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Aliphatic Amide from Seeds of *Carica papaya* as Mosquito Larvicide, Pupicide, Adulticide, Repellent and Smoke Toxicant

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Abstract Crude and solvent extracts of seed extract of *Carica papaya* was investigated for anti-mosquito potential, including larvicidal, pupicidal, adulticidal, smoke toxicity and repellent activities against *Culex quinquefasciatus* and *Anopheles stephensi*, the vector of filaria and malaria, respectively. The mortality rate of 3rd larval instars of *Cx. quinquefasciatus* and *An. stephensi* at 0.5% concentration was significantly higher ($p < 0.05$) than the mortality rates at 0.1%, 0.2%, 0.3% and 0.4% concentrations of crude extract. Among the solvent extracts, the petroleum ether extract showed the highest mortality at 100 ppm with LC_{50} and LC_{90} values of 31.16 ppm and 341.86 ppm against *Cx. quinquefasciatus*; 18.39 ppm and 250.76 ppm against *An. stephensi*. In testing for pupicidal activity, this plant extract exhibited a slightly pupicidal potency with LC_{50} values of 86.53 ppm and 72.16 ppm against *Cx. quinquefasciatus* and *An. stephensi* respectively. It showed repellency against the adult females of both mosquito species with 78 % and 92 % protection respectively. It also provided biting protection time of 4 h and 5 h respectively against *Cx. quinquefasciatus* and *An. stephensi*. In adulticidal activity there is 70% and 63.3% death of adult mosquito against *Cx. quinquefasciatus* and *An. stephensi* after 72 h. The smoke toxicity test showed that out of 200 adult mosquitoes, 190 adult mosquitoes of *Cx. quinquefasciatus* and 186 mosquitoes of *An. stephensi* dropped down at the floor after 5 h of smoke. One toxic compounds was detected having $R_f = 0.853$ (80% and 83.3% mortality in 24 h respectively for *Cx. quinquefasciatus* and *An. stephensi*). IR analysis provided preliminary information about the polyhydroxy aliphatic amide nature of the active ingredient.

Keywords *Culex quinquefasciatus*; *Anopheles stephensi*, IR analysis; Larvicidal activity, *Carica papaya*; Pupicidal activity; Repellent; Smoke toxicity; Adulticidal activity

Introduction

Mosquitoes are one of the most significant vectors of parasites and pathogens which continue to have a devastating impact on human beings (Maheswaran et al., 2008). Control of vector mosquito species is essential as they transmit malaria, filariasis, and many arboviral diseases; and they constitute an intolerable biting nuisance (Youdeowei and Service, 1983; Chatterjee and Chandra, 2000; De and Chandra, 1994; Collins and Paskewitz, 1995). Mosquitoes also cause allergic responses that include local skin and systemic reactions such as angioedema in humans (Peng et al., 1999). Synthetic insecticides create a number of ecological problems, such as the development of resistant insect strains, biomagnification etc. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability (Prabakar and Jebanesan, 2004). Recently, the environment friendly and biodegradable natural insecticides of plants origin have been receiving attention as an alternative green measure for the control of arthropods of public health importance (Dewick, 2009; Rawani et al., 2009, 2010; Haldar et al., 2011; Banerjee et al., 2012). Plants may be an alternative source of mosquito larval control agents because they constitute a rich source of bioactive chemicals. Not only can medicinal plant extracts be effective but also they may greatly reduce the risk of adverse ecological effects and expenditure towards mosquito control.

Carica papaya, the sole species in the genus Carica of the plant family Caricaceae is widely cultivated. Papaya (or papaw or pawpaw) is a tropical fruit tree. The papaya is a large tree-like plant, with a single stem growing from 5 to 10 meters (16 to 33 feet) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50~70 centimeters (20~28 inch) diameter, deeply palmately lobed with 7 lobes. The tree is usually unbranched, unless lopped. The flowers are similar in shape to the flowers of the *Plumeria*, but are much smaller and wax-like. They appear on the axils of the leaves, maturing into the large 15~45 centimeters (5.9~18 inch) long, 10~30 centimeters (3.9~12 inch) diameter fruit. Papaya is used as a food, a cooking aid, and in traditional medicine. It is used as remedy against a variety of diseases (Mello et al., 2008; Munoz et al., 2000). The stem and bark may be used in rope production. Fruit and seed extracts have pronounced bactericidal activities (Emeruwa, 1982). Leaves have been poulticed into nervous pains, elephantoid growths and it has been smoked for asthma relief among tropical tribal communities. The hypoglycemic effect of ethanolic extract of unripe, mature fruits has been reported by Olagunju et al (1995).

The present study was made to establish the larvicidal, pupicidal, adulticidal, repelling and smoke toxicity activities of *Carica papaya* seed extract against *Culex quinquefasciatus* and *Anopheles stephensi*.

1 Results

The results of the present study indicate that the mortality rate of 3rd larval instars of *Cx. quinquefasciatus* and *An. stephensi* at 0.5% concentration was significantly higher ($p < 0.05$) than the mortality rates at 0.1%, 0.2%, 0.3% and 0.4% concentrations of crude plant extract at 24, 48 and 72 hours of exposure (Table 1). The effect of solvent extract on third-instar larvae of both mosquito species were presented in (Table 2 and table 3). Highest mortality was observed in petroleum ether solvent extract at 100 ppm at 24 h, and the results of regression analysis revealed that the mortality rate (Y) was positively correlated with the period of exposure (X) having a regression coefficient close to one in each case. The results of log probit analysis (95% confidence level) revealed that LC_{50} values gradually decreased with the exposure period (Table 4 and Table 5). The pupicidal activity of two mosquito species was presented in Table 6. Highest mortality recorded in 150 ppm dose of pupae of *Cx. quinquefasciatus* and *An. stephensi* having LC_{50} values 86.53 ppm and 72.16 ppm respectively after 24 h. Table 7 shows the repellent activity of petroleum ether extract against the both the mosquito species. The result of adulticidal activity revealed highest mortality at 150 ppm doses of both mosquito species with LC_{50} value of 21.48 ppm and 64.21 ppm respectively after 72 h (Table 8). The smoke toxicity effect on adult *Cx. quinquefasciatus* and *An. stephensi* mosquitoes were recorded in the following sequences: commercial mosquito coil > mosquito coil containing powder petroleum ether extract > mosquito coil without any plant materials (Table 9). After treatment with smoke on 200 adult mosquitoes of *Cx. quinquefasciatus*, 40 mosquitoes died while 190 mosquitoes were laid down in the floor after 5 h. In case of *An. stephensi* adult mosquitoes 48 mosquitoes died and 186 mosquitoes were laid down on floor after 5 h. Table 10 presents mortality of both the tested mosquito species by isolated bioactive compound. The highest mortality (at a concentration of 75 ppm) was recorded in the compound having R_f=0.853. IR analysis of the compound and their respective functional groups are shown in Figure 1. From IR spectroscopy we observed the CH_2 stretching, a C=O stretching, O-H and C=O stretching vibrations of amide group.

2 Discussions

In recent years the use of environment friendly and easily biodegradable natural insecticides of plant origin has received renewed importance for malaria and other diseases control. Interest in this field is based on the fact that these substances are least phototoxic and do not lead to the accumulation of chemical residues in flora, fauna, soil and the entire environment in general. Not only the crude extract, solvent extracts of different plant parts play important

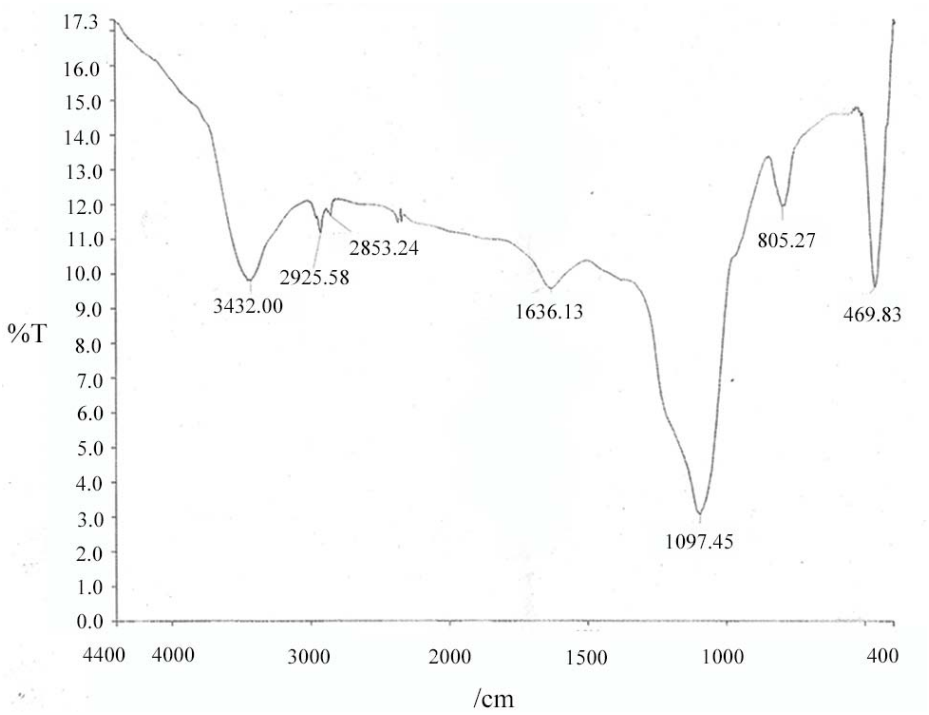

Figure 1 Infrared (IR) spectroscopy analysis of the compound and their respective functional groups

role in larvicidal, pupicidal, adulticidal, repellent and smoke toxicity activity (Chowdhury et al., 2007, 2008; Kihampa et al., 2009).

In the present study the activity of seed extract of *C. papaya* on different life forms of *Cx. quinquefasciatus* and *An. stephensi* were well established. Among the solvent extracts the petroleum ether extract showed highest mortality at 100 ppm doses at 72 hour against *Cx. quinquefasciatus* and *An. stephensi* followed by ethyl acetate, benzene, chloroform:methanol (1:1, v/v), acetone and ethanol solvent extract. Adhikari et al (2012) also observed the best result in petroleum ether extract among the solvent extracts, where the highest mortality was recorded at 50 ppm dose against 2nd instar larvae of *Cx. quinquefasciatus*. Several authors have also reported that petroleum ether extract show mosquito larvicidal activity (Nagella et al., 2012; Singha et al., 2012). The pupae of both mosquito species were also control by petroleum ether extract having LC_{50} value of 86.53 and 72.16 ppm respectively for *Cx. quinquefasciatus* and *An. stephensi* while Murugan et al (2012) studied on *Citrus sinensis*, the median lethal concentration values (LC_{50}) observed for the larvicidal and pupicidal activities against mosquito vector species *An. stephensi* first to fourth larval instars and pupae were 182.24, 227.93, 291.69, 398.00 and 490.84 ppm; *Aedes aegypti* values were 92.27, 106.60, 204.87, 264.26, 342.45, 436.93 and 497.41 ppm; and *Cx. quinquefasciatus* values were 244.70, 324.04, 385.32, 452.78 and 530.97 ppm, respectively. The investigation on repellent activity showed that, there is 5 h and 4 h protection from biting of *An. stephensi* and *Cx. quinquefasciatus* respectively when it applied at the dose of 2.5 mg/ cm^2 on the dorsal surface of the hand. Kumar et al (2012) showed that at 125, 250, 500 and 1 000 ppm dose of *Calotropis gigantea* leaves extract exhibits complete protection from mosquito bite for 60, 90, 90 and 240 minutes against *C. gelidus* and 60, 90, 90 and 120 minutes respectively against *Cx. tritaeniorhynchus*. In adulticidal activity the petroleum ether extract showed highest mortality in *Cx. quinquefasciatus* than *An. stephensi* after 72 h. There is 70% mortality of *Cx. quinquefasciatus* and 63.3 % mortality of *An. stephensi* was observed with LC_{50} value of 21.48 ppm and 64.21 ppm respectively. The root extract of *Valeriana jatamansi* which exhibited adulticidal activity of 90%, lethal concentration against adult *An. stephensi*, *An. culicifacies*, *A. aegypti*,

A. albopictus, and *Cx. quinquefasciatus* were 0.14, 0.16, 0.09, 0.08, 0.17, 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm^2, respectively (Dua et al., 2008). In smoke toxicity activity 40 adult mosquitoes of *Cx. quinquefasciatus* and 48 adult mosquitoes of *An. stephensi* were died after 5 h of smoke exposure. Murugan et al (2007) reported that smoke from *Albizza amara* was more toxic and effective repellent agent against *Ae. aegypti* than *Ocimum bascilicum*. Smoke produced from powder of *Azadirachta indica*, *Ocimum sanctum* and *Adhatoda rasica* leaves were used against *Cx. quinquefasciatus* and *Armigeres subalatus* biting activity for 6~8 h. However, the IR spectra of the bioactive compounds during the present study indicated that any polyhydroxy aliphatic amide compound might be responsible for mortality of mosquito larvae, pupae and adult mosquitoes. Kishore et al (2011) reviewed the chemical nature of several plant derived secondary materials, having larvicidal potentiality. The methanolic extract of leaves of *C. papaya* showed lethal effects against the first- to fourth- instar larvae and pupae of *Cx. quinquefasciatus* , the LC_{50} value of I instar was 51.76 ppm, II instar was 61.87 ppm, III instar was 74.07 ppm, and IV instar was 82.18 ppm, and pupae was 440.65 ppm, respectively (Kovendan et al., 2011).

The findings of the present investigation revealed that the leaf extract of *C. papaya* possess remarkable larvicidal, pupicidal, adulticidal and repellent activity against mosquito *Cx. quinquefasciatus* and *An. stephensi*. Further investigations are needed to elucidate this activity against a wide range of mosquito species and also the pin pointed active ingredient of the extract responsible for mosquitocidal activity should be identified and utilized, if possible, in preparing a commercial product.

3 Material and Methods

3.1 Preparation of crude extract

Fresh seeds of ripe fruits of *C. papaya* were randomly collected during March and April 2012. Crude extract of seeds were prepared in an electric blender and the plant juice was filtered by passing through the Whatman no. 1 filter paper. The filtrate was used as stock solution and required concentration (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) were prepared through mixing of stock extract with variable amount of distilled water.

3.2 Preparation of different solvent extracts

250 g dried seeds of *C. papaya* were put in a Soxhlet apparatus and the plant extracts were prepared using six different solvents namely petroleum-ether, benzene, ethyl acetate, chloroform:methanol (1:1 v/v), acetone and ethanol applying one after another on same seeds. The period of extraction for each solvent was 72 h. The extracts were collected separately, and the column of the Soxhlet apparatus was washed with 200 mL of water and 100 mL of a similar solvent as an eluent after each type of solvent extraction procedure. The eluted materials and each type of extract were concentrated in combination at 40℃ to 100 mL of extract by evaporation in a rotary evaporator. Then each of the extracts was filtered and dried. The yield of each solvent extract was noted separately.

3.3 Mosquito culture

Raft of *Cx. quinquefasciatus* eggs were collected from cemented drains surrounding the University campus. After hatching, first instar larvae were fed with small amount of flour until reaching the third instar form. The transformed pupae were separated manually with a glass dropper into a glass beaker (500 mL) containing tap water. The beaker was introduced into cages for emergence of adult mosquitoes. A cotton ball soaked in 10% glucose solution was used for glucose meal of adult mosquitoes and was periodically blood fed on immobilized pigeon. *An. stephensi* larvae were collected from underground and over head tanks of Kolkata metropolis and carried to the laboratory.

3.4 Larvicidal bioassay

The bioassay experiments were conducted according to standard WHO procedure (1981) with slight modifications. During experiments with crude extract, larvae of all the instars were used but only third instar larvae of *Cx. quinquefasciatus* and *An. stephensi* were used during bioassay experiments with solvent extracts. Each experiment was carried out in triplicate. The larvae were put in glass Petri-dishes (9 cm diameter/150 mL capacity) containing 100 mL of tap water. Five concentrations of aqueous extract (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) and three concentrations

of solvent extract (30 ppm, 50 ppm and 100 ppm) were applied into separate Petri-dishes to investigate the rate of larval mortalities. Tap water only was used in the control treatment. Larval mortalities were recorded after 24 h, 48 h and 72 h of exposure. The data of mortality in 48 h and 72 h were expressed by the addition of the mortality at 24 h and 48 h, respectively.

3.5 Dose-dependent pupicidal bioassay

The pupicidal bioassay followed the WHO standard protocols with suitable change. Each of the earlier prepared concentrations of petroleum ether solvent extract (50 ppm, 100 ppm and 150 ppm) was transferred into the sterile glass beaker (250 mL capacity). Ten early pupae of *Cx. quinquefasciatus* and *An. stephensi* were introduced into different beaker containing appropriate concentrations. Mortality rate were recorded after 24 h post-exposure. The experiments were replicated thrice on three different days.

3.6 Repellency activity

Repellent activity of plant compounds was tested with human volunteers. For the repellent activity of plant extract percentage protection in relation to dose method was adopted (Murugan et al., 2007). Three to four days old blood starved female adult mosquitoes (100) were kept in a net cage. The arms of the tested person were cleaned with isopropanol. After air-drying the arm only 25 cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves.

The plant extract was dissolved in isopropanol and this alcohol served as control. The plant extract at 1 mg/cm^2, 1.5 mg/cm^2, 2.5 mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The number of bites was counted over 5 min every 60 min, from 20.00 h to 6.00 h. The experiment was conducted five times. The percentage protection was calculated by using the following formula:

Protection (%)=[(Number of bites received by control arm − Number of bites received by treated arm)/ Number of bites received by control arm]×100

3.7 Adulticidal activity

The petroleum ether solvent extract of *C. papaya* was prepared to concentrations of 50 ppm, 100 ppm and 150 ppm respectively. 4 mL of each concentration were then impregnated on filter papers (140 mm × 115 mm). As for the control papers, they were impregnated with petroleum ether only. Impregnated papers were left to dry at room temperature overnight prior to testing.

The 3~7 days old adult female mosquitoes of *Cx. quinquefasciatus* and *An. stephensi* were used in batches of 15 using the WHO adult bioassay. Each test specimen was held for three hours continuously for these were natural compounds therefore the knockdown effect was assumed will be taking longer time to take place. Mortality was recorded every 10 minutes throughout the exposure period. At the end of the three hours exposure, the mosquitoes were placed in the holding tube and given 10% sugar solution enriched with vitamin B complex as the food. The test was replicated three times. Mortality was observed after 24 h and Abbott's formula was applied when minimal larval mortality was observed in control experiment.

3.8 Preparation of mosquito coil and smoke toxicity test

Mosquito coils were prepared following the methods of Saini et al (1986) with suitable modifications. The composition of mosquito coils were 2 g shade dried plant powder containing petroleum ether solvent extract, 2 g sawdust and 2 g charcoal powder. All the materials were thoroughly mixed with distilled water to form a semi-solid paste and the paste was used for the preparation of 0.4 cm thickness mosquito coils. The mosquito coils were dried in shade and used for further repellency experiment. The smoke toxicity experiment was conducted in a glass chamber measuring (140 cm×120 cm×60 cm) having a door at the front of the chamber. Two hundred blood-fed adult mosquitoes of both mosquito species were released into the chamber and the mosquitoes were exposed to the smoke of burning coils for 5 hours and the number of mosquitoes dropped down and/or died was recorded after every 30 minutes.

3.9 Preparation of samples for active part responsible for mortality

The photochemical analysis was carried out using petroleum ether solvent extract of *C. papaya* (as it exhibited highest mortality against both mosquito larvae) using the standard methods of Harbone (1984) and Stahl (1989). The petroleum ether solvent extract was chromatographed using silica gel 'G' TLC plates. The plates (thickness 0.5 mm) were prepared with silica gel G (Sigma, USA) and a thin-layer coating apparatus (Unoplan-Shandon, London). The mobile phase was petroleum ether. The thin layer chromatography (TLC) plates were sprayed with different spraying reagents for identification of active photochemical and the R_f value of positive spot was measured. Then purified fractions were made in different concentrations and treated against third instar larvae of *Cx. quinquefasciatus* and *An. stephensi* and larval death was recorded after 24 h, 48 h, and 72 h.

3.10 Bioassay with active ingredients

Preparative thin layer chromatography was done to separate the compounds of identified region of definite R_f values. Twelve numbers of plates were used for this purpose. Clear solutions were taken in conical flasks discarding the precipitate containing silica gel. The alcohol was evaporated and the solid mass present at the bottom of the conical flask was scrapped and weighed. The fractions were dissolved in distilled water to prepare different concentrations. Then third instar larvae of both mosquito species were introduced separately to different graded concentrations and the larval death rates were determined after 24 h, 48 h and 72 h, respectively.

3.11 Preparation of active ingredient for IR analysis

As the spots exhibited highest larval mortality during bioassay experiments, were scrapped from preparative silica gel 'G' plates and dissolved in absolute alcohol. Then the fraction was collected discarding the silica gel G and filtered through WhatmanNo.1 filter paper. After that the purified fraction was subjected to infrared (IR) spectroscopy. The IR spectroscopy analysis of the active spot was performed using KBr plates (JASCO FT-IR Model-420). All solvents and reagents used were of analytical grade and purchased from E. Merck, India.

3.12 Statistical analysis

The percentage mortality observed (M%) was corrected using Abbott's formula during the observation of the larvicidal potentiality of the plant extracts. Statistical analysis of the experimental data was performed using the computer software Statplus 2007 and MS EXCEL 2003 to find the LC_{50}, regression equations (Y = mortality; X = concentrations) and regression coefficient values.

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Table 1 Mean mortality of 3rd instars of *Cx. quinquefaciatus* and *An. stephensi* mosquitoes at different concentration of crude extracts of seeds of *C. papaya* (mean of three experiments)

	Concentrations	Mean Mortality (%)±SE		
		24 h	48 h	72 h
C. quinquefasciatus	0.1 %	43.33±0.33	46.67±0.58	63.33±0.33
	0.2 %	56.67±0.33	66.67±0.33	83.333±0.33
	0.3 %	70.00±0.00	73.33±0.33	90.00± 0.58
	0.4 %	80.00±0.58	83.33±1.20	93.33±0.33
	0.5 %	96.67±0.33	100.00± 0.33	100.00± 0.00
A. stephensi	0.1 %	46.67±0.33	50.00±0.00	66.67± 0.33
	0.2 %	53.33± 0.67	56.67±0.33	73.33± 0.33
	0.3 %	76.67±0.33	83.33±0.67	90.00± 0.00
	0.4 %	86.67± 0.33	90.00±0.58	100.00± 0.00
	0.5 %	100.00 ±0.00	100.0± 0.00	100.00± 0.00

Table 2 Mean larval mortality of mosquito larvae of 3rd instars of *Cx. quinquefaciatus* exposed to different concentration of solvent extracts of seeds of *C. papaya* (mean of three experiments)

	Concentration (ppm)	Mean Mortality (%)±SE		
		24h	48h	2h
Petroleum ether	30	60.00±0.58	70.00± 0.58	96.70± 0.33
	50	73.30± 0.58	80.00±0.58	100.00±0.00
	100	80.00±0.58	83.30±0.33	100.00±0.00
Benzene	30	26.70±0.33	40.00±0.58	60.00±0.58
	50	40.00±0.58	46.70± 0.33	73.30± 0.33
	100	60.00±0.58	66.70±0.68	83.30±0.67
Ethyl acetate	30	46.70±0.33	60.00 ±0.58	76.70±0.33
	50	56.70±0.88	63.33±0.33	80.00±0.58
	100	66.70±0.67	73.30± 0.33	93.30±0.33
Chloroform: Methanol (1:1 v/v)	30	23.30±0.88	30.00±0.58	46.70±0.33
	50	33.30±0.33	40.00±0.58	56.70± 0.33
	100	36.70±0.67	50.00±0.58	66.70±0.33
Acetone	30	23.30±0.33	36.70± 0.33	50.00±0.58
	50	30.00±0.58	36.70± 0.67	46.70±0.33
	100	36.70±0.67	46.70± 0.33	56.70±0.33
Absolute Alcohol	30	16.70±0.33	26.67± 0.33	36.67±0.33
	50	23.30±0.33	36.67±0.33	50.00±0.58
	100	36.70±0.33	50.00±0.58	50.67±0.33

Table 3 Mean larval mortality of mosquito larvae of 3rd instars of *An. stephensi* exposed to different concentration of solvent extracts of seeds of *C. papaya* (mean of three experiments)

Solvent extract	Concentration (ppm)	Mean Mortality (%)±SE		
		24h	48h	72h
Petroleum ether	30	70.00±0.58	76.67±0.67	86.67±0.33
	50	76.67±0.33	80.00±0.67	93.33± 0.33
	100	86.67±0.33	86.67± 0.33	96.67±0.33
Benzene	30	20.00±0.00	26.67±0.33	36.70±0.33
	50	33.30±0.33	40.00± 0.00	43.33±0.33
	100	33.30±0.33	63.33± 0.33	73.33±0.33
Ethyl acetate	30	53.33±0.67	63.33±0.33	73.33±033
	50	60.00±0.58	66.67± 0.33	76.67± 0.33
	100	56.67±0.33	66.67±0.33	83.33± 0.33
Chloroform: Methanol (1:1 v/v)	30	30.00±0.58	43.33±0.67	56.67±0.33
	50	36.67±0.33	50.00± 0.58	60.00±0.58
	100	53.33 ±0.88	66.67± 0.33	70.00±0.00
Acetone	30	33.33±0.33	43.33± 0.33	50.00±0.58
	50	43.33±0.67	56.67±0.33	60.00±0.58
	100	50.00±0.58	53.33±0.33	63.33±0.33
Absolute Alcohol	30	36.67±0.67	43.33± 0.33	50.00± 0.00
	50	43.33±0.88	46.67± 0.67	53.33± 0.33
	100	46.67±0.67	53.33±0.33	60.00± 0.00

Table 4 Log probit analysis and regression analysis of larvicidal activity of solvent extracts of seeds of *C. papaya* against 3rd instar larvae of *Cx. quinquefasciatus*

Solvent extract	Period of bioassay (h)	LC_{50} (ppm)	LC_{90} (ppm)	Regression Equation	R value
Petroleum ether	24 h	31.16	341.86	Y = 1.00X + 5.11	0.98
	48 h	13.73	309.00	Y = 0.66 X +6.45	0.96
	72 h	10.32	283.65	Y = 0.16 X +4.89	0.88
Benzene	24 h	119.09	622.53	Y = 1.66 X +0.89	0.99
	48 h	86.47	792.35	Y = 1.33 X +2.44	0.96
	72 h	34.33	262.64	Y = 1.16 X +4.89	0.99
Ethyl acetate	24 h	62.39	1040.87	Y = 1.15 X +3.62	0.98
	48 h	24.52	1559.07	Y = 0.66 X +5.22	0.96
	72 h	15.84	148.46	Y = 0.83 X +6.67	0.94
Chloroform: Methanol (1:1 v/v)	24 h	365.84	1305.57	Y = 0.67 X +1.77	0.96
	48 h	157.07	2418.75	Y = 0.90 X +2.27	0.99
	72 h	62.39	1040.87	Y = 0.20 X +3.62	0.99
Acetone	24 h	483.12	2166.23	Y = 0.01 X +1.77	0.96
	48 h	352.56	440.82	Y = 0.01 X +3.00	0.87
	72 h	72.39	244.69	Y = 0.01 X +4.44	0.65
Absolute Alcohol	24 h	305.17	300.01	Y = 0.02 X+0.67	0.97
	48 h	162.35	164.65	Y = 0.23 X +14.45	0.99
	72 h	102.79	168.97	Y = 0.02 X+2.78	0.98

Table 5 Log probit analysis and regression analysis of larvicidal activity of solvent extracts of seeds of *C. papaya* against 3rd instar larvae of *An. stephensi*

Solvent extract	Period of bioassay (h)	LC_{50} (ppm)	LC_{90} (ppm)	Regression Equation	R value
Petroleum ether	24	18.39	250.76	Y = 0.83X+6.11	0.99
	48	5.55	309.21	Y = 0.50X+7.11	0.98
	72	8.78	166.49	Y = 0.50X+8.22	0.98
Benzene	24	146.97	690.93	Y = 1.66X+0.22	0.99
	48	112.64	511.96	Y = 1.83X+0.67	0.98
	72	87.55	436.60	Y = 1.83X+1.45	0.93
Ethyl acetate	24	15.48	1128.75	Y = 0.17X+5.32	0.50
	48	0.93	2188.08	Y = 0.17X+6.22	0.87
	72	6.32	529.84	Y = 0.50X+6.77	0.98
Chloroform: Methanol (1:1 v/v)	24	148.90	1688.49	Y = 1.16X+1.67	0.97
	48	76.77	953.67	Y = 1.17X+2.99	0.97
	72	31.79	230.60	Y = 0.66X+ 4.89	0.96
Acetone	24	48.59	278.75	Y = 0.83X+2.55	0.99
	48	81.49	1211.75	Y = 0.50X+ 4.11	0.71
	72	48.59	278.75	Y = 0.66X+4.44	0.96
Absolute Alcohol	24	210.07	4851.95	Y = 0.50X+3.22	0.98
	48	117.54	4245.05	Y = 0.50X+3.77	0.98
	72	54.34	1930.92	Y = 0.50X+4.44	0.98

Table 6 Mean pupal mortality (%), probit analysis, and regression equation and regression co-efficient value of *Cx. quinquefasciatus* and *An. stephensi* mosquitoes exposed to different concentration of petroleum ether solvent extract of seeds of *C. papaya* (mean of three experiments)

Mosquito species	Concentration	Mortality (%) at 24 h	LC_{50}	LC_{90}	Regression equation	R value
Cx. quinquefasciatus	50	30.0±0.58	86.53	331.16	Y = 0.04x +1.22	0.98
	100	56.7±0.88				
	150	70.0±0.58				
An. stephensi	50	33.3±0.33	72.16	228.46	Y = 0.04x +1.66	0.96
	100	63.3±0.88				
	150	73.3±0.67				

Table 7 Result of repellency activity of petroleum ether extract seeds of *C. papaya* on *Cx. quinquefasciatus* and *An. stephensi*

Mosquito species	Concentration (mg/cm^2)	% protection Mean±SE	Average protection time (min)
Cx. quinquefasciatus	1	55	2.5 h
	1.5	69	3.5 h
	2.5	78	4 h
An. stephensi	1	65	2 h
	1.5	79	4 h
	2.5	92	5 h

Table 8 Adulticidal activity of *C. papaya* seed extract on two mosquito species

Mosquito species	Conc.	Mortality (%)			LC_{50}	LC_{90}	Regression equation	R value
		50 ppm	100 ppm	150 ppm				
Cx. quinquefasciatus	24 h	30.0±0.58	43.3±0.33	50.0± 0.58	135.81	1429.9	Y=0.03X+1.58	0.99
	48 h	43.3±0.58	56.7± 0.33	63.3 ±0.33	65.98	281.8	Y=0.03X+2.90	0.99
	72 h	60.0±0.58	63.3±0.33	70.0± 0.00	21.48	1654.9	Y=0.01X+5.29	0.91
An. stephensi	24 h	23.3±0.33	36.7±0.33	63.3± 0.33	106.04	326.41	Y=0.04X+0.11	0.98
	48 h	33.3±0.33	46.7±0.33	66.7± 0.33	97.15	543.4	Y=0.03X+1.55	0.99
	72 h	43.3±0.33	60.0±0.58	63.3± 0.33	64.21	736.2	Y=0.02X+3.55	0.93

Table 9 Smoke toxicity effect of petroleum ether extract of seeds of *C. papaya*, commercial mosquito coil and control mosquito coil without extract on *Cx. quinquefasciatus* and *An. stephensi*

Mosquito species	Time of exposure (h)	No. of smoke exposed mosquito dropped down at the bottom of glass chamber	No. of mosquito died by commercial mosquito coil	No. of mosquito died by control mosquito coil	Total mosquitoes present in glass chamber
C. quinquefasciatus	0.5	50	90	6	200
	1.0	80	100	13	
	1.3	100	150	20	
	2.0	120	200	25	
	2.3	140	200	28	
	3.0	150	200	30	
	4.0	180	200	35	
	5.0	190	200	40	
A. stephensi	0.5	60	80	5	200
	1.0	90	100	12	
	1.3	110	120	25	
	2.0	130	200	29	
	2.3	135	200	32	
	3.0	144	200	40	
	4.0	155	200	45	
	5.0	186	200	48	

Table 10 Susceptibility of 3rd instars of *Cx. quinquefaciatus* and *An. stephensi* mosquitoes to bioactive compound isolated from seeds of *C. papaya* (mean±standard error)

Mosquito species	Concentrations (ppm)	Mean Mortality (%)±SE		
		24 h	48 h	72 h
C. quinquefasciatus	25	33.67±0.33	42.67±0.33	53.33±0.67
	50	40.00±0.00	56.67±0.67	60.00±0.58
	75	66.67±0.33	73.33±0.33	80.00±0.58
A. stephensi	25	36.67±0.33	40.00±0.38	56.67±0.33
	50	46.33±0.67	53.33 ±0.33	66.67±0.33
	75	63.33±0.33	70.00±0.58	83.33±0.33