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The potency evaluation of the essential oil gotten from the plant, *Tagetes erecta,* against *Culex quinquefasciatus* and *Anopheles gambiae*

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Abstract
In recent times, filariasis and malaria which are vectored by *Culex* and *Anopheles* species respectively, remain a scourge and public health menace in Nigeria, in spite of concerted effort by health sector to reduce disease burden through treatment, campaigns, and vector control. Recently, research has shifted towards the development of insecticide against mosquito vectors from bioactive compounds of plant origin. This study aimed at determining the potency of the extracts, essential oil, gotten from *Tagetes erecta* leaves on two mosquito species, *Culex quinquefasciatus* and *Anopheles gambiae.* Using WHO test procedures, the mosquitocidal activities of *Tagetes erecta* were assessed. The samples that were observed to be susceptible to the essential oil with complete mortality of 100%, were exposed to different concentrations, including 62.5 µl/ml, 250 µl/ml, and 125 µl/ml, while samples exposed to 15.63 µl/ml and 31.25 µl/ml concentrations, reported resistance to the essential oil, after a period of 60 minutes. However, the difference in variation of susceptibility and resistance between these two species of mosquitoes was statistically insignificant (P= 0.8792; two-tailed t = 3.182, df = 3). The mosquitocidal potency was gotten for both species LC_{50} values with to be 10.26 µl/ml for *An. gambiae* and 10.42 µl/ml for *Cx. quinquefasciatus*, with the LC_{95} values as 31.73 µl/ml for both *An. gambie* and *Cx. quinquefasciatus* mosquito species. It was therefore concluded that the essential oil gotten from *Tagetes erecta*, showed marginally lesser activity on *Culex quinquefasciatus* compared to *Anopheles gambiae.*

Summary Statement
This study presents an alternative to synthetic insecticides through *Tagetes erecta* essential oil as an eco-friendly insecticide, offering valuable data on mosquito mortality and resistance for sustainable vector control strategies.

Keywords: Potency evaluation, essential oil, *Tagetes erecta, Culex quinquefasciatus, Anopheles gambiae.*

Introduction
Mosquitoes (*Diptera*) are insects that act as vectors for the transmission of infectious diseases and pathogens to both human beings and other mammals. They are said to be found almost in the whole world with exception of Antarctica and few Islands (World Health Organization, 2020) [24]. Mosquitoes are generally considered as nuisance, as their bite and noise around the ear while one is sleeping is annoying, and causes sleeplessness and restlessness. Mosquitoes are economically important as they reduce values from real estate and affect tourism adversely (CDC, 2020) [4]. Mosquito vector species of public health importance belong to the genera: *Hacmogogus, Sabethes, Anopheles, Culex, Aedes, Psorophora* and *Mansonia* (WHO, 2020) [24]. Over the years, malaria and filariasis which are vectored by *Anopheles* and *Culex* species respectively, remain a scourge and public health menace in Nigeria and other countries in Tropical Africa especially, in spite of concerted effort by health sector to reduce disease burden through treatment, campaigns, vector control. Other diseases of public importance transmitted by mosquitoes include Dengue fever, Zika fever, Rift valley fever, encephantitis, filariasis chukungunya and yellow fever (WHO, 2015) [23]. Although, malaria and filariasis can be treated and prevented, it has been a difficult battle for the total eradication of the disease.

Different prevention and control measure have been employed specially to destroy the insect vector. The measures include indoor Residual spray the use of Insecticide, Treated Nets (ITNs), elimination of breeding sites such as emptying and destruction of open cans, tins, pots and old tyres, clearing of gutter, clearing of bushes and wearing of protective wears during farming and outdoor night activities (Afolabi *et al.*, 2016) [1]. Use of drugs (chemoprophylaxis) was and is still one of the methods to prevent malaria and filariasis but the continuous development of resistance by the parasite is a challenge (WHO, 2022) [25]. Insecticide Treatment Nets were developed for malaria control and its vector but was not well adopted by the public (Ikpeida *et al.*, 2024) [9], due to the reason that some people are allergic to it, others stated that it was inconveniencing as it generates heat while some others have the fear that it can suffocate infants below 5 years of age (Nnanna and Ukpai, 2019) [15]. Due to susceptibility status of mosquito to insecticides, researches have shifted towards the development of insecticide against mosquito vectors from bioactive compounds of plant origin (Essien *et al.*, 2024) [7]. Reports show that essential oils from local plants and extracts have high efficacy against vectors of malaria parasites (Ejeta, 2019) [5]. Several studies have revealed the efficacy of some crude plant extracts, purified fractions and essential oil against mosquito vector species. Some of the examples includes the potential of *Tagetes erecta* leaf extract, where it was shown to be effective when used against mosquito species such as *Culex quinquefiasciatus* and *Aedes aegypti*, (Tri *et al.*, 2018) [22]. Similarly, Ramkumar *et al.* (2016) [18] confirmed the high efficacy of the leaf extract gotten from *Glycomus pentaphylla* against test specimens, namely *Anopheles stephens*, *Culex quinquefasciatus*, and *Aedes aegypti*. The efficacy of insecticides of plant origin against mosquitoes was also reported by Naghmouch *et al.* (2020) [14]. There is the need to explore some natural products of plant origin on these mosquito species. Natural products are eco-friendly, biodegradable and hence safer for human and environmental health. The present study therefore aims to determine the essential oil potency, that is obtained from *Tagetes erecta* leaves on two mosquito species, *Culex quinquefasciatus* and *Anopheles gambiae.*

Methodology

2.1 The Procedure of Collecting and Identifying the Plant Materials

The matured leaves of the plant species, *Tagetes erecta* were harvested at Trademore Housing Estate, Ubani, Umuahia North L.G.A, Abia State, in May, 2022. Identification and authentication of the plant specimens were conducted by Prof M. E. Bassey, a taxonomist at Botany and Ecological Studies Department of the University of Uyo (UUH 4095).

2.2 Sample Preparation and Oil Distillation

The fresh leaves were washed, sliced, weighed (1 kg) and introduced into a bottom flask with a 5 L round shape. An apparatus of the Clevenger-type, using the hydro-distillation method, extracted the essential oil for 4 hours. The process of distilling the extract was repeated with fresh sample four times to maximize the yield. The essential oils obtained were pooled together, measured and dried using sodium sulphate pellets. The oil was evaluated for percentage yield and stored in the refrigerator at 4 °C.

2.3 Test Mosquitoes

The test mosquitoes (*An. gambiae* and *C. quinquefasciatus*) used for this research were obtained from the Department of Insectary of Animal and Environmental Biology and the Malaria Vector Control Laboratory, in the Faculty of Science, University of Uyo (Uni-Uyo). Bioassays were further conducted on the specimens.

2.4 Essential Oil Susceptibility Bioassay

The bioassay for the determination of the susceptibility of *An. gambiae* and *C. quinquefasciatus* to essential oil of *T. erecta* was carried out according to the standard protocol that is outlined in the CONUS Manual for Evaluating Insecticide Resistance in Mosquitoes using the WHO tube Bioassay Kit (https://www.cdc.gov/zika/vector/insecticide-resistance.html., 2021). The use of specially designed WHO susceptibility tubes was involved in the process.

2.5 Preparation of Mosquitocides Solution for impregnated paper

Fifty millilitres (40 ml) of 20% tween 80 was measured using a pipette and transferred into a 50ml conical tube where the prepared essential oil of *Tagetes erecta* was diluted. The mixture was shaken vigorously, several times, after which the mixture inside the vial was transferred back to the conical tube. Five (5) concentrations of the oil were prepared, including 15.62 µl/ml, 31.05 µl/ml, 62.5 µl/ml, 125 µl/ml and 250 µl/ml. The conical tube containing the insecticide solution was thereafter labelled with the concentration of the insecticide. The test concentrations of the plant extract were repeated for four times. The marking of tubes used was done in a manner to ensure that the tube was associated with their respective concentrations. Two controls were set up for the experiments reported in the study. One control set up consisted of 40µg pirimiphos-methyl and another consisted of 20% tween 80 (1m). Each control set up was also replicated (4 times). The test filter papers were then separately impregnated with the test concentrations. The coated/impregnated filter papers were allowed to air dry, prior to use

2.5.1 Bioassay Procedure

Two to five days old mosquitoes, namely *Culex quinquefasciatus* and *Anopheles gambiae* were introduced separately into the WHO susceptibility test tubes. Each test and control experiments consisted of 25 adult mosquitoes of the two specimens. From the population in the cage, the mosquito species were aspirated and blown gently into the WHO susceptibility test tubes. The timer was started and susceptibility was determined by the number of dead mosquitoes. After 60 minutes, the number of mosquitoes that were alive and/or dead were recorded (CDC, 2010) [3]. Dead mosquitoes were assessed from their inability to fly or stand in a coordinated manner. The knock down at the diagnostic time (DT) for each insecticide (60 mins) was used as an indicator of the population susceptibility. According to Brogdon and Chan (2010), full susceptibility at the diagnostic time (DT) is indicated for mortality rates between 98 % -100 %; whereas, at mortality rates between 90%- 97% at DT, possible resistance was indicated, which

can be confirmed. A population resistant to the tested was therefore indicated at the mortality rates of <90%.

2.6 Statistical Analysis
Data obtained were pooled and analysed electronically using Microsoft Excel. Analysis involved determination of percentage mortality, computation of frequency tables. Frequency distributions were generated for all categories of variables. Level of significance was tested at 0.05 critical level. The mean mortality rate of the mosquito species was further computed and probit analysis was used to calculate the LC_{50} & LC_{95} (at 50% and 95% lethal concentrations). Paired sample t-test was also computed to establish differences in the values of mortality between *Culex quinquefasciatus* and *Anopheles gambiae* larvae.

3. Results
The potency of essential oil extracts from *Tagetes erecta* leaf, done by hydro-distillation against *An. gambiae* and *Cx. quinquefasciatus* are presented in the Figures 1 and 2. The rates of the mortality across both species were directly proportional to the concentrations of the extract used.

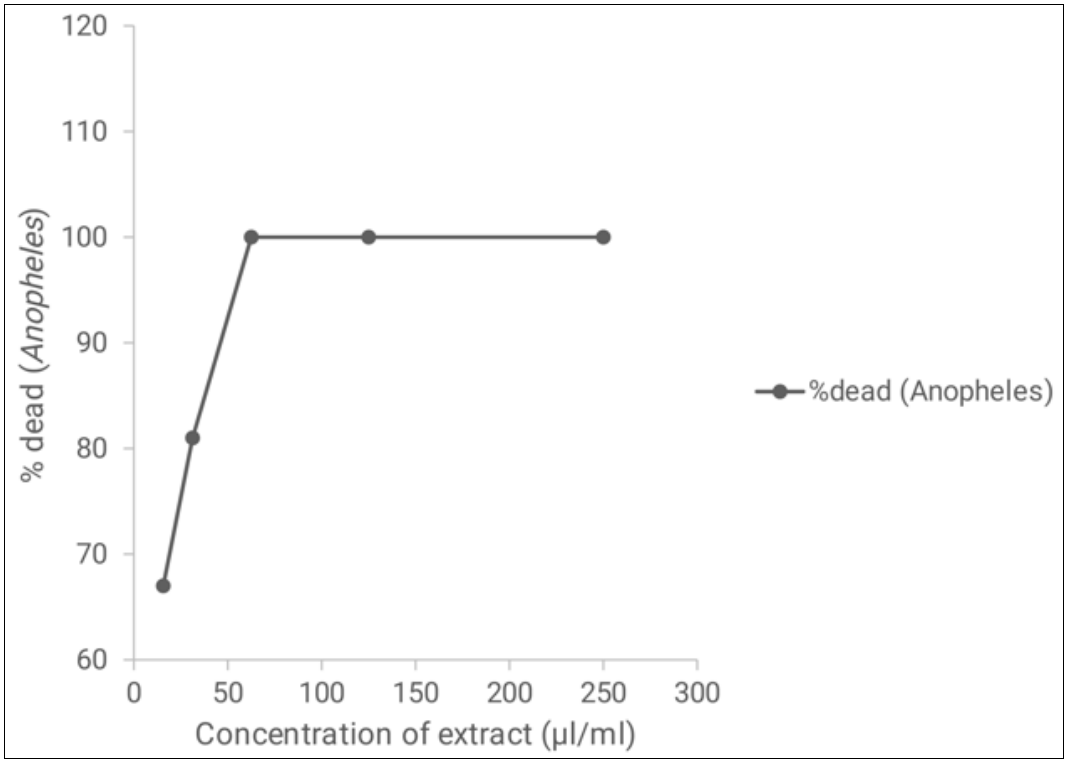

Fig 1: The percentage of the mortality rates of *An. gambiae* against concentrations of essential oil extracts from *Tagetes erecta*.

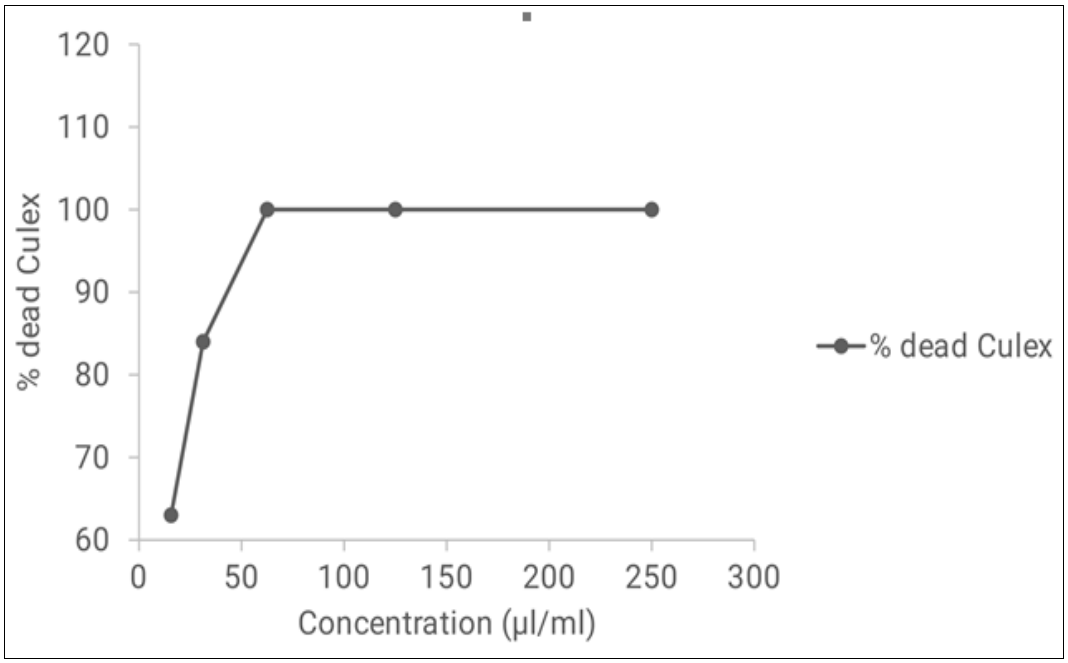

Fig 2: The percentage of the mortality rates of *Cx. quinquefasciatus* against concentrations of essential oil extracts from *Tagetes erecta*.

Table 1: The percentage of the mortality rates of *An. gambiae* and *Cx. quinquefasciatus* against concentrations of the essential oil extracted from *Tagetes erecta*.

Concentrations (µl/ml)	*An. gambiae* (% mortality)	*Cx. Quinquefasciatus* (% mortality)	P -value
250	100.0	100.0	0.8729
125	100.0	100.0	
62.5	100.0	100.0	
31.25	81.0	84.0	
15.63	67.0	63.0	
Control	0.0	0.0	

two-tailed t = 3.182, df = 3 = insignificant at 95% CI; paired sample t–test.

There was an insignificant interaction observed (p = 0.8729), with insignificantly lower mortality in *Cx. quinquefasciatus* compared to *An. gambiae*. Two-tailed t = 3.182, df = 3.

Table 1 indicated the results of the percentage of the mortality rates of *An. gambiae* and *Cx. quinquefasciatus* against concentrations of essential oil extracts from *Tagetes erecta*.

Samples exposed to different concentrations (62.5 µl/ml, 250 µl/ml, and 125 µl/ml) showed susceptibility to essential oil extracts with complete adult mortality of 100%, while samples exposed to other concentrations (15.63 µl/ml and 31.25 µl/ml) reported resistance to the plant extract used in the study. The diagnostic time was 60 minutes. However, the difference in variation of susceptibility and resistance between these two species of mosquitoes was statistically insignificant (P= 0.8792).

The susceptibility status of both mosquito species was exposed to WHO approved insecticide Pirimiphos-methyl which served as the control recorded 100% mortality at 60 minutes exposure time. Also, the diluent (20% tween 80 (1ml) also served as the control recorded 0% mortality at 60 minutes exposure time of both the *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes.

Table 2: Essential Oil Potential of *Tagetes erecta* leaf against *An. gambiae* and *Cx. quinquefasciatus* after 60 minutes exposure time

Mosquito species	LC_{50} (µl/ml)	LC_{95} (µl/ml)
An. gambiae	10.26	31.73
Cx. quinquefasciatus	10.42	31.73

The lethal concentration to cause 50% mortality was 10.26 µl/ml for *An. gambiae* and 10.42 µl/ml for *Cx. Quinquefasciatus*, while the lethal concentration of 95% mortality was 31.73 µl/ml for both *An. gambie* and *Cx. quinquefasciatus* mosquito species (Table 2).

Discussion

Asteraceae, Cladophoraceae, Lamiaceae, Meliaceae, Oocystaceae, Rutaceae and *Solanaceae* are members of the plant families, reported to possess adulticidal, repellent, and larvicidal activities against different species of mosquitoes (Sukumar *et al*., 2018; Shaalan *et al.*, 2005) [21, 20]. It is established that the discovery of the larvicidal and insecticidal potential of the extract of *Chrysanthemum cinerariaefolium*, belonging to the family *Asteraceae*, birthed the extensive screening/study for the insecticidal and larvicidal activity of other members of the plant family. It was observed in this present study, that essential oil gotten from *Tagetes erecta* did not show a significant potency on the mortality rates across the two important vector mosquitoes *Cx. quinquefasciatus* and *An. gambiae*. The essential oil extracts from the plant were effective at different concentrations (62.5 µl/ml, 125 µl/ml, and 250 µl/ml) for *Anopheles* mosquitoes and *Culex* mosquitoes after 60 minutes, respectively. The LC_{50} values was 10.26 µl/ml for *An. gambiae* and 10.42 µl/ml for *Cx. quinquefasciatus*. The essential oil extracted from the *Tagetes erecta*, therefore showed marginally lesser activity on *Culex quinquefasciatus* compared to *Anopheles gambiae*.

The results in this present study were further compared to other published studies. The findings agree with earlier studies by Murugesan *et al.* (2016) [13], who reported insecticidal effect of *Tagetes erecta* on *Aedes* and *Culex* mosquitoes. The study recorded 100% mortality at 100 mg/l concentration of the extract after 60 minutes of exposure. Also, the essential oil of *Tagetes erecta* leaves (LC_{50} 1.76ppm) against *Anopheles stephensi* was reported to be efficacious by Senthilkumar *et al.* (2009) [19].

The mosquitocidal property demonstrated by the essential oil extracted from *Tagetes on Culex quinquefasciatus* and *Anopheles gambiae* mosquitoes is a proof of the potency of this oil against the test mosquito species. *T. erecta L*., has also been demonstrated in other studies to have insecticidal activities against *Aedes aegypti* larvae (Marques *et al.*, 2011, Bhatt (2013) [12, 2], Jayaraman *et al.*, 2015 and Tri *et al.*, 2018) [11, 22]. A study reported by Green *et al.* (1991) [8], however, did not show mortality when tested on *Aedes aegypti* after the duration of 72h exposure to the crude extract from *T. erecta L*. It is also reported that *T. erecta L*. leaf extract proved potent inhibitory and adulticidal activities in *Culex tritaeniorhynchus* mosquitoes (Elango *et al.* 2012) [6]. Additionally, *T. erecta L.* flower extracts exhibited repellent and adulticidal properties against different mosquito species including *Culex quinquefasciatus, Anopheles stephensi*, and *Culex infulus* (Isman 2002, Pavitha and Poornima, 2014) [10, 17].

Conclusion

From the present study, it was observed that the concentrations of the essential oil (250 µl/ml, 125 µl/ml, and 62.5 µl/ml), extracted from *Tagetes erecta* demonstrated mosquitocidal activity against the test mosquitoes, *Anopheles gambiae* and *Culex quinquefasciatus*. Also, essential oil extracts of *Tagetes erecta*, showed marginally lesser activity on *Culex quinquefasciatus* compared to *Anopheles gambiae*. In comparison with 40 µg Pirimiphos-methyl, the mosquitocidal activity was also not superior. It is therefore important that more plant-based insecticides that are efficient be identified. These plant-based insecticides should also be suitable, adaptive to local ecological conditions and biodegradable. They should also possess the wide spread mosquitocidal property that will serve as an arsenal in the future, and as a suitable alternative product against mosquito-borne diseases.

Author's Contribution
Conceptualization: PMEU and EPN
Design: PMEU and EPN
Investigation: PMEU, EPN, IAU and ELI
Data Analysis: EPN, TA, and NMI
Drafting: PMEU and EPN,
Revised and approved: All authors

Conflict of Interest Statement
We declare we have no competing interests.

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