest

Field of Art

The present invention relates to a method for controlling termite pest.

Background Art

The termite fauna of Peninsular Malaysia is represented by three families, the Kalotermitidae, Rhinotermitidae and Termitidae, and comprises a total of about 175 species from 42 genera (Y.P Tho. 1992. Termites of Peninsular Malaysia. Forest Research Institute Malaysia, Kepong, Kuala Lumpur :1-224.). Termites are considered one of the most destructive insect pests of wooden structures throughout the world, especially in areas with warm, humid climates (Sanaa A Ibrahim, Gregg Henderson and Huixin Fei. 2003. Toxicity, Repellency, and Horizontal Transmission of Fipronil in The Formosan Subterranean Termite (Isoptera : Rhinotermitidae). *Journal Economic Entomology*. Vol. 96. No. 2: 461-467). Control of subterranean termites has always been an important concern of homeowners and the pest control industry.

Woodrow *et al.* (R. Joseph Woodrow, J. Kenneth Grace, And Robert J. Oshiro. 2006. Comparison of Localized Injections of Spinosad and Selected Insecticides for the Control of *Cryptotermes brevis* (Isoptera: Kalotermitidae) in Naturally Infested Structural Mesocosms. *J. Econ. Entomol*. Vol. 99 No. 4: 1354-1362) used six chemical insecticide treatments to compare their effectiveness against *Cryptotermes brevis* (Isoptera: Kalotermitidae). They used chlorpyrifos aerosol, aqueous disodium octaborate tetrahydrate (DOT), resmesthrin aerosol, distill water as a control, and two treatments of spinosad SC. In whole pallets, mean percentage mortality ranged from 53.3 to 58.7 % for the visual and AE spinosad treatments, respectively, whereas water averaged 6.8%. Remaining treatment mortalities were 33.2, 30.4, and 18.1 % for chlorpyrifos, DOT, and resmethrin, respectively.

Peterson et al. (C. J. Peterson and J. Ems-wilson. 2003. Catnip Essential Oil as a Barrier to Subterranean Termites (Isoptera: Rhinotermitidae) in the Laboratory. *Journal of Economic Entomology*. Vol. 96, No. 4: 1275-1282) evaluated a barrier to Subterranean Termites (Isoptera: Rhinotermitidae). The essential oil of catnip, *Nepeta cataria* (Lamiacae) was evaluated for behavioral effects on two populations of subterranean termite, *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) (Isoptera: Rhinotermitidae). For *R. flavipes*, the 24-h topical LD_{50} value was ≈ 8200 µg/g termite. Although tunneling ceased in these tests, mortality was not high, indicating that the termites avoided the treated sand.

LD_{50} of a mixture of the two major alkaloids, matrines and matrine from *Sophora flavescens* Aiton (Leguminosae), from Lixin and Henderson, 2007 (Lixin Mao and Gregg Henderson. 2007. Antifeedant Activity and Acute and Residual Toxicity of Alkaloids From *Sophora flavescens* (Leguminosae) Agains Formosan Subterranean Termites (Isoptera : Rhinotermitidae). *Journal of Economic Entomology*. Vol. 100. No.3: 866-870) study against *Coptotermes formosanus Shiraki* (Isoptera: Rhinotermitidae) was 12.3 and 8.6 µg per insect, respectively. After 24 h exposure it has remained effective at least 12 months after treatment under both light and dark storage conditions.

Zhu *et al.* (Betty C.R. Zhu, Gregg Henderson, Feng Chen, Huixin Fei and Roger A. Laine. 2001. Evaluation of Vetiver oil and Seven Insect-Active Essential oils Against the Formosan Subterranean Termite. *Journal of Chemical Ecology*. Vol 27. No.8: 1617-1625), a study of evaluation of vetiver oil and seven insect-active essential oil aginst Formosan Subterranean termite concluded that clove bud was the most toxic, killing 100 % termite in two days at 50 µg/ cm2 compared to the other 7 essential oil of *vetiver grass, cassia* *leaf, cedarwood, Eucalyptus globules, Eucalyptus citrodora,* lemongrass and geranium. Meanwhile Kwon and Chul (Park–II- Kwon and Shin Sang Chul. 2004. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove Japanese termite (*Reticulitermes speratus* Kolbe). *Journal of Agricultural and Food Chemistry Vol*. 42 (3). 2004) reported that clove bud and garlic oils kill 100 % Japanese termite (*Reticulitermes speratus* Kolbe) at 5.0 µL/L of air concentration.

Insect Pest management (IPM) has to face up to the economic and ecological consequences of the use of pest control measures. Sixty years of sustained struggle against harmful insects using synthetic and oil-derivative molecules has produced perverse secondary effects on mammalian toxicity, insect resistance and ecological hazards. The diversification of the approaches inherent in IPM is necessary for better environmental protection.

There has been a growing interest in the development of naturally produced plant compounds as alternative to synthetic insecticides (Maistrello, L., G. Henderson, and R. A. Laine. 2001. Efficacy of vetiver oil and nootkatone as soil barriers against Formosan subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* Vol. 94: 1532-1537), because chemicals produced naturally have less impact on environmental and human health.

Ginger has been used in Asia for thousands of years for relief from arthritis, rheumatism, muscular aches and pains, coughs, sinusitis, sore throats, diarrhea, colic, cramps, indigestion, loss of appetite, motion sickness, fever, flu, chills, and infectious diseases (College, J.N.M., 1985. The Dictionary of Traditional Chinese Medicine. Shanghai Sci-Tech Press, Shanghai). It has been tested against bacteria and funghi species (N.S. Alzoreky et al., International Journal of Food Microbiology (80) : 223-230, 2003; J. Nguefacka et al., International Journal of Food Microbiology (94) : 329-334, 2004)

Boesenbergia rotunda (L.) Mansf. Kulturpfl. (syn. *Boesenbergia pandurata* (Robx.) Schltr.) is a perennial herb belonging to the Zingiberaceae family. It is also known as ‘temu kunci’ in Malaysia. *Boesenbergia rotunda* is the most abundant *Boesenbergia* species in Malaysia. *Boesenbergia rotunda* is small herbaceous plant with short, fleshy or slender rhizomes, one to a few leaves. Fresh rhizomes have a characteristic aroma and a slightly pungent taste. It is commonly used in Southeast Asia as a food ingredient, a folk medicine for the treatment of several diseases such as aphthous ulcer, dry mouth, stomach discomfort, leucorrhea and dysentery. The rhizomes are given as tonics to women in mixtures after childbirth; added into lotions for rheumatism and muscular pains, and into pastes for application to the body after confinement (Burkill, I. H., 1935. A Dictionary of the Economic Products of the Malay Peninsula Volume I, pp 1078-1079. London: Government of the Straits Settlements & Federated Malay State by the Crown agents for the colonies). As regards its biological activities, *Boesenbergia rotunda* exhibits antimutagenic, antitumour, antibacterial, antifungal, analgesic, antipyretic, antispasmodic, anti-inflammatory and insecticidal activities (Cheenpracha, S., Karalai, C., Ponglimanont, C., Subhadhirasakul, S., and Tewtrakul, S., 2005. Anti-HIV-1 protease activity of compounds from *Boesenbergia pandurata*. *Bioorganic and Medicinal Chemistry* 16: 1710-1714). Previous investigations have revealed the isolation of pinostrobin (1), pinocembrin (2), cardamonin (3), alpinetin (4) and boesenbergin A (5) from the rhizomes of *Boesenbergia rotunda* (Jaipetch, T., Kanghae, S., Pancharoen, O., Patrick, V. A., Reutrakul, V., Tuntiwachwuttikul, P., and White, A. H., 1982. Constituents of *Boesenbergia pandurata* (syn. *Kaempferia pandurata*): Isolation, crystal structure and synthesis of *Boesenbergin* A. *Aust. J. Chem.* 35: 351-361). 2-D NMR technique was used for the structure elucidation of boesenbergin A to complement the data reported previously (Mahidol, C., Tuntiwachwuttikul, P., Reutrakul, V., and Taylor, W. C., 1984. Constituents of *Boesenbergia pandurata* (syn. *Kaempferia pandurata*). Isolation and synthesis of Boesenbergin B. *Aust. J. Chem.* 37: 1739-1745).

Disclosure of the Invention

The present invention relates to the use of *Boesenbergia rotunda* for termite pest control. More particularly, it relates to the use of essential oils of *Boesenbergia rotunda.*

In a preferred embodiment, the termite pest are *Reticulitermes flavipes* and *Macrotermes malaccensis.*

Boesenbergia rotunda extract or the essential oils are preferably used in the concentration range from 1,000 ppm to 10,000 ppm, more preferably from 3,000 ppm to 5,000 ppm.

Another object of the present invention is a method of controlling the termite pest, comprising administering *Boesenbergia rotunda* essential oil to said termite pest.

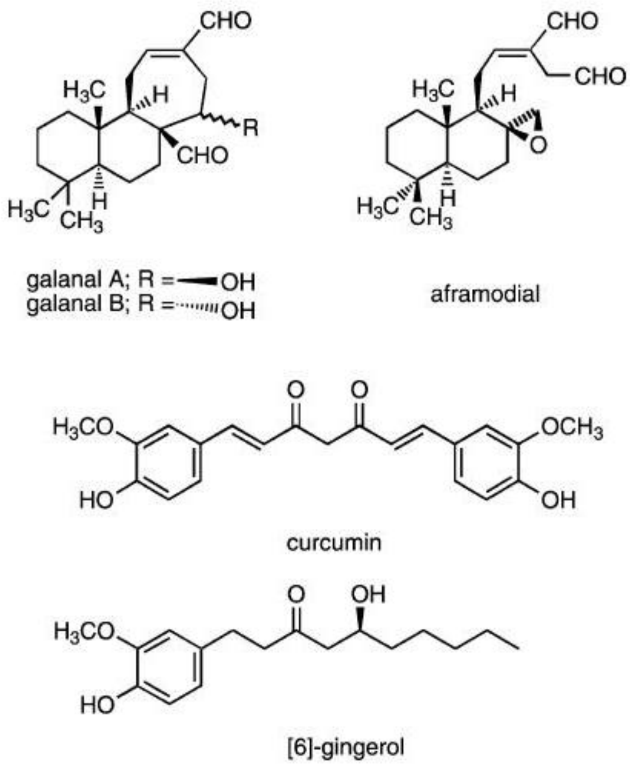
Of the two termite species, for the brown termite *Reticulitermes flavipes*, the LD_{50} value is 2,497 ppm/termite after 5 h application of *Boesenbergia rotunda* essential oils.

For the black termite *Marcotermes malaccensis*, the LD_{50} value is 3,757 ppm/termite after 6 h application of *Boesenbergia rotunda* (L.) essential oils.

Yogeshwer and Singh (2007) (Yogeshwer Shukla and Madhulika Singh, Cancer Preventive Properties of Ginger : A Brief Review, *Journal Food and Chemical Toxicology*, Vol 45(5): 683-690) reported that ginger from family Zingiberaceae essential oil contains gingerol, paradol, shagaol, zingerone as a non-volatile component. The mentioned chemicals possess cancer prevention properties. They found that ginger species contain biologically active constituents including the non-volatile pungent principles, such as the gingerols, shogaols, paradols and zingerone that produce a ‘‘hot’’ sensation in the mouth when consumed.

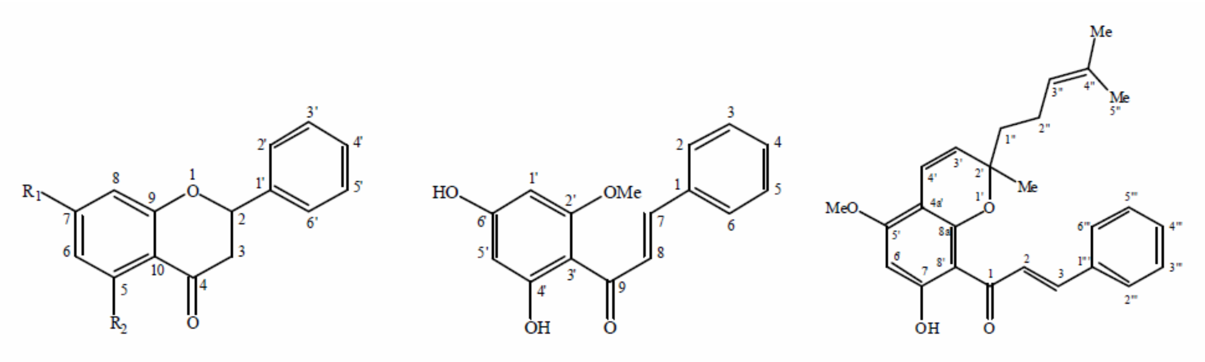
The odor of ginger depends mainly on its volatile oil, the yield of which varies from 1 % to 3 %. Over 50 components of the oil have been characterized and these are mainly monoterpenoids [b-phellandrene, (+)-camphene, cineole, geraniol, curcumene, citral, terpineol, borneol] and sesquiterpenoids [a-zingiberene (30–70%), b-sesquiphellandrene (15–20%), b-bisabolene (10–15%), (E-E)-a-farnesene, arcurcumene, zingiberol] (Badreldin H. Ali, Gerald Blunden, Musbah O. Tanira, Abderrahim Nemmar. 2007. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. *Food and Chemical Toxicology.* Vol. 46: 409–420)

The study from Prasad S. Variyar, A. S. Gholap & P. Thomas (Prasad S. Variyar, A. S. Gholap & P. Thomas. 1997. Effect of y-irradiation on the volatile oil constituents of fresh ginger (zingiber officinale) rhizome. *Food Research International.* Vol. 30, No. 1: 4143) on volatile oil constituents of fresh ginger (zingiber officinale) rhizome found that ginger oil major constituents were camphene, β-phellandrene, linalool, α-terpenoil, neral, geranial, ar-curcumene, zingebere, zingiberol, β-sesquiphellandrene, β-bisabolene and nerolidol



From Noriyuki Miyoshia *et al*., 2003.

Amy *et al*. 2007 (Amy Yap Li Ching, Tang Sook Wah, Mohd Aspollah Sukari, Gwendoline Ee Cheng Lian, Mawardi Rahmani1 and Kaida Khalid. 2007. Characterization of Flavonoid Derivatives from *Boesenbergia rotunda* (L.). *The Malaysian Journal of Analytical Sciences*. 11(1): 154-159) reported the extraction and separation on the hexane and chloroform extracts of *Boesenbergia rotunda* have led to the isolation and characterization of five flavonoids, which were identified and characterized as pinostrobin (1), pinocembrin (2), alpinetin (3), cardamonin (4) and boesenbergin A (5).



(1) R_1 = OMe, R_2 = OH
(2) R_1 = OH, R_2 = OH
(3) R_1 = OH, R_2 = OMe

(4)

(5)

From Amy *et al*., 2007

Example of carrying out the Invention

Termites

Two worker species of Peninsular Malaysia termite species were collected on August 2007 from field station of Forest Research institute Malaysia at Pasoh, Negeri Sembilan. The populations of black termite and brown termite were collected at different areas. Black termite species were collected in the soil 1km from the lab, while brown termites were found in the decay wood near the lab area at the FRIM field station. The soil and decay woods containing termites were stored at ambient conditions in the laboratory and termites were removed as needed. Termites were identified to species by examination of a soldier at Malaysia Agriculture Department, Kuala Lumpur. The species were *Reticulitermes flavipes* and *Macrotermes malaccensis***.**

Essential oil

30.5 ml of essential oil of *Boesenbergia rotunda* was obtained from 10kg dried *Boesenbergia rotunda* using steam distillation at chemistry lab in University of Malaya.

The essential oil of *Boesenbergia rotunda* was mixed with distilled water to obtain concentrations of 1000 ppm, 3000 ppm, 5000 ppm and 10 000 ppm concentrations.

Acute Toxicity Tests

Black termite and brown termite mortality observed and recorded at 15 min, 30 min, 45 min after application. After 45 minutes of observation, termite mortality were checked and recorded for every 1 h for 12 hours observation.

Bioassay

Filter paper with 9mm diameter was placed in petri dish. Each of 10 black termite workers were treated on the abdomen 10 µl of the appropriate dilution of *Boesenbergia rotunda* essential oil using a Hamilton PB-600 micro applicator

(Hamilton Co., Reno, NV). Each plate was closed using petri dish lid. Mortality was recorded at 15, 30, 45 minutes and in every 1 hour for 12 hours. 100 % mortality was used to calculate LD_{50} values by using ANOVA analysis.

For the bioassay test 10 replicates were done of 10 black termite workers treated using 10 µl of distilled water on the abdomen as above. Ten replicates for each test and control experiment were performed with black termite.

The above method was repeated using brown termite.

Percentage of average mortality was calculated from the numbers of living termites after 12 hr for each experiment and control.

Statistical Analysis

Finney Method (Lognormal Distribution) was used to determine LD_{50}. Meanwhile to evaluate the effect of *Boesenbergia rotunda* species essential oil on survival of two tested termite workers colonies, analysis of variance was using ANOVA. Workers from two tested colonies were compared with ANOVA using Duncan Multiple Range test.

Results

Results of this study showed that essential oil of *Boesenbergia rotunda* caused mortality to worker termites following by the time of exposure towards the termite body. From 12 hours of observation, mortality of the termite increased following by time. The treatments used ensure that the toxic constituents penetrate through the termite cuticle to the target site before they were getting toxified and dying.

Table 1. Mean Mortality of *Reticulitermes flavipes* after application of *Boesenbergia rotunda* (L.) with different dosage following by time of the treatment.

Concentrations	**Minutes**			**Hours**											
	15 m	**30 m**	**45 m**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1,000 ppm	2.10	2.80	2.90	3.70	4.90	5.20	6.20	6.40	6.40	6.50	6.60	7.00	7.50	7.60	7.70
3,000 ppm	2.60	2.90	3.40	3.90	5.30	5.40	6.40	6.60	6.60	6.70	6.90	7.20	7.90	8.00	8.10
5,000 ppm	3.20	4.60	4.90	5.20	6.10	6.40	7.00	7.20	7.20	7.60	8.00	8.30	8.90	8.90	9.00
10,000 ppm	4.40	5.80	6.40	7.30	8.20	8.40	8.80	9.00	9.00	9.20	9.40	9.60	9.80	10.00	10.00
Control	0.00	0.00	0.00	0.00	0.00	0.40	0.40	0.40	0.80	0.80	0.80	1.30	1.30	1.30	1.60

The results for the 12 h observation after application of *Boesenbergia rotunda* (L.) the mortality of *Reticulitermes flavipes* were significantly different following by time. After 15 min, 30 min, 45 min, 1 h to 7 h of application of *Boesenbergia rotunda* (L.), the mortality of the both termites were not significantly different. Mortality of *Reticulitermes flavipes* using *Boesenbergia rotunda* (L.) was significantly different after 5 h of application with LD_{50} value 2,497 ppm/termite.

Table 2. Mortality of *Macrotermes malaccensis* after application with different dosages of *Boesenbergia rotunda* (L.) following by time of the treatment.

Concentrations	**Minutes**			**Hours**											
	15 m	**30 m**	**45 m**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1,000 ppm	1.50	1.50	1.50	1.50	1.50	1.80	0.00	2.20	3.40	4.70	7.40	7.60	8.90	9.10	9.20
3,000 ppm	1.00	1.20	1.20	1.50	2.00	2.70	2.80	3.60	4.00	5.10	7.50	7.70	8.40	9.30	9.40
5,000 ppm	1.50	1.60	1.60	2.60	3.10	3.60	3.60	4.20	4.60	6.40	8.00	8.30	9.20	9.60	9.70
10,000 ppm	1.60	2.00	2.00	2.00	2.50	3.50	4.10	4.20	4.90	6.50	7.00	8.00	9.10	9.80	9.90
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.40	0.40	0.40	0.60	0.60	0.60

After direct application of *Boesenbergia rotunda* (L.) towards termites**,** the mean numbers of worker termites in the test arena for 15,30, 45 min and for 1 h to 12 h application using 1000 ppm, 3000 ppm, 5000 ppm and 10 000 ppm were significantly different between group of dosage.

The workers of *Marcotermes malaccensis* LD_{50} value was at 3,757 ppm/termite after 6 h applications with *Boesenbergia rotunda* (L.) essential oil. LD_{50} values for *Boesenbergia rotunda* (L.) ranged from 3,256 ppm to 7,951 ppm dosage with significant level at $P < 0.05$. An overall treatment *Boesenbergia rotunda* (L.) essential oil indicates that there was significant treatment effect mortality following the time.

Termites were treated using distilled water as control. Their mortality was also observed for 12 hours. After 12 hours of observations, (6%) (N=6) dies, possibly due to the unsuitable situation; lack of humidity because they have been removed from their natural habitate. The mortality of termites can be also caused by starvation for the 12 h of observation without food and soil.

Results obtained from our study also indicate that toxic constituents of *Boesenbergia rotunda* (L.) take 6 hours to reached the target site and slowly kill the termites.

CLAIMS

1. Use of *Boesenbergia rotunda* (L.) for termite pest control.

2. Use of essential oils of *Boesenbergia rotunda* for termite pest control.

3. Use according to any preceding claims, wherein *Boesenbergia rotunda* extract or its essential oils is used in the concentration range from 1,000 ppm to 10,000 ppm.

4. Use according to any preceding claim, wherein the termite pest are *Reticulitermes flavipes* and *Macrotermes malaccensis*

5. Method of controlling the termite pest, comprising administering *Boesenbergia rotunda* (L.) extract or its essential oil to said termite pest.

6. Method of claim 5, wherein the termite pest is exposed to the *Boesenbergia rotunda* (L.) extract or its essential oil for at least 5 hours, more preferably for at least 6 hours.

Abstract

Method for controlling termite pest

The present invention relates to the use of *Boesenbergia rotunda* (L.) for termite pest control and for method of controlling the termite pest.