ORIGINAL ARTICLE

Biocontrol of *Anopheles gambiae* larvae using fresh ripe and unripe fruit extracts of *Capsicum frutescens* var. *baccatum*

Sylvester Chibueze Izah[1], Saket Singh Chandel[2], Justice Oyindeinyefa Epidi[3], T. Venkatachalam[4], Roopam Devaliya[5]

[1]Department of Biological Sciences, Faculty of Science, Bayelsa Medical, University, Yenagoa, Bayelsa, Nigeria, [2]Department of Pharmacology, Siddhi Vinayaka Institute of Technology and Sciences, Bilaspur, Chhattisgarh, India, [3]Department of Biology, Isaac Jasper Boro College of Education, Sagbama, Bayelsa, Nigeria, [4]Department of Pharmaceutical Chemistry, JKKMMRF College of Pharmacy, Namakkal, Tamil Nadu, India, [5]Department of Pharmaceutical Chemistry, Mauli College of Pharmacy, Udgir, Maharashtra, India

Abstract

Purpose: This study investigated the biocontrol of *Anopheles gambiae* larvae using fresh ripe and unripe fruit extracts of *Capsicum frutescens* var. *baccatum*. **Materials and Methods:** Ripe and unripe fruit extracts of *C. frutescens* var. *baccatum* were tested against *A. gambiae* for 24 h. The *A. gambiae* used for the study was obtained from the wild and identified following standard procedure. **Results and Discussion:** Results of the bioassay showed that 0, 50, 100, 150, 200, and 250 ppm concentration, the percentage mortality was 0.00%, 16.67%, 38.33%, 46.67%, 70.00%, and 86.67%, respectively, for the unripe extracts, and 0%, 10.00%, 26.67%, 40.00%, 40.00%, and 66.67%, respectively, for the ripe extract. Analysis of variance showed that there was a significant difference at $P < 0.05$ across the concentrations. Between unripe and ripe extracts, there were no significant variations ($P > 0.05$) at 50, 100, and 150 ppm, but significant variations ($P < 0.05$) exist at 200 and 250 ppm. The LC_{50} value was 121.65 ppm for the unripe extract and 196.61 ppm for the ripe extracts. **Conclusion and Recommendation:** From the result of this study, there is the need for research to focus on the isolation and purification bioactive compounds that make the pepper confer activity against of larvae of *A. gambiae*.

Key words: *Capsicum frutescens* fruit, extracts, medicinal plants, mosquito, vector

INTRODUCTION

Within the past decades, the use of pesticides has increased in agricultural activities and household setting. The use of chemical was originally seen as a solution to many pests. Pesticides are classified based on their chemical compositions as well as the target organisms. On targets organisms, the notable classification includes insecticides, rodenticides, fungicides, herbicides, and fumigants. The use of these pesticides can cause pollution depending on the dose/concentration. Typically, these pesticides have the tendency to drift and they could end up in the aquatic ecosystem through spill during spraying, runoff of remain of cans, and/or farmland close to the aquatic ecosystem. On the aquatic ecosystem, studies have reported that many pesticides could affect hematological, enzymes, metabolites, electrolytes, behavioral, and mortality rate of fishes.[1-4]

Insecticides are substances that are used to eliminate insects specifically. Most commercially available insecticides have the tendency to kill both insects that undergo complete (mosquito) and incomplete (cockroaches) metamorphosis. As such, insecticides most have the tendency to act on the eggs, larvae, pupa, and adult of insects such as mosquito as well as egg, nymph, and adult of cockroaches. In agricultural activities, insecticides are very useful for increased productivity, especially in areas that insects threaten agricultural produce. Again on human health, insecticides are used to control some vector-borne diseases.

Address for Correspondence:
Sylvester Chibueze Izah, Department of Biological Sciences, Faculty of Science, Bayelsa Medical, University, Yenagoa, Bayelsa, Nigeria.
E-mail: chivestizah@gmail.com

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Different brands of insecticides are available in the market today. Like most pesticides, many of the insecticides are harmful to humans, and some have the tendency to concentrate along the food chain. Many insecticides can be grouped based on their activity through systemic (insecticides with long-term activity) and contact (insecticides with short-term effect or no residual activity). Before now, many of the insecticides available in the market are produced with inorganic compounds and due to technology organic-based insecticides were developed and it works mainly by contact.

Due to the toxicity of inorganic-based insecticides, research into the production of natural products for the control of insects has been carried out and a major breakthrough had been achieved. To these effects, many plants have been found to have insecticidal potential. Authors have reported that the pharmacological characteristics of the plant are associated with their bioactive compounds.[5-12] Some of the bioinsecticides that have been well established are pyrethrum and neem.[13] These natural insecticides are less toxic and could biodegrade compare the inorganic-based insecticides.

In Sub-Saharan Africa, malaria is among the major vector-borne diseases. In 2010, about 81% of global malaria infection occurred in Africa.[14] The reported also indicated that about 50% of world malaria cases occurred in Nigeria, Democratic Republic of Congo, Ethiopia, and Uganda causing millions of dead per annum. Besides malaria, mosquito also transmits filariasis, yellow fever, dengue fever, encephalitis, etc.[15-17] Among the many genera of mosquito, *Aedes*, *Culex*, *Anopheles,* and *Mansonia* are known to transmit important diseases in human and animals.[15] Specifically, the *Anopheles* mosquito transmits malaria. Of all the species of *Anopheles, Anopheles gambiae* and *Anopheles arabiensis* transmit most malaria cases in Africa.[15,18,19]

In many regions that malaria is endemic, the genus *Anopheles* is the most important iniquitous dipterans fly that transmits the disease.[11,12] In addition, *Aedes aegypti* transmits chikungunya, yellow and dengue fevers, while *Culex quinquefasciatus transmits lymphatic filariasis*.[15]

Based on the safe use of natural insecticides when compared with inorganic-based insecticides, research has well carried out to determine the activities of different plant extract against the mosquito. Several plants have been used to control the larvae of *A. gambiae* using different solvents.[15] Izah[11] reported the activities of ethanolic, acetone, and crude extracts of *Capsicum frutescens* var. *minima* fruit against *A. gambiae* larvae. However, information about the biocontrol of *A. gambiae* larvae using fresh ripe and unripe fruit extracts of *C. frutescens* var. *baccatum* is scanty in literature, hence, the need for this study.

MATERIALS AND METHODS

Plant Collection and Extraction

The fruit of fresh ripe and unripe *C. frutescens* var. *baccatum* was obtained from a smallholder farmer in Ndemili, Delta state, Nigeria. About 20 g of the pepper were macerated using pestle and mortar and 100 ml of water was added. The medium was allowed to extract for 48 h. Then, after the pepper was filter pressed using a double muslin cloth. The filtrate was reconstituted with distilled water to different concentration.

Culture of *A. gambiae*

A. gambiae larvae used for the study was obtained from the wild with the assistant of baits in abandoned plastic containers half-filled with water, cotton wool, and debris. The water was carefully removed from the container containing the bait and syringe without the needle section was used to transfer the larvae. Some of *A. gambiae* larvae obtained were allowed to develop into adults and identified based on the morphology previously presented by Ahmed and Ahmed (2011), and Gimba and Idris (2014).[20,21] The *A. gambiae* larva was fed with biscuit and yeast at a ratio of 3:1 at room temperature (27 ± 3°C).

Larvicidal Bioassay

The activities of the fruit of fresh ripe and unripe *C. frutescens* var. *baccatum* against larvae of *A. gambiae* were carried out based on the scheme of the WHO[22] cited by Rathy *et al.*[23] A concentration of 0, 50, 100, 125, 150, 200, and 250 ppm of the extracts was made with dechlorinated. Then, 20 larvae of *A. gambiae* were introduced into each of the concentration. At 24 h, after the commencement of the experiment, the mortality rate was determined. The larvae that remain motionless and did not respond to prodding with a soft brush were considered dead.

The percentage of mortality was calculated as

$$\frac{\text{Number of dead larva}}{\text{Total number of larva in the experimental group}} \times 100$$

Statistical Analysis

The percentage mortality between the various concentrations was subjected to one-way analysis of variance at $P = 0.05$, and the mean value was converted to charts using Microsoft Excel. Where significant variations occurred, Duncan multiple range test statistics was used to determine the source of the variation. Furthermore, *t*-test was used to show variations between ripe and unripe extract for each concentration. The LC_{50} value was calculated based on probit analysis using Finney's table.[24] Microsoft Excel Window 2010 was used to carry out the regression analysis, the probit value against log concentration from the equation of the study was determined and the LC_{50} was calculated by substitution of the probit value of 50 and then finding the antilogarithm of the value.

RESULTS AND DISCUSSION

Figure 1 presents the activities of fresh ripe and unripe fruit extracts of *C. frutescens* var. *baccatum* against larvae of *A. gambiae* at 24 h. At 0, 50, 100, 150, 200, and 250 ppm, the percentage mortality was 0%, 16.67%, 38.33%, 46.67%, 70.00%, and 86.67%, respectively, for the unripe extracts. Statistically, there was a significant difference at $P < 0.05$ across the various concentrations except for 100 and 150 ppm that mean separation showed that there was not significantly different. Percentage mortality was 0%, 10.00%, 26.67%, 40.00%, 40.00%, and 66.67% for 0, 50, 100, 150, 200, and 250 ppm, respectively, for ripe extract. There was a significant difference at $P < 0.05$ for the various concentrations. Pair-wise comparisons between the various comparisons showed that there is no significant difference between 0 and 50 ppm, and between 150 and 200 ppm. Between unripe and ripe extracts, there were no significant variations ($P > 0.05$) at 50, 100, and 150 ppm, but significant variations ($P < 0.05$) exist at 200 and 250 ppm [Table 1]. The findings of this study revealed that the stage of the development of the pepper (*C. frutescens* var. *baccatum*) can induce varying toxicity in the larvae of *A. gambiae.* In general, the unripe extract had a superior effect which was relatively higher at 50–150 ppm and statistically different at 200–250 ppm [Figure 1 and Table 1]. The variation in the toxicity could be due to differences in the moisture content of the pepper as well as the concentration of the active ingredients of the pepper that makes it conforms the larvicidal activities.

The LC_{50} value of *A. gambiae* larvae exposed to unripe fresh fruit extracts of *C. frutescens* var. *baccatum* fruit for 24 h is 121.65 ppm [Figure 2], while the LC_{50} values of *A. gambiae* larvae exposed to ripe fresh fruit extracts of *C. frutescens* var. *baccatum* fruit for 24 h are 196.61 ppm [Figure 3]. Again the LC_{50} value showed that the unripe extract had superior activity against the larvae of *A. gambiae.* The relative difference may be associated with the concentration of the active ingredients in each of the paper.[11,12] Some of the active compounds that have been reported in different varieties of *C. frutescens* include alkaloids, tannins, steroids, glycosides, saponins,

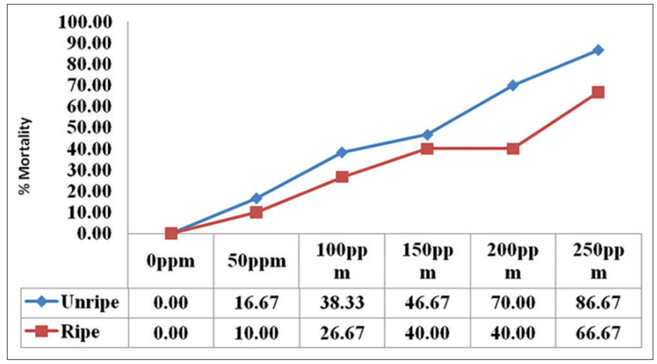

Figure 1: Activities of fresh ripe and unripe fruit extracts of *Capsicum frutescens* var. *baccatum* against larvae of *Anopheles gambiae* at 24 h

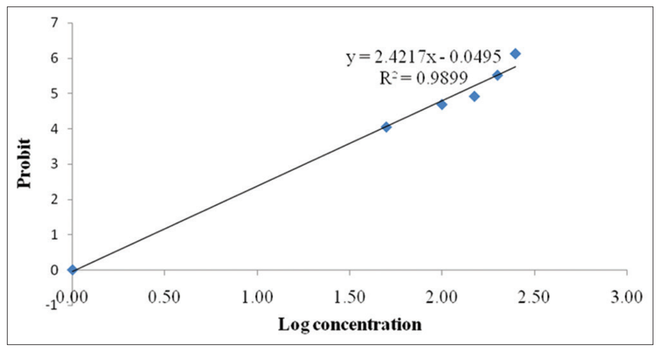

Figure 2: LC_{50} value of *Anopheles gambiae* larvae exposed to unripe fresh fruit extracts of *Capsicum frutescens* var. *baccatum* fruit for 24 h

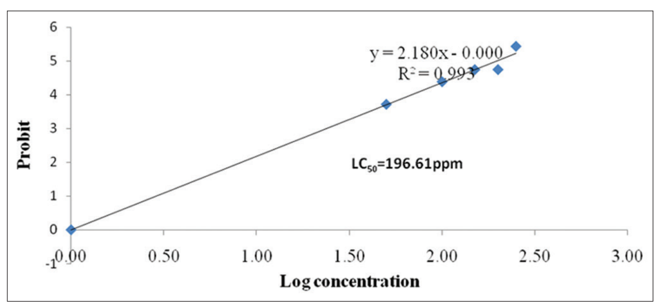

Figure 3: LC_{50} value of *Anopheles gambiae* larvae exposed to ripe fresh fruit extracts of *Capsicum frutescens* var. *baccatum* fruit for 24 h

Table 1: Comparative activities of fresh ripe and unripe fruit extracts of *Capsicum frutescens* var. *baccatum* against larvae of *Anopheles gambiae* at 24 h

Concentration, ppm	Unripe (*n*=3)	Ripe (*n*=3)	*t*-test	*P*-value
50	16.67±5.77	10.00±5.00	1.512	0.205
100	38.33±7.64	26.67±7.64	1.871	0.135
150	46.67±2.89	40.00±5.00	2.000	0.116
200	70.00±5.00	40.00±5.00	7.348	0.002
250	86.67±2.89	66.67±7.64	4.243	0.013

Data are expressed as mean±standard deviation flavonoids, phenol, carbohydrate, protein, reducing sugar, and capsaicin.[25-27] Basically, capsaicin accounts for the pungent smell of the pepper,[27] while alkaloids may account for its ability to wade of pests.[28] The LC_{50}values of this study vary from the LC_{50} value of 231.59 ppm (*A. aegypti*) and 300 ppm (*Aedes albopictus*) for crude extracts and 97.22 ppm (*A. aegypti*) and 41.74 ppm (*A. albopictus*) for ethyl acetate fraction of *C. frutescens* fruit as reported by Alvarez *et al*.[29] Youkparigha and Izah[12] reported LC_{50} value of 316.96 ppm, 176.20 ppm, and 136.14 ppm for cold water extracts, hot water extracts, and boiled extracts of ginger, *Zingiber officinale,* respectively, against *A. gambiae*, a malaria vector. Izah[11] reported LC_{50} value of 115.24 ppm, 173.16 ppm, and 265.19 ppm for ethanolic, acetone, and crude extracts of *C. frutescens* var. *minima* fruit, respectively, against *A. gambiae* larvae. The variation may be due to the biochemical/genetic makeup of the different mosquito as well the concentration of active compounds found in the pepper.[11,12]

CONCLUSION

This study evaluated the activities of fresh ripe and unripe fruit extracts of *C. frutescens* var. *baccatum* against larvae of *A. gambiae* at 24 h. The study found that unripe extract had LC_{50} value of 121.65 ppm being apparently superior compared to the ripe (with LC_{50} value of 196.61 ppm) extracts. The variations may be associated with the moisture content and difference in the phytochemical composition of the pepper that makes it confers larvicidal activity. As such, there is the need for research to focus on isolation and purification of the exact compound that makes the plant to kill the larvae of *A. gambiae.*

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