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Effects of Selected Leaf Extracts and their Repellency Activities against *Aedes Aegypti* (Mosquito)

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Abstract

Mosquito repellents play a crucial role in public health by protecting individuals from the bites of disease-carrying mosquitoes. This research aims to evaluate the repellent efficacy of selected plants; Azadirachta indica, Eucalyptus citriodora and Allium sativum, against Aedes mosquitoes. The procedure describe by were adopted to assess Repellency bioassay this involves the use of four volunteer arms for test concentration at 2.0, 3.0 and 4.0 mg/L with control. Both the control arms and treated arms were introduced simultaneously into the experimental cages. Results shows at 20ml serial dilution Azadirachta indica, Eucalyptus citriodora, and Allium sativum showed a very high rate of repellency to the mosquitoes, while at 30ml Azadirachta indica and Eucalyptus citriodora were able to repel mosquitoes with lower Repellency rate shown for Allium sativum. Also at 40ml Azadirachta indica exhibited high level of repellency when compare with control with CPT (10, 20, 30, 40 sec) respectively. There is significant different between extracts and mosquito species repelled at P>0.05. Thus, it can be concluded that repellency of mosquitocidal agent is effective against female Aedes mosquitoes having recorded complete protection time.

Keywords: Aza*dirachta indica, Allium sativum* and *Eucalyptus citriodora* Repellency, *Aedes sp.*

INTRODUCTION

Mosquito repellents play a crucial role in public health by protecting individuals from the bites of disease-carrying mosquitoes. These insects are vectors for various diseases, including malaria, dengue fever, Zika virus, and West Nile virus, posing significant health risks to populations worldwide (Harbach, 2018). The weaknesses of these tools, especially those with chemicals, are the high cost of production, and their negative effects on other beneficial. Repellency, in the context of mosquito control, refers to the use of substances that repel mosquitoes, preventing them from biting and potentially transmitting diseases. Mosquitoes, particularly *Aedes aegypti*, are major vectors of diseases like dengue fever, chikungunya, and

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Zika virus (WHO, 2020). Their bites not only cause discomfort but also pose a significant public health threat. Chemical repellents like DEET are effective, but concerns exist regarding their safety and potential environmental impact (Fradin *et al.*, 2002). This highlights the need for exploring alternative, plant-based repellents. The rich plant biodiversity of Nigeria, with an estimated 7,895 species, including 128 endemics Osawaru *et al*., (2013) holds immense potential for the development of eco-friendly insect repellent solutions. Embracing this natural resource can not only provide alternative pest control methods but also foster sustainable practices and economic opportunities within the country. The relevance as allied to human health can be harnessed in the production on repellents which are eco-friendly (Iqbal *et al.,* 2021). The repellent effect is attributed to the complex blend of bioactive compounds present in these oils, which can mask human odors attractive to mosquitoes or directly deter them (Molecules, 2020). Studies from The Malaria journal (2009), have identified promising repellent properties in plants like citronella, lemongrass, neem, and clove.

Resistance to insecticides can be both physiological and behavioural with most studies focusing on the former. In this thesis, the work is contributing to knowledge of behavioural resistance by developing and validating new assays to quantify the effects of this phenomenon in response to insecticide treated nets. The disease is most prevalent in tropical and subtropical regions because of rainfall, consistent high temperatures and high humidity (Jamieson, 2006). Sub-Saharan Africa malaria mosquitoes with be the focus of this report as this is where the largest number of malaria cases and deaths occur. Many researches are involved in developing innovative vector control approaches along with conventional vector mass.

However, these approaches need laboratory evaluation and artificial rearing of vectors in mass scale. In order to achieve this, it is essential to provide necessary conditions for them to grow, rather in an anthropogenic environment. Many epidemiologically important issues revolve around the feeding behavior of mosquitoes vectors (Gunathilaka *et al*., 2016). Selection of a suitable blood meal source and cost effective technique are the main challenges in an insectary. Membrane blood-feeding system as an artificial feeder to blood-feed *Aedes* mosquitoes is unique and suitable for multiple purposes. It is important to develop an inexpensive, convenient, and effective artificial membrane blood-feeding technique that takes animal welfare into consideration. Artificial feeding systems that have been developed differ in the composition of the meal, the nature of the membrane, and the method of temperature regulation. Several types of liquid diets including goose, chicken, bovine, pig, mice, rat, gerbil, hamster, rabbit, non-human primate blood types, and artificial diets are being used for the purpose of blood feeding mosquitoes (Yi-PeyLuo, 2014).

According to the available literature, *Aede s*prefers mammalian hosts (Turell *et al.,* 2005) and may preferentially feed on humans, even in the presence of alternative hosts (Saifur *et al*., 2012) they also feed multiple times during one *gonotrophic* (Scott, 2013) which has implications for disease transmission. The present study indicated that the blood meal source affects feeding rates and reproduction in colonized *Aedes aegypti* under laboratory conditions. Mosquitoes are nearly ubiquitous and inhabit most regions except Antarctica. They exist in regions more than five thousand metres above sea level and almost one thousand three hundred metres below sea level. Mosquitoes belong to family *Culicidae* that has about 3500 species belonging to 41 genera (Service, 1986). Only about 100 mosquito species have been implicated as intermediate hosts of vertebrate parasites since 1878 (Foster and Walker, 2002). Being important vector of several diseases, they pose serious threat to human and veterinary welfare more than any other insect group (Guzman *et al*., 2010). The common fear for

mosquitoes is their role as vectors that can spread diseases such as dengue, malaria, filariasis, yellow fever and Japanese encephalitis (Ramkumar *et al.*, 2014). The most common is the dengue fever virus which is transmitted to humans by the infected females of the family "Culicidae" i.e. *Aedes aegypti* Linn. and *Ae. albopictus* Skuse, (Koenraadt *et al.*, 2006).

Mosquito borne diseases are having economic impacts in form of health and commercial losses specifically in countries within the tropical and subtropical regions (Panneerselvam & Murugan, 2013). More than 100 countries are reported to be endemic to mosquito borne diseases and around two million people die because of these diseases every year (Subramaniam *et al.*, 2011). Four countries particularly Malaysia, Philippines, Vietnam and Cambodia are confronting annual epidemics which are more than 90 % of the total reported dengue cases of the region (Chang *et al.*, 2011). Dengue being endemic in some part of Africa, spread irrespective of the urbanization level (Saifur Rahman, 2012). The transmission of dengue fever is mainly caused by two species of *Aedes* mosquito; *Ae. aegypti* and *Ae. albopictus* (Smith, 1956). *Aedes albopictus* is a semi-domestic mosquito while *Ae. aegypti* is a domestic mosquito in 2 urban areas (Chan *et al.*, 1971). *Aedes aegypti* is anthropic in nature (Huber *et al.*, 2008) and like to rest and feed inside the houses and human dwellings (Ponlawat & Harrington, 2005). Most of these dengue vectors species rear in both natural and artificial containers such as gutters, pools, septic tanks, tree holds, leaf axils, fruit peels, discarded and unused tires, water jars, old boats and others (Aigbodion & Anyiwe, 2005; Cadena, 2013).

MATERIALS AND METHODS

Study Area:

The study was carried out in the Department of Biological sciences Laboratory, Faculty of Life Science, Bayero University, Kano

Collection of Mosquitoes Species

The larva of the mosquitoes were collected from a stagnant water at Biological Science department, and were identified by an insect taxonomist in the Department of Biological Science, Bayero University, Kano.

Mosquito Rearing Protocol

Healthy emerged adult females and males *Aedes sp* were reared and remain inside the insectary for at least 5days for mating to take place. Adults were fed with 10% sucrose before were fed with blood meal after 3-5 days (Des *et al.*, 2007). After each blood meal feeding exercises, successfully fed mosquitoes were engorge with red colorations abdomen (Clements, 1992) and lay eggs immediately overnight. Beaker was placed inside the cage containing water and a piece of filter paper for oviposition. Eggs were laid on filter paper over night. Filter paper containing eggs were placed in a plastic tray with 300ml of distilled water and allowed to hatch into larva (Des *et al.*, 2007). Developing larvae were fed with pinch yeast every day and the use of clean water is also important for refreshing the environment after every feed. Separation pupa from larvae was done daily and placed into a plastic bowl for adult to emerge after 2-3days, inside the insectary (Edillot *et al.*, 2007). Colonies would be maintained and all experiments were carried out at a constant temperature of 25 ± 2^{o}C and 80 ± 10% relative humidity (Clements, 1992).

Collection of plant materials

The leaves of *Azadirachta indica* and *Eucalyptus citriodora* were collected from trees in Bayero University, Kano Gwale L.G.A., which the coordinates being 11.981574°N, 8.479527°E. while *Allium sativum* was bought from Yankura market, Sabon Gari, Fagge L.G.A.

Identification of Plant Materials.

The plant materials were authenticated by plant taxonomist in the department of plant biology, Bayero University, Kano.

Processing of Plant Materials.

The leaves of these plants were allowed to dry under humid condition for 2weeks and crushed to powder using a grinder. The powdered plant materials were measured at ratio of 1:10 of the diluents. 100g was measured out using a weight scale and poured into a clean bottle in a distilled water of 1 litre and allowed to sit for 3 days, with subsequent shaking. The materials were then filtered with cheesecloth to separate the liquid extracts from the plants residue.

Maceration Extraction Method

Maceration of different plant extracts was conducted following the procedure of Frank *et al.,* 2020 where by healthy leaves were washed in tap water, cut into small pieces and air dried. After the leaves were completely dry, they were ground into powder. 1g of powder was diluted into 100mL of different solvent and then macerated at room temperature for 3 days and then filtered (Frank *et al.,* 2020). The combined filter was concentrated to dryness by rotary evaporation at 50^0c and was kept in a freezer. The prepared test concentration of each leaf extract was volumetrically diluted.

Serial Dilution of Stock Solution

The techniques was described by CDC (2022). Three clean dried containers were labelled with volumes of the diluents being 20ml, 30ml and 40ml respectively for each of the plant extracts. Using a graduated cylinder the MLS of the diluent distilled water was added to the labelled containers. 1 ml of the stock solution was added to labelled 20ml of the diluent, the solution was mixed thoroughly by swirling gently, after which 1 ml was transferred to the labelled 30ml diluent, which was then gently agitated and 1ml was transferred to the labelled 40ml of the diluent and mixed well. This was done for each plant stock solution.

Repellency Bioassay

Using Repellency test bioassay, the process describes by Marimuthu,(2011). A Cage testing repellency involving four volunteer arms was used for each test concentration. For each test concentration volunteer's skin were washed with unscented soap and serial dilute concentration of plant extracts (20, 30 and 40 mg/mL) was applied from elbow to the wrist covering fingers with gloves. The test was carried out in a dark room to make the female mosquitoes active. The control arms (washed with distilled water) and treated arms (rub with crude extracts) were introduced simultaneously into the experimental cages. A 30seconds, 40seconds and 60seconds landing counts were observed respectively for the different plant extracts concentrations, at 20ml, 30 ml and 40ml respectively. The mosquitoes that land or probe on the arm and the time of landing was recorded.

Percentage Determination of Repellency.

Percentage repellency was calculated using the formula (WHO, 2009):

$$\% \text{ Repellency} = \frac{\text{No. of mosquitoes landed on control arm group} - \text{No. of mosquitoe landed}}{\text{Number of mosquitoes tested}}$$

Statistical Analysis

All data analyzed were compute using SPSS (Microsoft Co.) Standard deviation was calculated based on the mean values of the experiments to compare between means treated and untreated arm tested on repellency with a control groups at $p<0.05$ by t-test. Comparison between means of treatment and control groups were use performed analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The results of the repellent activity of *Azadirachta indica, Eucalyptus citriodora and Allium sativum* leaf extracts at three different concentrations of 20, 30, and 40ml (Table 1). Concentrations of plant extract used exhibited repellency activity against female *Aedes Sp* which indicates that the *Azadirachta indica, Eucalyptus citriodora and Allium sativum* was effective at CPT 10.0%, 13.3% and 20.0% (32 sec) exhibiting high repellency action against the mosquito tested compared to control (Table 1). Similarly, the result showed percentage repellency of *Allium sativum* as well as *Eucalyptus citriodora* with high repellency action (figure 1).

However, repellent activity of *Azadirachta indica* against female *Aedes Sp* mosquitoes tested (figure 1) with low repellency actions when compared to control respectively. There is significant different tested between extracts and species repelled at $P>0.05$ tested (ANOVA). Previous study on the concentrations of plant extract used exhibited repellency activity against female *Aedes* mosquitoes which indicates that the methanol extraction of *Combretum micratum* was effective in exhibiting a repellency action against the mosquito tested compared to control.

The result shows that *C. micratum* had repellent activity against the female Anopheles mosquitoes tested. With strong repellency actions on volunteers A, B, and C with complete protection time (CPT) (341.0 sec, 307.0 sec and 210.0 sec) when compared to control with different concentrations 0.2, 0.3 and 0.4mL respectively (Aminu *et al,* 2019). Result of adult female *An. gambiae* mortality with effects of *X. Americana, C. micrathum* and *A. citrodora* leaf extracts were recorded subsequently to 24 h, 48 h and 72 h of exposure (Aminu et al, 2019). Ethanol extracts of *X. Americana* is measured as active mortality at 48h and 72h, as LC_{50} is greater than 750 mL, (26±1.0) and (73±1.2), while *C. micrathum* extracts at 72h (77±0.1) with high mortality effective as LC_{50} range from 200-750 mL. *A. citrodora* leaf extracts mortality was high at 30.0ml with 48h (62±1.3) and (22±1.0) respectively. Methanol leaf extracts of *X. Americana* also display significant effect on adult mortality with a mean of (77±1.2) and (55±5.2) respectively, as LC_{50} range from 100-200 mL (Aminu et al, 2019).

The extracts of *C. micrathum* and *A. citrodora* were not highly effective after 72h of exposure, with no mortality recorded at 28h, 48h and 72h as compare to control. There is significant difference between plant extract and solvent at $P>0.05$ when expose to time for mortality rate (Aminu et al, 2019). Ethyl-acetate extracts of *X. Americana* at 48h and 72h of exposure showed significant effects on adult mortality with (68±1.0) and (81±1.3). *C. micrathum* extracts at 72h (82±1.6) with LC_{50} of 200 mL against *Aedes* mosquitoes, as LC_{50} of 300mL compare to control (Aminu et al, 2019). Also at 48h mean (83±1.0) mortality was significantly high as well as *A. citrodora* recorded mortality rate at 24h (66±0.0), 48h (70±0.4) and 72h (85±0.1) respectively. These findings have reemphasized the need to explore the possibility of using herbal-based repellents as supplementary and complimentary measures for malaria control. This will reduce the chemical burden on the environment.

Table 1: Repellency effects of different leaf extracts against adult *Aedes* mosquitoes

Volunteers	Plant tasted	Conc.(mL)	Mean No. ± SD (treated arm)	Mean No. ±SD(control arm)	CPT (Sec)
A					
	Azadirachta indica	20.0	0.00±0.00	3.00±1.00	10.0
		30.0	0.00±0.00	5.00±1.66	13.3
		40.0	1.00±0.01	4.00±1.33	20.0
B	*Eucalyptus citriodora*	20.0	0.00±0.00	0.00±0.00	10.0
		30.0	0.00±0.00	7.00±2.33	13.3
		40.0	0.00±0.00	1.00±0.00	20.0
C	*Allium sativum*	20.0	0.00±0.00	4.00±1.33	10.0
		30.0	1.00±0.00	7.00±2.33	13.3
		40.0	0.00±0.00	6.00±2.00	20.0

CPT= Complete protection time; SD ±mean no of mosquitoes landed on both treated arm and control; There is significant different tested between extracts and species repelled at P>0.05 tested (ANOVA).

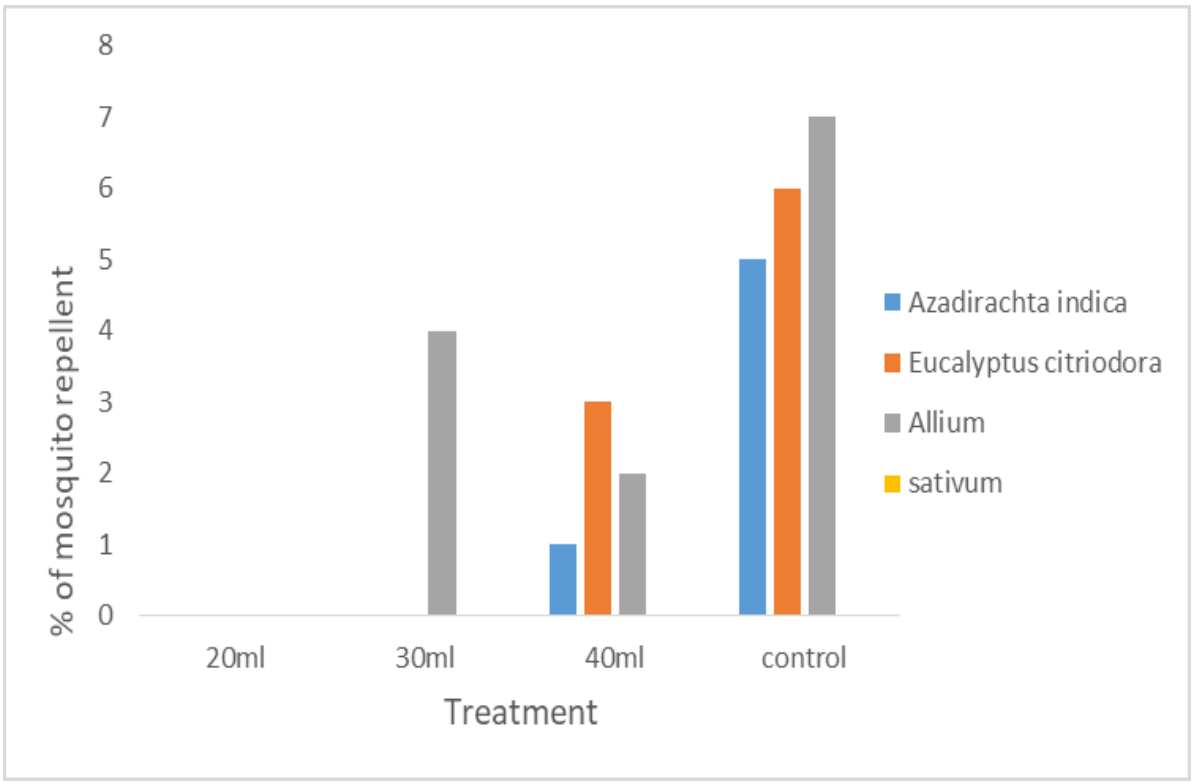

Figure.1: Showing percentage repellents of *Aedes* treated with different plant leaf extracts

CONCLUSION

Repellent activity of *Azadirachta indica, Eucalyptus citriodora* and *Allium sativum* extract of different concentrations exhibited repellency activity against female *Aedes sp* which indicates that the *Eucalyptus citriodora* and *Allium sativum* was effective exhibiting high of CPT (10 sec) with low repellency against female *Aedes* mosquitoes (20.0 sec) when compared to control respectively. There is significant different between extracts and mosquito species repelled at P>0.05. Due to the results obtained in this study, it can be concluded that based on the repellent effect that employed as effective mosquitocidal agent against female *Aedes*

mosquitoes having recorded complete protection time (CPT) 10 sec, 13.3 sec and 20 sec among three volunteers (A, B, C) in this study.

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