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Termicidal activity of *Azadirachta indica* and *Khaya ivorensis* extracts on subterranean termites in Mubi, Nigeria

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Abstract
This study was carried out to evaluate the efficacy of *Azadirachta indica* and *Khaya ivorensis* against subterranean termites. Ethanol and aqueous extracts of the plants were separately prepared in 200mg/ml and 400mg/ml concentrations. In the Laboratory mortality was observed for 72hrs, while antifeedant activity was observed in the field, where 20 pieces of woods of were exposed to termites vertically buried in the soil to a depth of 10cm. The results showed that the treatments at varying concentrations effectively controlled the termites. 100% mortality was recorded in all the treatment jars after 72hrs and none was recorded in the control. Similar trend was observed in the exposed woods. The treatments significantly reduced the weight loss to as low as 17.60g in *K. ivorensis* treated woods, compared to 153.40g in the control after 3 weeks. Hence, this further confirmed the potency of *A. indica* and *K. ivorensis* against insect pests.

Keywords: *Azadirachta indica*, *Khaya ivorensis*, mortality, Mubi, termites, weight loss

Introduction
Termites are considered as one of the major bio-deteriorating agents affecting woods [1]. They feed on organic wastes such as animal dung, living or dead woods etc., and as a result, can cause considerable damages in agriculture, constructions, forestry and housing [2]. They also feed on live plants materials when they do not find their desired food crops like maize, groundnuts, and millets [3]. Subterranean termites, especially the Coptotermes and Macrotermes have been implicated to cause huge damages domestically and in the field. Various agricultural crops, wooden portions of buildings, living trees, furniture, books, poles, logs, etc. have been reported to have been damaged by subterranean termites [4, 5, 6, 7, 8]. They cause serious material and monetary loss worth millions of dollars [9].
Wood is an essential commodity that can be used for both indoor and outdoor services [1]. Wood has a variety of usage especially for construction purposes like furniture, building, roofing in building, transmission poles for electricity, paper making, fuel, etc. [10]. However, despite the economic benefit derived from the use of woods, their usage is depleted by insect pest attack, which necessitates the application of chemical insecticides as treatment for preservation of woods whenever they are used, especially in the area of construction [11].
The use of conventional synthetic insecticides still remains the most effective means of controlling field and stored product insect pests despite their draw backs inclusive of high mammalian toxicity and environmental pollution [12, 13]. Currently, research on insecticide product development focuses on materials of plant origin which are effective, readily available, affordable and environmental friendly [14, 15].
Neem (*Azadirachta indica* A. Juss) and *Khaya ivorensis*, both belonging to the family Meliaceae are widely studied plants because of their varieties of usage. *A. indica* is widely known for its medicinal properties [16, 17], as well its insecticidal properties [18, 19]. *K. ivorensis* has also been reported by various authors as medicinal and insecticidal [20, 21, 22, 23, 24, 25]. Both plants have been reported to protect woods from termites by Adedeji *et al*. [8] in Ogun State, and Olufemi *et al*. [1] in Borno State, Nigeria. However, this study intends to evaluate the repellent effect of ethanol and aqueous extracts of *A. indica* and *K. ivorensis* against subterranean termites in Mubi, Adamawa State, Nigeria.

Materials and Methods

Description of Study Area

The study area is Mubi. It is located between latitude 10º12N and longitude 13º10'E, and has a tropical climate and is found within the Sudan savanna [26]. Average temperature is about 32 ^{0}C, with a minimum of 15.2 ºC, usually in December and January period [26]. The area has an average relative humidity from 28% to 45% and annual rainfall of about 1050 mm. The rainy season is between May and October, while the dry season between November and April [26, 27].

Collection of plants materials.

Azadirachta indica

The seeds of Azadirachta *indica* were collected from tree plantation within the main campus of Adamawa State University Mubi, between the month of August and September 2019. The seed's coats of *A. indica* were decorticated and dried at room temperature for about 2 to 3 weeks before they were grinded into powder form, with the aid of pestle and mortar. The powder sample was stored in a bottle with a screw cap.

Khaya ivorensis

Similarly, fresh stem barks of *Khaya ivorensis* were peeled from healthy trees from the same tree plantation in the Adamawa State University's main campus. They were subsequently air-dried at room temperature for about two weeks. The air-dried stem barks of *K. ivorensis* were thereafter, grinded and sieved using wire mesh of relatively smaller size. The powder sample was stored in dark bottle with screw cap for further use.

Preparation of ethanol and aqueous extracts of *A. indica* seed and *K. ivorensis* stem bark powder

Ethanol and aqueous extracts were prepared using maceration method as performed by Dahchar *et al*. [28]. 100g of each powder sample was soaked in 200 ml of ethanol (80% v/v). This was allowed to stand for 72 hours in a dark cupboard under room temperature. The content was shaken at a regular interval to ensure proper mixture. Thereafter, the mixture content was filtered through Whatman's Filter Paper (No. 42). After the filtrate was obtained, the ethanol content of the mixture was removed using a water bath at 60 to 65 °C. The stock solution obtained was however, used to constitute 200 and 400 mg/ml concentrations of the treatments that was used for the experiment. For aqueous extract, distilled water was used in place of ethanol and of the same quantity.

Collection of subterranean termites for laboratory toxicity study

The termites were collected behind the fisheries department at Adamawa State University Mubi, using a method described by Tamashiro *et al*. [29] and Menandro [30]. This was done by setting ''termite'' wooden trap to collect the termite. The infested stakes where covered by these wooden 'trap' boxes, then, collected after some days when the termites starts infesting the wood.

Collection and preparation of woods for field experiment

An unidentified soft wood plank was purchased from a timber shed at Mubi timber shade. The plank was cut into sizeable pieces by a carpenter, which was used as the experimental woods. The woods were dried to a constant weight in an oven, and the initial weight of each of the experimental woods was noted and recorded after labeling each prior to experiment proper.

Laboratory bioassay

One ml each of the treatment extract concentrations (200 and 400 mg/ml) was introduced into 300 cm^3 experimental jars. These were covered with filter papers (to avoid direct contact of the treatment with the termites), before 10 newly developed subterranean termites were introduced. Pieces of soft woods were place in the experimental jars as food before the jars were covered with muslin cloths with the aid of a rubber band immediately. This was to prevent the termites from escaping, and also to provide proper aeration. A control experiment was set up with only acetone and the feed added. The treatment jars and the control were replicated 4 times. Mortality counts were observed for 24, 48 and 72 hours post exposure.

Field experiment on anti-feedant Properties of *Azadirachta indica* and *Khaya ivorensis* against subterranean termites

The experimental woods of known weights were treated with 400 mg/ml and 200 mg/ml of both aqueous and ethanol extracts of *Azadirachta indica* and *Khaya ivorensis*, simply by rubbing the entire surface area of the woods with the aid of a paint brush. Different paint brushes were used for different treatment extracts, as well as the extract concentrations, in order to avoid possible contamination. The control experimental woods were treated with acetone. The experimental woods for each of the extract concentration and the control experiment were replicated four times. The experimental woods were thereafter buried in the soil partially, to a depth of 10cm around termite mound within Adamawa State University Main Campus. About 2.5 cm of the woods remained visible above the soil surface. Weekly observation for weight loss was done for four (4) weeks. At the weekly interval, woods were removed, weigh and recorded, and then subsequently put back in the same soil.

Data analysis

Analysis of variance (ANOVA) was employed in the analysis of data, and Duncan Range Multiple Test was used to separate the mean differences, at 5% level of significance (p>0.05). The analysis was performed using SPSS version 19.0.

Results and Discussion

The pressure exerted from the use of chemical insecticides against insect pests has led to environmental degradation and has caused harm to the ecosystem. As part of the global strategy in tapping plant products in the quest of finding an alternate control measure rather than the use of chemical insecticides against insect pests, *Azadirachta indica* and *Khaya ivorensis* extracts were evaluated for their termicidal potential in terms of toxicity and anti-feedancy in this study.

Mortality counts of termites exposed to ethanol and aqueous extracts of *Azadirachta indica* revealed a very high toxicity effect of the treatments. The result showed that there was no significant differences between the mortality recorded in the treatment jars when compared to the control (untreated experiment), where no mortality (0.0%) was recorded throughout the experiment. Meanwhile, there was no significant difference in the number of mortality recorded in the treatment jars at 24, 48, and 72 hours. The extracts at 200 mg/ml and 400 mg/ml recorded 100% mortality at 72 hours post exposure period (Table 1). *Khaya ivorensis* extracts recorded similar result as *A. indica*; as 100% mortality was

achieved in all the treatment jars at 72 hours of treatment exposure with no significant difference (p>0.05). This shows that as the concentration of each of the treatment increases, the toxicity effect also increases. Also, there was no significant difference in the toxicity effect of the treatment extracts at 24, 48 and 72 hours based on the mortalities recorded. However, there was a significant difference (P<0.01) between the mortality recorded in the treatment jars and the control jars as shown in Table 1.

This revealed that both *A. indica* and *K. ivorensis* are potentially promising agents against subterranean termites, which have continued to destroy human valuables both at home and in the field. This is in agreement with the findings by Ibe *et al*. [31] who reported that neem and other plant sourced treatments recorded 100% mortality of termites after 72 hours of treatment exposure in Imo State, Nigeria. Similarly, Daniel and Bekele [32] reported neem seed powder as a potential agent for the control of termites as 100% mortality was also achieved when termites were exposed to crude extracts of neem seed powder.

Termicidal activity of *A. indica* seed and *K. ivorensis* stem bark extracts against subterranean termites on treated woods and control (untreated woods), which were vertically buried in the soil around termite mounds in Mubi, was evaluated through weight loss observed on the exposed woods. The weight loss is assumed to have been caused by attack on the experimental wood samples, as a result of the invasion by termites when exposed to termite mound. The result revealed that the treatments had a strong antifeedant property against the subterranean termites. Both *A. indica* and *K. ivorensis* significantly reduced the feeding rate of the termites on the experimental woods when compared with the control experiment. However, ethanol extracts of *A. indica* and *K. ivorensis* proved to be more effective than the aqueous extracts of the same treatments, as they recorded a significant lower weight loss as shown in Tables 2.

K. ivorensis stem bark extracts performed better than *A. indica* seed extracts, since the former significantly reduced the weight loss more than the later. This indicates that better protection of woods is more swiftly produced by the *K. ivorensis* against subterranean termites compared to *A. indica*. This finding is in agreement with the findings by Adedeji *et al*. [8], who also reported *K. ivorensis* as a potential agent for termite control; the stem bark extracts of *K. ivorensis* significantly protected woods against termites in Ogun State, Nigeria. Neem has been reported for its inability to effectively prevent feeding on woods by termites because of its inability to penetrate the wood in its raw state, thereby recording high weight loss of woods [1]. The fact that the wood samples were only coated with treatments by simply rubbing the surface of the woods using paint brush, could be the reason why *K. ivorensis*, was superior to *A. indica* in preventing the wood samples from subterranean termites in Mubi. However, neem treatments have been reported to effectively control insect pests because of its toxicity, repellent or anti-feedant properties against organic vegetables [25, 33], *Sitophilus zeamais* on maize grains [34, 35], and *Callosobruchus maculatus* on cowpea seeds [36, 37].

The total weight loss recorded in wood samples treated with ethanol extracts at 400mg/ml (24.95g in *A. indica* and 17.60g in *K. ivorensis*) showed its superiority of effectiveness over aqueous extracts at the same concentration (81.30g in *A. indica* and 37.60g in *K. ivorensis*). This also revealed that ethanol treatment extracts are more effective than the aqueous extracts in controlling termites in Mubi.

Table 1: Mortality counts of termite exposed to *Khaya ivorensis* bark and *Azadirachta indica* seed extracts at 24hrs treatment interval.

Treatment	Conc. (mg/ml)	*Azadirachta indica*			*K. ivorensis*		
		Week1	Week2	Week3	Week1	Week2	Week3
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	0.00	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
Aqueous	200	5.00 ± 0.00 ^{b}	7.50 ± 0.71 ^{b}	10.00 ± 0.00 ^{b}	5.00 ± 0.00 ^{b}	8.00 ± 1.41 ^{b}	10.00 ± 0.00 ^{b}
	400	5.50 ± 0.71 ^{b}	9.00 ± 1.41 ^{b}	10.00 ± 0.00 ^{b}	5.50 ± 0.71 ^{b}	8.00 ± 0.00 ^{b}	10.00 ± 0.00 ^{b}
Ethanol	200	6.00 ± 1.41 ^{b}	9.00 ± 1.41 ^{b}	10.00 ± 0.00 ^{b}	8.00 ± 1.41 ^{b}	10.00 ± 0.00 ^{b}	10.00 ± 0.00 ^{b}
	400	5.00 ± 0.00 ^{b}	7.00 ± 1.41 ^{b}	10.00 ± 0.00 ^{b}	6.50 ± 0.71 ^{b}	10.00 ± 0.00 ^{b}	10.00 ± 0.00 ^{b}

SD – Standard Deviation. Values are means of four replicates. Means carrying the same superscript(s) along the column are not significantly different (P>0.05) at 5% level of significant

Table 2: Weight loss of woods treated with extracts of *Khaya ivorensis* bark and *Azadirachta indica* seed exposed to termite mound

Treatment	Conc. (mg/ml)	*Azadirachta indica*			*K. ivorensis*		
		Week1	Week2	Week3	Week1	Week2	Week3
		Mean ± SD (g)	Mean ± SD (g)	Mean ± SD (g)	Mean ± SD (g)	Mean ± SD (g)	Mean ± SD (g)
Control	0.00	47.90 ± 0.00 ^{c}	100.70 ± 0.00 ^{c}	177.10 ± 0.00 ^{c}	36.30 ± 0.00 ^{c}	84.10 ± 0.00 ^{c}	153.40 ± 0.00 ^{c}
Aqueous	200	27.95 ± 0.64 ^{b}	62.05 ± 0.64 ^{ab}	108.25 ± 2.74 ^{bc}	11.95 ± 0.98 ^{ab}	28.50 ± 2.89 ^{ab}	48.45 ± 5.02 ^{b}
	400	22.15 ± 2.37 ^{b}	48.15 ± 4.91 ^{ab}	81.30 ± 7.51 ^{b}	8.85 ± 0.40 ^{ab}	21.05 ± 0.58 ^{ab}	37.60 ± 1.62 ^{ab}
Ethanol	200	9.75 ± 0.40 ^{a}	21.80 ± 0.35 ^{a}	24.95 ± 0.87 ^{a}	4.00 ± 0.12 ^{a}	8.90 ± 1.96 ^{a}	18.75 ± 1.21^{a}
	400	3.45 ± 0.40 ^{a}	12.10 ± 0.35 ^{a}	38.35 ± 0.58 ^{a}	2.95 ± 1.91 ^{a}	8.40 ± 3.93 ^{a}	17.60 ± 6.80 ^{a}

SD – Standard Deviation. Values are means of four replicates. Means carrying the same superscript(s) along the column are not significantly different (P>0.05) at 5% level of significant.

Conclusion

In this study, *Azadirachta indica* and *Khaya ivorensis* offered desirable results in terms of toxicity and repellent properties measured in terms of mortality and weight loss, respectively against the subterranean termites in Mubi, Adamawa State. The ethanol extracts was more potent than the aqueous extracts, while *K. ivorensis* offered more protection to wood samples than *A. indica*. Therefore, sustainable use of this biopesticides, which are cheap and readily available, against insect pests like termites will go a long way in protecting our environment and the ecosystem at large against the dangerous termicides or insecticides, which are costly, non-biodegradable, and harmful to the user.

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