Comparative Studies on *Larvicidal* Efficacy of Ethanolic extracts of *Carica Papaya* Leaf and Leaf Stalk against *Aedes* sp

Igimoh, Michael Uduozemhe and Omoregie, Anthony Osarobo*

Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria.

ABSTRACT

Agents of mosquito vector control over the years have been more of chemical insecticides. In order to ensure safer environment, there is the need for a more environment-friendly substitutes. Studies and reports on trials of phyto-extracts of plants parts as competent alternatives in reducing disease burden, more target-specific and causing less deleterious effects on the environments are rife. This study investigated the larvicidal efficacy of the ethanolic extracts of leaf and leaf stalk of *Carica papaya* on *Aedes* sp. larvae at different concentrations of 500 ppm, 750 ppm and 1000 ppm, at 24, 48, and 72 h with mortalities recorded accordingly. Data were analysed statistically using Analysis of Variance (ANOVA), probit analysis and unpaired t-test. Results showed that mortality occurred across all concentrations. The highest concentration of 1000 ppm recorded 76.87% and 93.60% mortalities at 72 h for the extracts of *C. papaya* leaf and leaf stalk respectively. The calculated LC_{50} and LC_{90} values at 72 h exposure time were 543.46 ppm and 1643.84 ppm for *C. papaya* leaf and 442.01 ppm and 959.71 ppm for *C. papaya* leaf stalk. Variation in larval mortality between both extracts were significant (p<0.05) at the 24 and 48 h time and 750 and 1000 ppm concentration. Phytochemical screening of the plant materials revealed the presence of alkaloids, saponins and terpenoids in equal strength in both extracts except flavonoids which were present as well in only the leaf and not the stalk extracts. Both extracts showed high larvicidal potency against the *Aedes* mosquito species.

Keywords: *Carica papaya*, *Aedes* sp., ethanolic extracts, larvicides, phytochemicals

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***Correspondence:** Omoregie, Anthony Osarobo. Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria.*
***Tel:** +2348058413493*
***Email:** anthony.omoregie@uniben.edu*

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INTRODUCTION

Integrated Vector Management (IVM) is a vector control strategy that knits together multiple control tools to achieve a common control goal. This integrative method of control is being advocated for to intensify efficacy and cost-effectiveness of control outcomes and ease the over-reliance on insecticides (WHO, 2008). The use of larvicides constitutes an integral part of most control strategies directed against mosquito vectors

(Service, 2012). However, these insecticides are mostly of chemical compositions. Pertinent concerns with their use that instigates undesirable effects on humans, other animals and the environment and, the resulting increase in reported cases of insecticide resistance has led to calls for finding more environment-friendly insecticides (Brown, 1986; Russell *et al*., 2009; Service, 2012). With an array of investigations being done and documented, the use of botanicals are proving to be sustainable, safer and effective substitutes while also serving as additional agents of biological control in IVM (Ghosh *et al*., 2012).

The Papaya plant (*Carica papaya*) apart from the nutrition it gives, possesses anticancer (Pandey *et al*., 2016; Hossain *et al*., 2020), antibacterial (Mabley *et al*., 2011; Tarkang *et al*., 2013; Singh and Rawat, 2017), antioxidant (Ying *et al*., 2021) and insecticidal (Nayak *et al*., 2007; Prabhu *et al*., 2017; Sianipar *et al*., 2018) properties. There are a number of reports on the biological and larvicidal activities of the various parts of *C. papaya* including leaves, fruits and seeds against *Aedes* mosquito species (Kovendan *et al*., 2012; Malathi and Vasugi, 2015; Chandrasekaran *et al*., 2018; Adamu *et al*., 2019; IIham *et al*., 2019). However, reports on the investigations of the leaf stalk as potential larvicides are a handful or none existent. This present study was therefore conducted to evaluate and compare the larvicidal efficacy of ethanolic extracts of the leaf and leaf stalk of *C. papaya* against *Aedes* sp. of mosquitoes

MATERIALS AND METHODS

Plant collection, processing and extraction:

Fresh plant materials identified as leaves and leaf stalks of C. papaya were collected within the premises of the University of Benin, Ugbowo Campus, Benin City, Nigeria. They were washed and shade dried (27 37°C) for a maximum of 21 days and then pulverised to powder level. The actualised weight of leaves and stalk were 20g and 90g respectively. Each material was macerated mechanically with ethanol solvent; the leaves in 500 ml and stalk in 200 ml ethanol, in a chromatographic jar for 72 h. The setup was subsequently filtered and the resulting filtrate concentrated to paste level using crucible water bath to actualise the crude extracts. The extracts were collected and preserved in a sample bottle and, kept in a refrigerator before use.

Qualitative phytochemical screening:

The phytochemical constituents of the leaf and leaf stalk extracts of *C. papaya* were analysed qualitatively in line with Keay *et al*., (1964) and Ejikeme *et al*., (2014). Compounds and constituents ascertained include alkaloids, terpenoids, flavonoids and saponins.

Collection of mosquito larvae:

Eggs and larvae of Aedes sp. were collected from stagnant water in abandoned containers around the Faculty of Life Sciences, University of Benin. These samples were then transferred to the Department of Animal and Environmental Biology where they were raised into a colony according to WHO (1975) with some modifications. The collected larvae were introduced into plastic bowls containing water and fed with yeast. Pupae were subsequently reared to adults in a 0.4m x 0.4m x 0.4m (L x B x H) mosquito rearing cages. Adult mosquito populations were fed with sugar solution and blood meals. Healthy larval populations from the colonies were used to carry out the bioassay study.

***Preparation of stock solution*:**

Standard WHO (2005) procedure was adopted in this study with slight modifications. Solid extracts of 2g each of *C. papaya* leaf and *C. papaya* leaf stalk were dissolved in separate 100ml of water to make up 2% stock solution.

Larvicidal bioassay

From the stock solution, three different test

concentrations of 500 ppm, 750 ppm and 1000 ppm were prepared by adding 5 ml, 7.5 ml and 10 ml from stock solution to each plastic container and diluted to 100 ml respectively. A test solution without any extract was used as control (0 ppm). A mixture of 10 third and fourth instar larvae of *Aedes sp*. mosquitoes were introduced into each test bowls with the various concentrations for 72 h. For each experimental plastic bowl of a test concentration, three replicates were maintained. Larval mortality was observed and recorded at an interval of 24 h, 48 h, and 72 h post-exposure.

Statistical analysis:

Percentage mortality was calculated from the mortality data and when the control mortality ranged from five to twenty per cent, the observed mortality was corrected using the Abbott's formula (Abbott, 1925):

Percentage Mortality, %M = $(Nd_L \times 100) / Nd_t$

Where

Nd_L = Number of dead larvae or pupae;

Nd_t = Number of introduced larvae

The Abbott's formula used is

Corrected Mortality,

$M_{cor.} = [(M_T - M_C) / (100 - M_C)] \times 100$

Where

M_T = % test mortality;

M_C = % control mortality.

Larval mortality data were subjected to probit analysis to calculate the lethal concentrations (LC_{50} and LC_{90}) of each plant extract against *Aedes* sp. larvae at 95% confidence limits, utilizing the Statistical Package for the Social Sciences (SPSS) 16.0. Larvicidal mortality in relation to exposure time and test concentrations were also analysed using one-way Analysis of Variance (ANOVA) followed by the Duncan's Multiple Range test (DMRT) to determined source of significant difference. Difference in variation in mortalities between the study extracts at 24, 48 and 72 h intervals was determined using the unpaired t-test on Microsoft Excel 2010. Significance level was set at $p<0.05$.

RESULTS

Qualitative phytochemical constituents of the ethanolic extracts of C. papaya leaf and stalk

Phytochemical analysis revealed the presence alkaloids, flavonoids, terpenoids and saponins in varying degree in the ethanolic extracts of *C. papaya* leaf. The extracts of *C. papaya* stalk had similar phytochemical constituents and in similar strengths to that of the leaf except for flavonoids, which was absent (Table 1).

Table 1: Qualitative phytochemical content of the ethanolic extract of *C. papaya* leaf *and* Stalk

Plant Parts	Phytochemicals			
	Alkaloid	Flavonoids	Terpenoids	Saponins
***C. papaya* Leaf**	+++	+	++	+++
***C. papaya* stalk**	+++	-	++	+++

Key: +++ highly present, ++ moderately present + slightly present and - absent

Effect of time and concentration on larval mortality

There was no significant difference (p>0.05) in larval mortality for the test larvae exposed to all the different test concentrations of *C. papaya* stalk at all the time intervals and likewise *C. papaya* leaf except at 72 h wherein there was statistical significance (p<0.05). A clear steady increase in percentage mortality was observed as test concentrations increased at the 24 h and 72 h exposure time in *C. papaya* leaf bioassay. Highest mortalities per exposure time were always observed in larvae also exposed to highest test concentration of 1000 ppm (24 h = 60.17%; 48 h = 60.17%; 72 h = 76.87%) (Table 2). *C. papaya* Stalk bioassay also showed increase in larval mortalities with increasing concentrations in all the exposure time intervals of 24, 48 and 72 h. Highest mortalities in each exposure time interval was similarly observed in highest exposure test concentration of 1000 ppm (Table 2).

Table 2. Effect of conc. of *C. papaya* leaf and stalk on mortalities at 24 h, 48 h, and 72 h

Plant Parts	Conc. (ppm)	n	Mean±SD (Percentage Mortality)		
			24 h	48 h	72 h
***C. papaya* Leaf (CPL)**	0	3	0.33±0.58 (3.33)	0.33±0.58 (3.33)	0.33±0.58 (3.33)
	500	3	4.68±0.59 (46.84)	5.68±0.55 (56.84)	4.68±0.59^{b} (46.84)
	750	3	4.68±0.59 (46.84)	4.68±0.59 (46.84)	6.35±0.56^{ab} (63.54)
	1000	3	6.02±0.97 (60.17)	6.02±0.97 (60.17)	7.69±1.49^{a} (76.87)
	F-value		3.20	2.60	6.78
	p-value		0.11	0.15	0.03
***C. papaya* Stalk (CPS)**	0	3	0.33±0.58 (3.33)	0.33±0.58 (3.33)	0.33±0.58 (3.33)
	500	3	5.68±1.14 (56.84)	6.02±1.72^{b} 60.17)	6.02±1.72^{b} (60.17)
	750	3	6.68±1.50 (66.84)	7.35±1.12^{ab} (73.54)	7.69±1.49^{ab} (76.87)
	1000	3	7.69±1.49 (76.87)	9.03±0.96^{a} (90.27)	9.36±1.11^{a} (93.60)
	F-value		1.50	3.81	3.75
	p-value		0.30	0.09	0.09

Lethal concentrations (LC_{50} and LC_{90}) of *C. papaya* leaf and *C. papaya* stalk

The concentration of *C. papaya* leaf required to kill 50% and 90% of *Aedes* sp. in the study at the exposure time of 24, 48 and 72 h are given in table 3. LC_{50} and LC_{90} were correspondingly 677.63 ppm and 11499.00 ppm at 24 h; 155.25 ppm and $7.012x10^{9}$ ppm at 48 h and 543.46 ppm and 1643.84 ppm at 72 h. Moreover, *C. papaya* stalk had an LC_{50} and LC_{90} of 412.88 ppm and 2083.59 ppm; 431.42 ppm and 1093.36 ppm and, 442.01 ppm and 959.71 ppm at 24, 48 and 72 h of exposure respectively (Table 4).

Table 3: Lethal concentration of *C. papaya* leaf extract against *Aedes* larvae

Observation time		Lethal concentration
24 h	LC_{50}	677.63
	LC_{90}	11499.00
48 h	LC_{50}	155.25
	LC_{90}	7.012×10^{9}
72 h	LC_{50}	543.46
	LC_{90}	1643.84

Table 4: Lethal concentration of *C. papaya* stalk extract against *Aedes* larvae.

Observation time		Lethal concentration
24 h	LC_{50}	412.88
	LC_{90}	2083.59
48 h	LC_{50}	431.42
	LC_{90}	1093.36
72 h	LC_{50}	442.01
	LC_{90}	959.71

Variation in mortalities between C. papaya Leaf and C. papaya Stalk at 24, 48 and 72 hours

Difference in mortalities recorded in the *Aedes* mosquito population exposed to all the test concentrations of 500 ppm, 750 ppm and 1000 ppm of *C. papaya* leaf versus *C. papaya* stalk determined at the different exposure time were significant at 24 h and 48 h (p<0.05). Of the two extracts, highest larval mortality were observed in 1000 ppm of *C. papaya* stalk at both 24 h ($\bar{x}$ = 7.69) and 48 h ($\bar{x}$ = 9.03) of exposure while the least ($\bar{x}$ = 4.68) were seen in 500 ppm and 750 ppm at 24 h and 750 ppm at 48 h time of exposure of the larvae to *C. papaya* leaf. Although the leaf extracts had lower mortalities at 24 and 48 h of exposure showing a rather slow action and potency against the test larvae, it ultimately exercised a similar action and potency against the *Aedes* sp. by causing similar mortalities as the stalk extract at 72 h exposure time (p>0.05) (Table 5). *Also, recorded mortalities between C. papaya leaf and C. papaya stalk at the various test concentrations revealed a significant variation only at 750 and 1000 ppm concentrations (p<0.05) (Table 6).*

Table 5: Variation in mortalities between *C. papaya* leaf and *C. papaya* stalk at the various times of exposure

Test Conc.	24 h ($\bar{x}$)		48 h ($\bar{x}$)		72 h ($\bar{x}$)	
	CPL	**CPS**	**CPL**	**CPS**	**CPL**	**CPS**
500 ppm	4.68	5.68	5.68	6.02	4.68	6.02
750 ppm	4.68	6.68	4.68	7.35	6.35	7.69
1000 ppm	6.02	7.69	6.02	9.03	7.69	9.36
	***p*<0.05**		***p*<0.05**		***p*>0.05**	

Table 6: Variation in mortalities between *C. papaya* leaf and *C. papaya* stalk at the various test concentrations

Exposure Time	500 ppm ($\bar{x}$)		750 ppm ($\bar{x}$)		1000 ppm ($\bar{x}$)	
	CPL	**CPS**	**CPL**	**CPS**	**CPL**	**CPS**
24 h	4.68	5.68	4.68	6.68	6.02	7.69
48 h	5.68	6.02	4.68	7.35	6.02	9.03
72 h	4.68	6.02	6.35	7.69	7.69	9.36
	***p*>0.05**		***p*<0.05**		***p*<0.05**	

DISCUSSION

The phytochemicals analysed and found in the ethanolic extracts of the leaf *C. papaya* in this study including alkaloids, flavonoids, terpenoids and saponins, correspond to those of other reports (Malathi and Vasugi, 2015; Ilham *et al*., 2019; Wadekar *et al*., 2021). The secondary metabolites present in botanicals which may be responsible for the toxicities against mosquitoes and other animals that could lead to their death, are also in

charge of the chemical defence mechanism in the plants (Kovendan *et al*., 2012). Alkaloids have been reported to causes fertility setbacks in adult mosquito species (Saxena *et al*., 1993). Their compounds are renowned for exhibiting acetylcholinesterase AChE inhibitory activity that results in accumulation of acetylcholine in the synaptic cleft (Murray *et al*., 2013). Saponin which is a terpenoid compound functions in the digestive system by binding free sterols in the digestive system; thereby affecting the process of skin turnover in insects (Sukadana *et al*., 2008). Flavonoids act on the nerves of larvae and bring about difficulty in breathing in the insects and finally cause death (Anwar *et al.*, 2014). However, their absence in the leaf stalk extract of *C. papaya* of this study seemed not to have lowered the mortality rates in the *Aedes* population when compared the leaf extract.

A directly proportional relationship was observed between larval mortality rates and the test concentrations. Mortality was seen to increase as a result of corresponding increase in test concentration for both extracts implying that it took longer time for same number of the *Aedes* larvae exposed to lower test concentrations to die and shorter exposure time for those exposed to higher concentrations. This corroborates the findings in reports by Yuniar *et al*., (2017), Sunarti, (2019) and Adamu *et al*., (2019). Adamu *et al*., (2019) reported 100% mortality in the test mosquito larvae that were exposed to the lowest test concentration of 10mg/ml of *C. papaya* leaf extracts at the 24th hour exposure time which was longer compared to the shorter time of about 12 h it took to record same mortality for the mosquitoes exposed to the highest concentration of 50 mg/ml.

There was no record of 100% mortality rates for both study extracts. Highest mortality rates recorded were for larvae exposed to the highest test concentration and exposure time of 1000 ppm and 72 h respectively. The recorded mortalities were 76.9% for the leaf and 93.6% for the stalk extracts. Highest percentage mortality reported by Malathi and Vasugi (2015) for the 2nd and 4th larval stages of *Ae. aegypti* exposed to ethanolic extracts of *C. papaya* leaf for 24 h period of exposure was 33% and 36% respectively. Ethanolic extracts of the papaya seeds exhibited stronger larvicidal activity against the test larvae in that report, producing 100% mortality of the 4th instar larval stages at same 24 h exposure time. This case of extract dependent larvicidal activity was also recorded in this study as reflected in the significant difference in the larval mortalities between both extracts with the 750 ppm and 1000 ppm test concentrations. Although the highest mortality effected on the *Aedes* larvae by both extracts were similar, more studies need to be done to understand the reason behind the fairly higher mortality observed in the mosquito larvae exposed to the stalk extracts and also for influencing more larvicidal activity even when flavonoids were absent.

CONCLUSION

The findings of this study show that ethanolic extracts of *C. papaya* leaf and stalk have proven yet again to have high larvicidal potential against *Aedes* mosquito species. Worthy of emphasis is the fairly higher larvicidal activities that were observed in the mosquito species exposed to extracts of the leaf stalk than the leaf. This should increase the preference for them as agents of *Aedes* larval control. Both plant extracts when properly used, should contribute meaningfully to a cost effective and eco-friendly IVM control strategies of the *Aedes* vector.

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