Insecticidal potential of *Moringa oleifera* (Lamarck) root on workers of *Macrotermes bellicosus* (Smeathman)

Magdalene Ehinomen OMOREGIE[ab]*, Anthony Osarobo OMOREGIE[a], Beatrice Ngozi ILOBA[a]

[a]Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.
[b]Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City, Nigeria.

*** Corresponding Author**
E-mail: magdalene.omoregie@uniben.edu

Co-Authors:
A.O. Omoregie. E-mail: anthony.omoregie@uniben.edu
B.N. Iloba. E-mail: beniloba@yahoo.com

Abstract

The study was carried out to investigate the insecticidal potential of methanolic extract of Moringa oleifera (Lamarck) root on workers of Macrotermes bellicosus (Smeathman). The root extract was tested at different concentrations (1, 2, 4, 7 and 14%) on termite workers at 24, 48, 72 and 96 h in a soil bioassay. Data were analysed statistically using Analysis of Variance (ANOVA). Results showed that, at 96 h post treatment the percentage mean mortality of termites induced by the extract ranged from 13.35% to 56.65%. As concentration of root extract increased at 14%, mortality was significant ($P < 0.05$) after 96h of exposure. The phytochemicals identified in the root were: alkaloids, phenols, flavonoids, tannins, glycosides, steroids, triterpenoids, saponins and anthraquinones. Moringa root extract showed promising potency against Macrotermes bellicosus and may thus be useful for the control of this termite species.

Keywords: methanolic extract, phytochemicals, soil bioassay, mortality, soxhlet extractor

1. Introduction

Macrotermes sp. are members of the fungus-growing sub-family Macrotermitinae in the order Blattodea (Kumar *et al*. 2013; Beccaloni & Eggleton 2013) that cultivates symbiotic fungi in their nest (Binate *et al.*, 2008). There are about 330 species in the genus *Macrotermes*, spread over tropical Africa and Asia. *Macrotermes bellicosus* is considered a popular termite in Nigeria (Ekpo & Onigbinde 2007). Species of *Macrotermes* build large epigeal nests (mounds) from where they forage outwards to distances up to 50 m in galleries/runways. They attack plants at the base of the stem, ring-barks or cut them completely. In Africa, they are serious pests of some agricultural crops and tree plantations. They attack crops such as maize (Wood *et al.,* 1980), groundnut (Johnson & Gumel 1981), sugarcane (Boboye, 1986), rice (Alam 1992) and cocoa (Ndubuaku & Asogwa 2006). They are responsible for the majority of crop damage and 90% of tree mortality in forestry. In some part of Africa *Macrotermes* do cause a yield loss of 30-60% (UNEP & FAO 2000). A number of insecticides have been used in managing termites including organochlorines and chlorpyrifos with high mammalian toxicity (Lewis 1980; Su & Scheffrahn 1990b; Jitunari *et al*. 1995). This was followed by the pyrethroids (mainly served as repellents) which were stronger than chlorpyrifos but had less residual effects than the organochlorines (Lenz *et al.* 1990; Su & Scheffrahn 1990b; Pawson & Gold 1996). All the above termiticides degrade over time. Beginning in the year 2000, several new nonrepellent soil termiticides appeared on the market e.g., fipronil, imidacloprid, and chlorfenapyr. Currently, these three nonrepellent termiticides and the pyrethroids are the only chemicals available for the soil treatment against termites (Wagner *et al.* 2003). The impact of liquid termiticides on both human health and environment as well as the limitations of both the nonrepellent termiticides and pyrethroids has created a need for alternative methods in the control of subterranean termites. Plant extracts offer a vast, virtually untapped reservoir of chemical compounds with many potential uses. One of these uses is in agriculture to manage pests with less risk than with synthetic compounds that are toxicologically and environmentally undesirable. Plant products have been exploited as insecticides, insect-repellents, anti-feedants and insect growth and development regulators (Saxena 1998). They are possible alternatives against the synthetic insecticides for the control of termites (Sbeghen *et al*. 2002). Many plants have been recognised to have anti-termitic and repellent activities against termites, such as lemon grass (*Cymbopogon citratus* DC. Stapf), Cassia leaf (*Cinnamomum cassia* Linnaeus.), clove bud (*Syzgium aromaticum* Linnaeus.) (Zhu *et al*. 2001), *Plectranthus amboinicus* (Lour.) Sprengel (Singh *et al*. 2004), malabar nut (*Adhatoda vasica* Linnaenus), hop bush (*Dodonaea viscosa* Jacquin), drum stick (*Moringa oleifera* Lamarck.) (Mohammad 2012). *Moringa oleifera* Lam. (drumstick tree) is a valuable bio-pesticidal plant (Mamun & Ahmed 2011). It belongs to the single genus family-Moringaceae with 13 different species. *Moringa* is widely cultivated in Africa. In Nigeria, *Moringa oleifera* is widely used traditionally for nutritional and medicinal purposes against various disease conditions (Furo & Ambali 2012). It grows best in dry sandy soil; it tolerates poor soil including coastal areas and is drought-resistant. Its various parts have been identified for antimicrobial activity (Dahot 1998), analgesic activity (Marugandan *et al*. 2000), antihypertensive activity (Dangi *et al*. 2002) and insecticidal activity (Dahot 1998). The present study was conducted to investigate the potential of

Moringa oleifera Lam root extract against workers of *Macrotermes bellicosus* as a possible alternative for the control of this termite species.

2. Materials and Methods

2.1 Collection of roots and preparation of extract

Roots of *Moringa oleifera* (Lam) were collected from the Senior Staff Quarters in the premises of University of Benin, Benin City, Edo State. These were cut into pieces, air-dried, then oven-dried at 60˚C for 45 min to remove any moisture. The dried roots were ground with micro-plant grinding machine (model no. D6, No. 19 × 182) and sieved through a 1.0 mm pore size mesh sieve to obtain a fine powder. Using a soxhlet extractor, 1046.86 g of root powder was extracted in 7.5 l of 95% (analytical grade) methanol, after which the methanol was removed at 60 ˚C using rotary evaporator. Solid root (11.44 g) extract was obtained which was used for the study.

2.2 Collection of termites

Various castes of *M. bellicosus* were collected from a mound in the premises of University of Benin Teaching Hospital, Benin City, Edo State. The termites were maintained in plastic boxes (28.2 × 26.5 × 27.7 cm) lined with moist cotton wool and fungal comb was placed inside as food. The top of the plastic boxes were covered with muslin cloth allowing air ventilation. The population of termite was used within 24 to 48 h of collection. In the laboratory the workers were identified and separated for the experiment proper.

2.3 Preparation of stock solution and percent concentration

Stock solution (10%) was prepared by dissolving 1.25 g of solid extract in 12.5 ml of distilled water. Concentrations of 1%, 2%, 4%, 7% and 14% were prepared from the stock solution by serial dilution.

2.4 Bioassay

Soil for the bioassay was collected from around the termite mound with 78.6% sand, 5.00% silt and 16.4% clay composition. It had no known applications of pesticides. The soil was sieved and sterilized in an oven (model no. MC173467) at 150˚C. Moisture content of the soil was determined. 7 g of sterilised soil was measured into 9 cm Petri dishes and 0.5 g fungal comb was added to keep the termites alive. The soil in Petri dish was moistened with 1 ml of distilled water after which 2 ml of the different concentrations of the extract and control (distilled water) was sprayed on the soil in the Petri dish. Twenty active workers were introduced into each treated and control Petri dishes. The lids of the Petri dishes had three aeration holes (2.5 cm in diametre) covered with muslin cloth to ensure free flow of air but preventing the escape of termites. The Petri dishes were then placed in plastic boxes lined with moist cotton wool and the plastic boxes kept in the dark. The experiment was maintained at 25 – 28˚C temperature and 50 – 60% relative humidity. The treatment and control (distilled water) were replicated three times in completely randomised design (CRD).

2.5 Mortality calculation

Mortality of termites was recorded at 24 h, 48 h, 72 h and 96 h after application of treatment and percentage insect mortality calculated using the equation:

Mortality (%) = No. of dead insects/Total no. of insects × 100

2.6 Statistical analysis

The data recorded for mortality was analysed using one-way ANOVA and mean separation done using Student-Neumal-Kuels (SNK). Observations were considered significant where $P < 0.05$. These analyses were done using SPSS version 16.0.

3. Results and Discussion

A result of the phytochemical analysis of methanolic extract of *M. oleifera* (Lam) root is presented in Table 1. The percentage mean mortality of small workers of *M. bellicosus* exposed to various concentrations of the methanolic root extract of *M. oleifera* is summarised in Table 2. There was no significant difference ($P > 0.05$) in the mortalities recorded by the controls, 1%, 2%, 4% and 7% at the exposure times. Conversely, at 14% concentration, the observed mortality (3.33%, 11.67%, 35.00% and 56.67%) at the exposure times was significantly different ($P < 0.05$). The lowest mortality (3.33%) was recorded at 24 h while the highest mortality (56.67%) was recorded at 96 h.

Table 1: Phytochemical analysis of methanolic extract of *Moringa oleifera* root

Phytochemicals	Root
Alkaloids	+
Flavonoids	+
Tannins	+
Phenol	+
Glycoside	++
Steroids	++
Triterpenoids	++
Anthraquinones	+
Saponins	+

Key: + slightly present, ++ moderately present, +++ obviously present, - absent

Table 2: Percentage mean mortality of *Macrotermes bellicosus* exposed to *Moringa oleifera* root extract

Percentage Concentration	24 h $\bar{x}$±SD	48 h $\bar{x}$±SD	72 h $\bar{x}$±SD	96 h $\bar{x}$±SD	p-Value
Control	0.00±0.00	1.67±0.58	6.67±1.15	15.00±2.65	$p>0.05$
1%	1.67±0.58	5.00±1.00	8.33±2.08	13.33±3.79	$p>0.05$
2%	3.33±0.58	10.00±1.00	13.33±1.53	16.67±2.08	$p>0.05$
4%	1.67±0.58	6.67±1.53	11.67±1.15	13.33±1.53	$p>0.05$
7%	3.33±1.15	11.67±2.52	18.33±3.06	21.67±2.52	$p>0.05$
14%	3.33^{b} ±1.15	11.67^{ab} ±1.15	35.00^{ab} ±6.08	56.67^{a} ±4.73	$p<0.05$

$P>0.05$- Significant difference; $p<0.05$- No Significant difference, similar superscript row wise- No significant difference

Bioactive compounds are secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals. Virtually all plant tissues of *M. oleifera* Lam e.g. leaves, roots, stem and fruits contain phytochemicals (Siddqui *et al.* 2009). Results obtained from this study showed the bioactive constituent of the root extract to include; alkaloids, flavonoids, tannins, glycosides, steroids, triterpenoids, anthraquinones and saponins. This is similar to that reported by Patil & Rasika (2013) in India for methanol-extracted *Moringa oleifera* root. The presence of these phytochemicals indicate that the extract from root of *M. oleifera* possess good insecticidal potential (Nadi 2001; Kabaru & Gichia 2001).

Insecticidal effect of *Moringa oleifera* extract on termites has been reported by Paiva *et al.* (2010) and Muhammad (2012). The present study is in consonance with these findings where methanolic extract of *M. oleifera* root was found to have insecticidal effect against workers of *M. bellicosus*. In this study, the methanol extract of *M. oleifera* root recorded highest termite mortality of 56.67% at 14% concentration 96 h after treatment. Similarly, Okweche *et al.* (2015) observed that *M. oleifera* seed extract recorded 95% mortality after 60 h and 100% at 72 h on the wood termite *Cryptotermes cavifrons* Banks. Oyedokun *et al.* (2011) also reported bio-insecticidal activities of aqueous and ethanolic extract of *Phyllanthus amarus* Schumacher, *Acassia albida* Delile and *Tithonia diversifolia* leaves in concentrations of 12.5%, 25%, 50%, 66.7%, and 75% on workers of *Macrotermes bellicosus*. The aqueous extract of the three plants caused 40 - 56%, 24 - 60% and 42 - 88% mortality respectively after 140 min while the ethanol extract of the plant extracts resulted in a higher percentage mean mortality of 64 - 91%, 36.4 - 76% and 36 - 68% respectively after 140 min. Badshah *et al.* (2004) also investigated the effect of leaf and flower extract of *Calotropis procera* against two termite species i.e., *Heterotermes indicola* and *Coptotermes heimi* and the result of their study showed that the leaf and flower extract recorded highest mortality of 21.3% (leaf) and 45.3% (flower) on *Heterotermes indicola* and 24.0% (leaf) and 56.0% (flower) mortality on *Coptotermes heimi*. From the present study, the root extract was toxic to small workers of *M. bellicosus* as concentration increased at 14% (the point where mortality was significant). Similar results on concentration-dependent increase in mortality rates was observed for the same organism exposed to extract of *Zingiber officinale* Roscoe, *Allium sativum* Linnaeus, *Dennettia tripetala* Bak.f. and *Capsicum annum* Linnaeus (Ojianwuna *et al.* 2016), where concentration increase from 10 - 30% resulted in significant (P > 0.05) mortality of the termite species. This is also true in investigations made by Paiva *et al.* (2010) where preparations of *Moringa oleifera* seeds (WSMoL and cMoL) were only active at concentrations of 1.0 and 1.5mg/ml on workers and soldiers of *Nasutitermes corniger.* Mortality was also a function of time in this study as mortality was highest in the root (56.67%) at 96 h. There is need for further research to determine the effective concentration of methanolic root extract that will result in 100%, as significant mortality only resulted at 14%. Also, the precise mechanism of action of the extract needs to be studied.

4. Conclusion

The use of extracts from plants is one of the environmentally friendly alternatives to using chemical pesticides for termite control. In this study, it was found that methanolic extract of *Moringa oleifera* root possess some insecticidal potency as extract was effective on termite mortality. *Moringa* plant is readily available and easily cultivated in Nigeria, thus it may be useful for the control of this termite species.

5. References

Alam, M. S. (1992). A survey of rice insect pests in Nigeria. Tropical Pest Management, 38, 115 - 118.

Badshah**,** H., Farmanullah, Z., Salihah, A. & Shakur, M. (2004). Toxic effects of AK (*Calotropis procera*) plant extracts against termites (*Heterotermes indicola* and *Coptotermes heimi*) (Isoptera: Rhinotermitidae). Pakistan Journal of Biological Sciences, 7 (9), 1603 - 1606.

Beccaloni, G. & Eggleton, P. (2013). "Order Blattodea". Animal Biodiversity: In Z. Q. Zhang (Eds.), An Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa, 1 (3703), 46 - 48.

Binate, S., N'dri, D., Toka, M. & Kouame, L. P. (2008). Purification and Characterization of two beta-glucosidases from termite workers *Macrotermes bellicosus* (Termitidae: Macrotermitinae). Journal of Applied Biosciences, 10, 461 - 470.

Boboye, S. O. (1986). Insect pests of sugar cane in Nigeria and their control. A Lecture delivered at a one-week Intensive Sugar Cane Production Training Course Organised by the Nigerian Cereals Research Institute, Badeggi, Nigeria.

Dahot, U. (1998). Antimicrobial activity of small protein of *Moringa oleifera* leaves. Journal of Islamic Academy of Sciences, 11 (1), 111 - 113.

Dangi, S. Y., Jolly, C. I. & Narayana, S. (2002). Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera*. Pharmaceutical Biology, 40 (2), 144 - 148.

Ekpo, K. E. & Onigbinde, A. O. (2007). Characterization of lipids in winged reproductives of the termite *Macrotermes bellicosus*. Pakistan Journal of Nutrition, 6 (3), 247 - 251.

Furo, N. A. & Ambali, A. G. (2012). An investigation into the phytochemical constituents of *Moringa oleifera* aqueous root extracts. Centrepoint Journal (Science Edition), 18 (1), 1 - 7.

Jitunari, F., Asakawa, F., Takeda, N., Suna, S. & Manabe, Y. (1995). Chlordane compounds and metabolite residues in termite workers blood. Bulletin of Environmental Contamination and Toxicology, 54, 855 - 862.

Kabaru, J. M. & Girchia, L. (2001). Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. African Journal of Science and Technology. Science and Engineering Series, 2 (2), 44 - 49.

Kumar, K., David, G. A., Valerie, K. & Michael, E. S. (2013). Treatise on the Isoptera of the World. Bulletin of the American Museum of Natural History, 377, 1 - 2704.

Lenz, M., Watson, J. A. L., Barrett, R. A. & Runko, S. (1990). The effectiveness of insecticidal soil barriers against subterranean termites in Australia. Sociobiology, 17, 9 - 35.

Lewis, D. L. (1980). Environmental and health aspects of termite control chemicals. Sociobiology, 5, 197 - 203.

Mamun, M. S. A. & Ahmed, M. (2011). Prospect of Indigenous Plant Extracts in Tea Pest Management. International Journal of Agricultural Research Innovation and Technology, 1 (1&2), 16 - 23.

Marugandan, S., Srinivasan, K., Tandon, S. K. & Hasan, H. A. (2000). Anti-inflammatory and analgesic activity of some medicinal plants. Journal of Medicinal and Aromatic Plant Science, 22, 56 - 58.

Muhammad, S. N. (2012). Effects of plant extracts on the behaviour and physiology of the *Odontotermes obesus* (Ramb.) (Isoptera: Termitidae). PhD thesis, University of Agriculture, Faisalabad, Pakistan. 129pp.

Nadi (2001). Toxixity of plant extracts to *T. granarium*. Pakistan Journal of Science, 4 (12), 1503 - 1505.

Ndubuaku, T. C. N. & Asogwa, E. U. (2006). Strategies for the control of pests and diseases for sustainable cocoa production in Nigeria. African Scientist, 7, 209 - 216.

Ojianwuna, C. C., Olisedeme, P. & Ossai, S. L. (2016). The toxicity and repellency of some plant extracts applied as individual and mixed extracts against termites *(Macrotermes bellicosus).* Journal of Entomology and Zoology Studies, 4 (1), 406 - 418.

Okweche, S. I., Hilili, P. M. & Ha, P. B. (2015). Comparative efficacy of some insecticidal plant materials against dry wood termite (*Cryptotermes cavifrons* Banks (Insecta: Isoptera: Kalotermitidae) infestation. Greener Journal of Agricultural Sciences, 5 (6), 210 - 216.

Oyedokun, A. V., Anikwe, J. C., Okelana, F. A., Mokwunye, I. U. & Azeez, O. M. (2011). Pesticidal efficacy of three tropical herbal plants' leaf extracts against *Macrotermes bellicosus*, an emerging pest of cocoa, *Theobroma cacao* L. Journal of Biopesticides, 4 (2), 131 - 137.

Paiva, P. M. G., Santana, G. M. S., Souza, I. F. A. C., Albuquerque, L. P., Agra-Neto, A. C., Albuquerque, A. C., Luz. L. A., Napoleão, T. H. & Coelho, L. C. B. B. (2010). Effect of lectins from *Opuntia ficus indica* cladodes and *Moringa oleifera* seeds on survival of *Nasutitermes corniger*. International Biodetermination and Biodegradation, 30, 1 - 8.

Patil, S. D. & Rasika, J. (2013). Antimicrobial activity of *Moringa oleifera* and its synergism with *Cleome viscosa*. International Journal of Life Sciences, 1 (3), 182 - 189.

Pawson, B. M. & Gold, R. E. (1996). Evaluation of baits for termites (Isoptera: Rhinotermitidae) in Texas. Sociobiology, 28, 485 - 491.

Saxena, R.C. (1998). Botanical pest control, In G. S. Dhaliwal, & E. A. Heinrichs (Eds.), Critical issues in Insect Pest Management (pp. 115-179). Commonwealth Publishers, New Delhi, India. Pp.

Sbeghen, A. C., Dalfovov, V., Serafini, L. A. & De-Barros, N. M. (2002). Repellence and toxicity of basil, citronella, ho-sho and rosemary oils for the control of the termite, *Cryptotermes brevis* (Isoptera: Kalotermitidae). Sociobiology, 40 (3), 585 - 594.

Singh, G, Singh, O. P., Prasad, Y. R., De-Lampasona, M. P. & Catalan, C. (2004). Chemical and insecticidal investigations in leaf oil of *Coleus amboinicus* Lour. Journal of Flavour Fragrance**,** 17 (6), 440 - 442.

Su, N. Y. & Scheffrahn, R. H. (1990b). Comparison of eleven soil termiticides against the Formosan subterranean termite and the eastern subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology, 83, 1918 - 1924.

UNEP & FAO (2000). Report of the UNEP/FAO/Global IPM facility termite biology and management workshop 1-3February 2000, Geneva, Switzerland. http://www.chem.unep.ch/pops/pdf/termrpt/termiterpt.pdf.

Wagner, T., Mulrooney, J., Peterson, C. & Shelton, T. (2003). Reduced risk products steal spotlight. Pest Control 71 (2), 16-18, 20-23. http://www.pestcontrolmag.com/pestcontrol/issue/ issueDetail.jsp?id=1833.

Wood, T. G., Johnson, R. A. & Ohiagu, C. E. (1980). Termite damage and crop loss studies in Nigeria – a review of termite damage to maize and estimation of damage, loss of yield and *Microtermes* abundance at Mokwa. Tropical Pest Management, 26, 241 - 253.

Zhu, B. C., Henderson, G., Chen, F., Fei, H. & Laine, R. A. (2001a). Evaluation of vetiver oil and seven insect active essential oils against the Formosan subterranean termite. Journal of Chemistry and Ecology**,** 27 (8), 1617 - 1625.

Zhu, B. C. R., Henderson, G., Chen, F., Maistrello, L. & Laine, R. A. (2001b). Nootkatone is a repellent for Formosan subterranean termite (*Coptotermes formosanus*). Journal of Chemistry and Ecology, 27 (3), 523 - 531.