

Preliminary investigation into the repellency effect of leaf oil of *Cymbopogon citratus* (lemon grass)

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ABSTRACT

The use of chemicals either as food or drug preservatives or as pesticides in household and agricultural produce has been on the rise. These chemicals, which are mostly synthetic, have been implicated as contributors to global warming. Hence, safety concerns over their usage are provoking intense studies to find alternative application as bio-pesticides in disease and pest control and in agriculture. This study was aimed at investigating the repellent efficacy and suitability of *Cymbopogon citratus* (Lemongrass) for the development of natural product-based mosquito repellent. *Cymbopogon* oil (75ml) was extracted from 500g of fresh lemongrass leaves using Soxhlet extraction method with n-hexane as solvent and dried by rotary evaporator. Extract was serially diluted and observed for 180 seconds. Mosquitoes were harvested from their breeding sites and kept in various insect boxes for the experiment. Human subjects with eucalyptus oil applied on hands served as Positive Control while Negative Control had acetone applied on hands. The Test Control had *Cymbopogon* oil diluted in 10mls ethanol. The Positive Control was freed from mosquito bites while mosquitoes' landing was observed in the Negative Control. Results showed a 100% repellency activity for positive control; 60% for negative control; 75% for 1ml/mg; 85% for 2.5ml/mg; 90% for 5ml/mg and 90% for 7.5ml/mg of repellency were recorded after 180 seconds. Findings suggest that *Cymbopogon citratus* extracts could be used for the production of mosquito repellent insecticide that is free from synthetic chemical which are harmful to humans and non-environment friendly. However, further studies and qualitative analyses are necessary to determine suitable concentrations for human topical application.

Keywords: *Cymbopogon citratus*, mosquito repellent, *Cymbopogon* oil, lemongrass, global warming

INTRODUCTION

Most plants rich in phyto-chemicals are used to prevent attacks from phytophagus (plant eating) insects. Though the primary function of these chemicals is defense against phytophagus insects; they also are effective against mosquitoes [1]. Insects detect odours when such bind to their odorant receptor (OR) proteins [2]. These OR proteins are displayed on ciliated dendrites of specialized odour receptor neurons (ORNs) that are exposed to the insect's external environment located on their antennae and maxillary palps [3].

Most phytochemicals are deterrent or insect-

repellants because of their high vapour toxicity to insects [4]. Plant-based repellents are still extensively used traditional across most rural communities in the tropical region [5] of southeastern Nigeria, and serves as the only means of protection from mosquito bites for some of these communities [6].

Essential oils (EOs) of plants have been widely used for many years due to their antimicrobial properties in foods and pharmaceutical products [7]. These oils are natural products obtained from plants. A large number of essential oils extracted from different families have been shown to

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have high repellence against arthropod species [8]. Among the essential oil producing plants such as *Cymbopogon* spp, eucalyptus spp and *Ocimum* spp. have been widely studied. *Cymbopogon* plants have been traditionally used to repel mosquitoes in jungle regions such as the Bolivian Amazon [9]. *Cymbopogon* produces the most natural repellents in the world [10].

Cymbopogon citratus (lemongrass) essential leaf oil is obtained from the aerial parts of the plants. The plant has been widely recognized for its ethnobotanical and medicinal usefulness [11]. *Cymbopogon citratus* is widely distributed in tropical and subtropical regions of Africa, Asia and America. It is a plant of great interest due to its commercially valuable essential oils used in food technology and traditional medicine [12]. It is known to contain important photochemicals that possess insecticidal properties [13]. *Cymbopogon citratus* oil contains geranial (alpha-citral) and neral (beta-citral) as the two main active components with geraniol and citronellol (known as repellents) present in small amounts [14]. Lemongrass essential oil has repellent effect, alone or in combination, against different species of disease-transmitting mosquitoes and the housefly *Musca domestica* [15, 16, 17].

Study Area

The study was carried out in the Industrial Biotechnology Laboratory at the Enugu State University of Science and Technology, Agbani, Enugu State. The study area is abundant with the specie of *Cymbopogon citratus* lemongrass used for the analysis.

Procurement of Lemongrass

Cymbopogon citratus leaves were harvested and collected freshly from the surroundings of Enugu State University of Science and Technology. Leaves were identified by Prof. J. C. Okafor, a taxonomist with the Applied Biology and Biotechnology Department, Enugu State University of Science and Technology. Identified leaves were harvested whole and dried at room temperature for 4 days and later

Eze-Steven and Michael *citratus* (lemongrass) oil has been known as effective insect repellent; its effectiveness in mosquito repellence has also been documented [18] [19].

Statement of the Problem

Mosquito-transmitted illness like malaria is on the rise, especially in the tropics. Use of synthetic chemical-based compounds in repelling these vectors is contributing to global warming. Hence the search for natural bio-repellent against mosquito vectors to stop malaria transmission.

Leaves of *Cymbopogon citratus* have claims of medical, nutritional and mosquito repellent properties, among local inhabitants in southeast Nigeria. These claims have not been scientifically verified. This research aimed at investigating the mosquito repellent potential in *Cymbopogon citratus* leaves and its suitability as natural product based mosquito repellent.

Aim and Objective of the Study

This study is aimed at assessing the repellency effect of leaf oil of *Cymbopogon citratus*. The specific objectives are:

1. To examine the mosquito-repellent activity of leaf oil of *Cymbopogon citratus*.
2. To estimate how long this repellency can be effective.

MATERIALS AND METHODS

pulverized to course powder and stored in air-tight container.

***Cymbopogon citratus* preparation**

A 500g of the powdered leaves were extracted in a Soxhlet extractor using n-hexane as solvent. Extract was dried using a rotary evaporator. The oil recovered was used for the analysis.

Collection of mosquito specimen

Mosquitoes were harvested from their breeding sites using a sieve net and safely transferred into an insect box. This was repeated severally to obtain the desired number of mosquito samples.

Test for repellent activity of *Cymbopogon citratus* on mosquitoes in captivity

The laboratory experiment was conducted using an insect box with a surface area of

2756cm² and an open surface covered with net. The box had six chambers and twenty (20) freshly caught, live mosquitoes were carefully transferred into the chamber of the box. A 10mls of *Cymbopogon citratus* oil was diluted with 10mls of ethanol making it 20mls of stock solution. It was diluted serial into test tubes as 1ml, 2.5mls, 5mls and 7.5mls. The experiment was repeated six times using 1ml, 2.5mls, 5mls and 7.5mls which were rubbed on each of the arm. The

Eze-Steven and Michael controls were Eucalyptus oil, which served as the positive control and Acetone as negative control. Timed observations of the reaction of mosquitoes were recorded. The experiment was observed for 180 seconds. Results were recorded with respect to mosquito landing attempts/bites, number of bites and time interval between bites.

Ethical considerations

Ethical approval for the study was sought for all that participated in the research.

RESULTS

Table 1: Result showing Time-dependent mosquito repellency by *Cymbopogon citratus* leaf oil on mosquitoes in captivity.

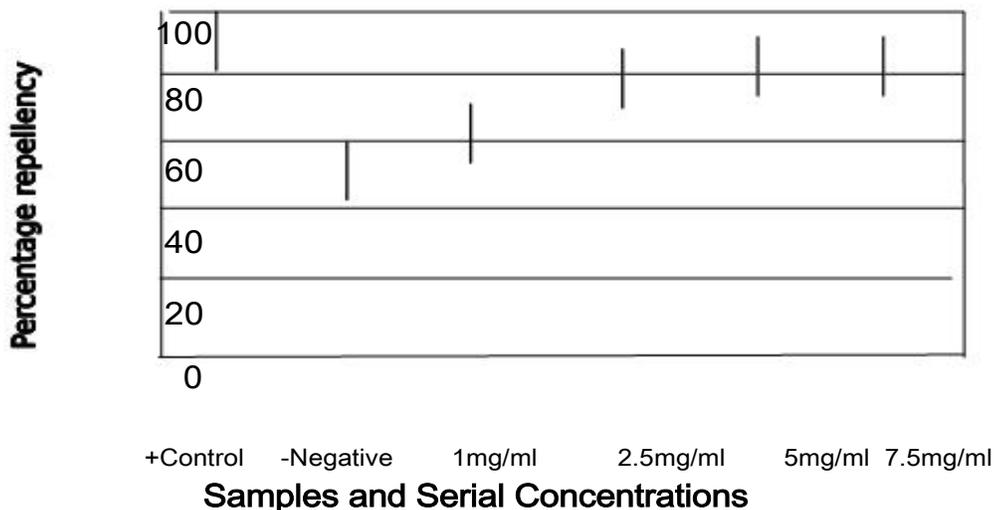
Quality of oil applied	Number of landing attempts/bites in time (seconds)				
	30	60	120	180	TOTAL
Positive Control	0	0	0	0	0
Negative Control	2	1	3	2	8
1mg/ml	0	2	1	2	5
2.5mg/ml	0	1	2	0	3
5mg/ml	0	1	0	1	2
7.5mg/ml	0	0	1	1	2
TOTAL	2	5	7	6	20

Table 2: Result showing the repellency effect of different concentrations of *Cymbopogon citratus* leaf oil on mosquitoes in captivity.

Extract Concentration (%) exposed	Number of mosquito landing	Number of mosquito repelled	Number of mosquito (%)	Percentage repellency	Percentage landing (%)
Positive Control	20	0	20	100	0
Negative Control	20	8	12	60	40
1mg/ml	20	5	15	75	25
2.5mg/ml	20	3	17	85	15
5mg/ml	20	2	18	90	10
7.5mg/ml	20	2	18	90	10

Figure 1: Bar chart showing relationship between percentage repellency and concentration of *Cymbopogon citratus* leaf oil with positive and negative control.

Concentration of *Cymbopogon citratus* leaf oil.



RESULT INTERPRETATION

The test experienced zero mosquito landing attempts and bites until 60 seconds, while the positive control as Eucalyptus oil within the period of the experiment, the oil repelled all the mosquitoes and within some hours after the experiment some mosquitoes died but using Acetone as negative control, mosquitoes landed showing that Acetone did not have repellent properties. The 1ml *Cymbopogon citratus* leaves oil had a bite at

60 seconds into the experiment, another 2.5ml at 60 seconds, and 5ml at 60 seconds while 7.5mls at 120 seconds from start of experiment. Of the 20 mosquitoes landing attempts/bites experienced during the study, 0(0%) were on the positive control, 8 (40%) were on the negative control, 5(25%) with 1ml of oil, 3(15%) with 2.5ml of oil, 2(10%) with 5ml of oil and 2(10%) with 7.5ml of oil.

DISCUSSION

The study with human arm provided a real case situation for confirmatory observations on the repellent activity of *Cymbopogon citratus* leaf oil against mosquitoes. Mosquitoes usually make surreptitious landings on exposed skin to feed; reason why the wearing of protective clothing stands as a good measure of preventing man-vector contact, which is a preliminary method of malaria prevention. Application of *Cymbopogon citratus* leaf oil prevented landing attempts and bites by mosquitoes and this verifies already documented evidence that *Cymbopogon citratus* extract is a potent mosquito repellent [20] [21]. However, the interval of protection is a matter of concern and calls for detailed analyses. In this study, oil from leaves of *Cymbopogon citratus* provided anti-mosquito protection for 60 seconds after which landing attempts and

bites were experienced. This observation would corroborate previous records of plants essential oils [22]; hence the duration of repellence might be undoubtedly connected with the concentration of oil. Using different volumes (1ml, 2.5ml, 5ml and 7.5ml) of the oil gave a suggestive result to consider that the intensity of oil could have relevance with its retention time on the skin and hence interval of protection. Only two bites were experienced with 7.5mls of oil and at 120 and 180 seconds of experiment, two bites were experienced with 5mls at 60 and 180 seconds of experiment. Three bites were experienced with 2.5mls at 60 and 120 seconds of experiment and five bites were experienced with 1ml at 60, 120 and 180 seconds. While the difference in volume is not equal to difference in concentration, it is assumed that at a higher level, the essential

oil would persist longer. Further quantitative studies would beam on the issue of interval of protection and provide useful guide for commercial production of *Cymbopogon citratus* leaf oil repellent for topical application against mosquito vectors. It would also provide reliable information for use of the extract in local mosquito prevention and control practices. Considering the high volatility, commercial formulation of plant-

The use of nature-based plant produce as insect repellent should be used in the fight against mosquitoes vectors in the transmission of malaria. This will reduce the

There is need for further research by scientists to establish the active components of oil of *Cymbopogon citratus* leaf oil responsible for this repellent activity. Also, following the finding from this study, it is imperative that

CONCLUSION

Eze-Steven and Michael based repellents from essential oils would have to use fixatives to maintain longevity of their action [23]. The study with live mosquitoes confirmed the insecticidal property of *Cymbopogon citratus* leaf oil [24] [25] [25]. Compared with chemical based pesticides [26] [27], *Cymbopogon citratus* leaf oil would serve as a very effective natural product based repellent and insecticide.

impact synthetic chemicals have on the global warming which further affect climatic and agricultural activities.

RECOMENDATION

agrochemical industries analyses the volatile oil of *Cymbopogon citratus* leaf oil and exploit its vast potential for use as a control measure against other disease vectors.

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