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Insecticidal efficacy of lemon grass (*Cymbopogon citratus*) and orange peel (*Citrus sinensis)* on mosquitoes

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ABSTRACT

Aim: Main purpose of the study was to determine insecticidal activities of essential oil extracts from orange peel (*Citrus sinensis)* and lemon grass (*Cymbopogon citratus*) against adult stage of the mosquito *Anopheles* and *Culex* spp..
Method and materials: The essential oil extracts were obtained from processed air-dried and powdered citrus fruit peels and lemon grass by soaking the extraction. The insecticidal activities of the extracts were tested at 1.5%, 3.0%, 4.5% concentrations following incubation periods of 30 min, 1, 6, 12 and 24 hours.
Results: The greatest insecticidal activity (65 and 60%) was exhibited at 4.5 % concentration with a 24-h exposure by *C. sinensis* and *C. citratus* respectively.
Conclusion: It was concluded that essential oil extracts from *C. sinensis* and *C. citratus* have the potential to be used as an eco-friendly pesticide to control the mosquito vector.

Keywords: Insecticides, Mosquitoes, *Citrus sinensis, Cymbopogon citratus,* Nigeria.

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Introduction

Mosquitoes can transmit more life-threatening diseases such as malaria, dengue, chikungunya, zika, west Nile, yellow fever, encephalitis and filariasis and make life at risk millions of people particularly in subtropical/tropical world (Ghosh *et al*. 2012; Curtis and Lines, 2000). To prevent occurrence of mosquito-borne diseases and promote public health, it is necessary to control their population. The use of organochlorine and organophosphorus insecticides is in first line of fighting against mosquitoes, but in recent years, due to high cost of these insecticides, their stability in environment, increase in resistance to insecticides, harmful effects on human health and other non-target populations, they have been used of them is limited (Russell *et al*. 2009). These reasons have prompted researchers to look for alternative methods, especially use of natural substances to fight and reduce the mosquito population, which is compatible with environment. One of most effective alternative methods is use of materials of plant origin, which is a simple and sustainable method for mosquito control. In addition, they are coordinated with behavioral and physiological processes and also create little resistance (Baran *et al*. 2020; Norouzi *et al*. 2021). More than 2000 plant species have been identified that have valuable metabolites in pesticide application. Members of plant family Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have been used against various mosquito species (Shaalan *et al*. 2005).

Cymbopogon citratus belongs to the Poaceae family. It is a tropical herb and one of the main medicinal and aromatic plants in Asia and Africa, as an antiseptic, antimicrobial, anti-inflammatory, antitussive, anti-rheumatic, to treat back pain, sprains and hemoptysis in some countries.African it is used to treat diabetes (Gaba *et al*. 2020; Kamaruddin *et al*. 2022).

Citrus sinensis and other citrus wastes cannot be used in landfills due to their low pH and high water and organic matter content, however, they have great potential as raw materials for

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fermentation and as a source of carbohydrate polymers and enzymes. There are various bioactive compounds with medicinal applications (Siddiqui *et al*. 2022).

In Nigeria inorganic mosquito repellent such as Raid, Mobil etc are not accepted in closed houses especially those having infants and young children due to their side effects and high cost, rather, people use smoke of some particular types of herbs such as leaves of Eucylyptus, lemon grass and orange peel in community to keep mosquitoes away. Therefore, this study was planned to evaluate insecticidal efficacy of orange peel (*C. sinensis)* and lemon grass (*C. citratus*) on mosquitoes.

Materials and Methods

The study was carried out in Usmanu Danfodiyo University, Sokoto at biological garden and also indoor of the female hostel of the University Area Sokoto State, Nigeria. The area lies at 11.30 to 13.3N and 4 to 6.50 and fall within the Sudan savannah ecological zone characterized by long dry season (October to May) and short rainy season of 300 to 650mm is experienced in the area and minimum and maximum temperature of 13^0c to 42^0c respectively. Relative humidity ranged from 14% during dry season (October to May) to 72%in rainy season (June to September), Usmanu Danfodiyo University permanent site (main campus) is located along Kware road, Sokoto.

Sample collection

Live Mosquitoes were collected from their natural breeding sites around the University campus and Biological Garden of Usmanu Danfodiyo University, Sokoto, using simple drag net for mosquitoes and were transported to the laboratory for identification. From collected groups of mosquitoes, *Anopheles* mosquitoes were separated based on features like- having long palps with white-bands and 3 dark and 2 white spots on wing vein, the maxillary palps and a heavy footstep. *Culex* mosquito were small, brown, with blunt abdomens and without band legs. Mosquito selected for this study were *Anopheles* spp.

Collection and preparation of plant materials

C. sinensis were obtained from fruits of orange, cut into small pieces, spread on clean white cardboard papers and air-dried at room temperature for 7 days. The dried peel was pounded using a mortar and pestle into powdered form. One hundred gram (100g) of the powder (each) was transferred into beaker separately and soaked in 500ml of water steered occasionally and left overnight. The extract was filtered using muslin cloth and served as stock solution. Three different concentrations (1.5%, 3.0% and 4.5%) were prepared using serial dilution procedure, from each extract. They were then spread on filter paper and the filter paper was placed inside the beaker. Ten mosquitoes were introduced in each beaker using a funnel from the captured stock and covered by a fine wire net. Control mosquitoes were kept in similar condition but filter paper was treated with distilled water only. Each set of experiment was replicated three times. The observations were recorded visually from outside at 30 minutes, 1, 6, 12 and 24 hrs intervals. No movement in mosquito's body was taken as evidence of death.

Results and Discussion

The results of the study showed that 1.5% concentration of *C. sinensis* extracts had 20% mortality within 30 min; while same concentration caused 40% mortality within 24 hrs exposure. The 4.5% concentration of the extract produced maximum mortality at all exposure periods. No mortality was recorded among control group. In all cases mortality was time and dose dependent (Table 1).

It was showed that 1.5% concentration of extract of *C. citrates* caused 5 % mortality in treated mosquitoes within 30 min. of exposure and 25% at 24 hrs exposure period; while 4.5% concentration of the extract produced 25% mortality within 30 min. and 60 % mortality was recorded within 24 hrs exposure. No mortality was recorded among control group (Table 2).

Mosquito-borne diseases are considered major threats to global health in developing and developed countries. Their tendency to spread outside their geographic range and cause large epidemics is clearly demonstrated (Traboulsi *et al*. 2002; Sowndarya *et al*. 2017). Although the use of chemical insecticides is very effective against mosquitoes, but due to development of resistance, it leads to a rebounding vectorial capacity (Li *et al*. 2007). In recent years, the use of insecticides containing natural substances, especially of plant origin, which are compatible with the environment, has been considered for vector control, because they are rich in bioactive chemicals, active against a limited number of species, including specific target insects and were biodegradable (Kamaraj *et al*. 2010). The mosquito detects the odor when the volatile odor of the plant is repelled.

Table 1: Mortality (%) among treated mosquitoes as affected by aqueous extract of *C. sinensis*

Concentrations	Exposure period				
	30mins	1hr	6hrs	12hrs	24hrs
1.5%	20.00	25.00	25.00	30.00	40.00
3.0%	25.00	30.00	45.00	45.00	50.00
4.5%	40.00	45.00	50.00	60.00	65.00
Control	0	0	0	0	0

Table 2: Mortality among treated mosquitoes as affected by aqueous extract of *C. citratus*

Concentrations	Exposure period				
	30mins	1hr	6hrs	12hrs	24hrs
1.5%	5.00	10.00	20.00	20.00	25.00
3.0%	10.00	25.00	30.00	45.00	45.00
4.5%	25.00	40.00	40.00	45.00	60.00
Control	0	0	0	0	0

The olfactory receptor protein, which is expressed on the ciliated dendrites of olfactory receptor neurons exposed to the external environment, is often displayed on the antennae and mandibular floor of mosquitoes, causing mosquito mortality. In each of the plants, the higher the escape rate, the higher the mortality, and the lower the escape, the lower the mortality.

The results of this study showed that both plants *C. citrates* and *C. citratus* possess insecticidal efficacy against tested mosquitoes, but *C. sinensis* caused more mortality than *C. citratus* extract. In both cases observed effect was time and dose dependent which can be due to the fact that active moieties present in these extract get accumulated with time or continuous exposure to the active moieties over prolonged period cause a cumulative effect in mosquito's body. The extract *C. citrates* was more potent at short exposure period which revealed that active moieties present in it are more volatile and thus absorbed rapidly while *C. citratus*showed persistent increase in mortality of mosquitoes which probably shows that the active moieties present in this extract had slow but continuous release.

Castillo *et al*. (2017) examined the pupicidal, adulticidal and repellent activities of essential oils from 7 other plants included *Cymbopogon citratus* and *Citrus sinensis* against *Aedes aegypti* under laboratory conditions. The greatest pupicidal activity was exhibited at 390 ppm with a 48-h exposure by *C. sinensis*. of exposure (Castillo*et al*. 2017). In our study the greatest insecticidalactivity (65 and 60%) was exhibited at 4.5 % concentration with a 24-h exposure by *C. sinensis* and *C. citratus* respectively. Of course, because the units of measurement and the type of extract are different in the fields, it is not possible to make a complete comparison.

Ezeonu *et al*. (2001) investigated the effect of extracts of two types of orange peel *Citrus sinensis* (sweet orange) and *Citrus aurantifolia* (lime) on mosquitoes and concluded that these two extracts have very low biological performance, but this fact should be c onsidered that a large amount of these peels are produced by orange juice industries.Extraction of skin oils is a suitable option to recover resources from waste. These facts show enough optimism for investment (Ezeonu *et al*. 2001). Dua *et al*. (2008) showed the effect of *Valeriana jatamansi* root extract on larvicidal and adulticidal activity against different mosquito species. The results of their investigation showed that the average lethal concentration of the extract against the larvae of *Anopheles stephensi*, *Anopheles culicifacies*, *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* was 68.1, 42.8, 51.2, 53.8 and 80 mg/liter respectively (Dua *et al*. 2008). Harve and Kamath (2004) tested the larvicidal activity of acetone and petroleum ether extracts of four plants *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafoetida*, *Trigonella foenum graceum* in combination against *Aedes aegypti* larvae in laboratory conditions. All plants showed potential synergistic activity, although they showed relatively weak larvicidal activity when tested individually (Harve and Kamath. 2004). Thekkevilayil *et al*. (2004) studied the in vitro bioassay of the essential oil extracted from *Ipomoea cairica* against the larvae of four mosquito vector species. The biometric test showed that the essential oil of the plant can cause 100% death in *Culex tritaeniorhynchus* larvae. It was found that the essential oil is very toxic to the larvae of *Culex tritaeniorhynchus* following mosquitoes *Aedesaegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Thekkevilayil *et al*., 2004).

Cavalcanti *et al.* (2004) showed that *Ocimum americanum* and *Ocimum gratissimum* have LC50 ppm of 67 and 60 ppm, respectively, and the essential oils of these two plants have potential to control *A. aegypti* (Cavalcanti *et al*., 2004). Vivekanandhan *et al*. (2022) investigated the ethyl acetate extract of *Metarhizium anisopliae* (mycelia) on larvae, pupae and adults of *Anopheles Stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* and concluded that in 24 hours after treatment *M. anisopliae* extract had significant toxicity on allIt had mosquito cheeks. The chemical compounds derived from *M. anisopliae* are effective on the target pests, are non-polluting, target specific and are an alternative chemical insecticide (Vivekanandhan *et al*., 2022). Inocente *et al*. (2019) tested the hypothesis that the extract of *Cinnamosma* spp. enriched with drimane sesquiterpenes, it is toxic to the *Aedes aegypti* mosquito. It showed that bark and root extracts, which contain higher abundances of drimane sesquiterpenes compared to leaves, were the most effective, and drimane sesquiterpenes provide valuable chemical substrates for the development of insecticides and repellents to control mosquito vectors (Inocente *et al*. 2019).The biological effectiveness of *Solanum xanthocarpum* leaf extract on the larvae and pupae of *Culex quinquefasciatus* was investigated in laboratory conditions. Therefore, the present research clearly showed that *B. thuringiensis* can act as a high potential in the mortality of mosquito larvae. It is an eco-friendly biopesticide for the control of mosquito vector management program (Kumar *et al*., 2012). Of course, the units of measurement, the type of extract, the type of plants, and the test stage were different in the various other studies.

Conclusion

It was concluded that *C. sinensis* and *C. citratus* possess insecticidal efficacy against tested mosquitoes, but *C. sinensis* caused more mortality than *C. citratus* extract. In both cases observed effect was time and dose dependent as waste of these plants is abundant in the industry, the insecticidal properties of the essential oils of these two plants should be evaluated by identifying the active components causing mortality and then using them in field experiments to evaluate their potential as an alternative to chemical larvicides.

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