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Evaluation of the Larvicidal Activity of Acetone Extract of Cleome Viscosa Pod on the Larvae of Female Anopheles Gambiae

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ABSTRACT

Mosquitoes constitute a serious Public Health menace, resulting in millions of death worldwide each year. Emergence of insecticide resistant strains of the mosquitoes poses a serious threat and hence calls for alternative control measures. This study assessed the larvicidal efficacy of the Acetone extracts of the pod of Cleome viscosa against the 4th instar larvae of the malaria vector *Anopheles gambiae*. Larvicidal activities of the pod of the plant were therefore studied on laboratory reared larvae of *A. gambiae* at concentration ranges of 0.5 mg/ml to 5.0 mg/ml. The present scenario for commanding the mosquito is aimed at application of target and stage - specific and cost effective phyto - products. The continuous use of synthetic insecticides and its toxicity together with growing incidence of insect resistance has called to the need of novel insecticide. Plant extract may be alternative source that constitute a rich bioactive compounds that are biodegradable and environment friendly. World health organization protocol was adopted for the Larvicidal bioassay. Four (4) group of third and fourth (3rd and 4th) instar larvae were exposed to various concentrations of 500ppm, 1000ppm, 1500ppm and 2000ppm and also a control group to compare its mortality rate. Mortality rate was observed and recorded in twelve hours. The result reveals that after twenty four hours exposure to the extract, at 2000ppm, Cleome viscosa pod extract exhibited the highest mortality rate of ninety-five percent (95%). The findings also show that mortality rate was concentration dependent. The extract contained some potent larvicidal activity which might be suitable alternative to chemical larvicide.

Keywords: Evaluation, larvicidal, acetone, cleome, viscose, anopheles gambiae.

INTRODUCTION

Among the various infectious diseases, vector borne diseases are the main burden today and may be expected to represent the highest proportionate disease burden in the near future. Mainly, the insect-transmitted diseases remain a major cause of illness and death worldwide [1]. Mosquitoes (class- Insecta, order- Diptera) are the most important single group of insects which cause millions of death every year by transmitting various diseases like Dengue, Chikungunya, Yellow Fever, Lymphatic Filariasis, Japanese Encephalitis, Malaria etc. [2].

According to World Health Organization, mosquitoes are 'Public Enemy No. l' [4]. There are more than 4500 species of mosquitoes distributed throughout the world in 34 general; but mostly belongs to Aedes, Anopheles and Culex [5]. Malaria is transmitted by female Anopheles mosquito; Aedes aegypti and some other species mosquito responsible for the transmission of Dengue and Chikungunya while Culex mosquito have been incriminated for the transmission of Lymphatic Filariasis [6]. These diseases not only cause mortality or morbidity among the human but also cause social, cultural, environmental and economic loss of the society [7]. They are found throughout the world except in that places which are permanently frozen. In nearly all mosquito species, the female individuals feed on vertebrate blood to obtain their needed protein for the development of their eggs. During their feeding, a complex type of salivary

secretion occurs and the fluid is directly injected into the capillaries to enable several life forms such as viruses, protozoa, and nematode worms for the exploitation of mosquitoes as a means of transfer between vertebrate hosts. In almost all cases, within the insect, there is an obligatory phase in which pathogens multiply prodigiously in the salivary glands and can be inoculated into a new host during a later blood meal [8].

The mosquitoes anopheles gambiae, has been incriminated with the disease parasite plasmodium, responsible for the notorious malaria scourge. WHO/UNICEF (2005) in their first comprehensive report of the roll back malaria partnership stated that malaria is endemic in 117 countries with some 3.2billion people living in risk areas. It further states that each year, there are about 350 - 800 million clinical cases of malaria worldwide with over 1 million death. About 59% of all clinical cases occur in Africa, 38% in Asia, and 3% in America. Malaria mortality is also highest in Africa with 89% of all deaths where 10% occurs in Asia and less than 1% in Americas. Of all malaria cases caused by plasmodium falciparum, the most deadly human malaria parasite, 74% are in Africa, 25% in Asia and 1% in the Americas. Even where people survive malaria, the disease causes numerous health and cognitive problems. It is associated with maternal anemia during pregnancy, with low birth weight for babies, and it is a major cause of childhood anemia. Severe disease episodes (i.e., "cerebral" malaria) have been shown to cause severe long-term physical and neurological disability. There is no clear evidence on the cognitive impact of malaria on individuals who contract less severe cases of the disease, although there are reasons to suspect non-trivial effects on learning among school children.

There is no effective vaccine or inoculation to prevent malaria. However, the disease can be treated at relatively low cost (at least in its milder forms) with drugs or even simple measures to reduce the severity of symptoms. Prevention measures are also relatively inexpensive. For example, mosquito nets impregnated with insecticides, available for $5-$10 (#1700 - #3000 equivalent) each (or less), can significantly reduce exposure to mosquitoes and thereby limit malaria morbidity and mortality. But these measures may require careful implementation and recurring maintenance, which may not be feasible for many affected families.

At present, many of the world's poor countries face high rates of malaria endemism. By one estimate, about 40 percent of the world's population lives in areas where malaria is endemic, and these people are on average very poor.

According to the United Nations Children's Fund (UNICEF), "Malaria is truly a disease of poverty. It afflicts primarily the poor, who tend to live in malaria-proneareas in dwellings that offer few, if any, barriers against mosquitoes" [9]. [10] argue that "as a general rule of thumb, where malaria prospers most, human societies have prospered least. The extent of the correlation suggests that malaria and poverty are intimately related." The causality of this relationship is complicated, however. Does malaria cause poverty? Or does poverty cause malaria? Both channels of causation seem reasonable. It is also possible, as noted by [11] that the correlation could be spurious, caused perhaps by some other direct connection between climate and geography with growth rates or income levels. In spite of the difficulties involved, two widely publicized papers have found that malaria appears to slow economic growth in poor countries. Both papers use cross-country regression techniques and attempt to use instruments or controls to address the obvious causality problems. McCarthy, Wolf, and Wu (1999) find that malaria prevalence is negatively related to growth of per capita income. In turn, they find that malaria morbidity is linked to climatic differences across countries. The magnitude of malaria's effect on growth is substantial: they find that Sub- Saharan African countries experience a reduction in income growth of 0.55 percent annually because of malaria. Using a relatively similar methodology, [12] find that countries with "intensive" malaria experience a

reduction in per capita income growth of 1.3%annually. They suggest that, everything else being equal, a country experiencing intensive malaria would have its long-term level of income per capita reduced by one-third, compared with the same country in the absence of malaria.

Based on this analysis, Sachs and other authors have suggested increasing current spending on malaria control by more than an order of magnitude. Global spending on malaria prevention and control is currently around $100-200 million annually.

But based in large part on his estimates of the economic impacts of the disease, [13] has estimated that $2-3 billion in annual spending would be needed to control the disease effectively in Africa alone. These larger sums are clearly within the capacity of the international community, but they would represent a substantial fraction of total aid disbursements by rich countries. As a result, the increases would either require significant reallocation of existing aid portfolios or increases in the total quantities of foreign assistance given by rich countries with no pathognomonic findings, it is critical that emergency clinicians in nonendemic areas maintain a high index of suspicion, conduct a thorough history /travel history, and interpret indirect findings to initiate prompt and appropriate treatment. This review gathers the best evidence from international public health resources, surveillance studies, guidelines, and academic research to give emergency clinicians tools to combat these potentially lethal infections.

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors.

In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non-biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale. Thus, the Environmental Protection Act in 1969 has framed a number of rules and regulations to check the application of chemical control agents in nature. It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control. These factors have resulted in an urge to look for environment friendly, cost-effective, biodegradable and target specific insecticides against mosquito species. Considering these, the application of eco-friendly alternatives such as biological control of vectors has become the central focus of the control programme in lieu of the chemical insecticides. One of the most effective alternative approaches under the biological control programme is to explore the floralbiodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act conceitedly on both behavioral and physiological processes. Thus there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases.

Roark described approximately 1,200 plant species having potential

insecticidal value, while [14] listed and discussed 344 plant species that only exhibited mosquitocidal activity. [15] reviewed the current state of knowledge on larvicidal plant species, extraction processes, growth and reproduction inhibiting phytochemicals, botanical ovicides, synergistic, additive and antagonistic joint action effects of mixtures, residual capacity, effects on non-target organisms, resistance and screening methodologies, and discussed some promising advances made in phytochemical research. Table I summarized the mosquitocidal activities of various herbal products from edible crops, ornamental plants, trees, shrubs, herbs, grasses and marine plants according to the exaction procedure developed in eleven different solvent systems and the nature of mosquitocidal activities against different life stages of different vector species as a ready reference for further studies. Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920s, but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme.

After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make up to 1 per cent of world's pesticide market. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots, pods etc ., of larger plants or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control. Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species havebeen known to produce chemical factors and metabolites of value in pest control programme. Members of the plant families- Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various types of larval, adulticidal or repellent activities against different species of mosquitoes.

Aim of the Study

The aim of the study is to investigate and evaluate the larvicidal effect of cleome viscosa pod on the larva of anopheles gambiae.

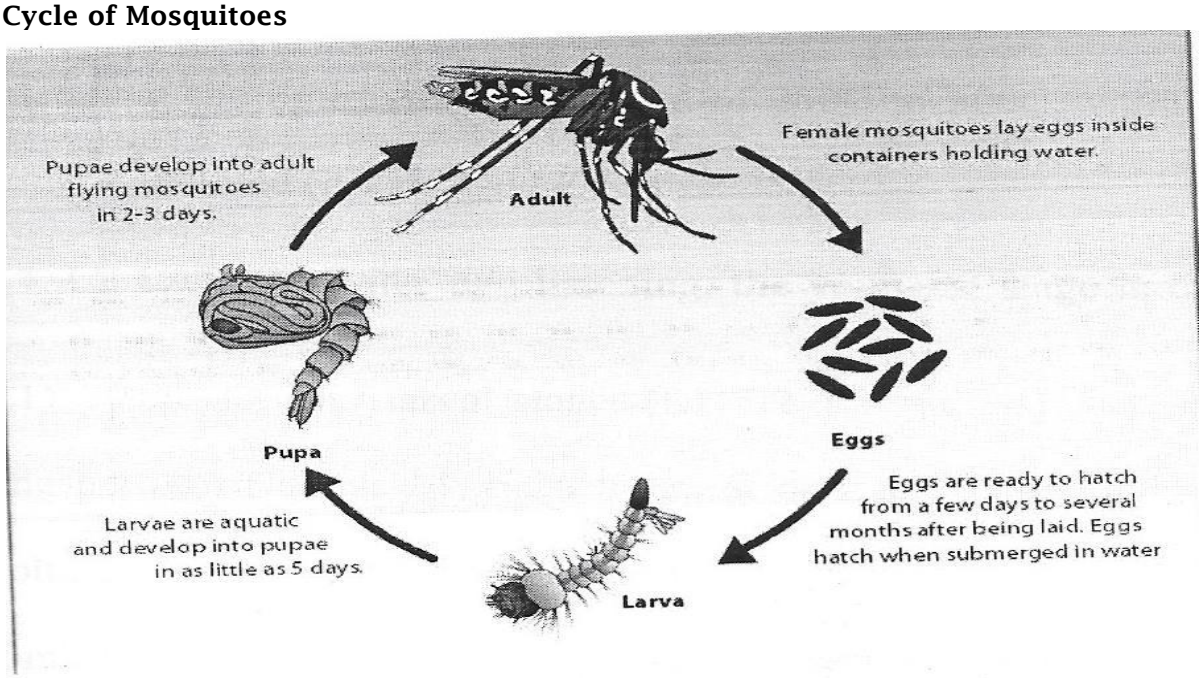
Significance of the Study

The research work will give a clear view of the larvicidal effect of cleome viscosa pod on mosquito larvae. Cleome viscosa pod is one of uncommonly used larvicide because the plant is considered as roadside weed and is irrelevant. The result of this work will nevertheless place this plant in an actual position of relevance as larvicide and mosquito control in both Nigeria and beyond and also suggest impossible means of reducing the drawbacks while increasing the benefits.

Limitations of the Study

Certain difficulties were encountered in the course of this study, among them is financial constraints; the cost of reagents was high. I also encountered bad road while conveying to and fro the area of sample collection. Time factor was also another difficulty encountered in the course of the study.

Cycle of Mosquitoes



(National Centre for Emerging and Zoonotic Infectious Diseases Division of vector - borne diseases Ghana)

MATERIALS AND METHODS

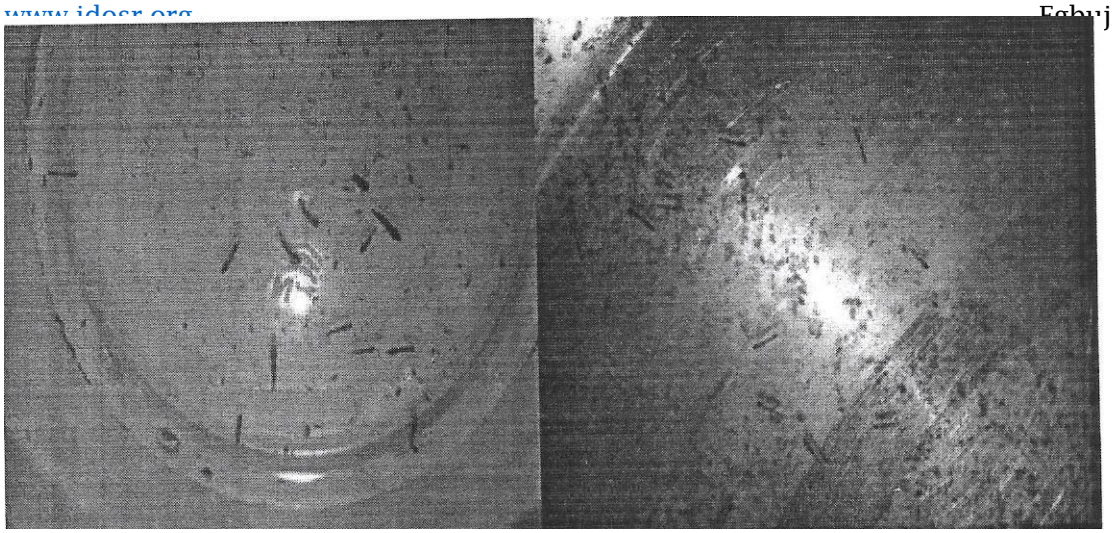
Plant Source

The plant *Cleome viscosa pods* was collected from Institute of Management and Technology (IMT) Enugu. The identification of the plant leaves was confirmed by a Botanist of the University of Nigeria Nsukka. The collected leaves were washed repeatedly with water and finally with distilled water. The leaves were sliced into small pieces and were shade dried for the period of 1 month. No mould growth was observed during the period of drying. The dried pod of Cleome viscosa were powdered with a sterile mortar and pestle. The powder was stored in an air tight container and kept in a cool dark and dry place prior to extraction process.

Anopheles Mosquito Larvae source, and Precautionary Measures to Prevent Larval Mortality before Experimental Test

The Anopheles mosquito larvae where collected from a stagnant water at various locations at Obinagu Idodo and Eziobodo Idodo all in Amechi Idodo in Nkanu East Local Government Area in Enugu State using dipper and pipette as described [16]. The collected larvae were free from tadpoles which may cause its mortality. In the laboratory, the larvae were transferred to enamel larval trays until adult emergence. After emergence, the mosquitoes were identified by a professional Parasitologist, Dr Goddy Ngwu of the Department of Zoology, University of Nigeria, Nsukka. Cyclic generations of A. gambiae were maintained in a 29 cm x 21.5 cm x 56.5 cm cages with potted plants. Mean room temperature of(27± 2°C) and a relative humidity of 70-80 percent were maintained in the insectary.

The adult mosquitoes were fed on ten per cent glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with laboratory reared albino mice. Ovitraps were placed inside the cages for egg laying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (Quaker oat and yeast in the ratio 3:1). 3rd and 4th instar larvae were then picked for larvicidal bioassay.



The instar anopheles' larvae following incubation

Preparation of Crude Extract

Extraction

The crude extract of the grounded *Cleome* viscosa pod (20g) was carefully weigh into a 250ml Conical flask and shaken vigorously intermittently for 72hrs. The coloured mixture was filtered into empty conical flask then concentrated to l/5th it's initial volume over a boiling water bath. The concentrated extract was stored in a 100ml wide mouthed amber coloured reagent bottle, stoppered, labelled, and kept carefully until used.

Preparation of test Concentration

The stock solution of the extract was prepared by dissolving 10ml of the extract in little volume of distilled water, then made up to 1000ml with same distilled water.

The working solution containing the following concentrations, 500ppm, 1000ppm, 1500ppm, 2000ppm was prepared by serial dilution. Treated control was prepared by 1ml of acetone to 200ml of distilled water and to another glass beaker 200ml of Acetone as control. The subsequent concentrations were stirred gently to distribute evenly. They are labeled adequately and kept safe prior to larvicidal bioassay.

Larvicidal Bioassay

Larvicidal bioassay of individual plant extracts was tested against 4th instar larvae of A. gambiae. The tests were conducted in 100ml glass beakers, in accordance with [17] protocol with slight modification. Three replicates and a control were run simultaneously during each trial. For control, 5ml of 20% DMSO in 995ml of distilled water was used. Twenty healthy larvae were introduced into each glass beaker and mortality was observed at 24, 48 and 72 hrs after treatment with extract concentrations of 10, 15, 20, and 25mg/ml. The treatments were maintained at room temperature. Larvicidal activity of each extract was determined, by counting the number of dead larvae on daily basis (24hrs interval). The moribund and dead larvae in the three replicates were combined and expressed as percentage mortality for each concentration. Dead larvae were recorded when they failed to move after probing with a needle. Moribund larvae were those unable to rise to the surface within reasonable period of time. The percentage mortality was calculated and analysis of data was carried out by employing probit analysis.

$$\% \text{ Mortality} = \frac{\text{Number of Dead Larvae}}{\text{Number of Larvae Introduced}} \times \frac{100}{1}$$

Determination of LC50 and LC95

The 24, 48 and 72h lethal concentration values (LC50 and LC95) will be determined by probit analysis as described by [18]. SPSS version 16 will be employed in the analysis.

Statistical Analysis

Using probit regression analysis table, regression times was plotted for dose response to Acetone Extract of Cleome viscosa pod treatment on *Anopheles Gambiae* larvae. The lethal dose to cause 50% mortality on the population (LD_{50}) was measured at 1000ppm.

RESULTS

Table **1: A Result Showing the Effect of Different Concentrations of Acetone Extract of Cleome Viscosa Pod on Anopheles Gambiae Larvae at Different time Interval**

Extract concentration (ppm)	Number of larvae exposed	Number of larvae died in 24hours	Number of larvae died in 48hours	Number of larvae died in 72hours
Treated control	20	0	0	0
Untreated control	20	0	0	0
500ppm	20	8	10	14
1000ppm	20	11	12	16
1500ppm	20	12	17	20
2000ppm	20	15	20	20

Figure **1: Result Showing Mortality Rate of Different Concentrations of Acetone Extract of Cleome Viscosa Pod on Anopheles Mosquito Larvae at Different Time Interval**

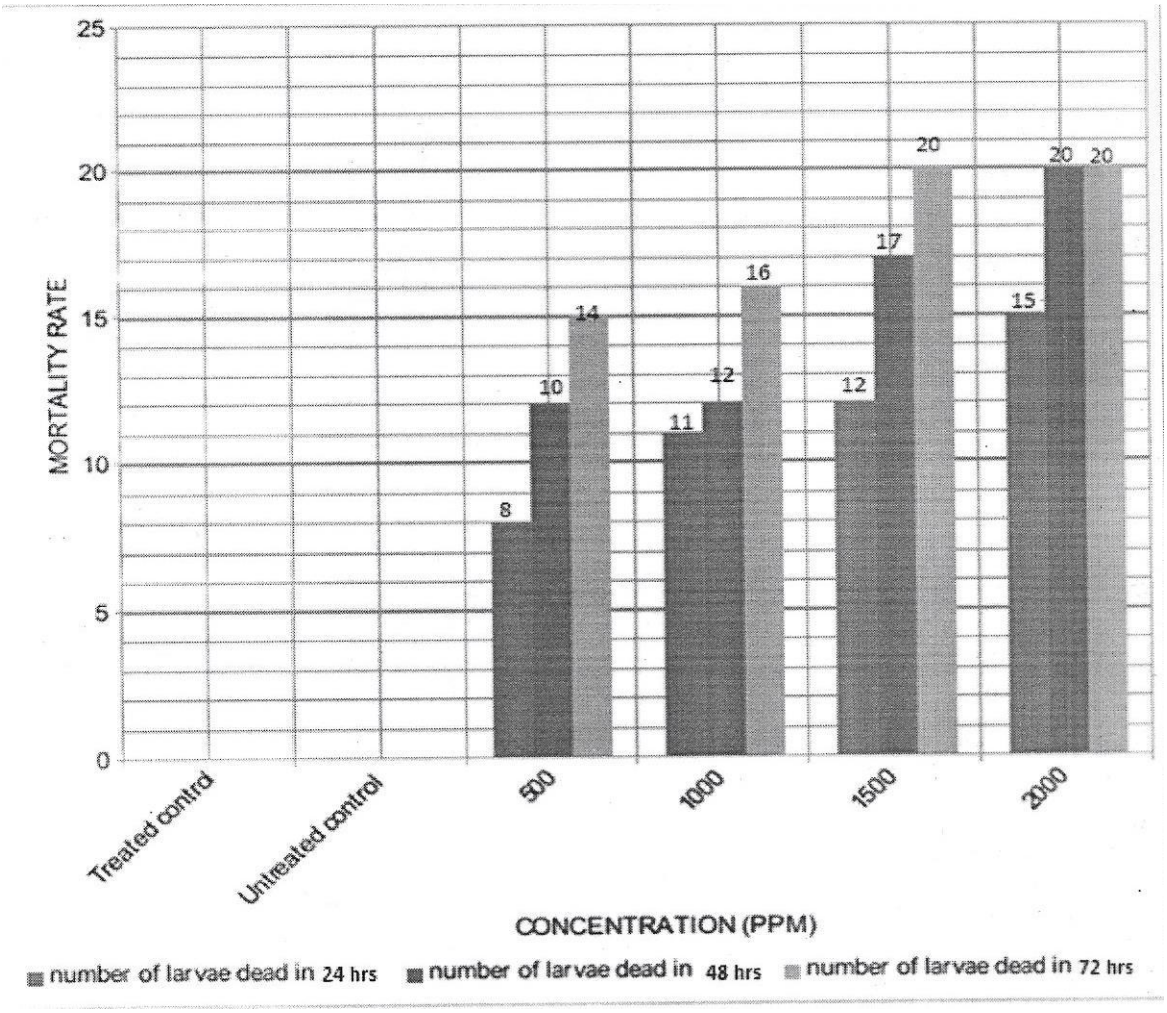


Table **2: Result Showing the Different Concentrations of Extract (PPM) and percentage (%) Dead and alive in 24 hours**.

Extract concentration (ppm)	Number of larvae exposed	Number of larvae died in 24hours	Percentage (%) dead	Percentage (%) alive
Treated Control	20	0	0	0
Untreated control	20	0	0	0
500ppm	20	8	40	60
1000ppm	20	11	55	45
1500ppm	20	12	60	40
2000ppm	20	15	75	25

Figure **2: A Chart Showing Percentage (%) Dead and Alive Within 24 hours against Concentration Extract (PPM).**

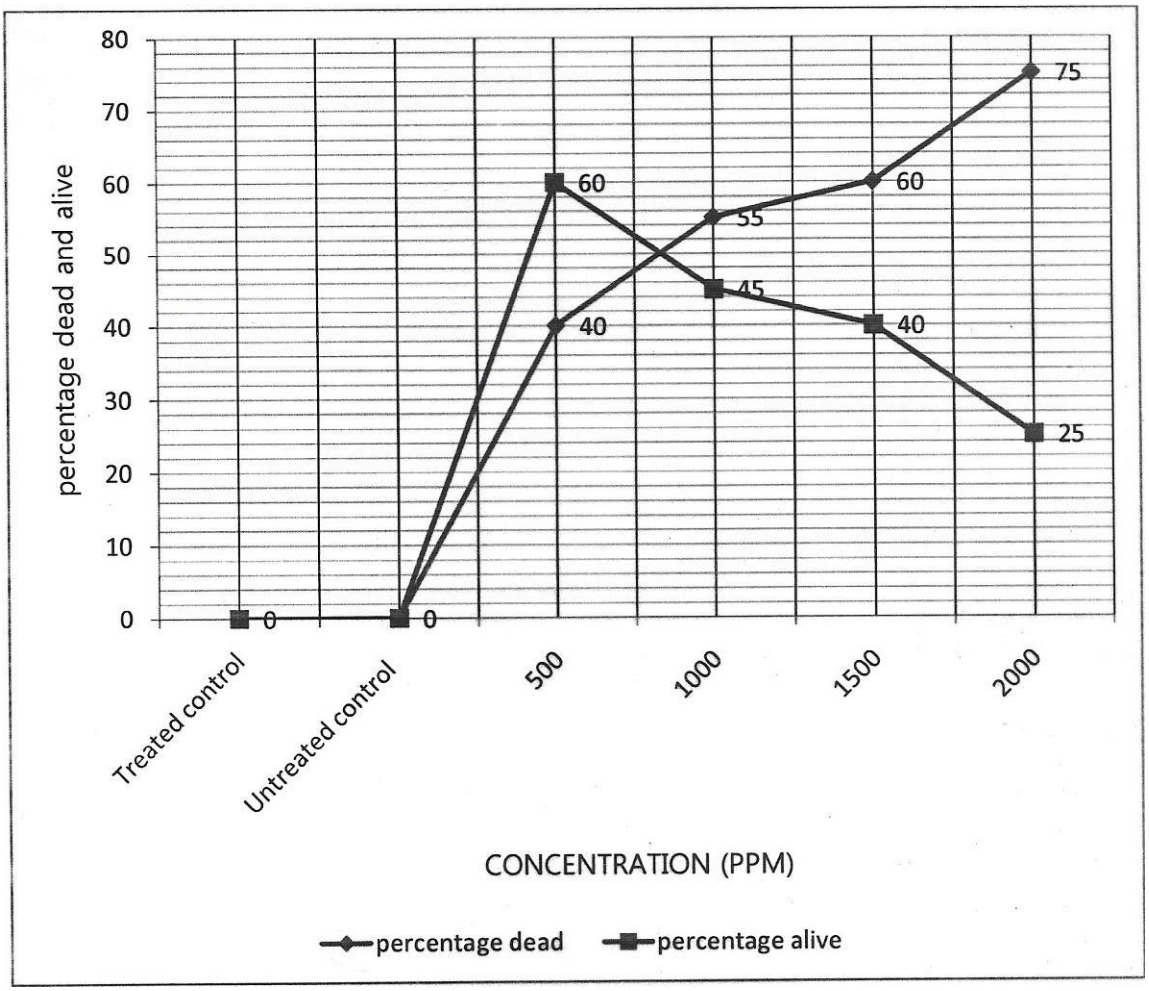


Table 3: **Result showing the different concentrations of extract (PPM) and percentage (%) Dead and Alive in 48 Hours**

Extract concentration (ppm)	Number of larvae exposed	Number of larvae died in 48hours	Percentage (%) dead	Percentage (%) alive
Treated control	20	0	0	0
Untreated control	20	0	0	0
500ppm	20	10	50	50
1000pm	20	12	60	40
1500ppm	20	17	85	15
2000ppm	20	20	100	0

Figure **3: Chart showing percentage (%) dead and alive within 48 hours against concentration of extract (PPM)**

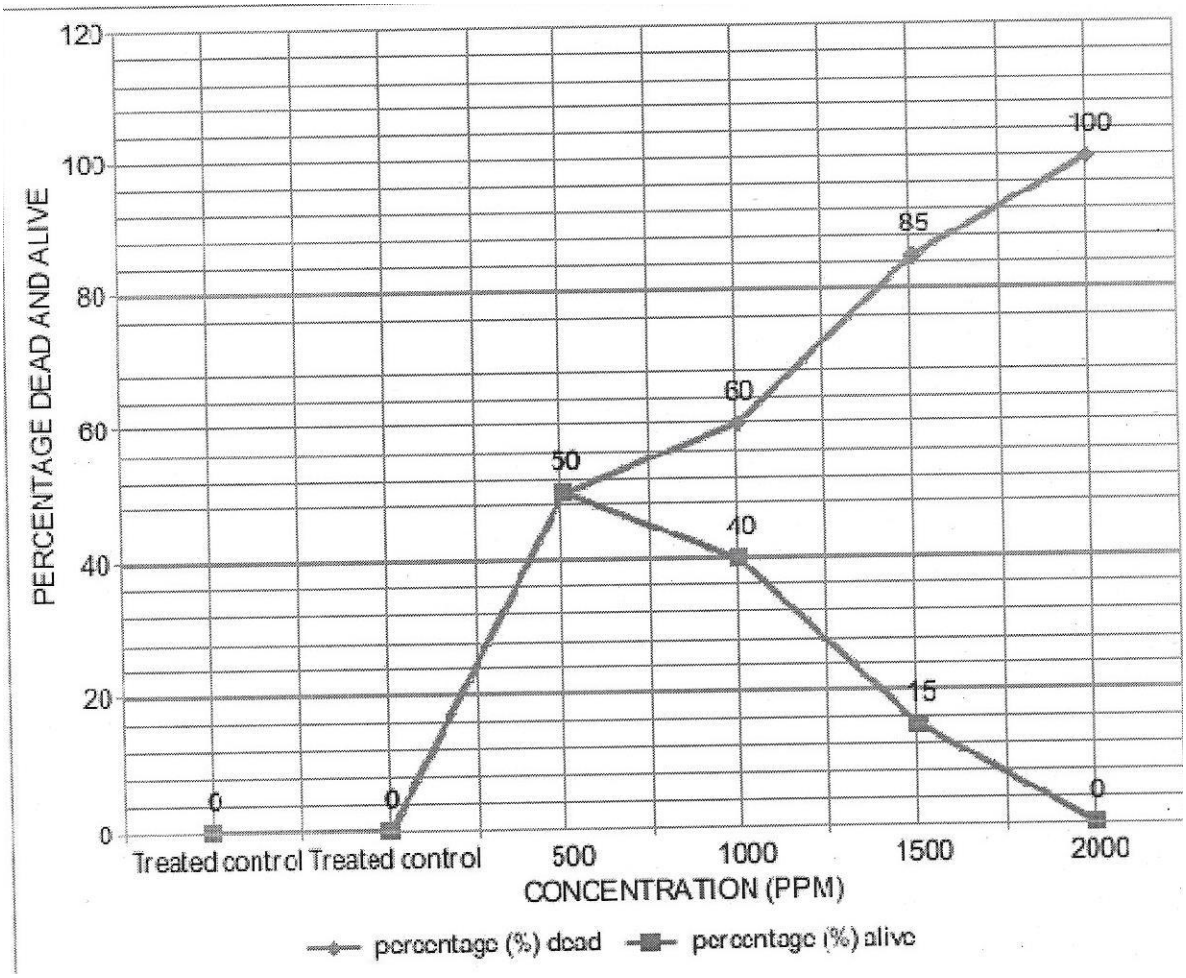
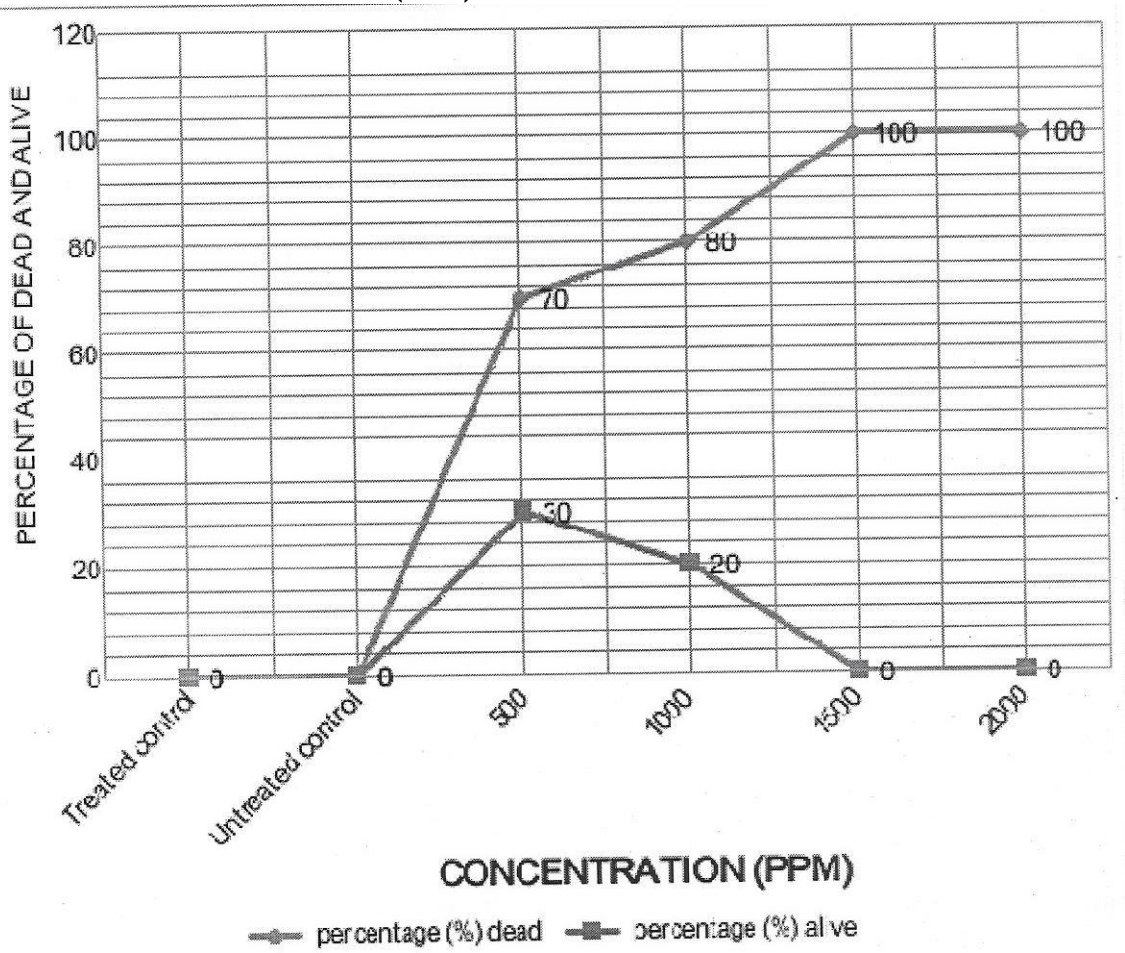


Table 4: **Result showing the different concentrations of extract (PPM) and percentage (%) dead and alive in 72 hours.**

Extract concentration (ppm)	Number of larvae exposed	Number of larvae died in 48hours	Percentage (%) dead	Percentage (%) alive
Treated control	20	0	0	0
Untreated control	20	0	0	0
500ppm	20	14	70	30
1000ppm	20	16	80	20
1500ppm	20	20	100	0
2000ppm	20	20	100	0

Figure **4: Chart showing percentage (%) dead and alive within 72 Hours against concentration of the extract (PPM)**



DISCUSSIONS

The plant kingdom has proved to be a reliable reservoir of potent phytochemicals which can serve as suitable, efficient, readily available and eco-friendly alternatives in the fight against insect pest. In line with the search for compounds with excellent activity against insect pest, this work evaluates the larvicidal potentials of crude acetone extract of the pod of the plant Cleome viscosa on the malaria vector, Anopheles gambiae. It is pertinent to state that no previous work on the larvicidal potential of the pod sample of this plant has been conducted.

Mosquitoes in the larval stage are attractive and target for pesticides because they breed in water. However, they introduce many risks to people and the environment due to the continuous increase in their presence. Better alternative means were sorted out [19]. Natural pesticides especially those derived from plants are more promising in this aspect [20]. In this study, Cleome viscosa pod were found to have some larvicidal activity against mosquito larvae at different concentration rates in part per million (ppm) as shown in tables 1. The result obtained as shown in chapter 4 showed the change in mortality rate of larvae with increase in the concentration of Cleome viscosa pod extract compared to control. Twenty motile larvae each per three hours was introduced into a glass beaker containing different extract concentration (ppm) of Cleome viscosa pod.

Twenty larvae was introduced into a glass beaker containing distilled water treated with 5ml of acetone used as treated control and twenty larvae was introduced into a distilled water and used as untreated control.

On day one, (after 24 hours),at a concentration of 500ppm,8 larvae

representing 40%,lost activity and did not respond to stimulus of touch with pasture pipette. At 1000ppm, 11 larvae lost activity representing 55% of the entire larvae population. At 1500ppm, 12 larvae out of 20 died representing 60% and at 2000ppm, 15 larvae died representing 75% of the total larvae population.

At bar chart presentation of percentage mortality against concentration was progressive after 24 hours. After 48 hours on day 2, at a concentration of 500ppm, 10 larvae representing 50% lost activity and did not respond to stimulus of touch with pastures pipette. At l000ppm, 12 larvae lost activity representing 60% of the entire larval population. At 1500pm, 17 larvae out of 20 larvae died representing 85% and at 2000ppm, 20 larvae died representing 100% mortality rate.

A bar chart presentation of percentage mortality against concentration was equally progressive after 48 hours. The total number of mortality for all the concentration within 48 hours was the sum of individual mortalities at respective concentrations, so 59 larvae out of 80 larvae exposed to different concentrations died on day 2 (48 hours' exposure).

The control had an extra at CT concentration of Zero. 20 larvae were exposed to acetone to which the feed materials were added. There was no death recorded, authenticating the above observation that the mortality was due to exposure to of Cleome viscosa pod.

On day 3 at the same concentration of 500ppm ,14 out of 20 larvae exposed died, representing 70% mortality rate. 16 larvae died as the concentration increased to l000ppm,representing 80%.At 1500ppm and 2000ppm, 20 larvae died representing 100% respectively. Since higher mortality was recorded at the same concentration as the days go by, it could then be said that mortality was time dependent also. The longer the time of exposure and higher the concentration of extract, the higher the mortality. This observation agrees favourably with different studies by independent researchers [21]. On day 3 being the last day, a total number of mortality rate recorded for all concentration was 70 out of 80 larvae exposed representing 87% mortality.

If the mortality at 72 hours at 2000ppm was compared with that of 48 hours, (87%) and 48 hours and 24 hours (75%). It will be observed that time was a major determinant factor.

The study shows that acetone extract of Cleome viscosa pod has larvicidal activity against the larvae of Anopheles gambiae and that the activities depend both on concentration and time of exposure.

Lethal dose concentration (LD50) was determined and its graph was plotted. The larvicidal activity was found to be higher against the larvae at the highest concentration rate of 2000ppm.

CONCLUSION

Results obtained from this study showed that Acetone extract of Cleome viscosa pod may serve as an alternative to synthetic insecticides in the control of the deadly malaria vector, A. gambiae. Phytochemicals are environmentally friendly, readily available and inexpensive and hence could serve as a more favourable option in the eradication of mosquitoes and other insect pests from our environment.

It is important to highlight that this work is a preliminary assay and hence calls for extensive work to be done especially in phytochemical analysis of the extracts. Also, the application of column chromatography and thin layer chromatography (TLC) to purify and isolate specific toxic phytochemical with bioactive potentials requires urgent attention. Determination of the structure of active ingredients by Infra-Red (IR) Spectroscopy, Nuclear Magnetic Resonance (NMR), Gas Chromatography and Mass Spectroscopy (GCMS) analysis and studies on the effects of active ingredient on non-target organisms and field evaluation of the active principle before its recommendation in vector control programme and commercial production. Again, it is also pertinent to state that changes in environmental factors such as turbidity of the water, the pH, water temperature etc arising from the introduction of extracts might have altered the ecological balance of the habitat, thereby contributing to the

death of the larvae. Hence subsequent research should standardize these factors to its ambient levels before carrying out the bioassay.

RECOMMENDATION

I recommend that more effort would be made to make the environment friendly for the plant growth and in-tum would give a viable extract for both field use and for small/large scale vector control operations. However further studies will be made necessary to isolate and characterize the action of the active principle which are responsible for larvicidal effect and to understand exactly its mechanism of action. Also further research should be carried out at higher time than 24hours, 48hours and 72hours so as to determine its potency.

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