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LABORATORY EVALUATION OF TERMITICIDAL EFFICACY OF *Cymbopogon citratus* STAPF LEAF EXTRACT AGAINST TERMITES, *Macrotermes bellicosus* (BLATTODEA: TERMITIDAE) IN OBIO AKPA COMMUNITY, ORUK ANAM AKWA IBOM STATE- NIGERIA

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ABSTRACT

The study was conducted in the laboratory to determine the termiticidal efficacy of *Cymbopogoncitratus* leaf extract on Termites (*Macrotermes bellicosus*). The experiment consisted of five treatments with three replicates. The concentrations 2g, 4g, 6g, standard control {Rambo insect powder (0.60% Permethrin) and experimental control. Termites were collected from the Termitarium found within the Akwa Ibom State University, Obio Akpa campus. Fifty *Macrotermes bellicosus* were introduced into Petri dishes each containing the different levels of *C. citratus* extract, observations were made for 30minutes, 1hour, 1hour 30 minutes, 2hours,and 3hours respectively, to test for mortality, repellency and survival duration of *Macrotermes bellicosus*. The results of the phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, steroids, saponins, glycosides, and citrals. Mortality test on *Macrotermes bellicosus* exposed to the different levels of treatments showed that mortality was extract concentration dependent. Maximum mortality was observed at standard control (insect powder),followed by the different concentrations of *C. citratus* extract during 3 hours exposure of termites to the extract as compared to experimental control where no mortality was recorded. There was a significant difference ($p<0.05$) in the number of termites that died at the different extract concentrations. The extract of *C. citratus* against *Macrotermes bellicosus* caused repellency in all the concentrations from 2g (65%), 4g (70%), 6g (85%) and standard control (100%). There was significant difference ($p<0.05$) in the movement of *Macrotermesbellicosus* from the extract baited filter paper at different concentration. Survival duration showed that all tested extract concentrations significantly ($p< 0.05$) reduced the survival duration of *Macrotermes bellicosus*. The study therefore, revealed the antitermitic activities of *C. citratus* which could be used as an alternative to synthetic insecticide for the control of Termites.
Keywords: *Cymbopogoncitratus, Macrotermes bellicosus,* Mortality, Repellency

INTRODUCTION

Termites are considered a serious pests to crops, rangeland and forestry in tropical Africa. Some of the deleterious species belong to the family Termitidae, which comprises of four subfamilies such as Macrotermitinae, Nasutitermitinae, Termitinae and Apictotermitinae (Alamu *et al.,* 2018; Mugerwa *et al.,* 2014). Being aware of the hazards associated with the use of synthetic pesticides, the need to explore suitable alternative method for termite control is crucial. Farmers use different plant materials to protect their crops from termites; several natural products either in their crude form or as plant powder provide unlimited opportunity as termiticide but this is not well documented. These plant derived pesticides are ecofriendly, nontoxic to non-target organisms, non- persistent in nature, besides they do not promote resistance (Nagare and Pardeshi, 2019; Liu *et al.,* 2000). *Macrotemes bellicosus* are serious pest of some agricultural crops, rangelands, wooden portions of buildings, furniture, books, utility poles and fence posts in several parts of Africa (Mitchell, 2002). *Macrotermes bellicosus* are a member of the fungus growing subfamily which are mostly mound builders and the largest termite species (Osipitan and Oseyemi, 2012). Different methods have been used by farmers in Africa for the control of termites, these includes the destruction of termite mounds (Mugerwa *et al.,* 2011c), application of wood ash (Mugerwa *et al.,* 2011b; Nyeko and Olubayo, 2005) and animal excreta (Mugerwa *et al.,* 2008; Banjo *et al.,* 2003) which are applied directly to termite mound or the wood materials are added to the soil. Termite management costs billions of dollars every year (Tsunoda and Yoshumura, 2004). This species of termite has been known to completely destroy agricultural crops and a variety of residential products around the world (Michael, 2000; UNEP and FAO, 2000 and Sekamatte, 2001). Chemicals, particularly synthetic insecticides like Organochlorine (OC) and Organophosphate (OP), have been used to control termites in the past (Paul et al., 2018). Some of the difficulties that prevent widespread use of pesticides include maximum residual effects, insecticide resistance in target pests, bad effects on human health, and environmental concerns (Coats, 1994). Termite control methods differ greatly from country to country. Providing physical and chemical barriers to termites is the best way to keep them out (Su et al., 2004).

Chemical treatment of the soil region, as well as the application of preservatives and bait to protect timber and buildings, are all part of the chemical control procedures. Insecticides used to treat soil to make it toxic or repellent to termites, as well as impregnation of timbers prior to use, are effective against both subterranean and dry wood termites. Because of the problems associated with pesticide use, there is a growing interest in developing alternative termite control strategies, and plants with termiticidal capabilities could be one of them. Termites are destructive and a persistent threat to the properties of individuals and governments, thus efforts to restrict their activities have been undertaken incessantly. Termiticides, chemical barriers, and baiting systems are among the several control strategies employed (Culliney and Grace, 2000; Davis and Kamble, 1992). Beginning scientific research into plant-based natural compounds with anti-termitic effects is worthwhile and appropriate.

MATERIALS AND METHODS

Collection of Plant Materials

The plant leaves of *C. citratus* were procured in Obio Akpa village, Oruk Anam Local Government Area, Akwa Ibom State and was identified in the Department of Botany and Ecological Studies, University of Uyo. Voucher specimen (UUH 2314) was obtained and deposited in their herbarium for further referencing.

Collection of Termites for Laboratory Experiment

The worker caste of *Macrotermes bellicosus* were collected from mounds in some farms at Obio Akpa Community, Oruk Anam Local Government Area of Akwa Ibom State, Nigeria. The termites were maintained in the laboratory on sugar cane strips for 72 hours prior to use. The moribund termites were removed and only active ones were used for the experiments.

Preparation of Aqueous Extracts

Five hundred grams (500g) of the powdered leaves was soaked in 1000 ml of distilled water for 24 hours. Thereafter, the mixture was filtered using Whatman No.1 filter. The filtrate obtained was evaporated to dryness in a vacuum using rotary evaporator and the extract was stored in sealed vials kept in refrigerator until used (Oboho *et al.,* 2016a; Manzoor *et al.,* 2011).

Phytochemical Analysis of *C. citratus*

The phytochemical analysis of *C. citratus* was carried out in the Department of Chemistry Laboratory, University of Uyo, Akwa Ibom State. Aqueous extracts were used for preliminary phytochemical analyses using standard procedures as described by Evans (2002) and Prashant *et al.* (2009).

Termiticidal bioassay

The method used was no-choice bioassay according to Alamu *et al.* (2018) and Nobuhiro *etal*. (2009), to evaluate the termiticidal activity of *C. citratus* extracts. The tests were carried out in a Petri dish (inner diameter 4.8cm) filled with 1g sand. 0.5/ml concentration of aqueous extracts of leaves of C. *citratus* was prepared by diluting extract. Filter paper discs (diameter 4.5cm) were treated with one ml of the extract. Discs were air dried and kept in Petri dishes. Experimental control set contains only water while Rambo insect powder (PERMETHRIN-0.06% with INERT CARRIERS-99.40%) was used as standard control with a dose rate of 10g. Ten adult workers were then released into the center of the Petri dishes and kept in BOD incubator (28±2°c and 80%±10% RH). Mortality of termites was observed after regular time interval for 30minutes, 1hour, 1:30 minutes, 2hours and 3hours respectively. Dead termites were removed after every count to avoid fungi or other infestations. Each treatment was replicated three times and arranged in a Completely Randomized Design. Mortality data was analyzed and percentage mortality was determined using Abbott (1925) formula:

$$\text{Mortality (\%)} = \frac{\text{Number of death determines}}{\text{Number of initials termites in the test}} X \frac{100}{1}$$

$$\text{Corrected percent mortality} = \frac{\text{Treatment - Control}}{\text{100 - Control}} X \frac{100}{1}$$

Repellency bioassay

This experiment was carried out in a laboratory setting with standard room temperature and relative humidity. Oboho *et al*. (2016b) described the procedure. The test regions in this investigation were 22cm Whatman no.1 filter sheets that had been sliced in half. On a half filter paper disc, one gram of the extract was put. Only the other half of the filter paper was treated with deionized water as a control. The treated and control half discs were air dried for one hour, and entire discs were re-made by using Sellotape to join the treated halves. 10 M. bellicosus was placed in the center of each filter paper in a Petri dish and covered with perforated lids lined with white muslin cloth. Three times each therapy was carried out. The number of termites present in the control (NC) and the treated (NT) strips were recorded every 30minutes and up to 48hours. Percent repellency (PR) values were computed with the formula:

$$PR = \frac{Nc - Nt}{Nc + Nt} X \quad 100$$

where:

PR = percent repellency; Nc = insect number present on control strip

Nt = insect number present on treated strip and negative PR values were treated as zero

Determination of Survival Duration

The survival duration of termites was determined according to the method by Krebs, (1999).

$$sd = \frac{\Sigma Nti - ti}{\Sigma ti} \quad x\,100$$

where:

Sd = Survival duration; Nli = Number of living termites at each checking time; Ti = checking time

Survival rate was determined using the formula

$$sr = \frac{sdi}{sdc} \quad x \quad 100$$

Where:

Sr = survival reduction rate; Sdi = means survival duration obtained with each tested concentration and 3 different replicate; Sdc = means survival duration obtained in control.

The rate of survival reduction was also determined with the survival rate: Rr = 100 – Sr

Rr = survival reduction rate.

Statistical Analysis

The data obtained from calculation of survival duration and the rate of survival reduction were subjected to an analysis of variance (ANOVA) at 2%, 4%, 6%, and the means were discriminated with the Student Newman-Keul (SNK) test.

RESULTS

The result of the phytochemical screening of this plant showed the presence of alkaloids, cardiac glycosides, flavonoids, saponnins and phenolics as shown in Table 1

Table 1: preliminary phytochemical screening of *Cymbopogon citratus* Leaves.

Plant constituents	Extract (Aqueous)	Name of test
Alkaloids	+	Wagner's test
Flavonoids	+	Shimoda test
Phenolics & tannins	+	Ferric chloride test
Steriods & sterols	+	Salkowski test
Saponin	+	Stable foam test
Glycosides	+	Glycosides test
Anthraqunone	-	Borntragers test
Citral	+	Pb acetate, shimoda test

Keys: + Presence - Absence

Repellent effect of *C.citratus* Extract against *Macrotermes sp.*

The result in **Table** 2 showed all tested extract of *C.citratus* have repellent effect against termites in all concentrations. The repellent effect of this extract against *Macrotermes* sp is shown in table 3 above. The extract caused repellency in all the concentrations except in experimental control. There are significant difference ($p<0.05$) amongst the extract in affecting the movement of *Macrotermes* sp.

Table 2: Repellent effect of *C. citratus* Extract against *Macrotermes sp.*

Concentration	Percentage repellency (PR) %
2g	65
4g	70
6g	85
Experimental control	0
Standard control	100

Table 3: Mortality of *M. bellicosus when exposed to different concentrations of C. citratus Extract.*

Concentration (g)	Mean mortality hours after treatment				
	30 minutes	1 hour	1:30mins	2 hours	3 hours
2	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}
4	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}	0.33 ± 0.33^{a}	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}
6	1.00 ± 0.33^{b}	1.00 ± 0.00^{b}	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}	0.33 ± 0.33^{a}
Experimental Control	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}
Standard control	1.33 ± 0.33^{b}	2.00 ± 0.00^{b}	1.67 ± 0.33^{b}	2.00 ± 0.00^{b}	2.67 ± 0.33^{b}

Means with means with different superscripts along the same columns are significantly different ($p < 0.05$)
Means with the same superscripts along the same row are not significantly different ($p < 0.05$)

As shown in Table 4 above, the activity of lemon grass extract against *Macrotermes sp* was found to be both time and concentrations dependent. The highest and lowest mortality of termites was observed in the highest and lowest concentrations of the extract respectively. All the exposed termites (50) were dead after 3hours in all concentrations except in experimental control (water) which did not record any mortality. Comparing number of termites that died at 2g, 4g, 6g and standard control after 30minutes, 1hour, 1 hour 30minutes,2hours and 3hours using Student Newman Keul (SNK) test, showed that there was a significant variation in the number of termites that died at the different concentrations ($p<0.05$). This implies that changes in concentration can vary the number of dead termites. The experimental control (water) did not record any mortality (Table 4).

Table 4: Survival Duration of *Macrotermes* when exposed to different Concentrations of *C. citratus* Extract

Concentration (g)	Mean survival duration hours after treatment				
	30 mins	1 hr	1 hr: 30 Mins	2 hrs	3 hrs
2	0.33 ± 0.33^{a}	1.00 ± 0.33^{b}	0.33 ± 0.33^{a}	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}
4	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}
6	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}	0.33 ± 0.33^{a}	1.00 ± 0.33^{b}	1.00 ± 0.33^{b}
Exptal Ctrl	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}
Standard control	1.33 ± 0.33^{b}	2.00 ± 0.00^{b}	1.67 ± 0.33^{b}	1.33 ± 0.33^{b}	2.00 ± 0.58^{b}

± = standard error

Means with different superscripts along the same columns are significantly different (p < 0.05)

Means with the same superscripts along the same row are not significantly different (p < 0.05).

Survival Duration of *M. bellicosus* when exposed to different Concentrations of *C. citratus* Extract.

All the tested concentrations significantly ($p < 0.05$) reduced the survival duration of *Macrotermes sp*. At 2g, the aqueous extract of *C. citratus* affected termite's survival with 30% reduction and at standard control (0.60 permethrin); it caused the highest reduction rate (100%). *Cymbopogon citratus* aqueous extract is the most effective on *Macrotermes* especially from 2g, 4g, 6g, and standard control. The survival duration in experimental control (water) is high, because there was no mortality recorded (Table 5). Survival duration increased as mortality decreased. This study also revealed that, all the concentrations except experimental control (water) reduced the survival duration of *Macrotermes*. The highest reduction of survival duration was recorded at standard control (0.60 permethrin) with different time intervals. The survival duration in experimental control (water) is high because no mortality was recorded at different time intervals.

DISCUSSION

The results in this study showed that *Cymbopogon citratus* contains some secondary metabolites such as flavonoids, saponins, tannins, alkaloids, phenols and citrals. Similar elements were found in the leaves of *C. citratus* by Oboho *et al.* (2016a). The existence of metabolites in this study backs up the findings of Ameh *et al.* (2010), who conducted phytochemical screening on a methanolic extract of *Allium sativum* and found abundant alkaloids, steroids, and flavonoids. These secondary metabolites, which include tannins, saponins, flavonoids, alkaloids, phenols, and citral, are a mixture of terpenoids that affect the neuron, axons, synapses, respiration, hormonal balance, and termite reproduction. This is consistent with previous research by Lee *et al.,* (2001), Mulunga *et al.,* (2007), Edelduok *et al*., (2012), and Oboho *et al*. (2016b), who attributed the insecticidal activities of plant botanicals to their major chemical constituents, as the presence of multiple compounds in plants has been known to be beneficial in pest control. Tannins, alkaloids, and saponins were found in significant concentrations in the current investigation, suggesting that they may be responsible for antitermitic activity. Tellez *et al.*, (2001) found antitermitic activity in tarbush (*Flouresia cernva*) leaves hexanes diethyl ether, and ethanol extracts, while Kareru *et al*., (2010) found antitermitic activity in *Thevetia peroviana* seed oil utilized in surface point. Even at lesser concentrations (2g) and at varied time intervals, the impacts of those phytochemical compositions of *C. citratus* leaf were highly dramatic. This is most likely owing to the leaf of lemon grass having a higher concentration of alkaloids, saponin, tannins, flavonoids, and glycosides. The extract proved quite efficient, especially when used in high doses. It has been claimed that the phytochemicals in this plant decrease oxidative phosphorylation and oxygen consumption in fish (Tiwari and Singh, 2003). It's not surprising, then, that *C. citratus* has strong termiticidal properties.

Macrotermes were repelled by a lemon grass extract. Antifeedant characteristics are strongly suggested by the plant's repelling actions (Moore *et al*., 2006; Karunamorrti *et al*., 2009; Maia *et al*., 2011). The termiticidal property of *Cymbopogon* is ascribed to its high geraniol concentration. Citral, a fragrant plant ingredient, may serve as a termite deterrent (insects). Olaifa *et al.* (1987) employed *Cymbopogon* extract against *Dyderous superstitiosus, Ootheca mutabilis*, and *Riptortus dentipes,* and found repellency due to the influence of geraniol and citral.

This study revealed that *Macrotermes* treated with extract of *Cymbopogon citratus* at different concentrations gave promising potential in the control of termites in terms of mortality except in experimental control which suggest that there was no mortality recorded.the mortality in this study increased as the concentration increases. This is similar to the work by Addisu *et al.* (2014) who reported that extracts of neem and *Jatropha* seeds was an effective termiticides at higher concentration. Olugbemi (2012), also reported that African locust bean, *Parkia biglobosa* exhited anti-termitic property against *Coptotermes intermedius.Ogunlowu and Idowu (1994)*, *observed a high mortality of termites when exposed to C. citratus aqueous extract.* The plant extract showed antitermitic activity in a dose (concentration) dependent manner, and exhibited a significant activity after 3 hours of exposure; the highest termite (100%) mortality was found at standard control (0.60 permethrin) with respect to time (table 4). The result also shows that *C. citratus* aqueous leaf extract have promising good termiticidal activities. The toxic effects of the extract on *Macrotermes* in this study may likely be as a result of cumulative effects of the various constituents present in the plant. Similar result was reported by Abolade *et al. (*2000)*,* Verena and Hertel *(*2001), who observed that some plant extracts are used for termite control. Elango *et al.* (2012) also reported that the hexane dried leaf extracts of *Tagetes erecta* possess termiticidal activity against Formosan subterranean termites, *Coptotermes formosanus.* This study also shows that *C. citratus* aqueous extract has good termiticidal activities where lower concentration (2g) also gave better results. After 3hour, the *Macrotermes treated* at 2g, 4g, 6g, and standard control (0.60 permethrin) were killed. The findings of this study also show that the leaf extract of *Cymbopogon citratus* provides a source of naturally occurring chemicals that could be used as termite control agents, and this activity is attempted with the presence of phytochemicals of various chemical structures that had repellent, survival, antifeedant, or toxic effects on termites in feeding assays (Ahmed et al., 2016). Several higher plants have been evaluated in the field and in the shop for their ability to combat insect pests and disease of various crops. *C. citratus* is a widespread weed in Nigeria.

CONCLUSION

This study revealed the antitermitic activity of *Cymbopogon citratus.* The plant derived material could be useful as an alternative for synthetic insecticides controlling field populations of *Macrotermes bellicosus* sine this plant is available, accessible affordable and easy to cultivate. Therefore it usage should be promoted among the local farmers. *C. citratus* may be used as sustainable antitermitic agent; the leaf extract could be exploited to develop new wood preservatives to protect wooden structures, agricultural crops, plants and trees. The cultivation of *C. citratus* as edges at home and fields for termite control should be promoted. Furthermore, there is need to conduct field studies to use this plant as antitermitic agent against termites' infestation of farms and farm products.

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