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Evaluation of the Bio-insecticidal Potential of Some Tropical Plant Extracts Against Termite (Termitidae:Isoptera) in Ogun State, Nigeria

A.A. Osipitan and A.E. Oseyemi
Department of Crop Protection, University of Agriculture, Abeokuta Ogun State, Nigeria

Corresponding Author: A.A. Osipitan, Department of Crop Protection, University of Agriculture, Abeokuta Ogun State, Nigeria

ABSTRACT

Macrotermes spp. are members of the fungus-growing sub-family Macrotermitinae. They are mostly mound building and are the largest termite species. *Macrotermes* spp. build large epigeal nests (mounds) from where they forage outwards to distances up to 50 m in galleries/runways. In Africa, Macrotermes are serious pest of some agricultural crops and tree plantations that is responsible for the majority of crop damage and 90% of tree mortality in forestry. Damage by termite to store products also provides entry for secondary infection by pathogens especially *Aspergillus*, which cause indirect yield loss and contamination of products with aflatoxins. Many plants have however, developed effective defenses against termites. This study evaluated the bio-activity of aqueous extracts from citrus: *Citrus sinensis*, cocoa: *Theobroma cacao*, sunflower: *Tithonia diversifolia* and cashew: *Anacardium occidentale* for the management of *Macrotermes bellicosus* on the field and in the laboratory. The results showed that extracts from the plants caused 80-100% mean insect mortality 10 h after insect exposure and have repellence values between 26.67 and 60%. *T. cacao, A. occidentale, T. diversifolia* and *C. sinensis* are viable options for environmentally friendly management of *M. bellicosus* on the field.

Key words: *Aspergillus*, aqueous extract, *Macrotermes bellicosus*, mortality, termitaria

INTRODUCTION

Termites are an important component of tropical and sub-tropical ecosystems. They are group of social insects that belong to the order Isoptera and family "termitidae" (Mitchell, 2002). About 2650 species of termites have been documented worldwide (Kambhampati and Eggleton, 2000). The most important termite pest genera in Africa include: *Odontotermes*, *Macrotermes*, *Pseudacanthotermes*, *Microtermes*, *Ancistrotermes*, *Allodontermes*, *Amitermes*, *Trinervitermes* and *Hodotermes* (Mitchell, 2002). Termites mostly feed on dead plant materials generally in the form of wood, leaf litter, soil or animal dung and about 10% of the estimated 4000 species of the insect are economically significant as pests that can cause serious structural damage to buildings, crop or plantation forest. Bong *et al.* (2012) reported that wood feeder especially *Coptotermes curvignathus* are the main termite species that infests palm plantation and living agricultural plants. They are prime example of insects that display decentralized, self organized system, swarm intelligence and co-operation among colony members to exploit food sources and environment that could not be available to any single insect acting alone.

A typical colony contains nymphs (semi matured young), workers, soldiers and reproductive individuals, sometimes containing several egg laying queens. A worker termite undertake the labour of foraging, food storage, brood, nest maintains and some of the defense effort in certain species (Watson *et al*., 1985). They are the main caste in the colony for digestion of cellulose in food and are the most likely to be found in infested wood (Krishnak and Weesner, 1970). Termites thrive in every type of terrestrial environment where enough food is present. They are soil miners, soil engineers and soil architects and in Africa, Asian, Australian and South America build various impressive nests and mounds that provide protection from predators and help in thermoregulation within the nest (Gay and Calaby, 1970; Lavelle *et al*., 1997). They are highly voracious and destructive and cause substantial damage to homes and other wooden structures in our environment, in severe infestation, structural integrity of a building and the safety of the occupiers could be threatened. Termites live a very cryptic life in the soil or wood, building tunnels, aggregate soil into mounds and transferring particles from different layer, a behaviour that alter the physical structure of agricultural lands and wood structure (Bong *et al.,* 2012).

Although, termites play beneficial roles in ecology; they are also destructive and are a major threat to crops and household properties (Edwards and Mill, 1986). Crops such as yam and cassava, sugar cane, groundnuts, sorghum and maize (Sands, 1977; Wood *et al.* 1980; Logan *et al*., 1990) are prone to infestation and damage by termites. Termites also attack grain stores and are commonly responsible for mortality of tree seedlings in forestry and cause considerable damage to buildings and other wooden structures like fence posts and utility poles.

Macrotermes spp. are members of the fungus-growing sub-family Macrotermitinae. They are mostly mound building and are the largest termite species. The queen could attain a length of nearly 6 inches (15 cm) in *Macrotermes natalensis*. There are about 330 species in the *Macrotermes* genus, spread over tropical Africa and Asia. *Macrotermes* spp. 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-barking or cutting them completely. The huge mounds of *Macrotermes* termites are complex structures with ventilation, air ducts, heating, cooling systems and chambers containing fungus gardens which the termites cultivate. In Africa, *Macrotermes* has been a serious pest of some agricultural crops and tree plantations. They are responsible for the majority of crop damage and 90% of tree mortality in forestry. Seedlings in the nurseries and newly planted trees are particularly susceptible to attack during the first 6-9 months after planting. Mortalities vary between 19-78%, occasionally approaching 100% in some areas. Bigger (1966) and Munthali *et al.* (1992) reported *Macrotermes* as the major pest in cassava. Damage by termite to stored products also provides entry for secondary infection by pathogens especially *Aspergillus*, which cause indirect yield loss and contamination of products with aflatoxins (Lynch *et al*., 1991).

Plant and plant products were reported to be useful and desirable tools in pest management programs because they are effective and complement for the actions of the natural enemies (Schmutterer, 1990; Ascher, 1993). The potential pesticide activities of neem, pyrethrum, *Tephrosia*, *Chrysanthemum cinerariaefolium*, *Anchomanes difformis*, *Aframomum melegueta*, *Zingiber officinale*, *Jatropha curcas* and *Annona muricata* products have been reported (Iloba and Ekrakene, 2006; Akinkurolere, 2007; Ukeh, 2008; Mulungu *et al*., 2011; Asmanizar *et al*., 2012). Many plants have developed effective defences against termites and in most ecosystems, there is an observable balance between the growth of plants and the feeding of termites (Xie *et al*., 1995). Defence is typically achieved by secreting antifeedant chemicals (such as oils, resins and lignins) into the woody cell walls. This reduces the ability of termites to efficiently digest the cellulose. Many of the strongly termite-resistant tree species have heartwood timber that is extremely dense (such as *Eucalyptus camaldulensis*) due to accretion of these resins. Over the years there has been considerable research into these natural defensive chemicals with scientists seeking to add them to timbers from susceptible trees. A commercial product, "Blockaid", has been developed in Australia and uses a range of plant extracts to create a paint-on non-toxic termite barrier for buildings. Termites are strongly repelled by some toxic materials to the extent that they become disoriented and eventually die from starvation rather than consume cross treated samples (Xie *et al.*, 1995; Peterson and Wilson, 2003). The control of termite with synthetic insecticides is prone to pollution of environment and underground water, killing of beneficial pest, pest resistance and resurgence etc. Hence, there is the need for continuous research into testing of extract from natural occurring plants that are cheap, readily available, eco-friendly and effective at managing the population of termite on the field. This study evaluated the bio-activity of aqueous extracts from citrus (*C. sinensis*), cocoa (*T. cacao*), sunflower (*T. diversifolia*) and cashew (*A. occidentale*) in the management of termite.

MATERIALS AND METHODS

Sources of plant materials and preparation of extract: The leaves of citrus (*C. sinensis*), cocoa (*T. cacao*), sunflower (*T. diversifolia*) and cashew (*A. occidentale*) were collected from farms at the University of Agriculture, Abeokuta, Ogun State. The plant parts were sufficiently air dried at room temperature until constant weights were obtained. The plant parts were shredded and ground using an electronic blender and 60 g each of the milled parts were weighed and poured into 200 mL of water and left for three days. Thereafter, the extracts were decanted and sieved using a muslin cloth. The resultant solvent was used for the laboratory and field study.

Laboratory study

Contact toxicity by topical application: Worker of *Macrotermes bellicosus* were used for the laboratory study. They were sourced from a termite mound in the premises of the University of Agriculture, Abeokuta. The test of contact toxicity of the plant extracts to the termites by topical application was carried out in the laboratory of the Department of Crop Protection, University of Agriculture, Abeokuta using the standard method described by McDonald *et al.* (1970). Ten worker termites of undertermined age and sex were placed in a petri dish lined with moist filter paper, thereafter, eight (8 mL) of each extract or the synthetic insecticide (0.1% Chlorpyrifos) were applied to the dorsal surface of the thorax of each insect individually with a microapplicator (Obeng-Ofori and Reichmutu, 1997; Ebenezer, 2000). The insects were examined at 2 h intervals after application for 10 h and the insect mortality was noted. Those that did not move or respond to three probing with a blunt probe were considered dead (Obeng-Ofori and Reichmutu, 1997). The treatments were arranged on a work table in the laboratory using Complete Randomized Design (CRD). The percentage insect mortality was calculated according to Niber (1994):

$$\text{Mortality (\%)} = \frac{\text{No. of dead insect}}{\text{Total No. of insects}} \times 100$$

Repellency study by treated paper method.

The repellency of the extracts by termites was conducted using treated filter paper. The method used was based on an area of preference test described by Landani *et al.* (1955) and McDonald *et al.* (1970). The test area was 11 cm Whatman number 1 filter paper cut into equal halves that have no contact with each other to prevent exchange of content. One half was deep into each of the treatments with forceps and allowed to drain before being placed into the plastic petri dish. The other half was placed into distilled water (control) allowed to drain and placed sideway with the first half. Ten termites were released separately into the center of each filter paper in the petri dish. The covers of the petri dishes were perforated, covered with nylon mesh and tightly held in place with rubber bands. Each treatment was replicated four times and arranged on the work table in the laboratory using complete randomized design. The number of insects present on control (NC) and treatment (NT) halves were recorded after 1 h exposure. The Percent Repellency (PR) values were computed using the method of Hossanah *et al.* (1990):

$$PR = \frac{NC\text{-}NT}{NC+NT} \times 100$$

All negative values were treated as zero.

Residual effects of extracts: The persistence of the extracts after application on a treated surface was studied. Five milliliters each of the extract and synthetic insecticide were used to soak filter papers placed in petri dishes. The petri dishes were covered for 24 h. Thereafter, ten termites were separately introduced into the petri dishes and left for 24 h. The number of dead insects was noted and the percentage mortality was calculated.

Field study: The field trial of the plant extracts was conducted on two field locations at the University of Agriculture, Abeokuta, Ogun State. Six average-sized termitaria of between 0.8 m and 1 m height were located and used for the study. Each of the termitaria was demolished and dug to a depth of 40-50 cm below ground level to expose the termites to application of the extracts. The king and queen were not injured or killed during the digging. Thereafter, 5 L each of the extracts was evenly applied to the dug termitaria with aid of a Cooper Pegler (CP15) Knapsack sprayer. One of the demolished termitaria was sprayed with 5 L of 0.1% Chlorpyrifos (synthetic insecticide) and another with 5 L of distilled water and these served as controls. The termitaria were observed for re-built and/or termite resurgence from the second day after application of the extracts. The effectiveness of the treatments at managing the population of termites on the field was rated according to Osipitan *et al.* (2008) as follows:

Upsurge/rebuilt of termitaria (Days after application of treatments)	Ratings
1-20	Not effective
21-40	Slightly effective
40-60	Effective

Statistical analysis: Statistical analysis of data was based on SAS's general linear models procedure (Statistical Application for Sciences). The data were subjected to analysis of variance (ANOVA). Means separation was done at $p<0.05$ with Student Newman-Keuls Test (SNK).

RESULTS

Effect of topical application of plant extracts on percentage mortality of termites: The mean percentage mortality of termites topically exposed to the plant extracts is presented in

Table 1: Effect of topical application of plant extracts on mortality of termites

	Mortality+SE (%) Periods after application of the extracts (h)				
Extracts	2	4	6	8	10
Citrus sinensis	53.33[b]	66.67[b]	86.67[a]	86.67[a]	86.67[a]
Theobroma cacao	13.33[c]	40.00[b]	53.33[ab]	73.33[a]	86.67[a]
Anacardium occidentale	46.67[b]	53.33[b]	73.33[a]	80.00[a]	80.00[a]
Tithonia diversifolia	26.67[bc]	40.00[b]	60.00[ab]	66.67[a]	80.00[a]
0.1% chlorpyrifos	100±0.0[a]	100±0.0[a]	100±0.0[a]	100±0.0[a]	100±0.0[a]

Means followed by the same letter are not significantly different from each other at ($p<0.05$), Significant means were separated using Student's Newman-Keuls Test (SNK)

Table 2: Mean percentage repellency of termite by some plant extracts

Treatments	Repellency (%)
Citrus sinensis	26.67[a]
Theobroma cacao	53.33[a]
Anacardium occidentale	60.00[a]
Tithonia diversifolia	26.67[a]
0.1% chlorpyrifos	66.67[a]

Means followed by the same letter are not significantly different from each other at ($p<0.05$), Significant means were separated using Student's Newman-Keuls Test (SNK)

Table 1. At 2 h After Application of the Treatments (AAT) the synthetic insecticide (0.1% Chlorpyrifos) induced the highest insect mortality of 100 % and it was significantly ($p<0.05$) higher than the mortality induced by other plant extracts. At 4 h AAT, the mortality caused by the plant extracts ranged between 40 and 60.67%. They were however, not significantly ($p>0.05$) different from each other. However, at 10 h AAT, the mortality induced by all the treatments ranged between 80 and 100%. They were, however, not significantly ($p>0.05$) different from each other.

Repellency effect of plant extracts on termite: The repellency effect of the plant extracts on termite is presented in Table 2. The highest (66.67%) insect repellency was induced by the synthetic insecticide (0.1% Chlorpyrifos), followed by the extract of *A. occidentale* (60%), *Theobroma cacao* (53.33%), *C. sinensis* (26.67%) and *T. diversifolia* (26.67%). The mean repellency caused by the extracts were however, not significantly ($p>0.05$) different from the mean insect pest repellency caused by the synthetic insecticide.

Residual effects of treatments on mortality of termite: The synthetic insecticide and *T. cacao* each induced 100% residual mortality of the termites. The residual mortality of termites due to the other plant extracts was comparable to each other, ranging from 63.33 to 83.33% (Table 3).

Field trial of treatments: As shown in Table 4, the synthetic insecticide, extracts of *T. cacao* and *A. occidentale* were highly effective. The termitaria treated with these treatments were not rebuilt after 60 days. The extracts from *T. diversifolia* and *C. sinensis* were slightly effective as the termite population resurged and rebuilt the demolished termitaria treated with these extracts only 21 days after application of the treatments.

Table 3: Residual effects of treatments on mortality of termite

Treatments	Mean mortality (%)
Citrus sinensis	63.33^{b}
Theobroma cacao	100.00^{a}
Anacardium occidentale	83.33^{b}
Tithonia diversifolia	66.67^{b}
0.1% chlorpyrifos	100.00^{a}

Means followed by the same letter are not significantly different from each other at ($p<0.05$), Significant means were separated using Student's Newman-Keuls Test (SNK)

Table 4: Rating of treatment effectiveness at managing termite populations in the field

Treatments	Rating
Citrus sinensis	Slightly effective
Theobroma cacao	Highly effective
Anacardium occidentale	Highly effective
Tithonia diversifolia	Slightly effective
0.1% chlorpyrifos	Highly effective
Distilled water	Not effectively

Means followed by the same letter are not significantly different from each other at ($p<0.05$), Significant means were separated using Student's Newman-Keuls Test (SNK)

DISCUSSION

In this study, the synthetic insecticide (0.1% Chlorpyrifos) caused a significantly higher mortality of the termites in the laboratory. This is similar to the findings of Akhtar and Mushtaq (1997) who reported that tenekil, thiodan and chlorpyrifos are effective at controlling termites. Of the botanicals, the mortality caused by the extract of *T. cacao* was significantly comparable to the mortality caused by chlorpyrifos. The effectiveness of the extract of *T. cacao* may be attributed to the action of theobromine and caffeine, the active ingredient in extract. Nathanson (1984) reported that caffeine acts as natural pesticide that paralyzes and kill certain insects that feed on beans as well as leaves and fruits of some plants that contains caffeine. The extract from other plant extracts induced significant mortality of the termite. This is in consonance with the findings of Zhu *et al.* (2001) and Peterson and Wilson (2003), which indicated that naturally occurring anti-termite compounds extracted from locally available plants have potentials for managing the population of termites. The use of plant parts and derivatives to control insect pest of stored products and backyard vegetables, as well as termites and mosquitoes has been an age long practice in African agriculture (Maistrello *et al.*, 2003).

In this study, all the extracts were repellant to the termites. Repellents offer protection and keep away insect pests from treated materials. Talukder and House (1994) and Khan and Gumbs (2003) reported that repellents are safe in pest control operations as they minimize residues, ensure safety of food, environment and wildlife. Jembere *et al.* (1995) tested the efficacy of *Ocimum kilimandscharicum* against infestation of *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier) and *Rhyzopertha dominica* (Fabricius) and reported that the extract repels the three major stored product insect pests. Natural occurring materials in plants have been discovered to possess substances that repel termites. In central Queensland, Sydney, observation of a lone fence post in a paddock that was not attacked by termite for 50-75 years prompted a two-year testing of the post which revealed that the post contains a plant extract called false sandalwood that repels the insects. Likewise, a commercial product, "Blockaid," that uses a range of plant extracts was developed in Australia to create a paint of non-toxic termite barrier for buildings. In 2005, a group of Australian scientists "discovered" a treatment based on an extract of a species of *Eremophila* that repels termites. It was reported that termites are strongly repelled by the toxic material to the extent that they will starve rather than consume cross treated samples and when kept close to the extract, they become disoriented and eventually die.

In this study, mortality caused by the extracts was directly related to the duration of exposure to the treatments as from 2 h after exposure until 10 h after as against the effect of the synthetic insecticide (0.1% Chlorpyrifos) that was immediate. The generally accepted method of termite control over the years has been chemical insecticides. The use of insecticide is however, detrimental to the environment and beneficial organisms among other negative effects. In the last two decades, much attention has been directed at exploring the potentials of insecticides of botanical origin as alternatives to the use of synthetic insecticides (Khaire *et al*., 1992; Jembere *et al*., 1995; Ewete *et al*., 1996; Umeh and Ivbijaro, 1999; Enobakhare and Law-Ogbomo, 2002) The plant extracts in this study demonstrated wide spectrum at curtailing the population of termites in the field. The effectiveness cut across the composite castes to which the extracts were applied in the field (Queen, King, Soldier, Workers and Nymphs) versus a single caste (Worker) trial in the laboratory study. The comparable effectiveness of the extract of *T. cacao* with the synthetic insecticide (0.1% Chlorpyrifos) is a good development for the management of termites as the extract is easily available and the technology for its extraction could be easily adopted. Likewise, the extract is environmentally friendly, constitutes no threat to underground water and the environment and is not hazardous. It is also safe from other drawbacks such as high cost, technicality of mixing and application among others negative attributes associated with the use of agrochemicals.

Many plants have developed effective defenses against termites and there is an observable balance between the growth of plants and the feeding of termites in most ecosystems. The use of extracts from plants is one of the environmentally friendly alternatives to using chemical pesticides for termite control. It is also cheaper than applying chemical pesticides because it utilizes products and materials that are available in homes and around the farms. In this study, considering the bio-activity of extracts from *C. sinensis*, *T. cacao*, *T. diversifolia* and *A. occidentale* at managing termite in the laboratory and on the field, these plant extracts could be considered for exploration in management of termite on the field.

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