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TERMITICIDAL POTENTIALS OF STEM BARK EXTRACT OF *ZANTHOXYLUM ZANTHOXYLOIDES* (CANDLEWOOD) AGAINST *MACROTERMES SUBHYALINUS* (ISOPTERA:TERMINIDAE)

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ABSTRACT

This study was conducted in Biology Laboratory, Federal Polytechnic Bida to evaluate the efficacy of stem bark extract of *Zanthoxylum zanthoxyloides* against *Macrotermes subhyalinus* at ambient temperature of 28±2°C and relative humidity of

Introduction

Termites belong to the family terminidae and insect order isoptera which are social insects living in colonies. It is one of the most important insect pests, which plays a vital and integral role in the destruction of many woods and wood products. It is a rural and urban insect pest because it can be found in almost every terrestrial habitat (Adeyemo, *et al.*, 2015). *Macrotermes* species impact the economy negatively by causing damage to various agricultural crops, wooden portions of buildings, furniture, utility poles and fence posts in several parts of Africa and the world at large (Nyeko *et*

70±5%. Parameters assessed were, phytochemical constituents of plant material, mortality of termites and repellency activities of plant extract. Three different concentrations of the plant extract were formulated (1%, 2% and 3%). The results of the phytochemical constituents revealed the presence of saponins, flavonoid, anthraquinone, cardiac glycosides, and tannins. The extract of stem bark of *Z. zanthoxyloides* caused 80%, 86.7% and 100% mortality of *M. subyalinus* at 1%, 2% and 3% respectively after 24hours treatment, there effect is significantly different with the control (P<0.05). The repellency activities of plant extract indicated mean % repellency of 30.0% , 33.3% and 43.3% against termites at concentrations of 1%, 2% and 3% respectively. The stem bark extract of *Z. zanthoxyloides* is recommended for the control of *M. subhyalinus* and the development of new insecticides from plant extracts sources can be an alternative for the control of *M. subyalinus.*

Keywords: Termiticidal, *Z. zanthoxyloides, Macromtermes subyalinus,* Mortaliy, Repellency

al., 2010). Outdoor infestations by *Macrotermes* species has profound effects on the agricultural enterprise before and after harvest due to their foraging activities on plants (Fenemore and Prakash,2006). Termites also cause serious damage to young tree seedlings especially in the coconut-growing areas since they are often subjected to moisture stress and transplanting (Bong *et al*., 2012). Ekpo and Onigbinde (2007) reported *M. subhyalinus* as a destructive insect pest that attacks not only the forest products but as well attacks agricultural produce and products such as maize, sugarcane, cocoa, groundnut, beans and vegetables.

In India 300 species of termite have been recorded and pestilence (in ecosystems) is caused by 35 species (Verma *et al.,* 2009) and about 15 to 25% maize crop loss has been reported due to termite infestation resulting in a 2.46-billion-rupee economic loss. Furthermore, synthetic insecticides cause toxicological and environmental problems such as their residual effects in food, soil water and adverse effects on non target organism and other beneficial organisms as well (Amoako, 2010; Fening *et al.*, 2013). Due to the serious negative impacts of the use of synthetic insecticides, search for alternative approaches to managing insect pests have to be considered. Consequently, Pesticides obtained from plant materials are known to be very effective in insect-pest control as they have repellent and antifeedant properties among others and are less hazardous compared to the conventional pesticides (Mishra, 2013), there is a need to control insect pests such as *Macrotermes* species with affordable and locally available plant materials which are biodegradable, readily available, easy to prepare and easy to apply (Muhammad, 2009). However, it has been found that certain plants have evolved specific defence mechanisms whereby the secondary metabolic pathways yield certain metabolites that could be toxic to parasites or function as repellents or deterrents to pest invasion (Krupal *et al.,* 2017). It is generally accepted that replacing the recalcitrant synthetic pesticides with biodegradable compounds that originate in living systems can prevent the adverse effects of synthetic pesticides on environment (Krupal *et al.,* 2017). Discovery of plant defence systems and phytochemicals generated a new vista in the fields of medicine and pestology (War *et al.,* 2012). The use of leaf, bark and root of *Z. zanthoxyloides* have been effective in protecting grains from storage product pests such as *Callosobruchus maculatus*, *Sitophilus zeamais* and *Prostephanus truncates* (Udo, 2000; Owusu *et al.,* 2007). This research work therefore, investigated the

termiticidal potentials of stem bark extract of *Z. zanthoxyloides* against termites.

MATERIALS AND METHODS

Experimental site

The study was conducted at the Biology laboratory in the Department of the Biological Sciences Federal Polytechnic Bida, Niger State, Nigeria lie on latitude 9°6' North and longitude 6°1' East

Collection and preparation of plant material

The stem bark of *Zanthoxylum zanthoxyloides* were collected from botanical section of the Biological garden, Federal Polytechnic Bida Niger State, Nigeria. The plant materials were transported to the Biology Laboratory, Federal Polytechnic Bida and identified by a botanist, the voucher specimens were deposited in the Laboratory herbarium for reference purposes. The plant materials was washed with distilled water to removed the dust and soil particles and cut into smaller pieces before air dried in the Laboratory, then pulverised into fine powdered using electric blender and sieved with mesh size 0.1mm in order to standardised the particles.

Preparation of ethanolic extract

The sample was prepared by soaking 200g of dried powdered in 600ml of ethanol for 24 hours and the mixture was stirred with rod glass at interval of 12 hours to ensure uniform coating. The mixture was filtered using Whatman No. 1 filter paper and the filtrates was exposed to rotary evaporator so that the remaining solvent evaporated and obtained residues were re-dissolved in water for experiment and for further analysis.

Phytochemical Screening

The phytochemical screening of stem bark extract of *Zanthoxylum zanthoxyloides* was carried out following the standard procedure and parameters assessed were, alkaloid, flavonoids, tannin, saponins, carbohydrates, anthraquinones and cardiac glycosides

Insect collection

The eggs of *Macrotermes subhyalinus* were collected from a termitarium behind Biological garden, Federal Polytechnic Bida, Niger state, Nigeria using camel hair brush and placed in a plastic container with cover lid been perforated to ensure supply of oxygen and prevent entry or exit of insects, then transported to the laboratory to allowed the eggs to hatched into nymph at ambient temperature of 28± 2°C and relative humidity of 70± 5% and used for the experiment.

Bioassay

Three different concentrations of the extracts were formulated, 1%, 2% and 3% (w/v). Five pairs of termites were placed in petri dishes and plant extracts of different concentrations was sprayed to them and distilled water served as control. All the treated and untreated control termites were transferred into plastic containers after five minutes and replicated three times. Number of dead termites was recorded after 1, 6, 12 and 24 hours treatment, insects were considered dead when they fail to respond to probing by blunt probes (Osabutey *et al.*, 2015) and percentage mortality of termites was calculated by using the formular (Uyi *et al*; 2019).

$$\% \text{ Mortality} = \frac{\text{Number of dead termites}}{\text{Total of number of termites}} \times 100$$

Repellency test

The Whatman No. 1 filter paper (8cm diameter) was divided into two equal parts, half part was treated with 1ml of each of the

concentrations (1%, 2% and 3% w/v) of the extracts and half was immersed with distilled water which serve as control using micro pipette. The two halves of filter paper was air dried to evaporate the solvent completely (Jeyasankar *et al*., 2016) and placed in the plastic containers of 9cm in diameter. The treated filter paper strips was attached lengthwise, edge to edge to untreated filter paper strips of similar size with cellophone tape paper (Hinazafer *et al*., 2020). Five pairs of termites were released at the center, between the treated filter paper and untreated filter paper and petri disc was covered with muslin cloth to prevent the exit and entry of any insects and placed in a darkness to reduced light effect on the insect (Uyi *et al*., 2019). All the experiments were replicated three times, arranged in a completely randomised design and the numbers of termites present on control (Nc) and treated (Nt) were recorded after 15, 30, 45 and 60 minutes of the treatment. The percentage repellency (PR) was calculated according to Oni *et al*. (2018).

$$\% PR = \frac{N_C - N_T}{N_C + N_T} \times 100$$

Where N_C is the number of insects present in the untreated filter paper and N_T is the number of insects present in the treated filter paper.
The average values was classified according to the repellency classes proposed by (Jeyasankar*et al*; 2016) as shown in Table 1

Table 1: Repellency classes according to Jeyasankar *et al* ; 2016

Repellency rate (%)	Class	Interpretation
>0.01- < 0.1 0	0	Non repellency
0.1 – 20	I	Very weak repellent
20.1 – 40	II	Moderate repellent
40.1 –60	III	Average repellent
60.1 – 80	IV	Fairly repellent

80.1 – 100 V Very repellent

STATISTICAL ANALYSIS

Data obtained were subjected to analysis of variance (ANOVA) and where significantly differences existed, means were compared at 0.05 significance level using New Ducan Multiple Range Test (NDMRT).

Results and Discussions

The result of phytochemical constituents of stem bark extract of *Z. zanthoxyloides* are presented in Table 2. The result revealed the following constituents of the plant extracts, saponins, cardiac glycosides, flavonoids, anthraquinone, carbohydrate and tannins, while steroid and alkaloids are absent. The result shows that flavonoids (+++) was highly present, carbohydrates (++) was moderately present, while saponins, cardiac glycosides, anthraquinones and tannins (+) were slightly present. This result is in agreement with that of Mann *et al.* (2013), who reported that some active constituents were present in plant extract of *Z. zanthoxyloides.*

Table 2: Result of Phytochemical constituents of stem bark extract of *Z. zanthoxyloides*

Phytochemical constituents	Test	Results
Saponins	Frothing	+
Cardiac glycosides	Keller-killian/Salkowski	+
Flavoniods	NaOH	+++
Anthraquinone	Borntrager	+
Alkaloids	Dragendoff	-
Carbohydrates	Molish's	++
Tannins	Ferric Chloride	+
Steroid	Liebermann-Burchard	-

Key: Highly present (+++): Moderately present (++): Slightly present (+): Absent (-)

Effect of stem bark extract of *Z. Zanthoxyloides* on the mortality of *M. subhyalinus*

Table 3 shows the mean percent mortality of termites treated with different concentrations of stem bark extract of *Z. zanthoxyloides.* The result indicated that as the concentration increases, the rate of mortality also increases with increased in time duration. The stem bark extract caused 13.3%, 26.7% and 30.0% mortality of termites at concentrations of 1%, 2%, and 3% respectively after 1 hour treatment while untreated control recorded 0.00% mortality of termites. There was significant different between all the concentrations of the extract and control ($P<0.05$). At 12 hrs after treatment the percentage mortality of termites recorded were 46.7%, 70% and 80% at concentrations of 1%, 2% and 3% respectively. The results of this investigation is consistent with that of Mann *et al.* (2013), who reported that 76.6% mortality of termites was recorded as a result of treatment with the stem bark extract of *Z. zanthoxyloides*. At 24hours after treatment the percentage mortality of termites were 80%, 86.7% and 100% at concentrations of 1%, 2% and 3% respectively. This shows that the concentration of 3% was more effective among all the concentrations ($P<0.05$) and all concentrations were more effective than control which showed 0.00% mortality of termites. The presence of secondary metabolites such as saponins, flavonoids, tannins and anthraquinone may be responsible for high mortality of termites after 24hours treatment. The results of this research work corroborates with that of Adesina (2005), who reported that most of the *Zanthoxylum* species contains lignans specifically diarylbutirolactones and 2,6-diaryl-3, 7-dioxabicyclo (3.3.0) octanes which are responsible

for the biological activities including the insecticidal and inhibitory effects on certain enzymes.

Table 3: Mean % Mortality of *M. subhyalinus* treated with ethanolic Stem bark extract of *Zanthoxylum zanthoxyloides*

Conc. % (W/V)	Mean % mortality of termites at different hours exposure (±S.E) 1	6	12	24
1	13.3±0.94^{c}	33.3±0.47^{c}	46.7±0.82^{c}	80.0±0.47^{c}
2	26.7±0.84^{b}	46.7±0.82^{b}	70.0±0.47^{b}	86.7±0.47^{b}
3	30.0±0.46^{a}	60.0±0.47^{a}	80.0±0.47^{a}	100.0±0.00^{a}
Control	0.00±0.0^{d} 0.00±0.0^{d}	0.00±0.0^{d}	0.00±0.0^{d}	

Each data is the mean ± standard error of triplicate determination. Different letters within the same column are significantly different (P<0.05) using Ducan's Multiple Range Test (D.M.R.T)

Effect of ethanolic stem bark extract of *Z. zanthoxyloides* on repellency against termites

Table: 4 shows the mean percentage of repellency of ethanolic stem bark extract of *Zanthoxylum zanthoxyloides* on *M. subhyalinus*. Repellency was seen in the number of termites found in the treated filter paper were significantly smaller than the number in the control. The results revealed that after 15minutes of treatment, the following percentage of repellency were recorded, 6.67%, 20% and 20% at concs. of 1%, 2% and 3% respectively and the control did not show any repellency. At 30minutes after treatment, the following percentage of repellency were recorded, 33.3%, 20% and 60% at concs. of 1%, 2% and 3% respectively, and there was no repellency for the control. At 45minutes after treatment, the following percentage of repellency were recorded, 33.3%, 60% and 60% at concs. of 1%, 2% and 3%

respectively and at 60minutes after treatment, the percentage of repellency was 26.7%, 33.3% and 33.3% at concentrations of 1%, 2% and 3% respectively. The mean percentage repellency of the extract against termites were 30.0%, 33.3% and 43.3% at concentrations of 1%, 2% and 3% respectively, while the control shows no repellency. The concentrations of 1% and 2% falls under repellency class II which is moderately repellent and conc. of 3% falls in class 111 which is average repellent while control experiment falls in class 0 which is non repellency. The research work conforms with that of Oni *et al.* (2018) who reported 33.3% repellency on oil of *Acalyphagod seffiana* extract against termites.

Table 4: Mean % repellency of stem bark extract of *Z. zanthoxyloides* on *M. subhyalinus*

Conc.	Mean % Repellency of the extract at diff. mins.				Mean	Repellency
% (w/v)	15mins	30mins	45mins	60mins	rate(%)	class
1	6.67	33.3	33.3	26.7	30.0	II
2	20.0	20.0	60.0	33.3	33.3	II
3	20.0	60.0	60.0	33.3	43.3	III
Control	0.00	0.00	0.00	0.00	0.00	0

Conclusion and recommendations

This research work has shown that the extract of the stem bark of *Zanthoxylum zanthoxyloides* has a toxic effect on *Macrotermes subhyalinus* (termites), this indicates that botanicals might be useful as insect control agents in replace of synthetic chemicals which has effect on human health and to minimize the severe damages caused by termites, the traditional use of plant materials, proved to be highly effective, and it can be exploited as a renewable source of biopesticides. The extract of stem bark extract of *Zanthoxylum zanthoxyloides* is recommended for the control of *Macrotermes*

subhyalinus and these plant biopesticides should be encouraged as this will complement the existing synthetic pesticides.

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