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Larvicidal and Insect Growth Regulatory (IGR) Activities of Leaf-extract of *Carica papaya* against the Filariasis Vector Mosquito, *Culex pipiens pipiens* (Diptera: Culicidae)

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Abstract This study evaluated the potentials of the leaf-extract of *Carica papaya* as a larvicide and Insect Growth Regulator (IGR) against *Culex pipiens pipiens* mosquitoes. Extract of the plant material was obtained by solvent extraction and bio-assayed against 4th instar larvae of the mosquito species, using extract-test concentrations ranging from 5.00 – 60.00 mg/l, and following World Health Organisation's protocols. The results indicated larvicidal activity 24 hours post-exposure, with LC_{50} and LC_{90} of 25.49 and 49.68 mg/l, respectively. Sub-lethal concentration of the extract elicited significant (P<0.05) growth regulatory effects against the mosquitoes; as the duration of immature stages was more than doubled (9.97±0.74 days in the untreated, as against 21.78±7.72 days in the test mosquitoes); and survival rate was reduced by >80%. The treated mosquitoes were significantly (P<0.05) smaller (wing length = 3.12±0.40 mm) than their untreated counterpart (wing length = 3.81±0.17 mm). Likewise, daily survival rate and longevity of the adult mosquitoes were significantly reduced in the treated group. The sub-lethal concentration of the extract, however, had no significant (P>0.05) effect on wing symmetry. The findings of this study suggest that *C. papaya* is a promising source of lead compounds for sustainable mosquito vector control.

Keywords Bio-assay • larval development • longevity • sub-lethal concentration • vector

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Introduction

Owing to their cosmopolitan ecology, *Culex* mosquitoes are usually the most common culicidae insect vectors around human dwellings (Olayemi and Ande 2008a; Olayemi et al. 2010). This mosquito participates significantly in the transmission of filariasis, elephantiasis, encephalitis, dengue fever, etc. The health and socio-economic burdens associated with these diseases constitute serious impediment to development in many tropical countries (Awolola et al. 2004; Braise et al. 2003; Carter Centre 2008). Filariasis alone threatens the health of about 1.3 billion people in 83 countries, especially in the tropics, where it is responsible for 15 million cases of elephantiasis (WHO 2004). WHO (2011) further estimated that the disease presently incapacitates about 40 million people worldwide. Likewise, almost 2.5 billion people live under threat of dengue, with 50 million new infections and about 24,000 deaths reported annually (da Fonseca and Fonseca 2002).

The failure of chemotherapy in effectively controlling mosquito-borne diseases (Mittal and Subbarao 2003), has re-inforced the belief that the only way to sustainably reduce the burden of these diseases to tolerable levels is by attacking the vector mosquitoes. To this end, synthetic chemical insecticides have predominantly, though ineffectively, being used in controlling mosquitoes, especially the adult stage, either as aerial sprays or impregnated bednets (Shallan et al. 2005). This strategy has failed to produce the desired results for reasons including, inadequate bednet coverage (Sukumar et al. 1991), exophagic and exophilic mosquito behaviour (Braise et al. 2003), as well as problems of widespread development of resistance, high costs and environmental toxicity hazards associated with synthetic chemical insecticides (Mittal and Subbarao 2003; Sukumar et al. 1991).

Therefore, in recent years, the search for effective eco-friendly and affordable natural mosquito larvicides of plant origin, has been intensified (Ndung'u et al. 2004; Shallan et al. 2005). This efforts have led to the isolation of phytochemical compounds with great potentials as mosquito larvicides from plant families including, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae, Rutaceae, etc. (Anyanwu and Amefule 2001; Ndung'u et al. 2004; Oparaocha et al. 2010; Shallan et al. 2005). Much earlier, Sukumar et al. (1991) reviewed botanical derivatives in mosquito control.

Among rural Africans, the pawpaw plant, i.e., *Carica papaya*, is widely recognized as an important source of medicinal and insecticidal agents. *C. papaya*, belonging to the family Caricaceae, is a fast growing tree, distributed throughout Africa (Afolayan 2003). In folk medicine, *C. papaya* is popularly used in the treatment of different ailments: the latex as anti-septic for healing burns; the leaves for removing elephantoid growths; the roots for treating syphilis; the seeds as an anti-helminthic, etc (Hewitt et al. 2002). Ethnomedically, *C. papaya* is a strong amibicide (Reed 1976), and Anibijuwon and Udeze (2009) demonstrated high anti-microbial activities of the leaf and root extracts against some pathogenic organisms of clinical importance in Southwestern Nigeria. Imaga et al. (2009) reported the use of the plant in treating sickle cell anaemia; while the leaf extract serves as prophylaxis against malaria (Satrija et al. 1994). Peter (2011) found compounds with insecticidal activity against different insects such as mosquitoes, aphids and caterpillars, in the leaf extracts of *C. papaya*; phytochemical screening of such extracts for insecticidal agents revealed the presence of alkaloids, saponins, flavonoids, cardiac glycosides, tannins, etc (Imaga et al. 2010). From literature, preparations containing these phytochemicals as active constituents, have demonstrated high insecticidal activities (Chaeib 2010; Deore and Khadabadi 2009). Shallan et al. (2005) reviewed botanical phytochemicals with mosquitocidal activities. High larvicidal activity of saponins against *Aedes aegypti* was reported by Chapagain et al. (2008), while Quevedo et al. (2011) found moderate larvicidal activity of 1-Phenyllisoquinoline against *Culex quinquefaciatus*.

Although, the medicinal importance of *C. papaya* has been well documented, there is a dearth of information on its potential insecticidal activity, especially, against mosquitoes. This study was, therefore, carried out to elucidate the larvicidal and Insect Growth Regulator activities of *C. papaya* against *Culex pipiens pipiens*, a major vector of some arboviral and filarial diseases.

Materials and Methods

Source and Identification of Plant Material

Fresh leaves of C. Papaya were collected from a plant growing naturally in Minna (Long. 6^o 33´E and Lat. 9^o 37´N), Nigeria. The plant species was identified on site and its identity further authenticated by a Botanist in the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. A voucher specimen was deposited in the Herbarium of the Department for future reference.

Preparation of Leaf-extract

The fresh leaves collected were washed and air-dried in the shade to a constant weight. The dried leaves were then pulverized, using a Q-link electronic grinding machine. The pulverized leaf granules were extracted by exhaustive aqueous-methanolic extract, using the Soxhlet apparatus, according to the techniques of Imaga et al. (2010), with slight modifications. The extraction was done by suspending 20g of the leaf granules in 250 ml of 95% methanol in the Soxhlet apparatus at 75^{o}C, in a water bath for 2 hours. The resulting crude filtrate was concentrated *in vacuo*, using a rotary evaporator while maintaining the bath temperature at 40^{o}C. The crude extract was then stored in the refrigerator at 4^{o}C until used.

Mosquito Larvae

The *Cx. p. pipiens* mosquito larvae used in this study were obtained from an established colony maintained in the Laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. The mosquito colony was reared following standard operating procedures for mosquito maintenance (Das 2007).

Larvicidal Bio-assay

The larvicidal bio-assay was performed according to World Health Organisation's standard method for testing the susceptibility of mosquito larvae to insecticides (WHO 1981). To produce graded series of extract concentrations, the crude extract was reconstituted in 95% methanol to produce a stock solution of 500mg/l (Vasudevan et al. 2009). The stock solution was then volumetrically diluted to 250ml distilled water to obtain the test solutions, ranging from 5 to 60 mg/ml, by doubling each previous concentration to obtain the next. Then, 4th instar larvae of the *Cx. p. pipiens* mosquitoes were exposed to different extract concentrations, in plastic bowls (1.25 litre capacity). There were four replicates of 25 larvae (a total of 100 larvae) for both the test and control experiments for each extract concentration. The control experiment contained only alcohol (blank). The experiments were maintained under laboratory conditions at 28.00±1.00^{o}C, 82.00±2.00% relative humidity and 12:12hr (light:dark) photoperiod. Mortality rates were observed and recorded after 24hr post-exposure period. The larvae were considered dead, if no movement was made even after probing the siphon region with a glass rod. The whole experiment was repeated again within 2 weeks.

Insect Growth Regulator Bio-assay

The growth regulatory effects of *C. Papaya* leaf-extract on the larvae, was preceded by series of larvicidal tests, to determine the highest sub-lethal extract concentration that will not produce significantly different mortality rates from the control experiments. This concentration was determined to be 5mg/l.

Then, approximately one-day old larvae were raised in culture media of the sub-lethal extract concentration, following established standard procedures for mosquito feeding and colony maintenance (Das et al. 2007; Olayemi and Ande 2008b). Both the control and test experiments were performed in six replicates and were maintained under same laboratory conditions as the larvicidal bio-assay experiments. The experiments were monitored daily at 0900hr, for dead specimens and those that that have metamorphosed to the next life stage. The emerged adult mosquitoes were maintained following an earlier described technique (Olayemi and Ande 2008b). The adult mosquitoes were monitored for daily mortality until the last specimen in a cage died. The dead adult mosquitoes had their wings detached and measured for wing length (i.e., index of adult body size) and symmetry (i.e., reflection of adult fitness), following the method of Olayemi (2008). The whole experiment was also repeated after the conclusion of the first experiment.

Data Analysis

Mortality rates and duration of the life stages were determined according to the methods of Olayemi and Ande (2009). Survival rates were calculated using the formula:

$$S_i = n_i/(X_{ni-1}) \text{ x } 100$$

where, S_i = Survival rate of life stage; n_i = number of individuals entering a life stage; X_{ni-1} = number of individuals that entered the preceding life stage.

For estimation of duration of life stages, the following formula was used:

$$D_i = T_i - (t_{i-1})$$

where, D_i = duration of life stage; T_i = present mean age; t_{i-1} = previous mean age at moulting. Daily survival rates and longevity of the adult mosquitoes were determined by calculating the proportion of individuals alive, post-emergence, on a daily basis and the total number of days lived by an adult mosquito.

The mortality data obtained post-24hr exposure from the larvicidal tests, as well as, those of various life stages from IGR experiment, were corrected by Abbot's formula

Table 1 – Larvicidal activity of methanolic leaf-extract of *Carica papaya* against *Culex pipiens pipiens* mosquito larvae

Mosquito Species	LC_{50} (95% Confidence Limit)	LC_{90} (95% Confidence Limit)	Regression Equation	χ^2
Culex pipiens pipiens	25.49 (22.76 – 29.88)	49.68 (46.38 – 54.25)	Y = 23.117 + 0.924	18.141

All values are in mg/l.

Table 2 – Effects of sub-lethal concentration of methanolic leaf-extract of *Carica papaya* on survival rates (%) and duration (days) of immature stages of *Culex pipiens pipiens* mosquitoes

Mosquito Life Stage	Survival Rates (%)		Development Duration (days)	
	Control Experiment	Test Experiment	Control Experiment	Test Experiment
Larval	$93.22 \pm 4.25^{b*}$	37.90 ± 6.11^{a}	8.26 ± 0.84^{a}	17.95 ± 5.20^{b}
Pupal	97.57 ± 1.49^{b}	21.14 ± 4.36^{a}	1.68 ± 0.59^{a}	3.83 ± 2.52^{b}
Total Immature	95.40 ± 2.87^{b}	29.52 ± 5.24^{a}	9.97 ± 0.74^{a}	21.78 ± 7.72^{b}

*Values followed by same superscript alphabets in a row are not significantly different at P = 0.05.

Table 3 – Effects of sub-lethal concentration of methanolic leaf-extract of *Carica papaya* on adult vectorial fitness of *Culex pipiens pipiens* mosquitoes

Adult Vectorial Fitness Parameters	Control Experiment	Test Experiment
Daily survival Rate (%)	$76.32 \pm 2.54^{b**}$	27.26 ± 3.48^{a}
Longevity (days)	18.56 ± 4.24^{b}	3.97 ± 3.75^{a}
Adult Body Size (Wing Length in mm)	3.81 ± 0.17^{b}	3.12 ± 0.40^{a}
Wing Symmetry (RtW : LtW)*	$1.01 : 1.00^{a}$	$1.00 : 0.98^{a}$

*RtW = Right Wing; LtW = Left Wing
**Values followed by same superscript alphabets in a row are not significantly different at P = 0.05.

(Abbot 1925), if control mortality was between 5 and 25%, while those above 20% were repeated. The LC_{50} and LC_{90} values for the extract concentration were obtained by Probit Analysis (Finney 1971). Mean data for IGR experiment between Control and test mosquito groups were compared by student t-tests, using SAS software.

Results

The results of the larvicidal efficacy of methanolic leaf-extract of *C. papaya* against the 4th instar larvae of *Cx. p. pipiens* are shown in Table 1. The toxic activity of the extract was reflected as LC_{50} = 25.49 mg/l and LC_{90} = 49.68 mg/l, respectively, representing the concentrations that induced 50 and 90% mortality post-24hr exposure.

Table 2 highlights IGR activities of the extract against immature stages of *Cx. p. pipiens*. Both immature survivorship and developmental rates were significantly (P<0.05) altered. Overall, immature survival rate between Control (i.e., untreated mosquitoes) and Test (i.e., mosquitoes exposed to sub-lethal concentration of extract) experiments, was reduced by >80% while, survival rates in the larval and pupal stages of the Control group of mosquitoes were above 90%, those of the test group were 37.90±6.11 and 21.14±4.36%, respectively. The durations of development of the larval and pupal stages were more than doubled in the test experiment compared with the Control. On the whole, while the development of the immature stages took 9.97±0.74 days in the Control group of mosquitoes, the test individuals took 21.78±7.72 days to complete larval and pupal development.

The effects of sub-lethal concentration of the extract on adult vectorial fitness are presented in Table 3. Daily survival rate of the adult mosquitoes dropped significantly (P<0.05) from 76.32±2.54% in the Control mosquitoes to a paltry 27.32±3.48% in the test group. Also, the untreated mosquitoes lived significantly (P<0.05) longer (18.56±4.24 days) than the test group (3.97±3.75 days). The sub-lethal extract concentration, however, had no significant (P>0.05) effect on wing symmetry though, the test mosquitoes were much smaller.

Discussion

The methanolic leaf-extract of *C. papaya* showed significant larvicidal activity against 4th instar larvae of *Cx. p. pipiens* , and such activity increased as the concentration of the test sample was raised. The larvicidal activity of the extract may be due to the insecticidal potencies for which the inherent phytochemicals of *C. papaya* leaves have been reported. Imaga et al. (2010) indicated the presence of alkaloid, flavonoid, saponins, glycosides, etc, in the extract of *C. papaya* leaves; and these compounds have been found to possess high larvicidal activities against different species of mosquitoes (Chapagain et al. 2008; Quevedo et al. 2011; Shallan et al. 2005).

In addition to larvicidal activity, the sub-lethal concentration of the extract indicated good Insect Growth Regulator (IGR) efficacy against *Cx. p. pipiens*, by eliciting significant alterations in immature developmental and adult vectorial fitness attributes. The sub-lethal concentration of the extract more than doubled the duration of immature development and reduced survival rate by >80%. These effects may be due to disruption of the endocrine mechanisms that regulate ecdysis and metamorphosis, as previously suggested for neem seed kernel extracts (Zibitz 1986). The effect of delayed immature development and reduced survival suggest that, aside the larvicidal activity of *C. papaya* leaf-extract against *Cx. p. pipiens*, as observed in this study, may mean that when applied in the field, the significant elongation of duration of immature stages and suppression of metamorphosis to successive life stages elicited by the extract could ensure that the resultant population density reduction is substantial; with attendant population reduction below the threshold required for sustaining disease transmission.

The significant reduction in longevity of *Cx. p. pipiens* mosquitoes that successfully emerged as adults indicates that the sub-lethal effects of *C. papaya* leaf-extract was successfully carried over from the immature to adult stage. This finding is very important with respect to the vectorial capacity of the species, as significant reduction in the average life-span of a mosquito population equally reduces its life-time disease transmission potential, perhaps, to a level that can no longer sustain transmission (Olayemi and Ande 2008c). The sub-lethal concentration of the extract also induced significant reduction in mosquito adult body size, probably, indicating that larval feeding activity of the mosquitoes was disrupted by the extract. According to Blanckenhorn (2000), nutritional reserve, for mosquito adult biomass and egg production, is acquired during the larval stage. However, phytochemical compounds with anti-feedant properties may interfere with optimum feeding activity of mosquito larvae (Shallan et al. 2005) hence, reduced adult body size. This finding has an important epidemiological implication for disease transmission by mosquitoes. Mosquitoes must attain a threshold adult body size to successfully transmit pathogens (Beerntsen 2000). Therefore, the production of significantly smaller mosquitoes by *C. papaya* leaf-extract may serve to complement its observed larvicidal efficacy and, thus, stand it in good stead as a source of lead substances for effective, sustainable eco-friendly mosquitocide.

Interestingly, however, the sub-lethal concentration of the extract had no significant effect on wing symmetry, probably, indicating that the body organs of the test mosquitoes were optimally formed, despite exposure to the extract. Wing symmetry is a reflection of quality of insect body organs (Leung and Forbes 1997), including those directly related to vector competence and potential, such as permeability of the walls of the salivary glands, viability of the ovaries, etc.

Conclusion

The findings of this study indicated that methanolic leaf-extract of *C. papaya* possess larvicidal and significant IGR activities against *Cx. p. pipiens* and may, therefore, serve as a viable source of effective eco-friendly lead compounds for mosquito vector control. However, further studies are necessary to evaluate the efficacies of extracts from other flora parts of *C. papaya* such as the roots, seeds etc, as well as, employ multiple extraction methods. Also, additional investigations are needed to determine exactly the constituents of *C. papaya* extracts responsible for larvicidal and IGR activities through bio-assay directed fraction, as well as, assess the non-target effects of the phytochemicals on other aquatic organisms.

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