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Susceptibility of *Aedes aegypti* L. to essential oils of *Eryngium foetidum* L. and *Plectranthus amboinicus* (Lour) Spreng.

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ABSTRACT

Background:
Aedes aegypti is a major vector of yellow fever, dengue, and lymphatic filariasis. Increasing resistance to synthetic insecticides has necessitated the search for safer, plant-based alternatives. This study evaluated the susceptibility of adult female *Aedes aegypti* to the essential oils of *Eryngium foetidum* and *Plectranthus amboinicus*.

Methods:
Essential oils were extracted by hydrodistillation using a Clevenger apparatus. Susceptibility was assessed based on knockdown effects at concentrations of 6.25, 12.50, 25.00, 50.00, and 100.00 µl/ml. The negative control was 1 ml of 10% Tween 80, while deltamethrin served as the positive control. Each concentration and control was tested in quadruplicate, with 25 adult female mosquitoes per replicate (100 per concentration). Bioassays were conducted using Wheaton bottles coated with the respective test solutions.

Results:
Eryngium foetidum oil produced a concentration- and time-dependent knockdown effect, achieving 100% and 96% knockdown at 50.00 µl/ml and 100.00 µl/ml, respectively. *Plectranthus amboinicus* oil showed a non-linear concentration–response relationship. At 30 minutes, KD_{50} values estimated by log-time probit analysis were 15.236 µl/ml for *P. amboinicus* and 59.311 µl/ml for *E. foetidum*, indicating greater susceptibility of *Ae. aegypti* to *P. amboininicus* oil. Only the positive control elicited knockdown among controls.

Conclusion:
Both essential oils demonstrated adulticidal activity, with *P. amboinicus* showing greater potency. These findings support further investigation of these plant-derived oils as potential eco-friendly mosquito control agents.

Keywords: Disease-vector, *Eryngium-foetidum,* Knockdown, *Plectranthus-amboinicus*

1. INTRODUCTION

The culicine mosquito *Aedes aegypti* (Linnaeus) is widely distributed across tropical and subtropical regions of the world. Adult mosquitoes of this species are morphologically distinguished by the characteristic lyre-shaped white scales on the dorsal surface of the thorax. Female *Ae. aegypti* preferentially oviposit in natural and artificial water-holding containers such as tree holes, discarded tyres, flower pots, water storage cisterns, and other abandoned receptacles, typically located in close proximity to human dwellings [1]. This species exhibits strong anthropophilic behaviour, demonstrating a marked preference for human blood over that of domestic animals [2,3]. Its close association with humans, coupled with its daytime biting habit, makes *Ae. aegypti* an efficient vector of several medically important arboviruses. It is responsible for the transmission of pathogens that cause yellow fever, dengue fever and dengue haemorrhagic fever, Zika virus disease, and chikungunya [1,4–6]. Additionally, it has been implicated in the transmission of certain filarial nematodes associated with lymphatic filariasis. Diseases transmitted by *Ae. aegypti* constitute a major public health burden globally, particularly in tropical Africa. They contribute substantially to morbidity and mortality, and some, such as lymphatic filariasis, may lead to chronic disfigurement and social stigmatization of affected individuals. Beyond their health consequences, these diseases impose significant socio-economic burdens on families and communities. Transmission occurs during blood feeding, when the mosquito injects saliva

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containing anticoagulant and immunomodulatory proteins into the host. These salivary components may provoke allergic cutaneous reactions in sensitive individuals. Furthermore, high biting densities may result in appreciable blood loss in heavily exposed populations [1]. The persistent buzzing sound produced by *Ae. aegypti* and other mosquito species can also disrupt sleep and reduce overall well-being. The absence of effective vaccines or chemoprophylactic measures for several of the viruses transmitted by *Ae. aegypti* underscores the importance of vector control as a primary preventive strategy [7]. Synthetic insecticides have historically played a central role in vector control programmes. However, their prolonged and indiscriminate use has led to the development of physiological resistance in mosquito populations, as well as adverse effects on non-target organisms, human health, and the environment [8]. These limitations highlight the urgent need for alternative, environmentally sustainable control approaches. Plant-derived natural products offer promising prospects for safer and eco-friendly mosquito control. Such products are generally considered to possess lower mammalian toxicity and greater environmental biodegradability compared with conventional synthetic insecticides [9,10]. Numerous studies have reported the bioefficacy of plant extracts and essential oils against mosquito vectors and other insect pests [11–19]. Previous investigations have demonstrated the bioactivity of *Eryngium foetidum* and *Plectranthus amboinicus* against *Anopheles gambiae*, a principal vector of malaria and lymphatic filariasis. *Eryngium foetidum*, commonly known as coriander (culantro), is a perennial herb belonging to the family Apiaceae. It is cultivated widely in tropical and temperate regions [20]. Ethnomedicinally, it has been used in the treatment of burns, earache, malaria, febrile conditions, hypertension, constipation, asthma, seizures, and gastrointestinal disorders [20]. Its larvicidal activity against *Aedes albopictus*, as well as the insecticidal and bioactive properties of its essential oil against stored-product pests such as the red flour beetle, have been documented [21,22]. Additional pharmacological and biological activities of the plant have also been reported [21–24]. *Plectranthus amboinicus* (Indian borage), a highly aromatic perennial herb of the family Lamiaceae, is widely used in traditional medicine for the treatment of colds, asthma, cough, fever, constipation, headache, and dermatological conditions [25]. The leaves are commonly used as flavouring agents in traditional cuisine [26]. Owing to its attractive heart-shaped foliage and characteristic aroma, it is also cultivated as an ornamental plant [25]. Annaduri and Venugoplan [27] reported the chemical composition and larvicidal activity of the essential oil of *P. amboinicus* against *Anopheles stephensi*. In view of the documented bioactivity of these plant species against insect vectors, the present study was designed to evaluate the susceptibility of female *Aedes aegypti* to the essential oils of *Eryngium foetidum* and *Plectranthus amboinicus*, with a view to exploring their potential as alternative botanical insecticides for vector control.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological materials

Biological materials used for this research were plants of the species *Eryngium foetidum* (aerial parts) and the leaves of *Plectranthus amboinicus.* Test biological vectors used were adult female insects of the species *Aedes aegypti.*

2.1.2 Chemicals and Reagents

The following chemicals and reagents were used: sodium sulphate (AnalaR grade), deltamethrin, acetone, ten percent (10%) tween 80.

2.1.3 Equipment and other Materials

Materials used in the course of this research were Clevenger apparatus, Wheaton assay bottles (CDC bottles), rearing cages, Whatman No.1 filter paper, cotton wool and aspirator

2. Methods

2.2.1 Collection and Authentication of Experimental Plants

E. foetidum and *P. amboinicus* that were used for this research were obtained from the medicinal plant garden of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. A plant taxonomist, Professor Margaret E. Bassey in the Department of Botany and Ecological Studies, Faculty of Biological Sciences, University of Uyo, Nigeria, did the authentication and assigned herbarium numbers UUH40026 and UUH40019 for *E. foetidum* and *P. amboinicus* respectively.

2.2.2 Oil Distillation

The parts used for the experiments reported in this research were the aerial parts of *E. foetidum* and leaves of *P. amboinicus.* They were separately processed and processing involved washing and shade drying on laboratory benches for two hours. This was to allow water to drain. Shade drying was followed by shredding and then each plant sample was separately weighed using a triple beam balance and kept in sterile, labelled sample bags. Hydrodistillation using a Clevenger apparatus was the process adopted for oil extraction. This was in accordance with British Pharmacopoeia [28]. A quantity of 1000 g of each plant sample was used. *E. foetidum* sample was placed in the flask of the Clevenger apparatus and then 2.5 litres of water was added. This was heated over a heating mantle maintained at 70 ^{0}C for four hours. The same was repeated for the leaves of *P. amboinicus.* Labelled glass sample bottles were used for collection of the oils. Oil obtained from each plant sample was separately dried over sodium sulphate and stored at a temperature of -4^{0}C in a freezer, prior to use.

2.2.3 Test Mosquito Species

Mosquitoes used for this study were adult females of *Ae. aegypti.* They were obtained (as eggs on pads) from the insect colony of Entomology and Parasitology Unit of the National Arbovirus and Vectors Research Centre, Enugu, Nigeria.

Rearing commenced by immersing the eggs in troughs containing water and placed inside cages in the insectary. As they metamorphosed into larvae they were provided with larval nutrients. When the fourth instar larvae metamorphosed into pupae, the pupae were transferred into water in fresh troughs in the cages. Feeding was suspended for the pupae. As adults emerged, females of test mosquitoes were collected using an aspirator and transferred to separate cages in preparation for the assays reported in this study. The procedure of Ejeta *et al.* [29] was adopted for the rearing of the test mosquito species.

2.2.4 Test Oil Concentrations

Stock solutions of the oils of *E. foetidum* and *P. amboinicus* were separately prepared using 10% tween 80 solution as the diluent. From the stock solution, five concentrations (6.25, 12.50, 25, 50 and 100µl/ml) of each oil were separately prepared and used for this study. Each test oil consisted of 1ml of the oil and each had four replicates. The positive control reported in this study consisted of a stock solution prepared by adding 0.625ml of deltamethrin to 50ml acetone according to the protocol of CDC. Then 1ml of the stock served as the positive control for the experiment. The negative control consisted of 10% of tween 80 solution (1ml). Each control was also replicated four times. The test bottles and their caps were properly labelled using a masking tape to indicate the oil used as well as the concentration. The same was done for the control bottles.

2.2.5 Susceptibility Test

Wheaton bottles (250 ml capacity each) were used for this study as described by Centres for Disease Control [30]. Four of the Wheaton bottles were coated with each oil concentration. In each case, when the test oils were introduced into the capped bottles, they were swirled to ensure even coating. This was done by turning the bottles to their sides and tilting the bottles back and forth to ensure that all sides of the bottles were coated. The bottle caps were afterward removed and the bottles were laid on their sides and rolled on the table for 1 hour until all visible signs of the oils, deltamethrin and tween 80 solution (10%) were gone and the bottles completely dry. Adult female *Ae. aegypti* mosquitoes (2 to 5 days old), were introduced into the bottles as quickly as possible and a timer started immediately. Susceptibility was determined by the knockdown effect of the oils on the mosquitoes. Observations for knockdown effect were made and recorded at 0, 5, 10, 15 and 30 minutes after introduction of the test mosquitoes, as described by Parker [31]. Mosquitoes were considered susceptible if they were unable to stand right by themselves during the observation period [31]. Data obtained were recorded separately for each oil concentration and the control.

2.3 Statistical Analysis

Data obtained from this study were used for the determination of means and standard errors of the means. Data were also subjected to One-way Analysis of Variance as well as Log-time Probit model, for the determination of the 30-minute median knockdown (KD_{50}) values of the oils. All analyses were carried out using SPSS version 20.

3. RESULTS

On exposure of the test mosquitoes to the lowest concentration (6.25µl/ml) of the oil of *E. foetidum,* 58% of the mosquitoes were knocked down after 15-minutes. At the end of 30-minutes exposure period, the same concentration resulted in 62% knockdown effect on the mosquitoes. After 30-minutes of exposure to 50µl/ml concentration of *E. foetidum* oil, 100% knockdown effect was observed on the test mosquitoes. However, the highest concentration of the test oil (100µl/ml) recorded 96% knockdown effect on the mosquito species after 30 minutes exposure period (Table 1 and 2). In the positive control experiment, 100% knockdown effect was observed on exposure of the test mosquitoes to the deltamethrin treated bottles after 5 minutes, while there was no knockdown effect recorded on exposure of the test mosquitoes to the 10% tween-80 treated bottle (negative control) after 30 minutes (Tables 1 and 2).

Table 1: Knockdown Effect of Different Concentrations of *E. foetidum* on *Ae. aegypti*

Conc. (µl/ml)	No. of Mosquitoes (25x4 replicates)	Percentage knockdown/time of exposure (minutes)				
		0	5	10	15	30
6.25	100	0	18	30	58	62
12.50	100	0	22	32	64	70
25.00	100	0	24	34	70	78
50.00	100	0	28	42	80	100
100.00	100	0	32	46	88	96
Positive control (deltamethrin)	100	0	100	100	100	100
Negative control (10% tween 80)	100	0	0	0	0	0

Table 2: Mean Knockdown values of *Ae. aegypti* exposed to different concentrations of *E. foetidum* oil

Concentrations (µl/ml)	0 min	5 min	10 min	15 min	30 min
6.25	0.00±0.00	4.50±0.29^{d}	7.50±0.29^{d}	14.50±0.29^{d}	15.50±0.29^{c}
12.50	0.00±0.00	5.50±0.29^{cd}	8.00±0.00^{d}	16.00±0.00^{cd}	17.50±0.29^{b}
25.00	0.00±0.00	6.00±0.00^{cd}	8.50±0.29^{cd}	17.50±0.29^{cd}	24.50±0.29^{a}
50.00	0.00±0.00	7.00±0.58^{bc}	10.50±0.87^{bc}	20.00±1.73^{bc}	25.00±0.00^{a}
100.00	0.00±0.00	8.00±0.58^{b}	11.50±0.87^{b}	22.00±1.73^{ab}	24.00±0.58^{a}
Positive control (deltamethrin)	0.00±0.00	25.00±0.00^{a}	25.00±0.00^{a}	25.00±0.00^{a}	25.00±0.00^{a}
Negative control	0.00±0.00	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{d}
Overall Mean	0.00±0.00	8.00±1.42	10.14±1.35	16.43±1.47	18.76±1.63
p-Value	NA	<0.001*	<0.001*	<0.001*	<0.001*

Values of knockdown are Mean ± SE. Means along the same column with the same superscript =not significantly different. Means along the same column with different superscripts = significant

The knockdown effect of *P. amboinicus* oil on *Ae. aegypti* in this study reveals that on exposure to the least concentration (6.25µl/ml) of oil, complete knockdown (100%) effect was observed after an exposure period of 30-minutes. There was however an unusual trend observed in the course of the test as the knockdown effect decreased with increase in concentration (Table 3 and 4). However, on exposure to the highest concentration (100µl/ml) of the oil, 100% knockdown effect was observed after 15-minutes exposure of the mosquitoes to the test oil. The mosquitoes exposed to the positive control and replicates were all knocked down after an exposure period of 5-minutes while mosquitoes exposed to 10% tween 80 (negative control) were not knocked down throughout the duration of the experiment (30-minutes).

Table 3: Knockdown-Effect of Different Concentrations of *P. amboinicus* oil on *Ae. Aegypti*

Conc. (µl/ml)	No. of Mosquitoes (25*4 replicates)	Percentage knockdown/time of exposure (minutes)				
		0	5	10	15	30
6.25	100	0	30	40	96	100
12.50	100	0	28	40	82	94
25.00	100	0	26	38	76	90
50.00	100	0	26	40	76	86
100.00	100	0	36	54	100	100
Positive control (deltamethrin)	100	0	100	100	100	100
Negative control	100	0	0	0	0	0

Table 4: Mean Knockdown values of *Ae. aegypti* exposed to different concentrations of *P. amboinicus* oil

Concentrations (µl/ml)	0-min	5-min	10-min	15-min	30-min
6.25	0.00±0.00	7.50±0.29^{c}	10.00±1.15^{c}	24.00±0.58^{a}	25.00±0.00^{a}
12.50	0.00±0.00	7.00±0.00^{c}	10.00±0.00^{c}	20.00±0.29^{b}	23.50±0.29^{a}
25.00	0.00±0.00	6.50±0.29^{c}	9.50±0.29^{c}	19.00±0.58^{b}	22.50±0.29^{a}
50.00	0.00±0.00	6.50±0.29^{c}	10.00±0.58^{c}	19.00±1.15^{b}	21.00±2.02^{a}
100.00	0.00±0.00	9.00±0.58^{b}	13.50±0.87^{b}	25.00±0.00^{a}	25.00±0.00^{a}
Positive control (deltamethrin)	0.00±0.00	25.00±0.00^{a}	25.00±0.00^{a}	25.00±0.00^{a}	25.00±0.00^{a}
Negative control (10% tween 80)	0.00±0.00	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{b}
Overall mean	0.00±0.00	8.79±1.37	11.14±1.33	18.93±1.57	20.36±1.64
p-Value	NA	<0.001*	<0.001*	<0.001*	<0.001*

Values of knockdown are Mean ± SE. Means along the same column with the same superscript = not significantly different. Means along the same column with different superscripts = significant.

3.1 The 30-minutes KD_{50} Values

The 30-minute median knockdown (KD_{50}) values obtained using the Log-time Probit model, for *E. foetidum* and *P. amboinicus* essential oils tested on adult female *Ae. aegypti* mosquitoes were 59.311 and 15.236 respectively.

4.0 DISCUSSION

The present study evaluated the susceptibility of adult female *Aedes aegypti* to essential oils of *Eryngium foetidum* and *Plectranthus amboinicus*, using knockdown response as the bioassay endpoint. Both oils

demonstrated appreciable adulticidal activity; however, *P. amboinicus* exhibited greater potency than *E. foetidum*, as evidenced by the lower 30-minute KD_{50} value (15.236 µl/ml versus 59.311 µl/ml). These findings indicate a higher intrinsic toxicity of *P. amboinicus* oil to *Ae. aegypti* under the experimental conditions employed. Interestingly, this pattern contrasts with the findings of Ubulom et al. [32], who reported greater efficacy of *E. foetidum* oil against adult *Anopheles gambiae*. Such differences may be attributed to interspecific variation in mosquito physiology, detoxification enzyme systems, cuticular permeability, or target-site sensitivity. Species-specific susceptibility is well documented in vector control research and highlights the importance of testing botanical insecticides against multiple vector species. The essential oil of *E. foetidum* produced a clear concentration- and time-dependent knockdown effect. Notably, 100% knockdown was achieved at 50 µl/ml, whereas 96% was recorded at 100 µl/ml. This apparent non-proportional response has been reported with other natural products and may reflect partial agonistic effects or complex phytochemical interactions. Similar trends have been observed in studies involving plant extracts such as *Citrus aurantifolia* and *Berberis aristata* [33,34]. The bioactivity of *E. foetidum* oil has been linked to its aliphatic and aromatic constituents [35], which may act synergistically or competitively at neurotoxic target sites. In contrast, *P. amboinicus* exhibited a non-linear concentration–response relationship. Essential oils are complex mixtures containing multiple bioactive and inactive constituents, and their biological effects may be influenced by synergistic or antagonistic interactions among components [36]. Threshold effects may also occur, whereby increasing concentration does not proportionally enhance activity beyond a certain point. These factors likely explain the irregular knockdown progression observed with *P. amboinicus*. The negative control (10% Tween 80) produced no knockdown effect, confirming that the observed activity was attributable to the test oils. As expected, deltamethrin elicited marked susceptibility, validating the experimental design.

5.0 CONCLUSION

Adult female *Aedes aegypti*, a principal vector of yellow fever, dengue, Zika, chikungunya, and lymphatic filariasis, demonstrated susceptibility to the essential oils of *Eryngium foetidum* and *Plectranthus amboinicus*. The comparatively lower KD_{50} value of *P. amboinicus* indicates greater potency under laboratory conditions. Given the growing limitations associated with synthetic insecticides—particularly resistance development and environmental concerns—these plant-derived essential oils represent promising candidates for incorporation into integrated vector management programmes. Further investigations into their chemical composition, mode of action, safety profile, and field efficacy are warranted to fully establish their potential as eco-friendly mosquito control agents.

DECLARATIONS

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the research, authorship, and/or publication of this article.

Authors' Contributions

All authors contributed to the study conception, research design, and overall framework. Paul Thomas was responsible for the collection and authentication of plant materials and for essential oil extraction by hydrodistillation. Peace Ubulom conducted mosquito rearing and susceptibility bioassays. Akaninyene Akpan performed the statistical analyses. Peace Ubulom and Edidiong Udofa prepared the manuscript, including drafting and formatting for publication. Edidiong Udofa handled all correspondence related to the manuscript. All authors reviewed and approved the final version of the manuscript.

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