Journal of Biological Studies **JBS**
https://onlinejbs.com/ Vol.1 (3):98-105, 2018
ISSN 2209-2560

Research Article

Termiticidal Efficacy of *Citrus* Peel Extracts against Termites (*Macrotermes bellicosus*)

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Received 07 July 2018; Accepted 01 August 2018; Published online 01 September 2018

Abstract

Termiticidal efficacy of *Citrus sinensis*, *Citrus limon* and *Citrus aurantifolia* peels extracts against termites (*Macrotermes bellicocus*) was carried out in an attempt to ascertain the mortality and LC_{50}, of the termites using 0.21% of distilled water as a negative control. The experiment was replicated trice with distilled water as an untreated control. The termiticidal effects (mortality) of the *Citrus* plant extracts on the termites showed no significant difference ($p>0.05$; $F = 1.57$) as the concentration increased from 10 - 30%. Also there was significant difference ($p<0.05$; $F = 29.39$) in the mortality of the termites as the time of exposure increased from 24 - 72 hrs. Extract of *C. aurantifolia* caused more mortality that *C. sinensis* and *C. limon*. *C. aurantifolia*, *C. sinensis* and *C. limon* exhibited an LC_{50} of 17.50, 17.50 and 12.90 mgL^{-1}. These plant extracts could serve as alternatives to synthetic insecticides in termite management practices because they are biodegradable, cost effective and non-toxic to environment and human health, easy to prepare and readily available in local markets in Delta State, Nigeria.

Key words: Termiticide, *Citrus*, Termites, *Macrotermes bellicocus*, Mortality and LC_{50}

Introduction

Termites are among the most devastating insect pests, severely damaging agricultural crops and urban infrastructure (Verma *et al.*, 2016). They belong to the isopteran order of insects, so named because of the equal shape and venation of the fore and hind wings (Ito and Ighere, 2017a). Geographically, termites are found primarily in the tropical regions of the world, where they play an important role in the recycling of wood and other cellulose-based materials (Abdurahaman, 1990). The distribution pattern of termites is majorly determined by temperature, water conditions and human activities such as movement of wood products from termite-infested to termite-free locations. Out of 300 species of termites known so far from Africa, about 35 species have been reported damaging agricultural crops and buildings. The major mound-building species in Africa are *Odontotermes obesus, Odontotermes redemanni*, and *Odontotermes wallonensis*, and the subterranean species are *Heterotermes indicola, Coptotermes ceylonicus, C.*

heimi, Odontotermes horni, Microtermes obese, Trinervitermes biformis, and *Microcerotermes beesoni* (Rajagopal, 2002; Engel and Krishna 2004).

Termites are the most destructive pest of plants, trees and wooden structures (Ranjith *et al.*, 2015). Their ecological aggressiveness, agility, structural and agricultural destructiveness cannot be over emphasized. Termites cause significant damage when they attack wood in quest for cellulose, their staple food. *Macrotermes bellicosus* species of termites are mostly mound builders and are one of the largest termite species (Osipitan *et al*., 2013). This species of macrotermes is one of the most important agricultural, forestry and household pest (Ojianwuna *et al*., 2016). It has been reported that *Macrotermes* termites cause serious damage (100% loss) to agricultural crops such as maize, sugarcane, millets rice, yam and groundnuts and various domestic wooden products such as furniture and farm structures (Nyeko *et al*., 2010; Addisu *et al*., 2014; Ito and Ighere, 2017a). In some parts of Africa, such as East Africa, *Macrotermes* cause yield loss of 30-60% (UNEP and FAO, 2000). The loss caused on various crops and tree species due to termites vary ranging from 50-100% (Sakasegawa *et al*., 2003). In Nigeria, reports from Abraka, Mokwa, Lokoja, Onisha and Aba showed yield losses in yams as a result of soil pests and only termites *Macrotermes* species caused 29% of the crop damage (Ojianwuna *et al*., 2016).

Damages and economic losses due to termite invasion are enormous, therefore there is need for the control of these pests. Termites could be controlled by synthetic insecticides which remain the primary method used to prevent termite attack on wooden structures (Abdullah *et al*., 2011). However, the persistent use of synthetic insecticides in the control of termites is known to cause aquatic and environmental pollution, lethal effect on non-target organism (Sohail *et al*., 2011) and has resulted in the need to search for plant-derived compounds as an alternative for termite control. Botanical extracts based on locally available plants, have frequently been claimed to be effective in termite management (Tascioglu *et al.*, 2012). Plant extracts affects insect pest in several ways including altering the behaviour of the insect, growth retardation, toxicity, oviposition deterrence, feeding inhibition and reduction of fecundity and fertility (Ito and Utebor, 2018; Ito and Ighere, 2017a and b).

The goal of the study is to establish a termiticidal agents which can be used to control termite infestation. Human inhabitants from these regions and beyond will be able to use *Citrus* peels of orange (*C. sinensis*), lemon (*C. limon*) and lime (*C. aurantifolia*) on termites in Oghara. This will help protect agro-farms and furniture from termite invasion in tropical communities. In addition, the usefulness of these plants peels found in the area will be appreciated and this may enhance their usage. This study is focus on the comparative effectiveness of termiticidal activities of *Citrus* plants peels of orange (*C. sinensis*), lemon (*C. limon*) and lime (*C. aurantifolia*) on *Macrotermes bellicosus* in Oghara. The specific objectives for this study are: (i) to evaluate the insecticidal potencies of the five pulverized plants materials on the pests (ii) to determine the LC_{50} of the three extracts in 72 hours exposure periods and (iii) establish the relationship between mortality and lethal concentration (LC_{50}) of *C. sinensis*, *C. limon* and *C. aurantifolia* on termites.

Materials and Methods

Source of plant materials

Three locally available plant materials were sought for in Oghara, Delta North District, Delta State, Nigeria and identified as; orange (*Citrus sinensis*), lemon (*Citrus limon)* and lime (*Citrus aurantifolia*)

Preparation of extracts

The citrus peels were chopped into bits and air dried for several days. They were grinded with micro plant grinding machine and were sieved through a 0.25mm pore size to obtain a uniform fine dust particle (Ito and Ighere, 2017b; Selase, *et al.,* 2009). The resulting powders were stored in separate containers with screw cap at room temperature prior to use. The amount of powder mixed with the 100ml of water were calculated on weight by volume, ie: weight of powder/volume of water 10, 20, 30g of each grinded plant materials were soaked in 100ml of water to obtain crude extracts of three concentration levels of 10, 20 and 30% (w/v). Each mixture was filtered with cheese cloth after 24 hours.

Collection and establishment of test organism

Population of termites were collected from a termitarium within Delta State Polytechnic Otefe-Oghara, Delta State. Termite mound was dug up using shovel and soil containing termites was put on plastic sheets. Termites were collected from the plastic sheets using camel hair brush and placed in plastic containers as described by (Addisu *et al.,* 2014). Termites were fed with dry wood inside the container and the top of the container was covered with muslin cloth to allow free flow of air also to prevent the termites from escaping. The containers carrying the termites were carried to the laboratory of Animal and Environmental Biology and placed in a cool dark area until needed.

Bioassay procedures for extracts toxicity

The prepared plant extracts were weighed and taken into containers containing 100ml of sterile distilled water. The containers containing the botanical powder were shaken thoroughly for about 5 minutes to ensure uniform distribution of the solute. Whatman No. 1 filter paper was placed in each Petri dish and treated with 2ml of each plant extract. 20 worker termites were randomly selected from stock population and kept into the Petri dish containing the treated filter papers. Each treatment was tested at 3 concentration (10, 20, 30% w/v). The experiment was replicated thrice and in all the setup, 0.21% of water served as a standard check and negative control respectively. Mortality of termite was recorded at 24hour interval for 72 hours post treatment application. The experiment was conducted under laboratory condition (25 ^{0}C and 60 – 70% Relative Humidity) (This method was adopted from Addisu *et al.,* (2014) with slight modification by Ojianwuna *et al.,* (2016). Life and dead termites were counted and percentage mortality was calculated according to the following equation;

$$\text{Percentage mortality} = \frac{\text{No of dead termite}}{\text{Total no of termite}} \times \frac{100}{1}$$

Statistical Analysis

The data recorded for different response variables in the study were analyzed statistically using Microsoft Excel 2014 for analysis of variance (ANOVA) and descriptive statistics. Data collected was subjected to probit analysis to determine the median lethal concentration (Selase *et al.,* 2009).

Results

The results of the termiticidal efficacy of orange (*Citrus sinensis*), lemon (*Citrus limon)* and Lime (*Citrus aurantifolia*) at different concentrations exposed for 72 hours is presented in Table 1. All plant species investigated had termiticidal properties at different level of concentration and hour of exposure. The control experimental set-up for this study showed no mortality.
The termiticidal effects (mortality) of the *Citrus* plant extracts on the termites showed no significant difference (p>0.05; F = 1.57) as the concentration increased from 10 - 30%. Contrarily, there was significant difference (p<0.05; F = 29.39) in the mortality of the termites as the time of exposure increased from 24 - 72 hrs. Table 1 showed that the highest mortality of 100 % was observed in *C. aurantifolia* at 30% concentration.
Table 1. Percentage mortality of *Macrotermes bellicosus* exposed to 24 – 72 hours toxicity of *C. sinensis*, *C. limon* and *C. aurantifolia* in 10 – 30% concentration

conc. of *Citrus*	**Total No. of termites**	**Botanicals**	**Exposure periods (hours)**			**Total No. of dead termites**	**% Mortality**
			24h	**48h**	**72h**		
Control (2ml)	20	Distilled water	0	0	0	0	0.00
10	20	*C. sinensis*	6	4	0	10	50.00
	20	*C. limon*	8	3	1	12	60.00
	20	*C. aurantifolia*	7	5	3	14	70.00
20	20	*C. sinensis*	8	5	1	14	70.00
	20	*C. limon*	9	4	3	16	80.00
	20	*C. aurantifolia*	7	7	3	17	85.00
30	20	*C. sinensis*	7	6	3	16	80.00
	20	*C. limon*	8	6	4	18	90.00
	20	*C. aurantifolia*	9	5	6	20	100.00

Based on exposure period, the first 24h exhibited the highest mortality at all concentrations of *C. sinensis*. This was closely followed by 48h which exhibited a mortality of 4, 5, and 6 termites at the application of 10, 20 and 30% concentration of *C. sinensis* (orange) extracts (Table 1). The percentage mortality of termites as a results of *C. sinensis* was 50, 70 and 80%. This gave an LC_{50} of 17.50 mgL^{-1} (Fig. 1).

C. limon which ranked second in mortality hierarchy had an LC_{50} of 17.50 mgL^{-1} mgL^{-1} (Fig. 2). It recorded its highest percentage mortality (90.00%) at the application rate of 30% of lemon extract. The respective percentage mortality *C. limon* produced were 60, 80 and 90% termites at a concentration of 10, 20 and 30% respectively (Table 1). At 24h exposure, *C. limon* recorded a 8, 9 and 8 dead termites of the respective concentration. The least mortality for *C. limon* was observed at 72h of exposure period which gave 1, 3 and 4 termite mortality.

Unlike *C. sinensis* and *C. limon*, *C. aurantifolia* exhibited the strongest/highest termiticidal activity against termite at all concentration. Its effect on termites gave 70.0, 85.0 and 100 % (percentage) mortality at the application of 10, 20 and 30% concentration of the extract (Table 1). It also exhibited 7, 7 and 9 mortality of termites at 24h exposure period. The hourly mortality for *C. aurantifolia* was recorded after 72h like just as *C. sinensis* and *C. limon* (Table 1) with the LC_{50} being 12.90 mgL^{-1} (Fig. 3).

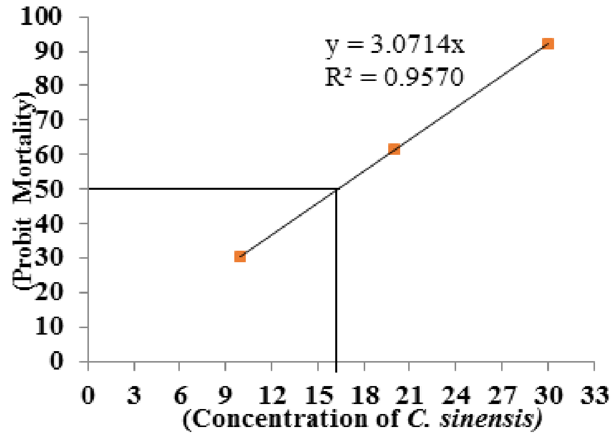

Figure. 1. Mortality and Concentration relationship of orange (*C. sinensis*) mgL^{-1} at LC_{50}.

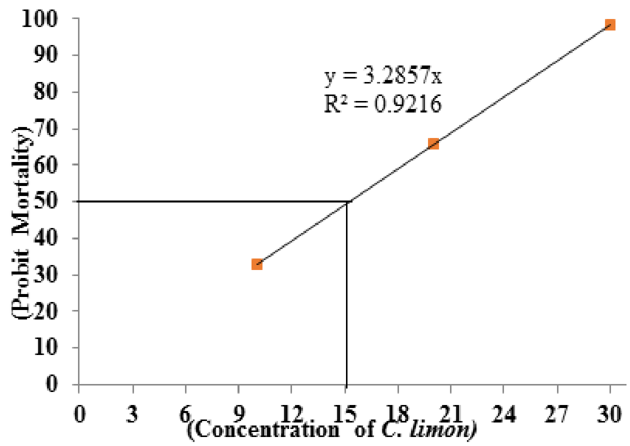

Figure. 2. Mortality and Concentration relationship of lemon (*C. limon*) mgL^{-1} at LC_{50}.

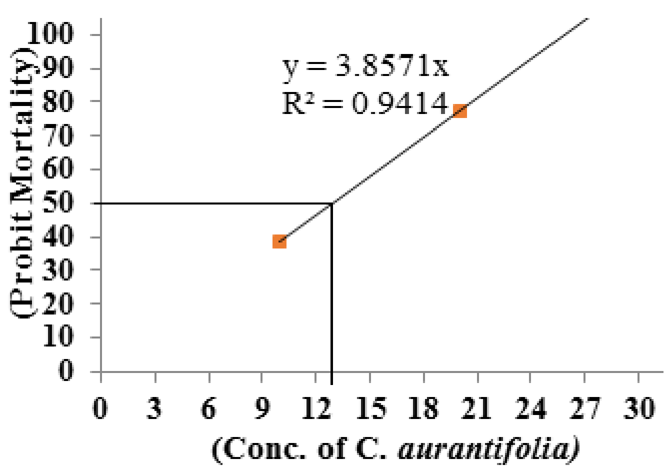

Figure. 3. Mortality and Concentration relationship of lime (*C. aurantifolia*) mgL^{-1} at LC_{50}.

Discussion

The termiticidal toxicity peels of *C. sinensis*, *C. limon* and *C. aurantifolia* applied as extracts against termites (*M. bellicocus*) was carried out in an attempt to ascertain the mortality and LC_{50}, of the termites using 0.21% of distilled water as a negative control. The termiticidal effects (mortality) of the *Citrus* plant extracts on the termites showed no significant difference ($p>0.05$; F = 1.57) as the concentration increased from 10 - 30% also there was significant difference ($p<0.05$; F = 29.39) in the mortality of the termites as the time of exposure increased from 24 - 72 hrs. The termiticidal activity exhibited by all *Citrus* species in this study is cause by the presence of, limonene, β-pinene, and fenchone.

C. aurantifolia caused the highest mortality of termites at different concentrations compared to the mortality caused by *C. sinensis* and *C. limon.* This is due to the active ingredient (A.I) found in lime (*C. aurantifolia*). Lime flesh and peel contain diverse phytochemicals, including polyphenols and terpenes, citral, limonene, β-pinene, and fenchone (*Loizzo et al., 2012) which are believed to have caused this high mortality observed. Citral is the main active AI in lime. Lime (C. aurantifolia) extract in this study* gave 70.0, 85.0 and 100 as percentage mortality at the application of 10, 20 and 30% concentration (Table 1) with the LC_{50} being 12.90 mgL^{-1} (Fig. 3).

C. limon ranked second in the termiticidal properties of the plants used in this experiment gave an LC_{50} of 15.50 mgL^{-1}. The respective percentage mortality of termites caused by *C. limon* were 60, 80 and 90% at a concentration of 10, 20 and 30% respectively). At 24h exposure, *C. limon* recorded a 8, 9 and 8 dead termites of the respective concentration. The least mortality for *C. limon* was observed at 72h of exposure period which gave 1, 3 and 4 termite mortality. This mortality is due to the limonenes and β-pinenes which are the active ingredient responsible for the mortality caused by lemon. This indicates that the efficacy of the plant materials depends on the plants' active ingredients.

The percentage mortality of termites as a results of *C. sinensis* application was 50, 70 and 80%. This gave an LC_{50} of 17.50 mgL^{-1} Emeasor and Okorie (2008) used sweet orange (*C. sinensis*) for controlling maize weevil (*S. zeamais*). They confirmed no significant mortality effect of orange rind powder on adult *S. zeamais* resulting in severe damage of the grains and weight loss while rind oil showed significant reduction of grain damage protecting grain damage. Thus they inferred that *C. sinensis* rind oil, (not the powder) can be used for the storage of maize grains. Oranges (*C. sinensis*) contain carotenoids (beta-carotene, lutein and beta-cryptoxanthin), flavonoids (e.g. naringenin) and numerous volatile organic compounds producing orange aroma, including aldehydes, esters, terpenes, alcohols, and ketones which causes mortality in insects including termites. Abdullahi *et al* (2011) tested citrus peel powder against stored products infesting pest *Tribolium castaneum* (Coleoptera: Tenebionidae). The result of their study indicated that the highest dose of the citrus peel powders recorded the maximum mortality of the insect after every 24hours interval of exposure. The results and supporting studies suggested that orange oil have potent insecticidal activity and could be used for termite management. The mode of action might be through contact of the water extract with the body wall of the insects. Contact causing irritation of the skin (Williams *et al*., 2004), metabolic disruption, membrane damage and nervous system dysfunction.

Conclusion

This present study revealed the termiticidal activity of orange (*C. sinensis*), lemon (*C. limon)* and lime (*C. aurantifolia*) peels extract against *M. bellicosus* termite. *C. aurantifolia* extract showed better termiticidal (mortality) than *C. sinensis* and *C. limon*. These plant extracts could serve as alternatives to synthetic insecticides in termite management practices because they are biodegradable, cost effective and non-toxic to environment and human health, easy to prepare and readily available in local markets in Delta State, Nigeria.

Recommendation

Consequently, in further investigations eco-friendly termiticidal formulations can be developed using lime peel extract as well as in combinations with the other citrus (if need be). The active components of these extracts can also be isolated and used for the enhanced activity of plant based termiticides.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgment

We would like to thank the management of Delta State University, Abraka particularly Department of Animal and Environmental Biology and Delta State Polytechnic, Otefe-Oghara for providing facilities and enabling environments for this research.

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