

# *Capsicum chinensis* (Hot Pepper) Powder Larvicidal Activity Against Mosquitoes Larvae in Lafia Local Government Area, Nasarawa State, Nigeria

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## ABSTRACT

Mosquitoes spread more diseases than other known arthropod and have shown resistance to conventional insecticides despite several control efforts, prompting the need to explore alternative control measures such as the use of bio-larvicides which are environmentally friendly. To this end, a study on larvicidal activity of the powder of *Capsicum chinensis* against mosquitoes larvae in Lafia Local Government Area, Nasarawa State, Nigeria was carried out through collection of wild field mosquitoes larvae from April to July 2018. Fresh *Capsicum chinensis* fruits were collected from farmlands and dried under room temperature and further processed to fine powder from which varying concentrations were used against the larvae. The larvae were exposed at 24, 48 and 72 hours, respectively. *Anopheles gambiae* larvae were susceptible (100% mortality) to the various concentrations of the powder at the end of the 72 hours exposure period while *Culex quinquefasciatus* were resistant (0% mortality). There was a very high significant difference ( $P < 0.0001$ ) in mortality rate of *An. gambiae* larvae in relation to concentrations while there was no significant difference ( $P = 1$ ) in mortality rate of *Cx. quinquefasciatus* across concentrations. LD<sub>50</sub> and LD<sub>90</sub> values for *An. gambiae* at 24 hours were 21.88mg/mL and 52.48mg/mL respectively; 16.98mg/mL and 38.90mg/mL respectively at 48 hours and 13.80mg/mL and 30.19mg/mL respectively at 72 hours. This study shows that *Capsicum chinensis* is a promising bio-larvicide for controlling *An. gambiae*. Also, *Cx. quinquefasciatus* may possibly require higher multiple doses of *Capsicum chinensis* to influence mortality.

## Introduction

Mosquitoes are considered insects of public health importance due to their ability to transmit a variety of diseases including West

Nile, dengue, yellow fever, lymphatic filariasis (elephantiasis) and malaria which is the leading cause of morbidity and mortality in

Nigeria [1]. Malaria, which causes about 1.2 million deaths (majorly among pregnant women and children under five years old) is caused by *Anopheles gambiae* s. l. [2]. Despite several efforts in controlling this vector, the medical and economic burdens caused by it continue to grow [3]. Currently, mosquito control strategies are fixated on the use of synthetic insecticides as constituents for Long Lasting Insecticide Treated Bed Nets (LLINs) and Indoor Residual Sprays (IRS) as methods recommended by the World Health Organization (WHO) [4]. Unfortunately, the success recorded by this strategy in terms of reduction of morbidity and mortality [5] is short-lived as mosquito resistance to insecticide has increased through time [6-11]. The failure in current control measures and the growing insecticide resistance is necessitating the search for newer and more effective control strategies [12]. The use of botanicals (plant-based products) is one of the best alternatives to synthetic insecticides [13, 14]. This is because they offer a more environmentally friendly method of mosquito control [15] in that they have very weak adverse effect on non-target subjects [16] and are easily biodegradable [17]. Consequently, several plant species have been employed, worldwide, to control mosquito populations.

*Capsicum chinensis* (hot pepper) has both medicinal and insecticidal value and is used traditionally as medicine for treatment of various illnesses [18] including asthma, pneumonia, diarrhea, cramps, indigestion and toothache and it has been reported to possess repellent activity against insect pests of stored grains [19]. Extracts of *Capsicum* species have been proven as repellants to some species of some stored product beetles such as *Sitophilus zeamais* Motschulsky (Coleopteran: Curculinidae) and *Tribolium castaneum* (Herbst) (Coleopteran: Tenebrionidae) [20]. There are reports of using *Capsicum* as biopesticides against Alfalfa weevil larvae *Hyper brunneipennis* [21]. The toxicity of *Capsicum* species against insects is thought to be the effects of secondary metabolites such as alkaloids, saponins and flavonoid compounds [22]. Against this backdrop, this study was carried out to determine the larvicidal efficacy of the powder of *Capsicum chinensis* on mosquitoes' larvae in Lafia Local Government Area, Nasarawa State, Nigeria.

## Materials and Methods

### Study Area

The study was carried out in Lafia Metropolis with the following coordinate's 8°29'30"N 8°31'0"E/8°49'16"N 8°51'66"E. It is the capital and the largest town in Nasarawa State.

### Collection of Sample Plant

Fresh ripe fruits of *Capsicum* were collected from cultivated farmlands on 5th April 2018 along Agyaragu-Tofa road in Lafia, Nasarawa State. Taxonomic identification of the plant was carried out in the Laboratory of the Department of Botany, Federal University of Lafia by Mr. Markus Musa. The plant samples were rinsed in clean water to remove dirt/debris, sliced into parts,

then air dried under room temperature in the Laboratory of the Department of Zoology, Federal University of Lafia for 3 weeks. The dried plant was ground into fine powder in an electric grinder [23] then weighed on a weighing balance to get 20mg, 40mg, 60mg, 80mg and 100mg doses, respectively.

### Mosquitoes Larvae Collection

First and second in star larvae of *Anopheles* and *Culex* mosquitoes were collected between April and July, 2018 from different breeding sites in Lafia using standard dippers and identified using identification keys by Gillies and de Meillon [24] and Burges and Cowan [25]. The larvae were kept in containers and allowed to acclimatize for 24 hours at room temperature before carrying out the test.

### Preparation of Bioassay

The bioassay was prepared according to the recommended protocol of World Health Organization [26] for susceptibility. The larvae of *Anopheles gambiae* and *Culex quinquefasciatus* were exposed to varying doses (20mg, 40mg, 60mg, 80mg, and 100mg) of the powder of *Capsicum chinensis*. Each treatment (concentration) had four replicates and a control of two replicates. In each of the replicates, 25 first and second in star larvae were released into disposable 250 ml bowls containing 100 ml of distilled water and thereafter treatment was added. Control (0mg) was prepared as 100 ml distilled water only. The knockdown rate of larvae was recorded after 5mins, 15mins, 20mins, 30mins and 60mins, respectively. Larval mortality was recorded in 24, 48 and 72 hours exposure period. Mortality was confirmed by gentle prick on the abdomen of the larvae with a needle [26, 27].

### Test Analysis

The interpretation of the mortality rate of *Anopheles gambiae* and *Culex quinquefasciatus* larvae is as described by WHO [26] where:

- Mortality rate between 98 – 100% within the diagnostic time indicates susceptible.
- Mortality rate between 80 – 97% suggest possible resistance.
- Mortality rate < 80% indicates resistance.

### Percentage Mortality

Unmoved and moribund larvae were recorded as dead.

% Mortality = No. of Dead Larvae x 100% / Total No. of Larvae Exposed

### Determination of LD<sub>50</sub> and LD<sub>90</sub>

The mortality data were further subjected to probit analysis for estimating lethal dose values (LD<sub>50</sub> and LD<sub>90</sub>) using Finney method [28]. The 24, 48 and 72 hours LD<sub>50</sub> and LD<sub>90</sub> were determined

through Microsoft excel regression analysis using the regression equation ( $Y = a + bx$ ).

## Data Analysis

Data obtained was analyzed using R Console version 3.2.2. Pearson's Chi Square test was used to compare mortality rate of mosquitoes' larvae in relation to concentrations of *C. chinensis* powder. Level of significance was set at  $P < 0.05$ .

## Results

### Mortality Rate of Mosquitoes' Larvae in Relation to Concentrations of *Capsicum chinensis* Powder and Exposure Periods

**A. *Anopheles gambiae*:** *An. gambiae* larvae at 24 hours exposure period had highest mortality rate of 92% at 20mg/ml, 80mg/ml and 100mg/ml concentrations and no mortality (0.00%) was observed in control 0mg/ml as shown in Table 1. Therefore,

there was a very high significant difference ( $\chi^2 = 91.442$ ,  $df = 5$ ,  $P < 0.0001$ ) in mortality rate of *An. gambiae* larvae in relation to concentrations of *C. chinensis* powder. At 48 hours exposure period mortality rate of *An. gambiae* larvae was highest 98% at 60mg/ml while no mortality 0% was recorded in the control 0mg/ml (Table 1). Thus, mortality rate of *An. gambiae* larvae in relation to concentrations of *C. chinensis* powder showed a very high significant difference ( $\chi^2 = 96.835$ ,  $df = 5$ ,  $P < 0.0001$ ). *An. gambiae* larvae mortality rate at 72 hours exposure period was 100% for all concentrations except for control 0mg/ml which had 0% (Table 1). Hence, mortality rate of *An. gambiae* larvae in relation to concentrations of *C. powder* showed a very high significant difference ( $\chi^2 = 100$ ,  $df = 5$ ,  $P < 0.0001$ ).

**B. *Culex quinquefasciatus*:** No mortality was observed at 24 hours, 48 hours and 72 hours exposure period respectively in the larvae of *Cx. quinquefasciatus* in relation to varying concentrations of *C. Powder* (Table 1).

**Table 1:** Mortality rate of mosquito's larvae in relation to concentrations of *Capsicum chinensis* powder and exposure periods.

Species	Time (Hours)	% Mortality across concentrations (mg/mL)						$\chi^2$	df	P value
		0	20	40	60	80	100			
<i>An. gambiae</i>	24	0	92	90	91	92	92	91.442	5	0.0001*
	48	0	97	96	98	96	97	96.835	5	0.0001*
	72	0	100	100	100	100	100	100	5	0.0001*
<i>Cx. quinquefasciatus</i>	24	0	0	0	0	0	0	0	5	1ns
	48	0	0	0	0	0	0	0	5	1ns
	72	0	0	0	0	0	0	0	5	1ns

\*: Significant

<sup>ns</sup>: Not significant

### Lethal Dose (LD<sub>50</sub> and LD<sub>90</sub>) of *Capsicum chinensis* Powder that Exhibits Larvicidal Activity Against *An. gambiae* Larvae in Relation to Exposure Periods

The lethal dose of *C. chinensis* that will exhibit larvicidal activity against 50% and 90% respectively of *An. gambiae* larvae at 24 hours exposure period is 21.88mg/ml and 52.48mg/ml (Table 2).

Table 2 also shows that the lethal dose of *C. chinensis* required at 48 hours exposure period to exhibit larvicidal activity against 50% and 90% respectively of the *An. gambiae* larvae is 16.98mg/ml and 38.90mg/ml. After 72 hours exposure period, the lethal dose of *C. chinensis* required to exhibit larvicidal activity against 50% and 90% respectively of the *An. gambiae* larvae is 13.80mg/ml and 30.19mg/ml (Table 2).

**Table 2:** Lethal dose (LD<sub>50</sub> and LD<sub>90</sub>) of *Capsicum chinensis* Powder that Exhibits Larvicidal Activity against *An. gambiae* Larvae in Relation to Exposure Periods.

Time (Hours)	Lethal dose (mg/mL)	
	LD <sub>50</sub>	LD <sub>90</sub>
24	21.88	52.48
48	16.98	38.90
72	13.80	30.19

### Resistance/Susceptibility Profiles of Mosquitoes Larvae Exposed to *C. chinensis* Powder in Relation to World Health Organization Indices

At 24 hours exposure period, *An. gambiae* showed possible

resistance across all concentrations mortality rate range from 91% to 92% while *Cx. quinquesfasciatus* were resistant having 0% mortality rate. At 72 hours exposure period, *An. gambiae* were susceptible while *Cx. quinquesfasciatus* were resistant.

## Discussion

The present investigation revealed that the powder of *C. chinensis* showed promising larvicidal efficacy against *An. gambiae* but may not be against *Cx. quinquefasciatus*. This agrees with the studies by Abok *et al.* [14] and Dalis [29] who recorded that the leaf extracts of *H. suaveolens* were potent against the larvae of *An. gambiae*. However, this result did not conform to that of Madhumathy *et al.* [30] in which the ethanol extract of *C. annum* proved to be more effective on *Cx. quinquefasciatus* than *An. stephensi* in India. This could be due to the difference in species of *Capsicum* (*C. chinensis* - powder form and *C. annum* - ethanolic extract) used in the respective studies. The highest knockdown rate was observed at 100mg/ml dose of *Capsicum chinensis* Powder used against *An. gambiae* larvae which possibly suggest that the highest concentration get to the target site of the larvae very fast to initiate larvicidal efficacy of *C. chinensis* powder. This is in accordance with the finding by Abok *et al.* [14] in a study on *Hyptis suaveolens* extract exhibits larvicidal activity against *Anopheles gambiae* larvae at the highest concentration. Knockdown was not recorded however for *Cx. quinquefasciatus* across all doses of *C. chinensis* powder which possibly suggests them as tolerant to *C. chinensis* powder and affirms their survival in polluted water bodies.

The results obtained after 24, 48 and 72 hours exposure time in relation to *An. gambiae* demonstrated progressive increase in percentage mortality as concentrations increased. This buttresses Finney [28] assertion that larvicidal efficacy increased with an increase in concentration. The findings agree with studies by Odey [31] who investigated the larvicidal potency of the leaf powder of *Milletia aboensis* on the larval stages of *An. gambiae* and *Cx. quinquefasciatus* and Kholhring [32] who investigated the mosquitocidal activity of *M. pachycarpa* on the larvae and eggs of *Ae. Aegypti*, recording peak mortality at the highest concentration after total exposure time. Chukwujekwu *et al.* [33] who investigated the anti-plasmodia diterpenoids from the leaves of *H. suaveolens* also recorded similar results. This result also coincides with findings of Olotuah [19] who conducted a laboratory evaluation of pesticidal activities of *H. suaveolens* against stored product pests *Sitophilus oryzae*, *Sitophilus zeamais* and *Callosobruchus maculatus* and recorded peak mortality at the highest concentration (100 mg/ml) after total exposure period.

The 100% mortality recorded in *An. gambiae* larvae in relation to the treatments after 72 hours exposure period possibly suggests time is a determining factor that should be considered to achieve a good susceptibility profile. This agrees with the findings by Abok *et al.* and Odey [14, 31] who showed that exposure duration played a major role in determining the resistance/susceptibility profiles in mosquitoes. After 24-hour exposure period, the larvae of *An. gambiae* showed possible resistance to the powder of *C. chinensis* across all doses. At 48-hour exposure period, they showed possible resistance at 20mg/ml, 40mg/ml and 80mg/ml and 100mg/ml

doses of the plant powder but were susceptible to the plant powder at 60mg/ml. However, the larvae were susceptible to all doses after 72-hour exposure period. The resistance/susceptibility profiles recorded in relation to exposure periods was like the finding of Abok *et al.* [14].

The powder was shown to be more effective after 72 hours exposure time, with LD<sub>50</sub> of 13.80mg/ml and LD<sub>90</sub> 30.19mg/ml. A possible reason for susceptibility recorded may be that the plant powder used up the dissolved oxygen available in the water, making it difficult for the mosquito species to survive [14] and also from the activity of capsaicin in the plant powder, which has significant lethal and anti-feedant effect on mosquito larvae [30]. Generally, *An. gambiae* larvae were completely susceptible to all doses while absolute resistance was observed in *Cx. quinquefasciatus* larvae. Consequently, the finding by Meenakshi and Jayaprakash [34] showed that *Anopheles* larvae were more susceptible to the leaf extract of *Rhizophora mucronata* than *Aedes* larvae. Also, Kemabonta *et al.* [35] reported mortality of *An. gambiae* in relation to the insecticidal activity of essential oils for both *P. nigrum* (black pepper) and *C. longa* (turmeric) to be a 100.0%. Furthermore, the habitat of *Culex* larvae suggests a possible reason for their resistance as they thrive in dirty, toxic and polluted water unlike *Anopheles* mosquitoes that prefer clean and clear water [25].

## Conclusion

The result of this study clearly shows that *Capsicum chinensis* possesses larvicidal properties against larvae of *An. gambiae* while very high concentration of the powder of *C. chinensis* would be required to kill *Cx. quinquefasciatus*. Hence, mosquitoes breeding success can be interrupted by pouring multiple doses of the powder of *C. chinensis* in stagnant water bodies as a direct control measure on vector populations. Also, it will be good to assess the impact of *C. chinensis* powder on non-target organisms.

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