DETERMINING THE REPELLENCY OF *Cleome viscosa* EXTRACTS ON *Aedes aegypti* IN CAPE COAST, GHANA

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DECLARATION

Candidate's declaration

I hereby declare that this report is the result of my original research and that no part of it has been presented for another degree in this university or elsewhere

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Supervisor's declaration

We hereby declare that the preparation and presentation of the dissertation were supervised by the guidelines on supervision of dissertation laid down by the University of Cape Coast.

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ABSTRACT

Aedes-borne viruses such as Zika, chikungunya, dengue fever, and yellow fever, among others are of global importance. The World Health Organization (WHO) estimated that the mortality burden of arbovirus diseases such as yellow fever in Africa, was 84,000-170,000 severe cases and 29,000-60,000 deaths in 2013. Although vaccine development for prevention of mosquito-borne arbovirus infections has been a focus, mitigation strategies continue to rely on vector control. However, vector control has failed to prevent recent epidemics and arrest expanding geographic distribution of key arboviruses, such as dengue. As a consequence, there has been increasing necessity to further optimize current strategies within integrated approaches and advance development of alternative, innovative natural strategies for the control of mosquito-borne arboviruses. This study aimed to determine the efficacy of Cleome viscosa extracts as repellent against *Aedes aegypti* mosquitoes. Volatile extraction method was used for the leaves of Cleome. Methylene Chloride extracts of *Cleome viscosa* was used for the test. *Aedes aegypti* larvae were collected from discarded tyres in Cape Coast. Larvae collected were reared to adults. Repellency test of Adult males and females was conducted using a Y-tube olfactometer. The results clearly indicated that the methanol extract of *C. viscosa* leaves have equal potential repellent activity against male and female *A. aegypti* mosquitoes and can be used to control Aedes aegypti populations in reducing the spread of arboviruses.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 **Importance of mosquitoes.**

Mosquitoes are vectors of several diseases affecting humans and domestic animals worldwide. Mosquitoes cause substantial mortality and morbidity among people living in tropical and sub-tropical zones (Ranson et. al 2010). The prevalence of vector-borne diseases significantly decreased in many parts of the world after the development of various control methods and synthetic pesticides for vector control in the 1940s and 1950s. However, the prevalence of diseases spread by vectors is rising. Global diseases like dengue and other viral infections spread by mosquitoes are currently a problem (Smith et al., 2016).

1.2 Aedes aegypti; Distribution and Aedes-borne diseases

Aedes aegypti (Linnaeus) is arguably one of the most important domestic mosquito vectors of several arboviruses including yellow fever, dengue fever, chikungunya and Zika virus (Farraudiere et al. 2017). *Ae. aegypti*, which has its origins in Africa, is now widespread throughout tropical and sub-tropical areas of the world through trade (Moore et.al 2013; Powel et.al 2013; Kraemer et.al 2015). Climate change is also impacting the distribution of Aedes mosquitoes and transmission patterns of Aedes-borne viruses (Ryan et al., 2019). *Aedes aegypti* feeds predominantly and frequently on humans (Harrington et.al 2001) and has caused a growing incidence of death globally for millennia by spreading fatal diseases. Immature stages develop preferentially in artificial containers, usually in close proximity to humans (Brown et.al 2014). However, human activities involving water storage and the disposal of potential water-holding containers greatly influence the breeding of Aedes in individual households and may lead to the provision of breeding sites year-round (Ngugi et.al 2017). The adaptation of these vectors to urban domestic habitats has led to their exploitation of a range of artificial containers and their capacity to exploit potential breeding water situated indoors or outdoors (Dom et.al 2013).

1.3 Implications for global health

Aedes-borne diseases are prevalent in more about 250 countries across the world (Figure 1 & 2), infecting millions of individuals every year at the global level and among the leading cause of human deaths (Ghosh et al., 2012). The frequency and magnitude of outbreaks of these *Aedes*-borne arboviruses, are increasing globally, fueled by the convergence of ecologic, economic, and social factors (WHO, 2022)

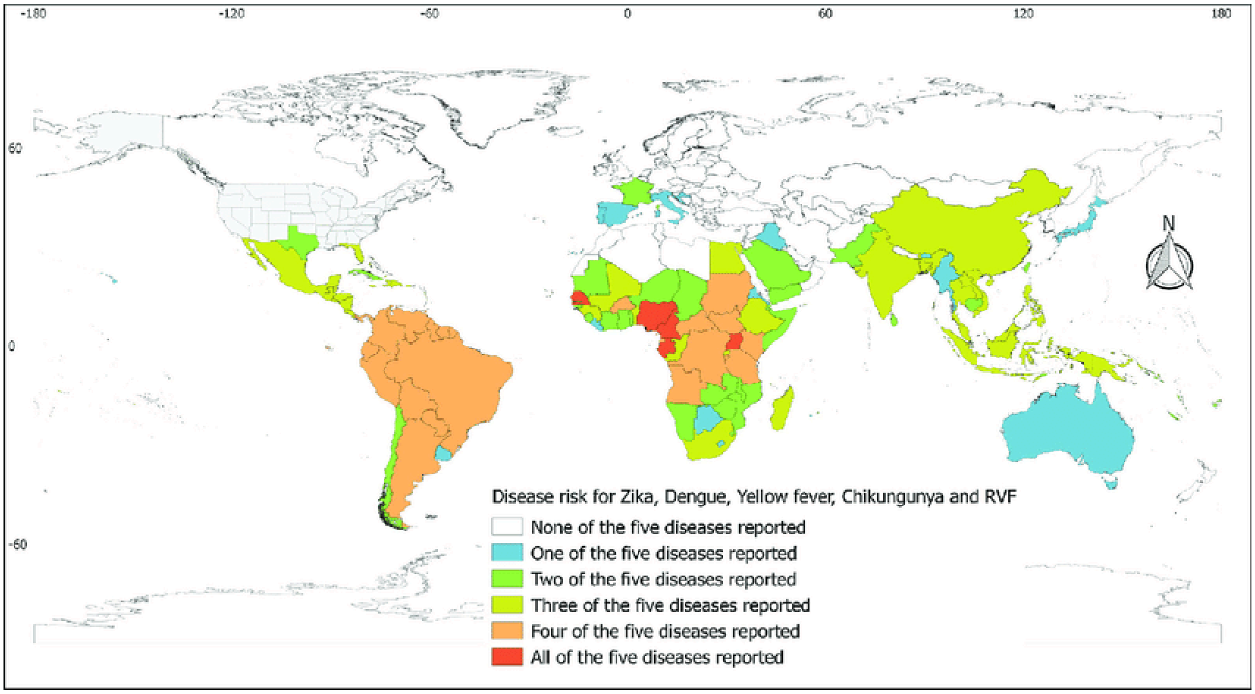

Figure 1. Global country-level occurrences of selected arboviral diseases (Leta et al., 2017)

Region	Number of countries/ territories	Number of countries/territories suitable for			Number of countries/territories affected by				
		Aedes aegypti	*Aedes albopictus*	Either vector	Zika	Dengue fever	Yellow fever	Chikungunya fever	RVF
Africa	58	56	56	57	14	36	30	26	36
Americas[a]	56	52	44	52	48	46	13	46	0
Asia	52	45	43	49	11	15	0	20	3
Europe	56	12	32	32	0	3	0	3	0
Oceania[b]	28	23	22	25	12	11	0	11	0
Overall number of countries/ territories	250	188	197	215	85	111	43	106	39

RVF, Rift Valley fever.
[a] Includes Central America, North America, the Caribbean, and South America.
[b] Includes Australia and the Pacific islands.

Figure 2. Number of countries/ territories suitable for the vectors and number of countries/territories affected by the diseases, by region (Leta et al, 2017).

1.4 Arboviral diseases in Africa

Dengue fever is among the most relevant vector-borne diseases, causing 100-400 million infections and 25,000 deaths per year (Brady & Hay, 2020; Guo et.al 2013). Yellow fever is endemic in tropical areas of Africa (Figure 2**).** The importance of Aedes in sub-Saharan Africa has increased recently because of outbreaks of these arboviral diseases in multiple countries there (Braack et al. 2018). Within the last five years in West Africa, there have been outbreaks of dengue in Burkina Faso (Tarnagda et al. 2018; Lee et al.2018; Quattara et al.2019), Cote d'Ivoire (Suzuki et al. 2017; Fofana et al. 2019), and Senegal (NEWS DESK 2018), yellow fever in Cote d'Ivoire (Zahouli et al. 2017) and Nigeria (Ajogbasile et al. 2020), and recent confirmed cases of dengue and outbreaks of yellow fever in Ghana (Amoako et al. 2018; Manu et al. 2019). Therefore, the risk of dengue, yellow fever and chikungunya outbreaks in Ghana appears to be high. Aedes-borne arboviral infections are a rising public health concern, but Ghana hasn't paid much attention to their control or prevention (Abdulai et al., 2023).

Figure 3: Yellow Fever endemic zones in Africa, shown in yellow (Princeton, 2017)

Taking into account the country's present state of arboviral infections, this study was carried out in Cape Coast, Central Region's capital city and a popular tourist destination, to evaluate the risk of a future outbreak of Aedes-borne arboviruses. The high volume of international visitors to the city brought on by the medieval castles and forests with big populations of primates functioning as tourist attractions (Deikumah & Kudom, 2010) could easily encourage the introduction and spread of arboviruses.

1.5 Control of Aedes mosquitoes and challenges

To prevent and reduce arboviral diseases, there is the need to first control the Aedes vector or prevent them from getting into contact with humans (Rather et al., 2017). Some of the current measures that have been applied to control *Ae. aegypti* since its domestication are environmental sanitation; removal of waste disposal and proper maintenance of sewage systems and canals, biological control; use of fungi and plants, and the use of chemical pesticides (Lacey et.al 2007; Kean et.al 2015). Amongst these, the use of chemical insecticides for controlling the mosquito population is the commonest. The products of these insecticides are mainly organophosphates, pyrethroids and growth regulators. Pyrethroids are used for the control of the adults, whereas temephos are used in immature stages (Bona et al., 2016; Moyes et al., 2017).

1.6 Challenges in controlling *Aedes aegypti*

Despite these alternatives above, chemical control has not yet been able to stop the increase of cases of viral diseases, nor the dispersion of the vectors to new areas, but it has generated mosquito populations resistant to the molecules used for its control and has increased pollution in the environment (Bona et al., 2016; Moyes et al., 2017; (Rivero et al., 2010).

1.6.1 Alternative strategies for controlling Aedes aegypti

Over the years, strategies for controlling *Aedes aegypti* populations have been solely dependent on the use of synthetic chemical insecticides and repellents, which are not only expensive but also pose threats to human health and the environment in general. The World Health Organization (WHO) highlights the necessity of developing novel control methods that may contribute to the reduction of the mosquito populations and disease burden and has therefore proposed innovative methods including the use of genetically modified mosquitoes, producing new baits, and finding new insecticide and repellent molecules that are safe and environmentally friendly (WHO, 2017).

Recently, the use of environment friendly and biodegradable natural insecticides of plant origin have received renewed attention because they are rich with bioactive compounds active against specific target insects and are less likely to cause resistance in target species. Novel clinically active drugs are discovered from the natural products isolated from higher plants (Singh et al., 2015). Plant-derived extracts and phytochemicals have been investigated on insect pests for the past 30 years to develop alternatives to conventional insecticides (Ca´ssia Seffrin *et al*., 2010).

1.7 Importance and of *Cleome viscosa*

Cleome viscosa Linn., commonly known as the Asian spider flower or wild or dog mustard, family (*Capparaceae*), is a sticky herb found as a common weed in plains of Africa, India, and throughout the tropical regions of the world annually (Singh et al., 2015). The whole plant and its parts that is; leaves, seeds and roots, are widely used in traditional medicine especially in the herbal industry (Taylor & Francis 2010). Traditionally, this plant is used in various disorders such as diarrhea, fever, inflammation, liver diseases, bronchitis, skin diseases and malarial fever. Extract has alkaloids, phenolics, flavonoids and tannins as major phytochemicals. A research showed that the methanol extract of *Cleome viscosa* whole plant appears to be non-toxic by oral administration at tested doses (Taiwo & Joel, 2015) which makes it safe for consumption. Moreover, another investigation on toxicity on albino rats has been stipulated the methanolic extracts of *Cleome viscosa* to be significant in wound healing activity as evidenced by the reduction in the number of days required, and the wound contraction compared to the standard (Panduraju et.al 2011). *C. viscosa* seed oil has peripheral analgesic and antiemetic activities (Ahmed et.al 2011). The smoke from its leaves is used by the locals to repel mosquitoes at night.

1.7.1 Insecticidal activities of *Cleome viscosa*

Cleome has demonstrated promising repellent potentials against various insect species. *Cleome viscosa* possesses insect killing properties, along with repellent and cytotoxic activities (Rimi et.al 2017). Potent contact and stomach activity of *C. viscosa* extracts against first instars of *Clavigralla tomentosicollis* (African pod bug) also confirmed the insecticidal activity of this plant (Upadhyay, 2015). Its extracts exhibited larvicidal activity against the second and fourth instar larvae of *Anopheles stephensi*, a vector of malaria (Saxena et.al 2000). Another study showed that *C. viscosa* seed extracts possess antifeedant and chronic toxic properties against *Helicoverpa armigera* (Sivaraman et al., 2014). Extracts from seeds of C. viscosa showed larvicidal potential against 3rd or early 4th stage larvae of *Anopheles stephensi* (Liston), *A. aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say) in different organic solvents (Bansal et al. 2014). Leaf extracts of C. viscosa L. (Capparidacae) were found active against *Spodoptera litura*. The plant contains some phytocidal chemical components that are responsible for toxic and repellent activity in insects from the host plants. Various solvent extracts such as acetone, chloroform, petroleum ether, methanol, hexane, and water also have shown a significant oviposition inhibition in *C. chinensis* (Upadhyay et.al 2012). *C. viscosa* showed strong insecticidal activity against *S. litura* (Phowichit et al. 2008).

1.8 Problem Statement and Justification

Aedes aegypti mosquitoes are important vectors of human viruses. The most widely used method of controlling these vectors is the use of synthetic chemicals. Data shows that *Aedes aegypti* has developed resistance to these chemicals, which are also toxic to the environment. Attention has been drawn to exploring botanical extracts to offer alternatives to synthetic insecticides (Ladhari et.al, 2013). Extracts of *C. viscosa* has been found to possess insect killing properties, along with repellent and cytotoxic activities (Rimi et al., 2017). However, the repellent effects of *Cleome viscosa* specifically on *Aedes aegypti* has not been extensively investigated. Exploring Cleome extracts for repellence against *Aedes aegypti* will help develop environmentally friendly measures of controlling Aedes population, reduce the reliance on synthetic chemicals and minimize the adverse negative impacts of using synthetic chemicals.

1.9 Aims and Objectives

1.9.1 Aim

To determine the efficacy of *Cleome viscosa* extracts as natural repellents against *Aedes aegypti*.

1.9.2 Objectives

1.To determine the efficacy of *Cleome viscosa* extracts as natural repellents against *Aedes aegypti*.

2. To compare the repellency rate of Cleome extracts between males and females *Aedes aegypti* mosquitoes

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study site

The study was carried out in Cape Coast (5°06′N 1°15′W; 5.1°N 1.25°W), the capital of the Central Region, located in southern Ghana and 165 km west of Accra the capital of Ghana, with the Gulf of Guinea situated to its south. Cape Coast is one of the country's most historic cities and it is known for its role in the transatlantic slave trade. The city has an area of 122.0 km^2 of land, population density of 1,557/ km^2 and a settlement population of 189,925 people (GSS, 2021). Cape Coast has a tropical savanna climate. The major rainy season is between May and July as well as a lighter rainy season from September to November, with dry seasons in January, February and August. The city has a mean monthly relative humidity ranging from 85 to 99%. Within the city are pockets of remnant natural forest, which contain several wild organisms (Deikumah & Kudom, 2010).

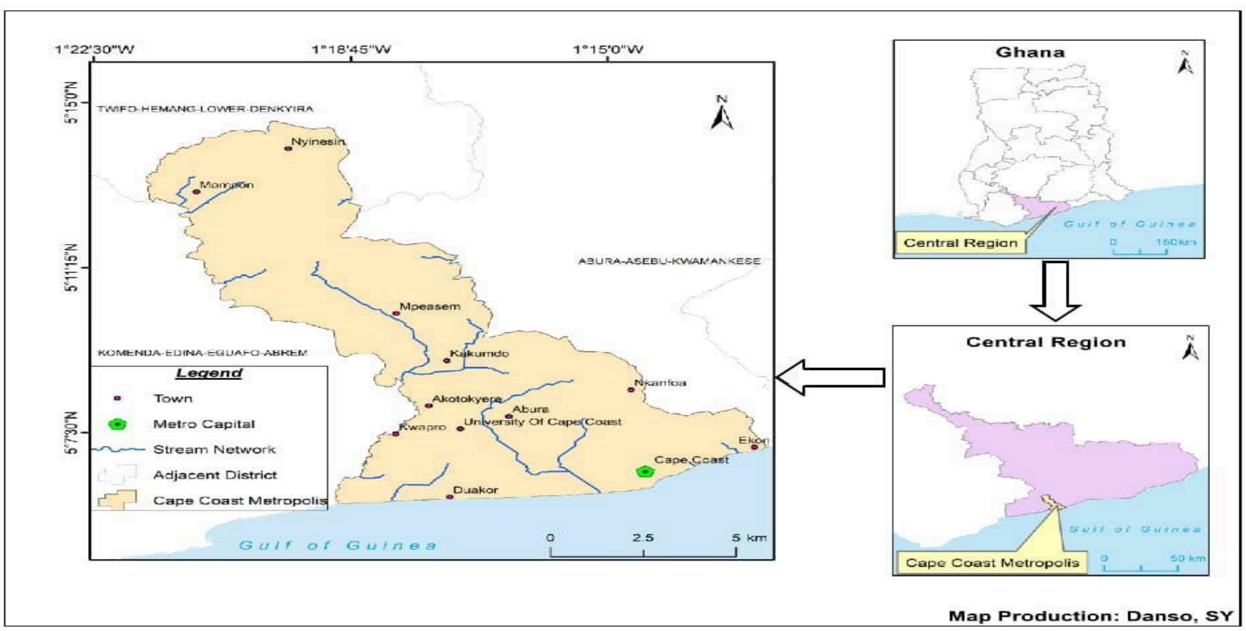

Figure 4: Map of Central Region showing Cape Coast Metropolis

2.2 Collection and Extraction of *Cleome viscosa*

Fresh leaves of *Cleome viscosa* were collected from UCC campus and validated by Dr. John Abraham, Lecturer, Department of Conservation Biology and Entomology, University of Cape Coast.

Volatile extraction method was used for the extraction. Fresh leaves of *Cleome viscosa* were chopped into small pieces, weighed and placed in an oven bag and set on a distillation apparatus. Steam was passed through the plant material, which caused the volatile compounds to vaporize and filtered by activated charcoal. The condensed vapor in the activated charcoal was then extracted with methylene chloride. The extracts were removed to glass vials and preserved in a refrigerator at 4°C.

2.3 Collection of *Aedes aegypti* larvae

Aedes aegypti larvae were sampled specifically from a number of discarded car tyres in Cape Coast. *Ae. aegypti* find tires to be particularly alluring due to the limited light and humidity inside (Dom et al., 2013).

Figure 5: Discarded car tyres

2.3.1 Rearing of larvae

The Aedes mosquito larvae collected were transported in well labelled plastic bottles to the insectary. Larvae were reared in different plastic trays to prevent the crowding of larvae in one tray. Larvae were fed on fish meal until they developed into pupae. Pupae were removed daily with a pipette into small rubber bowls and placed in Bug Dorm-1 cage for emergence. Adults were provided with 10 % sugar solution. The temperature and humidity in the laboratory were monitored regularly using a thermo-hygrometer.

2.4 Repellency test

A Y-tube Olfactometer was used for the repellency testing. The apparatus, as the name suggests, is a Y-shaped tube with two arms and a base. One arm was attached to a chamber containing the *Cleome viscosa* extract and was labelled as test whereas the other arm was attached to a chamber with no extract and labelled, the control chamber. 10 *Aedes aegypti* mosquitoes were introduced into the Y-tube olfactometer's base using an aspirator and allowed to advance towards one of the arms in a period of one minute. Mosquitoes that did not make any choice within the one minute was considered a no-choice. The number of mosquitoes that The percentage of mosquitoes that were repelled by the extract was determined by counting how many were found in the control chamber to the extract (test) chamber. The procedure was repeated nine times each for female and male mosquitoes.

2.4.1 Calculation of repellency percentage

$$\text{Repellency percentage} = \frac{\text{Number of mosquitoes in control} - \text{Number of mosquitoes in test}}{\text{Number of mosquitoes in control}} \times 100$$

2.5 Data Analysis

For each treatment, means of both female (Table 1) and male (Table 2) mosquitoes in the test chamber and control chamber were calculated for, as well as their corresponding standard deviations. The percentage repellency of male and female mosquitoes were also calculated for according to World Health Organization (WHO) protocol for evaluating mosquito repellents. Chi-square test was used to determine if there is any significant difference in the repellency rate of the *Aedes aegypti* observed in the *Cleome viscosa* extract used.

CHAPTER THREE

RESULTS

A total of 250 *Aedes aegypti* larvae were collected from discarded car tyres. Some of the discarded car tyres were colonized by *Culex* species instead of *Aedes aegypti*.

3.1 Repellency Test

Out of the 250 mosquitoes collected, 102 mosquitoes were males while 148 were females. A total of 90 mosquitoes each of females and males were used for the repellency test.

Table 1: Efficacy test of *Cleome viscosa* on female *Aedes aegypti* mosquitoes

Number of times	Test	Control	No choice
1	5	1	4
2	3	4	3
3	0	1	9
4	1	2	7
5	2	5	3
6	0	3	7
7	3	2	5
8	0	4	6
9	0	1	9
Total	14	23	53
Mean	1.555556	2.555556	5.888889
Standard Deviation	1.810463	1.509231	2.315407

Table 2: Efficacy test of *Cleome viscosa* on male *Aedes aegypti* mosquitoes

Number of times	Test	Control	No choice
1	4	3	3
2	2	5	3
3	2	0	8
4	0	1	9
5	1	2	7
6	1	3	6
7	0	2	8
8	0	1	9
9	0	0	10
Total	10	17	63
Mean	1.111111	1.888889	7
Standard Deviation	1.364225	1.615893	2.54951

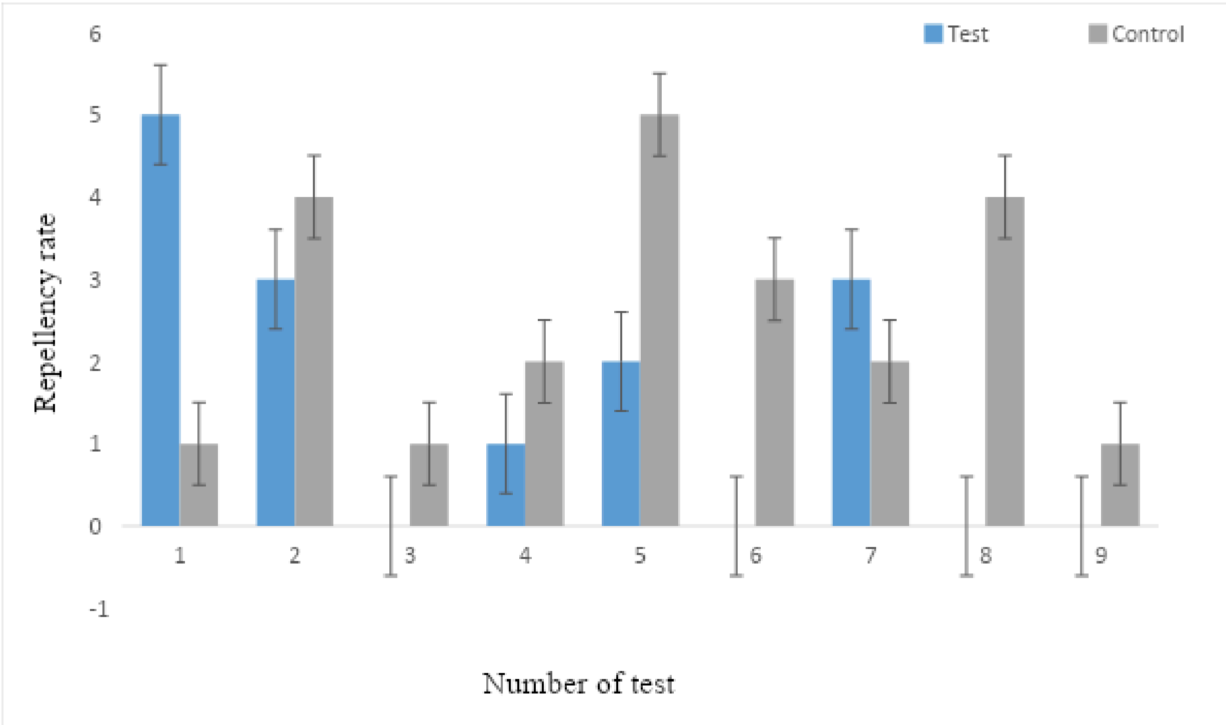

Figure 6. Repellency rate of female *Aedes aegypti*

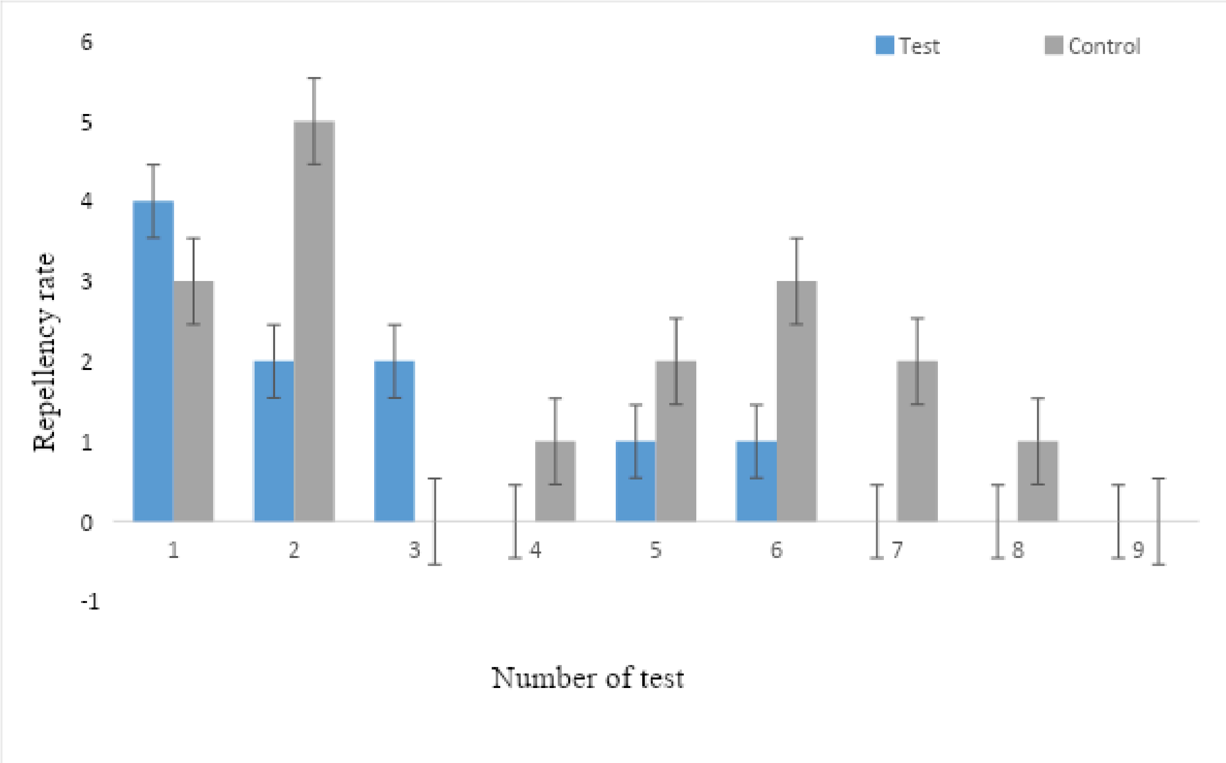

Figure 7. Repellency rate of male *Aedes aegypti*

Chi-square test of independence at 95% confidence interval was performed to determine if there is any significant difference between the repellency rate of males and females *Aedes aegypti* mosquitoes to *Cleome viscosa* extracts. At a degree of freedom of 1 and a Chi-square of 0.004271, the test showed a p-value of 0.947893 which is greater than the alpha value 0.05. A p-value > 0.05 indicates that there is no significant difference between the repellency rates of males and females *Aedes aegypti* mosquitoes to *Cleome viscosa*.

Table 3: Contingency results from Chi-square test at 95% confidence interval

	Test		Control		Total
	Observed	Expected	Observed	Expected	
| Female	14	13.875	23	23.125	37
Male	10	10.125	17	16.875	27

Total	24	40	64

CHAPTER FOUR

DISCUSSION

Arboviruses transmitted by Aedes mosquitoes represent major international public-health concerns that will surely require a range of integrated interventions to be effectively controlled. As the scope of arboviruses continues to grow, development and evaluation of alternative vector-control products and strategies are critical to pursue. Sole reliance on chemical insecticides in controlling Aedes mosquitoes poses threats to the environment and has also developed resistance in these mosquitoes. This study utilized extract of *C. viscosa* leaves as a bio-insecticide against *Aedes aegypti* in providing environmental friendly ways of controlling mosquitoes.

4.1 Larvae distribution

The high breeding rate of *Aedes aegypti* in discarded tyres reflects how conducive the tyre is for the laying of eggs by adult *Aedes aegypti* mosquitoes. This can be confirmed by a study that showed that *Ae. aegypti* find tires to be particularly alluring due to the limited light and humidity inside (Dom et al., 2013). The finding from this study can also be compared to a study conducted in Miami-Dade County, Florida whose findings demonstrate that vector mosquitoes, primarily *Aedes aegypti,* are being produced in tyre shops in Miami, indicating these habitats are highly favorable breeding environments for the production of vector mosquitoes. The study further showed that even small tyre shops with no more than 30 tyres in stock were producing *Aedes aegypti* in large numbers (Andre et al., 2019). Predominance of *Aedes aegypti* in breeding areas in the selected urban study site, Cape Coast, can be compared to a study by Suzuki et.al that *Aedes aegypti* was predominant in an urban site in Accra, indicating that Aedes thrive in urban centers.

4.2 Repellency status

The percentage repellency was 41.17% and 39.13% in males and females respectively. The lower rate of percentage is due to the high number of mosquitoes that did not make any choice. The Means for mosquitoes that made no choice, 5.888889 (±2.315407) and 7(±2.54951) in females and males respectively were higher than those in the controls and tests for both. This may be attributed to the time taken for the mosquitoes to make decisions between the two chambers. Effectiveness of the extract could have also been affected by environmental factors such as humidity and temperature. Further studies in extending the time of decision and varying concentrations of the extract may help get best results.

A Chi-square test of independence was performed to determine if there is any significant difference on the repellency rate of *Cleome viscosa* between male and female *Aedes aegypti* mosquitoes. With a 95% confidence level, the test gave the Chi-square value (x^2), p-value and a degree of freedom (0.004271, 0.947893 and 1), respectively. Since the p-value > 0.05, it indicates that there is no significant difference between the repellency rates of males and females *Aedes aegypti* mosquitoes to *Cleome viscosa* hence failing to reject the null hypothesis. This shows that extracts of *Cleome viscosa* has repellency potential on *Aedes aegypti*. The high p-value of 0.947893 suggests that the observed difference in repellency rates between male and female mosquitoes is likely due to chance or random variation. These findings imply that *Cleome viscosa* does not exhibit a differential repellency effect based on *A. aegypti* gender. Both male and female mosquitoes are equally affected by the repellent properties of Cleome, or lack thereof. These findings confirmed a study by (Rimi et.al 2017) that *Cleome viscosa* possesses insect killing properties, along with repellent and cytotoxic activities. Also, Crude extracts of *Cleome viscosa* is an excellent potential for controlling *Aedes aegypti* mosquitoes (K. Krishnappa et.al 2013). Similarly, *C. viscosa* showed insecticidal potential against pulse beetle *Callosobruchus chinensis*. Various solvent extracts such as acetone, chloroform, petroleum ether, methanol, hexane, and water have shown very high insecticidal potential as LD50 obtained in each was very low. These also have significantly repelled large number of insects at a very low dose (Upadhyay et.al 2012). All these effects in solvent extracts are due to the presence of toxic components in plant *C. viscosa* (Upadhyay et.al 2012).

4.3 Conclusion

The results of this study showed that *Cleome viscosa* extracts have significant repellent activity against *Aedes aegypti*. Based on the analysis, it can be concluded that both male and female *A. aegypti* mosquitoes are equally affected by the repellent properties of *Cleome viscosa*. Thus, further investigation is needed to isolate the exact compound which could be a great lead of insecticide potentials. Additionally, further research is recommended to confirm these findings and explore other potential factors that may influence the effectiveness of *Cleome viscosa* as a mosquito repellent.

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