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Biocidal Properties of *Cymbopogon Citratus* Extracts on Termite (*Microcerotermes Beesoni*)

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Abstract

The aim of this experiment was to provide a biological approach to termite control through the use of extracts of roots and shoots of Lemon grass (*Cymbopogon citratus*). This was a laboratory experiment that involved various treatments through in-vitro contact applications of plant extracts and a pre-screening experiment on solutions for lethal properties against termites. The experiment was conducted at the Laboratories of the Department of Plant Science and Biotechnology and the Chemical Sciences of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria between August, 2005 and March, 2006. The roots and shoots of *C. citratus* were obtained from its tuft, sundried for 4 weeks and chopped into match-stick sizes. Extracts were obtained from the plant through non-polar (n-hexane) and polar (ethanol) solvents. In the laboratory experiments, the efficacy of the obtained oil extracts in controlling termites were determined through contact tests on termite castes and mortality rate recorded. This was also compared to a control contact invitro experiments where water, concentrated n-hexane and ethanol were determined for efficacy in termite control. Means of the results obtained were separated using Tukey's Honestly Significant test. Analysis showed that there was no significant different at $P < 0.05$ between n-hexane shoots extract and n-hexane roots extract at 60seconds treatment applications. Observations showed that extracts obtained from all the parts were effective in the control of the different castes of termites with the n-hexane roots extract showing the fastest efficacy within 50seconds.

Keywords: Termites, Pest control, *Cymbopogon citratus*, Lemon grass, Plant extract.

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Ethical: This study follows all ethical practices during writing.

Contents

1. Introduction

Lemon grass is an aromatic plant belonging to the Poaceae family Akhila [1]. It is a tall, clumped perennial grass growing to a height of 1 m. The leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm. Conversely, flowering has never been observed under cultivation due to rapid harvesting time. The rhizome produces new suckers that extend vertically as tillers to form dense clumps. Lemongrass can tolerate a wide range of soils and climatic conditions. However, vigorous growth is obtained on well-drained sandy loam soil with high fertility and exposed to sunlight.

The insecticidal activities of various extracts of the plant on vectors of medical or veterinary interests or non-agricultural pests was reported Ratnadass and Wink [2] and which also encompasses mosquitoes, mites, cockroaches, houseflies, termites and water snails and a host of human or cattle parasites. The anticoagulant activity of the oil extract of lemongrass was detected while trying to examine its pesticidal properties. Due to the environmental concern and expensive synthetic insecticides, the demand for alternative sources of insect pest control had increased. Also, the pervasive use of these insecticides in granaries of small-scale farmers has also led to a number of shortcomings such as elimination of non-target species, user hazards, found residues, evolution of resistance to the chemicals, high cost of the chemicals and the destruction of the balance of the ecosystem.

Conversely, termites (*Odontotermes sp.*) had been reported to reduce crop yield and destroy stored grains. They are reported to be serious pests of various crops (wheat, sugarcane, groundnut and paddy) and cause significant yield losses in annual and perennial crops and damage wooden structures in buildings, especially in semi-arid and sub-humid tropics. The control of termites had initially centered on the use of synthetic termiticides but observed hazards from residue had informed the adoption of chloropyriphos in the treatment of soil against subterranean termite, but this application was not devoid of hazard to human and livestock as its chronic exposure had been linked to neurological effects and developmental disorders. Presently, continuous use of synthetic termiticides for soil as well as crop treatment has been allowed for the present time because of the lack of any effective substitute. Although, researches are on-going for a quest for an effective formulation, which can reduce the damage by termites and also be environmental friendly. As an alternative to synthetic insecticides, several biodegradable botanicals had also be employed in pest management and had been proven to be reliable. Sequel to this, this experiment was focused on the adoption of botanicals in pest control and it had centered primarily on the control of termites using extracts of *Cymbopogon citratus* obtained through polar and non-polar solvents in a laboratory contact experiment.

Provide a factual background, clearly defined problem, proposed solution, a brief literature survey and the scope and justification of the work done.

2. Material and Methods

The materials used during this experiment were: Lemongrass roots and shoots, Ethanol, n-hexane, cutlass, spade, hand trowel, Petri-dishes, syringe, stop watch, jar bottle, cover bowl, rotary evaporator, foil paper, filter paper and knife and matured shoot and root of *Cymbopogon citratus* were collected from the demonstration plot of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The research project was conducted at the Department of Plant Science and Biotechnology Laboratory, Adekunle Ajasin University Akungba Akoko. During extract preparation, obtained plant parts were washed and 400g of the fresh shoots was chopped into match-stick sizes and stepped in 700ml of ethanol and n-hexane into a 3litres of rubber bucket with a closed lid. This was kept intact with a paper tape securely wrapped around the cover of the bucket to prevent evaporation. This set up lasted for 7days using ethanol and n-hexane as the solvents in each experimental setup and then mixed with electric shaker for 2minutes and repeated 3times daily. It was then filtered using vacuum filtration through the Buchner funnel. The content of this bucket was immediately poured to the rotary evaporator and the temperature set at the boiling point of each of the solvents under reduced pressure to obtain the extract from the fresh shoots. The extract was preserved in a covered conical flask in the laboratory at ambient temperature and used throughout the experiment. This was similarly repeated for the fresh roots.

2.1. Collection of Termites

Collection of termites was done early in the morning from the termite hill on the field within the premises of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. The termite samples were collected from their natural habitat (termitarium) and transferred to Mc Cartney bottle which contained part of their termitarium. This was kept on the cabinet in the laboratory at ambient temperature throughout the experiment.

2.2. In-vitro Experiments

There were two In-vitro experiments as stated below.

2.2.1. Control in-Vitro Experiment Involving the Use of Solvents

The mortality experiment was performed by applying at different time intervals of 10seconds, 20seconds, 30seconds, 40seconds, 50seconds and 60seconds, 5ml each of water, n-hexane and ethanol differently on 15 workers and 15 soldiers of termite in a petri dish and placed in a growth chamber at ambient temperature. The experiment had three replicates.

2.2.2. In-Vitro Experiments Involving the Use of Plant Extracts

These were performed by applying 5ml of all the extracts of shoot and root differently prepared with n-hexane and ethanol with a syringe on the termite caste which contained 15 workers and 15 soldiers in a petri dish and placed in a growth chamber at ambient temperature. The extracts were tested for mortality on the selected castes of termites containing 15 soldiers and 15 workers and the experiment had three replicates. 5ml of the extracts was applied to the termite castle at different time interval 10seconds, 20seconds, 30seconds, 40seconds, 50seconds and 60seconds. The results obtained on the mortality rate at the different time intervals were cumulated and means

separated using Tukey's honestly significant test analysis. This experiment had three replicates and a control containing the castes of termites but without any treatment application.

3. Results and Discussion

It was observed that the application of the different solvents had no effect on the termites from 10 to 60 seconds of treatment application. This is presented in Table 1.

Table-1. Cumulative means of mortality rate of termite caste under different applications of solvents

Solvent	Time of Application					
	10 SEC	20 SEC	30 SEC	40 SEC	50 SEC	60 SEC
water	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a
n-hexane	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a
Ethanol	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a	0.00±0.0^a

Note: *Means sharing the same superscript along columns are not significantly different at $P < 0.05$ using Tukey´s Honestly Significant test.

Conversely, after 10seconds of application of all the extracts, n-hexane root extract recorded the highest number of mortality. While the least mortality was observed in ethanol shoot extract and followed by ethanol root extract. There were significant differences among all the plant extracts used at 10seconds of treatment application.

After 20seconds of application of all the extracts, the least mortality was observed in ethanol shoot extract replicate while the highest mortality was observed in n-hexane root extract replicate. This was followed by n-hexane roots extract. There was no significant difference between ethanol shoots and ethanol plant extracts at 20seconds of treatment application.

After 30 seconds of application, the extract proved to be more effective than the preceding treatments as higher mortality rate was observed. N-hexane root extract recorded highest mortality rate and the lowest mortality in ethanol shoot extract.

At 30seconds of treatment application of all the extracts, there were significant differences among all the plant extracts.

After 40seconds of application, it was observed that n-hexane roots extract recorded the highest mortality rate which was followed by n-hexane shoot plant extracts. The least mortality rate was observed in ethanol shoots extract but there was significant difference among all the plant extracts used at 40seconds of treatment application.

Between 50seconds and 60seconds of treatment application, all the extracts had almost been completely eliminated except in ethanol shoot extract. The highest mortality was eventually observed in n-hexane root extract, in which complete mortality was observed within 50seconds. There was no significant difference between n-hexane roots and shoots extracts at 60seconds of treatment application. This observation is presented in Table 2.

Table-2. Cumulative mean of mortality rate of termite caste under different application regimes

Extract Type	Time of Application					
	10 SEC	10 SEC	10 SEC	10 SEC	10 SEC	10 SEC
n-hexane shoot	9.00±1.6^c	11.66±0.7^b	18.33±0.7^c	22.33±0.9^c	26.33±0.7^c	30.00±0.0^c
n-hexane root	10.33±0.2^c	13.66±0.9^c	20.33±0.9^d	25.33±0.5^d	28.00±1.2^d	30.00±0.0^c
Ethanol Shoot	4.00±1.2^a	7.66±0.1^a	11.33±0.6^a	14.66±0.9^a	15.33±0.9^a	22.33±0.1^a
Ethanol Root	5.66±0.1^b	7.00±0.7^a	13.66±1.5^b	16.66±1.2^b	22.33±0.9^b	24.33±0.9^b

Note: *Means sharing the same superscript along columns are not significantly different at $P < 0.05$ using Tukey´s Honestly Significant test.

Pesticides had earlier been reported to be useful in the increase of farm yield products and farmers had depended heavily on the synthetic pesticides to control insects in their crops, which till today remains one of the most commonly used method in controlling insects. The use of synthetic pesticides in agriculture comes with a cost for the environment and the health of animals and humans. Sequel to the attendant problems associated with the use of synthetic pesticides, the use of biodegradable botanicals are now preferred being that botanicals are readily available from local plants with little or no processing. Zhu, et al. [3] opined that they should have low toxicity to non-target organisms, especially humans and beneficial insects. Consequently, in the determination of the efficiency of extracts, the norm of non-contributory lethal tendencies of the solutions was also observed in the control experiments. This had convincingly proven that the solvents were exclusive of the lethal effects observed in the extracts in termite control. Similar views on the adoptive use of botanicals in pest management, were shared by Keital [4] who reported that pulses stored in gunny bags treated with aqueous extract from leaves of *Melia azadirachta*, *Hyptis suaveolens*, tuber of *Cyprus rotundus* and *Chrysopogon zizanioides* were effectively protected against crop pests. This use of botanicals unequivocally corroborates the use of *Chrysopogon zizanioides* in this research. In addition, Keital [4] reported that seeds treated with botanical oil extracts did not lose their viability and also established the powder made from essential oil at different basics provided complete protection against *M. beesoni* and did not show significant effect on the potent on seed germination rate. This is an assertion of the use of morphological parts plants as being not restricted to any specific part. This also substantiates the use of the shoot and root of *Cymbopogon citratus* and the observed efficacy in termite control. Although Kim [5] had proven the potential insecticidal activity of extract from *Cinnamomum cassia* bark and oil, horse radish oil, mustard (*Brassica juneea*) oil against *Callosobruchus chinensis*, within one day after eucalyptus seed powder treatment caused the death of emerging adult of C. *chinensis*. On the control of termites Rahman, et al. [6] had made a reported on the toxicological study on termites. This was in support of the use of plant materials as surface protectant against termite's population growth by reducing the seed damage rate. In a similar observation, Isman [7] had previously reported that volatile oil extract from *Azadirachta indica* at high concentration could effectively protect agricultural crops, forestry from *Microcerotermes beesoni*. These are contentions that corroborate the use of *Cymbopogon citratus* in termite control in this research. In the preparation of natural pesticides, different solvents could be used. Report from Moein and Farrag [8] had specified the use of n-hexane and ethanol, and had also drawn a comparison on

their adoptive uses. The report of 0.5% concentration of extract inducing 50% mortality that later dropped to 14.28% with ethanol is in agreement with the methodology adopted in this research on the use of the solvents and the subsequent reduction in efficacy with ethanol. The efficacy of lemon grass is consequently in accordance with the report of Zue, et al. [9] on the use of lemongrass plant extracts as anti-termitic and repellent ingredient against termites where also a better performance was obtained in comparison to a synthetic termicide. The use of lemongrass extract in this research has provided safer alternative against the use of chemical termicides because of its low toxicity to non-target organisms, especially humans and beneficial insects and as reported by Zue, et al. [9] and also in agreement with Ratnadass and Wink [2] that various extracts of the lemongrass have been reported to have insecticidal activities on vectors of medical or interests or non-agricultural pests which encompasses mosquitoes, mites, cockroaches, houseflies, termites and water snails and a host of human or cattle parasites. All the plant extracts recorded high mortality rate of termites based on the allocated time, Therefore, in corroboration of Cunningham, et al. [10] assertion, *Cymbopogon citratus* could be used effectively as a substitute for synthetic pesticides as its use is not accompanied with deleterious environmental and public health effects by consuming foods cultivated with agrochemicals, by exposure during application and by consumption of intoxicated species.

4. Conclusion

The results from this study affirms the adoptive use of C*ymbopogon citratus* extracts in the control of termites. Consequently, its choice of solvent for formulation has a reduced effect on its efficacy. Thus, the cultivation of *Cymbopogon citratus* could be promoted as well as its use as edges at homes and fields in order to control termites.

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