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Termiticidal Activities of Some Medicinal Plants Extracts against Termites, *Macrotermes subhyalinus* Rambburi (Isoptera: Terminidae) Under Laboratory Conditions

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Abstract

The use of environmentally friendly chemicals for termite management has been of global concern. This study examined the effects of five naturally occurring plant extracts from *Azadirachta indica*, *Datura metel*, *Allium sativum*, *Zingiber officinale* and *Nicotiana tabacum* against the termite *Macrotermes subhyalinus*. Methanol extraction of the plant samples was carried out using cold extraction method. From the stock solutions, for each of the plant samples, different extract concentrations of 2%, 4%, 6%, 8% and 10% were prepared separately. The phytochemical screening of the test plant samples for various phytochemical constituents, such as phenols, terpenoids, flavonoids, alkaloids, glycosides, phenol, saponins and tannins was also conducted using Standard Methods. The results obtained showed that termite mortality increased with increased extract concentration and the number of days of exposure. All the tested plant extracts were able to evoke 100% mortality within four days of exposure at higher concentration of 10%, except in *A. indica* extract which evoked 100% mortality within four days of exposure at the concentration of 8%. Phytochemical screening of the test plant samples revealed the presence of the phytochemicals such as phenols, terpenoids, flavonoids, alkaloids, glycosides, saponins and tannins in all the plants except for *A. sativum* where phenol was not detected. All the test plants recorded significant termites mortalities within 4 days hence hence, *Azadirachta indica*, *Datura metel*, *Allium sativum*, *Zingiber officinale* and *Nicotiana tabacum* are hereby recommended to be used in the control of termite, *Macrotermes subhyalinus*. The plant extracts are viable alternatives to the synthetic chemical insecticides used in controlling the insect.

Keywords: Stock Solution, *Macrotermes subhyalinus*, Cold Extracts, Phytochemicals, Methanol Extraction, Concentration

Introduction

Termites are social insects having well-defined system of morphological castes that practice division of labour in their colonies (Hattori *et al*., 2013; Peralta *et al*., 2004) [15, 25]. Termites belong to the Order of insect Isoptera and they are reported to cause major agricultural and domestic problems in many countries (Silesh *et al*., 2013) [28]. A total of 2600 species of termites are reported all over the world. About 126 species of the termites are known to feed on the mammalian dungs and play a significant role in nutrient recycling resulting in increased soil nutrients and aeration as a result of their foraging activity. Tunneling and nesting activities of the termites enhance microbial activity as well as improve chemical and physical properties of the soil depending upon the soil type, organic matter and moisture contents (Freymann *et al*.,) [13].

Among the species of termites are *Macrotermes subhyalinus*, *Macrotermes bellicsus*, *Macrotermes natalensis*, *Macrotermes carbonarius*, *Macrotermes michaelseni*, *Macrotermes gilvus*, *Odontotermes obesus*, *Microtermes obesi*, *Odontotermes assamensis* and *Heterotermes indicola*. Macrotermes sp. which is one of the most agriculturally important insects and the largest termite species builds their nests or mounds from where they forage outwards to distances up to 50m in galleries (Osipitan *et al*., 2017; Sahay *et al*., 2014) [23, 27].

The damage caused by the termites on crops results into secondary infection by plant pathogens which cause indirect yield loss and contamination of products with the production of aflatoxins (Osipitan *et al*., 2017) [23].

Presently farmers depend on synthetic agro-chemicals for termite control in their agricultural crops and in urban settings. This extensive use of conventional synthetic insecticides usually results into various ecological problems including environmental contamination, development of resistant insect strains, ecological imbalance and damage to mammals, biodiversity eradication and human health hazards (Isman, 2008) [18].

Considering the aforementioned problems posed by conventional chemical insecticides, there is the need to look for new bio-rational and ecologically friendly strategies against these insect pests, such as plant-derived chemicals exhibiting termiticidal properties are safe alternatives to hazardous synthetic termiticides (Lima *et al*.,) [19]. Many previous studies have characterized the essential oils and extracts of different plant species for their potential insecticidal action against subterranean termites and other insect pests of economic importance (Isman, 2008; Majeed *et al*., 2018) [18, 20].

Insecticidal plants have received significant attention due to their effectiveness in the control of many harmful insects of economic importance and for environmental safety. In addition, plant products are considered as safer alternatives to toxic synthetic chemicals (Dipendra *et al*., 2012) [10]. Many plant extracts have been studied for their toxicities on termites and other insect species (Nazeer *et al*., 2012) [21]. Many medicinal plants contain bioactive compounds such as phenols, aldehydes, ketones, and other compounds that have a strong effect against microorganisms and termites (Abdesalam *et al.*, 2019; Adedire *et al*., 2011; Behiry *et al.*, 2019) [2, 4, 7]. Extracts of *Azadirachta indica, Datura metel, Cymbopogon citratus*, *Origanum vulgare*, *Rosmarinus cinalis Cinnamomum verum*, *Syzygium aromaticum*, *Eucalyptus globulus* and *Thymus vulgaris* have been reported to show strong termiticidal activity against *Odontotermes assamensis* (Majeed *et al*., 2018) [20].

Considering the destructive activities of termites and the environmental effects of the commonly used synthetic termiticides, this research was primarily intended to evaluate the effectiveness of the selected plant species under laboratory condition as a control agents of termites.

Materials and Methods

Collection of Termites

Workers of subterranean termites, *Macrotermes subhyalinus* were collected from a termitarium within the campus of Ekiti State University, Ado Ekiti. They were packed into a transparent plastic container covered with a net to prevent the escape of the insects. The containers were taken to the Laboratory of the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria where the study was conducted. Some decaying pieces of mango, *Magnifera indica* wood were introduced into the plastic containing the termites to serve as food. The culture which serves as reservoir was maintained at 28±2oC and 75±5% relative humidity for 96 hours. Afterwards, Carmel brush was used to separate the matured, active and healthy termite workers used for the toxicity from the other castes in the culture.

Plants Collection and Extraction

Five different plant samples, that is, *Azadirachta indica* (leaves), *Datura metel* (leaves), *Allium sativum* (leaves), *Zingiber officinale* (rhizoids) and *Nicotiana tabacum* (leaves) were used for the experiment. The plant part samples, *A. indica*, *D. metel* and *N. tabacum* were collected from the campus of Ekiti State University, Nigeria, while that of *Z. officinale* was collected from a farm at Olorunsogo community, Ado Ekiti, Nigeria and *A. sativum* were bought from Mojere Market inAdo Ekiti, Nigeria.

These plant materials were sliced into pieces and air dried on the laboratory table for 20 days and ground into very fine powder using an electric grinder. Afterward, methanol extraction of the plant samples was carried out using cold extraction method. Two hundred grams (200 g) of the powdered plant samples were soaked separately in an extraction bottle containing 150 mL absolute methanol. The mixture was stirred occasionally with a glass rod for 3 days after which the extraction was terminated. The extract was concentrated by using rotary evaporator. From the stock solutions, for each of the plant samples, different extract concentrations of 2%, 4%, 6%, 8% and 10% were prepared separately using the methods described by (Ashamo and Akinnawonu, 2012) [6].

Table 1: List of Plants and the parts evaluated under laboratory conditions against workers of subterranean termites (*Macrotermes subhyalinus*)

Botanical namet	Common name	Family	Plant part extracted
Azadirachta indica	Neem	Meliaceae	Leaves
Datura metel	Thorn apple	Solanaceae	Leaves
Allium sativum	Garlic	Amaryllidaceae	Leaves
Zingiber officinale	Ginger	Zingiberaceae	Rhizome
Nicotiana tabacum	Tobacco	Solanaceae	Leaves

Toxicity bioassay of extracts on termites

Nine centimeter (9 cm) diameter No 1 filter paper were placed in sterile Petri-plates and treated separately with 5 mL of extracts of each concentration (2%, 4%, 6%, 8% and 10%). Twenty (20) workers from the culture were collected termites were released to the plates to feed on the treated filter paper. Control experiment without plant extract was also set up. Each experiment was replicated four times and arranged in Complete Randomization Designed arrangement (CRD). Termite mortality was recorded at 1 day interval till 100% mortality of termites was achieved. Live and dead termites were counted and percentage mortality was calculated using the formular below:

$$\text{Percentage mortality} = \frac{\text{Number of dead termites}}{\text{Total number of termites}} \quad \frac{\text{x 100}}{1}$$

Phytochemical screening

Phytochemical screening of the test plant samples for various phytochemical constituents such as phenols, terpenoids, flavonoids, alkaloids, glycosides, saponins and tannins were conducted using Standard Methods as described by (Sofowora, 1993; Trease and Evans, 2002) [29, 30].

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA), and means were separated using the new Duncan's Multiple Range Test (DMRT)

Results

Toxicity of *A. indica* leaf extracts on Termites (*M. subhyalinus*)

Table 2 presented the termiticidal effect of *A. indica* leaf extract on termites. Termite mortality increased with increased in extract concentration and the number of days of exposure to treatments. None of the plant extracts was able to caused 100% mortality of termites in the first 2 days of exposure to treatments. However, the extract was able to cause 92.5% mortality when exposed to the same 2% extract concentration in the first 2 days treatmentof. The extracts evoked 100% termite mortality on exposure to concentration of 4, 6, 8 and 10% within 8, 6, 4 and 4 days of exposure respectively. The effects of the plant extracts were significantly effective (P>0.05) compared to controls.

Toxicity of *D. metel* leaf extracts on Termites (*M. subhyalinus*)

Table 3 presented the toxicity of *D. metel* leaf extract on termites. Termite mortality increased with in increased in extract concentration and the number of days of exposure. At 2% concentration, none of the plant extracts were able to caused 100% mortality of termites in the first 2 days of exposure. The extracts caused 100% termite mortality on exposure to concentration of 2, 4, 6, 8 and 10% within 8, 6, 6 and 4 days of exposure respectively. The effects of the plant extracts were significantly effective (P>0.05) compared to controls.

Toxicity of *A. sativum* leaf extracts on Termites (*M. subhyalinus*)

Table 4 presented the toxicity of *A. sativum* leaf extract on termites. Termite mortality increased with in increased in extract concentration and the number of days of exposure. At 2% concentration, none of the plant extracts was able to caused 100% mortality of termites in the first 2 days of exposure. The extracts caused 100% termite mortality on exposure to concentration of 6, 8 and 10% within 8, 6, and 4 days of exposure respectively. The effects of the plant extracts were significantly effective (P>0.05) compared to controls.

Toxicity of *Zingiber officinale* bulb extracts on Termites (*Macrotermes subhyalinus*)

Table 5 presented the toxicity of *Z. officinale* bulb extract on termites. Termite mortality increased with in increased in extract concentration and the number of days of exposure. At 2% concentration, none of the plant extracts were able to caused 100% mortality of termites in the first 2 days of exposure. The extracts caused 100% termite mortality on exposure to concentration of 6, 8 and 10% within 8, 6, and 4 days of exposure respectively. The effects of the plant extracts were significantly effective (P>0.05) compared to controls.

Toxicity of *N. tabacum* leaf extracts on Termites (*M. subhyalinus*)

Table 6 presented the toxicity of *N. tabacum* leaf extract on termites. Termite mortality increased with in increased in extract concentration and the number of days of exposure. At 2% concentration, none of the plant extracts was able to caused 100% mortality of termites in the first 2 days of exposure. The extracts caused 100% termite mortality on exposure to concentration of 8 and 10% within 6, and 4 days of exposure respectively. The effects of the plant extracts were significantly effective (P>0.05) compared to controls.

Phytochemical composition of the test plant samples

The phytochemical screening of the test plant samples revealed the presence of the phytochemicals tested for, that is, phenols, terpenoids, flavonoids, alkaloids, glycosides, saponins and tannins except for *A. sativum* leaves where phenol was not detected.

Table 2: Toxicity of *A. indica* Leaf extract on mortality of termites (*M. subhyalinus*)

	% mortality at	days post	Treatment	
Concentration (%)	2	4	6	8
2.0	42.35±2.33e	56.25±2.77d	74.82±3.17c	85.15±3.12b
4.0	65.20±3.44d	76.30±4.22c	93.50±4.11b	100.00±0.00a
6.0	75.36±4.61c	88.40±4.32b	100.00±0.00a	100.00±0.00a
8.0	87.25±3.92b	100.00±0.00a	100.00±0.00a	100.00±0.00a
10.0	92.50±3.93a	100.00±0.00a	100.00±0.00a	100.00±0.00a
Control	0.00±0.00f	0.00±0.00e	0.00±0.00d	0.00±0.00c

Each value is a mean ± standard error of four replicates. Mean in the same column with the similar superscripts are not significantly different at (p<0.05) using the New Duncan's Multiple. Range Test.

Table 3: Toxicity of *D. metel* Leaf extract on mortality of termites (*M. subhyalinus*)

	% mortality at	days post	Treatment	
Concentration (%)	2	4	6	8
2.0	55.16±2.14e	72.30±3.43d	88.70±3.71c	100.00±0.00a
4.0	63.25±3.12d	81.15±3.16c	95.18±4.18b	100.00±0.00a
6.0	75.60±3.14c	89.15±4.81bc	100.00±0.00a	100.00±0.00a
8.0	81.50±3.92b	92.15±3.87b	100.00±0.00a	100.00±0.00a
10.0	85.25±3.13a	100.00±0.00a	100.00±0.00a	100.00±0.00a
Control	0.00±0.00f	0.00±0.00e	0.00±0.00d	0.00±0.00b

Each value is a mean ± standard error of four replicates. Mean in the same column with the similar superscripts are not significantly different at ($p<0.05$) using the New Duncan's Multiple. Range Test.

Table 4: Toxicity of *A. sativum* leaves extract on mortality of termites (*M. subhyalinus*)

	% mortality at	days post	Treatment	
Concentration (%)	2	4	6	8
2.0	48.50±1.85e	57.75±2.33e	70.12±3.23d	82.95±3.82c
4.0	54.10±2.13d	68.55±2.75d	81.25±3.17c	92.42±4.18b
6.0	63.35±2.54c	77.50±3.17c	86.15±4.41b	100.00±0.00a
8.0	75.28±3.19b	83.25±4.15b	100.00±0.00a	100.00±0.00a
10.0	81.25±4.27a	100.00±0.00a	100.00±0.00a	100.00±0.00a
Control (0.0)	0.00±0.00f	0.00±0.00f	0.00±0.00e	0.00±0.00d

Each value is a mean ± standard error of four replicates. Mean in the same column with the similar superscripts are not significantly different at ($p<0.05$) using the New Duncan's Multiple Range Test.

Table 5: Toxicity of *Z. officinale* bulb extract on mortality of termites **(***subhyalinus*)

	% mortality at	days post	Treatment	
Concentration (%)	2	4	6	8
2.0	49.15±1.77e	67.65±2.51e	72.58±3.23d	82.50±3.18c
4.0	56.25±2.12d	73.35±3.71d	85.50±3.88c	98.10±3.67b
6.0	65.38±2.94c	83.76±3.76c	91.25±4.13b	100.00±0.00a
8.0	74.40±3.56b	88.25±3.77b	100.00±0.00a	100.00±0.00a
10.0	82.50±4.19a	100.00±0.00a	100.00±0.00a	100.00±0.00a
Control	0.00±0.00f	0.00±0.00f	0.00±00e	0.00±00d

Each value is a mean ± standard error of four replicates. Mean in the same column with the similar superscripts are not significantly different at ($p<0.05$) using the New Duncan's Multiple. Range Test.

Table 6: Toxicity of *N. tabacum* leaf extract on mortality of termites (*M. subhyalinus*)

	% mortality at	days post	Treatment	
Concentration (%)	2	4	6	8
2.0	51.72±2.22e	57.25±2.15e	77.20±3.16d	85.60±3.87d
4.0	66.16±2.17d	71.20±3.13d	82.81±4.18c	89.50±4.11c
6.0	73.25±3.31c	79.45±4.33c	87.75±3.16b	92.50±4.37b
8.0	88.47±4.14b	97.25±4.78b	100.00±0.00a	100.00±0.00a
10.0	93.40±4.96a	100.00±0.00a	100.00±0.00a	100.00±0.00a
Control (0.0)	0.00±0.00f	0.00±0.00f	0.00±0.00e	0.00±0.00e

Each value is a mean ± standard error of four replicates. Mean in the same column with the similar superscripts are not significantly different at ($p<0.05$) using the New Duncan's Multiple. Range Test.

Table 7: Qualitative phytochemical composition of the test plants' samples

			Phytochemical	Constituents			
Plant parts	Saponins	Alkaloids	Tannins	Flavonoids	Terpenoids	Glycosides	Phenols
A. indica	+	+	+	+	+	+	+
D. metel	+	+	+	+	+	+	+
A. sativum	+	+	+	+	+	+	_
Z. officinale	+	+	+	+	+	+	+
N. tabacum	+	+	+	+	+	+	+

Key: + = Present, while - = Absent

Discussion

Presently, the most common control strategy of termites is the use of synthetic chemical insecticides. Among various control measures, chemical control stood first in termite management (Vinothkumar *et al*., 2018) [31].

The continuous use of synthetic chemical insecticides in the control termites is known to cause environmental pollution, mammalian toxicity and the synthetic chemical insecticides have lethal effects on non-targeted organisms. The shortcomings associated with the use of synthetic chemical insecticides have resulted in the need to search for ecological friendly alternatives (Adedire *et al*., 2011; Hridayesh *et al*., 2016) [4, 16]. However, many plant species can be used as botanical insecticides due to the presence of some active compounds in them (Abbasipour *et al*., 2011a) [1]. These compounds act as fumigants, contact insecticides, repellents, and antifeedants (Zoubiri and Baaliouamer, 2014) [33].

Many plant compounds are effective in causing high mortality of termites as a result of their mechanism of action on the insect nervous system (Almeida *et al.*, 2015) [5]. Plant compounds may also have neurotoxic modes of action (Ebadollahi, 2013) [11]. According to (Cynthia *et al.*, 2016) [9] the active constituents present in the plants might probably get into the body system of the insects and interfere with their normal development and cause mortality.

The five plant species, namely, *A. indica*, *D. metel*, *A. sativum*, *Z. officinale* and *N. tabacum* used for this study were selected to evaluate their termiticidal potential and determine their efficacies as alternative control measures against the termite, *M. subhyalinus*, using adult termites mortality as indices.

All the plants extracts used in this study were effective in the control of termites as they evoke 100% termite mortality within the four days of treatment. It was also observed that as the time of exposure and concentrations of the botanicals increases the efficacy of the botanicals also increased. Higher mortality of termites due to the application of the plant extracts at different concentrations was observed on day 8. As the time of exposure increased, there was a progressive increase in the toxicity of the plant extracts, hence the termites mortality increased (Addisu *et al.*, 2014) [3]. Therefore, it can be said that the percentage mortality percentage of the tested termites were found to be concentration and time dependent.

Previous studies have demonstrated the efficacy of *A. indica* derivatives against a number of insect pests including subterranean termites (Obembe, 2017; Benelli *et al.*, 2017; Ghafoor *et al.*, 2019) [22, 8, 14]. The termites became insensitive towards the extracts upon a longer period of exposure. The results showed that extracts had an inhibitory effect on subterranean termites. The soils treated with the extracts did pose a hindrance to the tunneling activities of the termites, (Hu, 2005) [17].

The effects of *Z. officinale* and *A. sativum* on mortality of termites were reported in the studies carried out by (Cynthia *et al.*, 2016) [9]. The efficacies of the plant materials as insecticides have been reported to depend on the active ingredients present in the plant (Addisu *et al.*, 2914) [3]. The azadiractin present in *A. indica* has a wide range of activity which includes serving as feeding deterrent, insect-growth regulator and oviposition deterrent factor in insects (Isman, 2008) [18].

Garlic was tested against the termite, *M. subhyalinus* in the present study and 100 % mortality was recorded at 10% concentration within four days of exposure. Similar observation was reported by (Park and Shin, 2005) [24] that 3.5 micro litre/liter of garlic oil caused 100 % mortality on Japanese termite, *Reticulitermes spertus* Kolb after 24 hours of treatment.

Result from this study indicates that *D. metel* has the potential to act as a bio-termiticide due to its ability to cause 100% mortality at higher dosage within four days of exposure. This finding corroborated what was observed by (Ekesi, 2000) [12] that crude extracts from the leaves, stems, roots, and seeds of different plant species have growth-inhibiting, insecticidal, and/or anti-feedant properties. On the field, the extract of *D. metel* and the synthetic insecticide were effective at managing the population of termites (Osipitan *et al.*, 2017) [23]. The results obtained in this finding is similar to the findings of (Osipitan *et al.*, 2017) [23] that indicated the bio-activity of aqueous extract from citrus (*Citrus cinensis*), cocoa (*Theobroma cacao*), cashew (*Anarcardium occidentale*) and Mexican sunflower (*Tithonia diversifolia*) at managing the population of *macrotermes bellicosus* on the field.

N. tabacum leaf extract was able to cause high termite mortality as revealed by this work. *N. tabacum* leaf extract contains arrays of active substances such as, nicotine, anabasin (alkaloids), n-Hexadecanoic acid, Oleic acid (Fatty acid) and 4,8,13-duvatrine-1,3-diol, Phytol, B Linalool (terpenoid) and other compounds known to be insecticidal (Zhou *et al.*, 2011) [32]. The several phytochemicals present in *N. tabacum* are responsible for its bio-insecticidal activity which makes it to be effective against several pests and act as synaptic poisons in insect nerves that mimic the neurotransmitter acethylcholin, which causes symptoms of poisoning similar to organophosphate and carbamate insecticides (Regnault-Roger *et al.*, 2008) [26]. Nicotine, which is an active ingredient in *N. tabacum* is a nerve poison that works very quickly on insects and mammals (Regnault-Roger *et al.*, 2008) [26].

Conclusion

The results obtained from the present Studies show that extracts of the test plants; *A. indica*, *D. metel*, *A. sativum*, *Z. officinale* and *N. tabacum* exhibited termiticidal property. They caused significant mortality in termites after exposure, thus potentially offering a natural pest control option. Hence, the test plant species can be used as alternatives to replace the synthetic chemical insecticides for the control of termite.

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