ORIGINAL ARTICLE

The Efficacy of Carica Papaya Seed Extract on Mature Female *Aedes Aegypti* and *Aedes Albopictus* Populations (Diptera: Culicidae)

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ABSTRACT

Introduction: This paper aimed to examine the efficacy of *Carica* papaya seed extract (PSE) as the oral toxin component in two attractive toxic sugar bait (ATSB) formulations on the mortality of female *Aedes aegypti* and A*edes albopictus* in a laboratory setting**. Methods:** Toxicity bioassays were conducted to determine the PSE concentration needed in ATSB formulations by exposing female *Ae. aegypti* and *Ae. albopictus* to seven different PSE concentrations for 72 hours. Probit analysis, conducted at 24-hour intervals, provided the median lethal concentration (LC) required to kill 50% and 90% of both mosquito populations; LC_{50} and LC_{90}, respectively. In two separate cages, both populations of *Ae. aegypti* and *Ae. albopictus* female mosquitoes were exposed to ATSB A and ATSB B for 72 hours to determine the mortality rate. **Results:** The efficacy of 1% PSE was measured by comparing the mortality rates of ATSB A and ATSB B against a control population. After 72 hours of exposure, the results indicated that the *Ae. aegypti* mortality rate was significantly higher in ATSB A (82.50±3.2%) and ATSB B (80.00±2.0%) but there was no significant difference between *Ae. albopictus* mortality rates in ATSB A (90.00±2.0%) and the control group. However, the *Ae. albopictus* mortality rate was found to be significantly higher in ATSB B (96.25±2.4%). **Conclusion:** The study concluded that, the addition of 1% PSE increase mortality rates in both *Aedes* species, especially in the *Ae. albopictus* population.

Keywords: Mosquitoes; Attractive toxic sugar bait; *Aedes aegypti; Aedes albopictus*; Papaya seed extract; Fruit peel.

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INTRODUCTION

Carica papaya, or papaya, is a tropical fruit available throughout the year. Its fruit, leaves, seed and the latex derived from it are used as a cure for many tropical diseases, hence, its common name, the "medicine tree" or "melon of health"(1). Active ingredients such as carpine, chymopapain, papain, the enzyme myrosin, and carpasemine are found in papaya seeds while delicacies and high-demand nutrient-rich drinks are produced from the mesocarp; or fleshy middle part of the fruit. However, some of the active ingredients found in papayas, such as carpine and papain, are toxic (2). Trace amounts of carpine are present in black papaya seeds. Several studies have examined the phytochemical properties of papaya seed extract in ethanolic (3-6) and aqueous solutions (7) while plant-derived phytochemicals, such as alkaloids, saponin, flavanoids, essential oils and phenolics, have been investigated for insecticidal activity.

The development of metabolic and genotypic insecticide resistance in mosquito populations has thwarted vector control efforts (8). This global and well-documented insecticide resistance to pyrethroid, organophosphate, and organochlorine pesticides (9) has led to an increase in studies on plant-derived insecticides in recent years. While plant extracts, such as Persian chrysanthemum, American water lily (10), paddle weed (11), false daisy, and green chiretta (12) have been studied for their adulticide properties, papaya (13) and mangroves (15) have been studied for their larvicidal properties. This study aimed to assess the effectiveness of *Carica* papaya seed extract (PSE) as the oral toxin component in two

attractive toxic sugar bait (ATSB) formulations to control adult *Ae. aegypti* and *Ae. albopictus* populations. The ideal concentration of PSE for the ATSB formulations was selected based on a toxicity bioassay to identify the mean lethal concentration (LC) of PSE required to kill 50% and 90% of both mosquito populations in 72 hours

MATERIALS AND METHODS

Rearing of Mosquito Colonies

Eggs of both Aedes laboratory strains were obtained from the insectarium of Vector Control and Research Unit (VCRU), Universiti Sains Malaysia and maintained at 25°C to 28°C, a relative humidity of 60% to 70% and a light to dark photoperiod of 12-12 hours. To feed the larvae after hatching, an easily-digestible fine powder of 2:1:1:1 weight ratio of cat food, beef liver, yeast, and milk was applied on the water's surface once every two days. They were fed 0.8 mg/larva for the first and second instars and 1.6 mg/larva for the third and fourth instars (16). The newly-emerged pupae were then placed in a transpar¬ent plastic container filled with 700mL dechlorinated water. The opening of the container was covered with a net and a hole was made to insert or remove mosquitoes. The female mosquitoes were fed artificial blood consisting of 200 mg/mL bovine serum albumin (BSA) (Sigma–Aldrich Co., LLC) and 1 mM adenosine triphosphate (ATP) (Sigma–Aldrich Co., LLC) dissolved in phosphate-buffered saline (NaCl [137 mM], Na2HPO4 [10 mM], KH2-HPO4 [1.76 mM],KCl [2.68 mM], pH7.4) (17).

Preparation of Papaya Seeds

Twenty fresh papayas, at the commercial ripening stage, were purchased from selected supermarkets in Telipok, Sabah. The fruits were washed thoroughly to remove any mud or debris before a spoon was used to collect only the seeds. The seeds were washed again then spread out on an aluminium tray and microwaved (Panasonic® NN-ST25JB) for eight minutes on medium heat. This drying process is important to avoid degradation (18). The dried seeds were then blended (Panasonic® MX-GM1011 H) into a powder, for maximum contact with the 90% ethanol solution, then transferred to an airtight plastic bag and stored in a dry place. Since the 20 papayas yielded 43.35g of dried papaya seed powder, therefore, one papaya is estimated to yield 2.17g of dried papaya seed powder.

Preparation of Papaya Seed Extract (PSE) and Stock Solution

The seed extraction method was adapted and modified from Anuradha et al. (19). An analytical balance (Adam Equipment Inc) was used to measure out 5g to 10g of the papaya seed powder to be placed in a thimble made of a filter paper. Since the active ingredients in plants are more soluble in organic solvents, 90% ethanol was used in this study. The thimble was placed in an extraction chamber with 250mL of 90% ethanol in the boiling flask. The ethanol was then boiled over medium heat in a Soxhlet extractor for 4 hours. This seed extraction process was brief due to the small quantity of papaya seed powder in the Soxhlet extractor at that time. The resulting papaya seed extract (PSE) was then filtered through a 0.45µm filter paper (Whatman®), to remove any powder residue, before being placed in a rotary evaporator (Heidolph Hei-VAP Value) at 70°C to concentrate the extract. After the solvent was removed, the PSE was filtered again then stored in a glass bottle. Since 43.35g of papaya seed powder yielded 23.4mL of PSE, therefore, 1g of papaya seed powder is estimated to yield 0.5mL of PSE. A 2% PSE stock solution; 2mL PSE and 98mL acetone; was prepared for the toxicity bioassay and mortality test. For the toxicity bioassay, the 2% PSE stock solution was serial diluted with distilled water to produce six more PSE solutions containing 0.5%, 0.7%, 0.8%, 0.9%, 1.0%, and 1.5%, respectively. 10% simple syrup was then added to each of the seven PSE solutions to encourage mosquitoes to feed on the solutions (20).

Preparation of Attractive Sugar Bait (ASB) and Attractive Toxic Sugar Bait (ATSB)

Attractive Sugar Baits (ASBs): The ASBs consisted of either papaya peel or pineapple peel extract and 10% simple syrup. ASB A was created by adding 10mL simple syrup to a beaker of 90mL papaya peel extract then stirring, using a magnetic stirrer, to form a homogenised mixture. Simple syrup was prepared by heating a 1:1 weight/volume (w/v) ratio of white granulated sugar (Gula Prai, MSM Prai Berhad, Malaysia) and distilled water to 100°C (21). 10 drops of green food dye were added to ASB A before refrigeration at 4°C. The green dye enabled us to identify which mosquitoes had died after consuming this solution by examining the stomach region of each mosquito under a handheld microscope (Dino-Lite Digital Microscope). ASB B was produced similarly using pineapple peel extract instead of papaya peel extract. Two pieces of cotton wool were then separately soaked with each ASB solution and stored in glass bottles for use in the mortality test.

Attractive Toxic Sugar Baits (ATSBs): The ATSBs consisted of either papaya peel or pineapple peel extract, 10% simple syrup as an attractant; and 1% PSE as an oral toxin. ATSB A was created by combining ASB A (10mL simple syrup and 90mL papaya peel extract) and 100mL of 2% PSE stock solution. The present study used 1% PSE since the toxicity bioassay indicated that, in 72 hours, the LC_{50} and LC_{90} for *Ae. aegypti* were 0.79% and 1.16%, respectively, and the LC_{50} and LC_{90} for *Ae. albopictus* were 0.77% and 0.96%, respectively. 10 drops of orange food dye were added to ASTB A before refrigeration at 4°C. ASTB B was produced similarly using pineapple peel extract and red food dye instead. Two pieces of cotton wool were then soaked with each ATSB solution and stored in glass bottles. The food dyes enabled us to identify which mosquitoes had died after

ingesting which solution by examining the stomach region of each mosquito under a handheld microscope (Dino-Lite Digital Microscope). Papaya and pineapple peel extracts were selected as the attractants because previous research showed that *Ae. aegypti* and *Ae. albopictus* mosquitoes are attracted to them (25).

Experimental Design

5 to 7-day old adult, female *Aedes* mosquitoes were used in this study since this mosquito genus is said to exhibit both sugar feeding and blood feeding behaviours at this age (21). Only female *Aedes* mosquitoes were selected because the dyes used to label the ASB and ATSB solutions were more easily observable under a handheld microscope in the stomachs of female mosquitoes as opposed to male mosquitoes. This was established during our pilot study.

PSE Toxicity Bioassay

The PSE toxicity bioassay was conducted to verify the mean lethal concentration of PSE needed to kill 50% and 90% (LC_{50} and LC_{90}, respectively) of female *Ae. aegypti* and *Ae. albopictus* populations in 72 hours. Since the *Aedes* mosquitoes were to be exposed to seven varying PSE concentrations (0.5, 0.7, 0.8, 0.9, 1.0, 1.5 and 2.0%), seven mosquito cages, each measuring 15cm x 15cm x 15cm and containing 20 female *Ae. aegypti* mosquitoes, were prepared. Each of the seven varying PSE concentrations were soaked into separate pieces of cotton wool, placed into glass bottles and inserted into their respective mosquito cages. The PSE-soaked cotton wool piece in each cage was replaced daily with the exact same PSE concentration to maintain potency and freshness. Over the 72-hour observation period, the numbers of dead mosquitoes were recorded and removed daily. The experiment was then repeated using *Ae. albopictus* mosquitoes.

Mortality Rate of Mosquitoes in ATSB Solutions

The mortality test was conducted to determine the efficacy of 1% PSE as an oral toxin component in two ATSB formulations to kill Ae. albopictus and Ae. aegypti mosquitoes.The ASB solutions acted as a negative control in the mortality test since they did not contain any PSE and, therefore, would not affect the mortality rate of the mosquito populations. This made them ideal to elucidate the efficacy of 1% PSE as an adulticide in the ATSB solutions. The PSE concentrations used in this test were selected based on the 72-hour LC_{50} and LC_{90} results of the toxicity bioassay. Two 15cm x 15cm x 15cm mosquito cages (experiment and control), each containing 20 female *Ae. aegypti* mosquitoes, were prepared and labelled accordingly. The ATSB A and ASB A solutions were then placed in each cage and replaced daily to maintain potency and freshness. Throughout the observation period, the number of dead mosquitoes were recorded and removed daily. The mortality test was repeated using Ae. albopictus and the ATSB B and ASB B solutions. Table I summarises mortality test findings.

Table I: Mortality test of *Ae. aegypti* and *Ae. albopictus* in sugar baits

Mosquito species	Mortality test	
	Experiment	Control
Ae. aegypti	**ATSB A** (Papaya peel extract + 10% simple syrup + 1% papaya seed extract)	**ASB A** (Papaya peel extract + 10% simple syrup)
	ATSB B (Pineapple peel extract + 10% simple syrup + 1% papaya seed extract)	**ASB B** (Pineapple peel extract + 10% simple syrup)
Ae. albopictus	**ATSB A** (Papaya peel extract + 10% simple syrup + 1% papaya seed extract)	**ASB A** (Papaya peel extract + 10% simple syrup)
	ATSB B (Pineapple peel extract + 10% simple syrup + 1% papaya seed extract)	**ASB B** (Pineapple peel extract + 10% simple syrup)

Note: ATSB = attractive toxic sugar bait; ASB = attractive sugar bait.

Statistical Analysis

The toxicity bioassay and mortality test were each conducted four times to obtain mean data. For the toxicity bioassay, the 72-hour mosquito mortality rate and PSE concentration of each of the seven PSE concentrations was subjected to probit analysis. The probit-log(concentration) regression model was used to calculate the LC_{50}, LC_{90} and 95% confidence limits (95% CL) of *Ae. aegypti* and *Ae. albopictus* at 24-hour intervals (23). Levene's Test was used to assess the homogeneity of variance between the ATSB and ASB groups. Equal variance, across the data groups, was assumed when p values were more than 0.05. The independent t-test was used to determine if there was a statistically significant difference in mortality rates between the two ATSB solution groups. This was performed by comparing the mean percentage of mosquito mortality in the ATSB solution group (experiment) to the mean percentage of mosquito mortality in the ASB group (control) at 24-hours intervals (27). The independent t-test was run on the data with a 95% confidence interval (CI) and the mean percentage of mosquito mortality in the sugar baits was considered significantly different when the p values were less than 0.05. All statistical analyses were conducted using IBM SPSS Version 21.

RESULTS

PSE Toxicity Bioassay for *Ae. aegypti* and *Ae. albopictus*

The probit analysis revealed that the 24-hour LC_{50} and LC_{90} were 2.96% (95% CL= 0) and 12.11% (95% CL= 0), respectively, for *Ae. aegypti* and 2.78% (95%

Table II :Toxicity of papaya seed extract against *Ae. aegypti* and *Ae. albopictus* in every 24 hours interval

Mosquito species	*n*	After 24 hours		After 48 hours		After 72 hours	
		LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL	LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)	LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)
Ae. aegypti	20	2.96 (0)	12.11 (0)	1.61 (0)	9.50 (0)	0.79 (0.35-0.93)	1.16 (1.00-1.98)
Ae. albopictustus	20	2.78 (0)	9.06 (0)	0.90 (0.03-1.45)	2.13 (1.27-10.59)	0.77 (0.30-0.87)	0.96 (0.85-2.61)

Note: *n* = number of mosquitoes used in the bioassay; LC = lethal concentration; and 95% CL = 95% confidence limit.

CL= 0) and 9.06% (95% CL= 0), respectively, for *Ae. albopictus.* The 48-hour LC_{50} and LC_{90} were 1.61% (95% CL= 0) and 9.50% (95% CL= 0), respectively, for *Ae. aegypti* and 0.90% 95% CL= 0.03-1.45) and 2.13% (95% CL= 1.27-10.59), respectively, for *Ae. albopictus.* Lastly, the 72-hour LC_{50} and LC_{90} for *Ae. aegypti* were 0.79% (95% CL= 0.35-0.93) and 1.16% (95% CL= 1.00-1.98), respectively, while the 72-hour LC_{50} and LC_{90} for *Ae. albopictus* were 0.77% (95% CL= 0.30-0.87) and 0.96% (95% CL= 0.85-2.61), respectively. The analysis showed that every LC_{50} and LC_{90} value in the *Ae. aegypti* group was higher than the *Ae. albopictus* group. This indicated that PSE was more toxic to Ae. albopictus populations than to *Ae. aegypti* populations. Based on the mean lethal concentration values after 72-hour exposure to varying PSE concentrations, 1% PSE was chosen as the oral toxin in the ATSB formulation. Table II illustrates the lethal concentrations needed to kill 50% and 90% of *Aedes* mosquito populations at 24-hours interval.

The Mortality Rate of *Ae. aegypti* and *Ae. albopictus* in Sugar Baits

Ae. aegypti: Levene's test showed that an equal variance was assumed across the data groups (p> 0.05). The first independent t-test compared mosquito mortality rates between ATSB A and ASB A, and found that after 24 hours of exposure, ATSB A's mortality rate (40.00 ±6.1%) was significantly higher than ASB A's (13.75 ±6.3%) (t(6) = 3.000, p = 0.02, 95% CI = 4.84-47.66). Similar t-test results were obtained when the mosquito mortality rates between ATSBs and ASBs 48 hours after exposure were compared, i.e, ATSB A (73.75 ±4.3%) had significantly higher mosquito mortality rates than ASB A (36.25 ±7.2%) (t(6) = 4.489, p = 0.00, 95% CI = 17.06-57.94). Lastly, the mosquito mortality rate after 72 hours of exposure to ATSB A (82.50 ±3.2%) was also significantly higher than ASB A (55.00 ±2.0%) (t(6) = 7.201, p = 0.00, 95% CI = 18.16-37.84).

The second independent t-test compared mosquito mortality rates between ATSB B and ASB B. There was no significant difference between the mosquito mortality rate after 24 hours of exposure to ATSB B (22.50 ±2.5%) and ASB B (25.00 ±2.0%) (t(6) = -0.775, p = 0.47, 95% CI = -10.40-5.40). However, the mosquito mortality rate after 48 hours of exposure to ATSB B (60.00 ±2.0%) was significantly higher than ASB B (41.25 ±4.7%) (t(6) = 3.638, p = 0.01, 95% CI = 6.14-31.36). The mosquito mortality rate after 72 hours of exposure to ATSB B (80.00 ±2.0%) was also higher than ASB B (51.25 ±2.4%) (t(6) = 9.139, p = 0, 95% CI = 21.05-36.45). Therefore, the addition of 1% PSE in the ATSBs increased *Ae. aegypti* mortality rates of by more than 80% after 72 hours of exposure. Additionally, *Ae. aegypti* displayed toxicity to solutions containing only papaya and pineapple peels as exhibited by the more than 50% mortality rate after 72-hour exposure. Table III and Figure I show *Ae. aegypti* mortality rates in both ATSBs and ASBs.

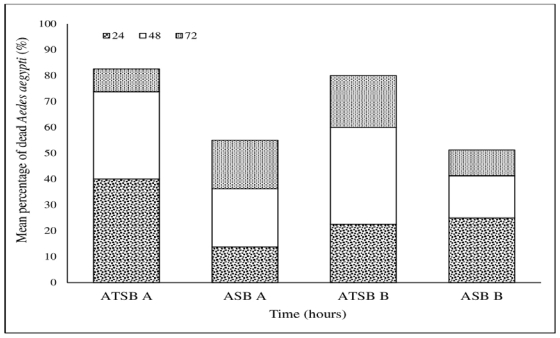

Figure I: The mortality of Ae. aegypti in ATSBs and ASBs. The stacked bar graph represent the cumulative mortality in 24 hours interval for three-day period of test.

Ae. albopictus: Levene's test showed that an equal variance was assumed across the data groups (p> 0.05). The mean mortality rate after 24-hour exposure to ATSB A (22.50±3.2%) was significantly higher than ASB A (mean mortality of 12.50 ±1.4% (t(6) = 2.828, p = 0.03, 95% CI = 1.35-18.65)). The mortality rate after 48 hours was also significantly higher in ATSB A (56.25 ±2.4%) than in ASB A (43.75 ±3.8%) (t(6) = 2.810, p = 0.03, 95% CI = 1.61-23.39). However, there was no significant difference in the mortality rates after 72 hours of exposure between ATSB A (90.00 ±2.0%) and ASB A (86.25 ±2.4%) (t(6) = 1.192, p = 0.28, 95% CI = -3.95-12.45).

The independent t-test results, however, indicated that there was significant difference in the mortality rate after 24 hours with ATSB B (32.50 ±4.3%) recording higher mortality than ASB B (10.00 ±2.0%) (t(6) = 4.700, p = 0.03, 95% CI = 10.79-34.21). The mortality rate after 48-hour exposure to ATSB B (82.50 ±3.2%) was also significantly higher than in ASB B (31.25 ±2.4%) (t(6) = 12.755, p = 0.00, 95% CI = 41.42-61.08). Finally, the mortality rate after 72 hours of exposure was higher in ATSB B (96.25 ±2.4%) than in ASB B (52.50 ±1.4% (t(6) = 15.652, p = 0.00, 95% CI = 39.61-50.59)). The result

Table III
Cumulative mortality of female *Ae. aegypti* after exposed to ATSBs and ASBs for 72 hours

Sugar Baits	Cumulative Mortality (%)					
	24 Hours		48 hours		72 hour	
	Mean (±SE)	*p* value	Mean (±SE)	*p* value	Mean (±SE)	*p* value
ATSB A (papaya peel extract + 10% simple syrup + 1% papaya seed extract)	40.00 (±6.1)	0.02	73.75 (±4.3)	0.00	82.50 (±3.2)	0.00
ASB A (papaya peel extract + 10% simple syrup)	13.75 (±6.3)		36.25 (±7.2)		55.00 (±2.0)	
ATSB B (pineapple peel extract + 10% simple syrup + 1% papaya seed extract)	22.5 (±2.5)	0.47	60.00 (±2.0)	0.01	80.00 (±2.0)	0.00
ASB B (pineapple peel extract + 10% simple syrup)	25.0 (±2.0)		41.25 (±4.7)		52.25 (±2.4)	

Note: the *p* values shown in the table are from the independent *t*-test analysis.

Table IV
Cumulative mortality of female *Ae. albopictus* after exposed to ATSBs and ASBs for 72 hours

Sugar Baits	Cumulative Mortality (%)					
	24 Hours		48 hours		72 hour	
	Mean (±SE)	*p* value	Mean (±SE)	*p* value	Mean (±SE)	*p* value
ATSB A (papaya peel extract + 10% simple syrup + 1% papaya seed extract)	22.50 (±3.2)	0.03	56.25 (±2.4)	0.03	90.00 (±2.0)	0.28
ASB A (papaya peel extract + 10% simple syrup)	12.50 (±1.4)		43.75 (±3.8)		86.25 (±2.4)	
ATSB B (pineapple peel extract + 10% simple syrup + 1% papaya seed extract)	32.50 (±4.3)	0.02	82.50 (±3.2)	0.01	96.25 (±2.4)	0.00
ASB B (pineapple peel extract + 10% simple syrup)	7.50 (±2.5)		32.50 (±2.5)		52.50 (±2.5)	

Note: the *p* values shown in the table are from the independent *t*-test analysis.

showed that papaya and pineapple peel extracts were toxic to Ae. albopictus, especially papaya peel extract which caused up to 80% mortality after 72 hours of exposure. The addition of 1% PSE, mainly to ATSB B, was found to increase *Aedes* mortality. Table IV and Figure II summarise *Ae. albopictus* mortality rates in both ATSBs and ASBs.

DISCUSSION

The probit analysis showed that, at every time interval,

FIGURE II

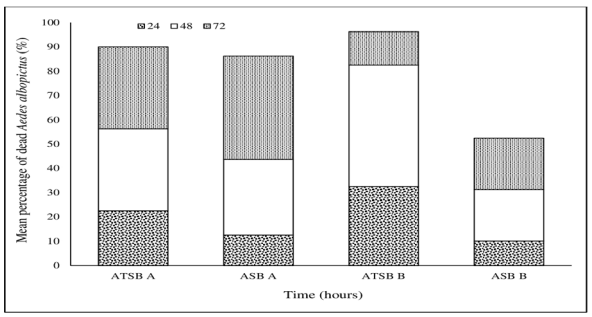

Figure II: The mortality of Ae. albopictus in ATSBs and ASBs. The stacked bar graph represent the cumulative mortality in 24 hours interval for three-day period of test.

the LC_{50} and LC_{90} values of the *Ae. albopictus* group were lower than the Ae. aegypti group. This may be because PSE is more toxic to adult female *Ae. albopictus*. The difference in toxicity also may be due to high insecticide resistance among *Ae. aegypti* populations that undergo high selection pressure due to liberal insecticide application in urban environments (25). The LC_{50} results of PSE have been compared to the LC_{50} results of other oral toxin, such as boric acid, to verify its efficacy. A study by Barbosa et. al. (24) attempted to determine the lethal concentration of boric acid needed to kill 50% (LC_{50}) of male and female *Ae. aegypti* mosquito populations at 24-hour and 48-hour intervals. They found that the adult male *Ae. aegypti* mosquito required an LC50 of 1.15% at 24 hours and 0.57% at 48 hours, while the adult female required 1.08% and 0.53%, respectively (26). Meanwhile, Xue and Donald (29) investigated the LC_{50} of boric acid needed at 24-hour and 48-hour intervals for male and female *Ae. albopictus p*opulations and discovered that while the adult males required only 0.174% at 24 hours and 0.078% at 48 hours, the females required 0.527% and 0.244%, respectively (29). The LC_{50} of PSE needed for the adult female *Ae. aegypti* and *Ae. albopictus* populations in this study were higher than the LC50 of boric acid. This indicates that both *Aedes* mosquitoes

were more susceptible to boric acid than PSE.

However, the mortality test results of this study showed that papaya and pineapple peel extracts alone were highly toxic to adult female *Ae. aegypti* and *Ae. albopictus* mosquitoes and that 1% PSE acted as an enhancer. This was true for both *Aedes* species, particularly *Ae. albopictus.* While ASB A caused more than 50% mortality in Ae. aegypti populations within 72 hours, it caused more than 80% mortality in *Ae. albopictus* populations while ASB B cause more than 50%. Conversely, both ATSB formulations caused more than 90% and 80% mortality in *Ae. aegypti* and *Ae. albopictus* populations within 72 hours.

A notable previous study involving ATSB formulations was carried out by Barbosa et al. (20). This study evaluated the mortality rate of male and female *Ae. aegypti* in three ATSB formulations within 24 hours. The ATSBs were prepared with three different fruits: mango (Mangifera indica), guava (Psidium guajava) and cupuaзu (Theobroma grandiflorum). Each fruit was then incorporated with 15% brown sugar and 4% boric acid to form the ATSB formulations while the ASB solutions (control) were formulated without boric acid. Their study showed that the general mosquito mortality rate in ATSB and ASB were 0.81 (81%) and 0.10 (10%) for males respectively; 0.61 (61%) and 0.12 (12%) for females. The ASB from Barbosa et. al. (24) had low mortality rates, therefore, the fruit composition of the ATSB formulation did not affect male and female mortalities. This was in contrast with our study, wherein papaya and pineapple peel extracts were toxic to adult female *Ae. aegypti* and *Ae. albopictus* populations. Moreover, the mortality rate of adult female *Ae. aegypti* after 24-hour exposure to 4% boric acid was higher than the mortality rate of adult female *Ae. aegypti* after 24-hour exposure to 1% PSE (≤ 40% mortality rate). Therefore, 4% boric acid is more toxic to adult female *Ae. aegypti* than 1% PSE used in the present study.Xue and Donald (26) aimed to determine the effect of 48-hour exposure to toxic sugar bait (1% boric acid + 10% sucrose solution) on the mortality rate of blood fed, gravid, and parous *Ae. albopictus.* Their study found a 98% mortality rate in blood fed, gravid, and parous *Ae. albopictus.* The mortality rate of adult female *Ae. albopictus* in both ATSB formulations at 48 hours is lower. This may be because adult female *Ae. albopictus* are more vulnerable to 1% boric acid than 1% PSE.

CONCLUSION

A 1% concentration of papaya seed extract (PSE) was selected as the oral toxin component of two ATSB formulations based on a 72-hour toxicity bioassay of A*e. aegypti* and *Ae. albopictus* mosquitoes. The mortality test results indicated that papaya and pineapple peel extract alone demonstrate toxicity and that the addition of PSE only enhanced their toxicity to both *Aedes* species, especially *Ae. albopictus.*

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REFERENCES

1. 1. Rosilawati R., Lee HL., Nazni WA., Nurulhusna AH, Roziah A, Khairul Asuad M, Siti Futri Farahininajua F, Mohd Farihan MY. Ropiah J. Pyrethroid resistance status of Aedes (Stegomyia) aegypti (Linneaus) from dengue endemic areas in Peninsular Malaysia. International Medical Journal Malaysia 2017; 16(2), 73-78.
2. Vythilingam I. Wan-Yusoff WS. Dengue vector control in Malaysia: are we moving in the right direction?. Tropical Biomedicine 2017; 34(4), 746-758.
3. Vinita C. Novel mosquito control: a natural approach to reducing and repelling mosquito populations. Journal of the South Carolina Academy of Science 2017; 15(2), 47-51.
4. Anuradha V, Syed Ali M. Yogananth N. Efficacy of mosquito repellent and adulticidal activities of Halophila ovalis extract against Filaria vectors. Journal of Tropical Disease 2016; 4(2), 56-59.
5. Fiorenzano JM, Fulcher AP, Seeger KE, Allan SA, Kline DL, Koehler PG, Mьller GC. Xue R. Evaluations of dual attractant toxic sugar baits for surveillance and control of Aedes aegypti and Aedes albopictus in Florida. Parasites & Vectors 2017; 10(9), 1-9.
6. Junnila A, Revay EE, Mьller GC, Kravchenko V, Qualls WA, Xue R, Allen SA, Beier JC. Schlein Y. Efficacy of attractive toxic sugar baits (ATSB) against Aedes albopictus with garlic oil encapsulated in beta-cyclodextrin as the active ingredient. Acta Tropica 2015; 152, 195–200.
7. Qualls WA, Naranjo DP, Subна MA, Ramon G, Cevallos V, Grijalva I, Gymez E, Arheart KL, Fuller DO, Beier JC. Movement of Aedes aegypti following a sugar meal and its implication in the development of control strategies in Durбn, Ecuador. Journal of Vector Ecology (2016b); 41(2), 224 – 231.
8. Ovie FO, Ndukwe GU, Oliver NL, Obi KC, Aguwa US, Olu SI. Effect of aqueous extract on Carica papaya seed and back on the Testes and sperm morphology of male wister rats. International Journal of Scientific and Research Publications 2019; 9(9), 671-676.
9. Eno AE, Owo OI, Itam EH, Konya RS. Blood pressure

depresssion by the fruit juice of Carica papaya (L.) in renal and DOCA-induced hypertension in the rat. Phytotherapy Research 2000; 14, 235- 239.
10. Dada FA, Nzewuji F, Esan A, Oyeleye S, Adegbola V. Phytochemical and antioxidant analysis pf aqueus extracts of unrope pawpaw (Carica papaya Linn.) fruit's peel and seed. International Journal of Research and Reviews in Applied Sciences 2015; 27(3), 1-4.
11. Asghar N, Syed Ali RN, Zaib H, Nasir Rasool Zulfiqar AK, Sohail AS, Tauqir AS, Muhammad Ramzan SAJ, Saeed AN, Muhammad Zia-Ul-Haq and Hawa ZJ. Compositional difference in antioxidant and antibacterial activity of all parts of the carica papaya using different solvents. Chemistry Central Journal 2016; 10(5), doi: 10.1186/s13065-016-0149-0.
12. Hayatie L, Biworo A. Suhartono E. Aqueous extracts of seed and peel of Carica papaya against Aedes aegypti. Journal of Medical and Bioengineering 2015; 4(5), 417-421.
13. Wahyuni D. New bioinsecticide granules toxin from ectract of papaya (Carica papaya) seed and leaf modified against Aedes aegypti larvae. Procedia Environmental Sciences 2015; 23,323 – 328.
14. Naggayi M, Mukiibi N, Iliya E. The protective effects of aqueous extract of Carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats. African Health Sciences 2015; 15(2), 598-605.
15. Liu N. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annual Review of Entomology 2015; 60, 537-559.
16. Vontas J, Kioulos E, Pavlidi N, Morou E, Torre A. Ranson H. Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. Pesticide Biochemistry and Physiology 2012; 104, 126 – 131.
17. Govindarajan M, Sivakumar R. Mosquito adulticidal and repellent activities of botanical extracts against malarial vector, Anopheles stephensi Liston (Diptera:Culicidae). Asian Pacific Journal of Tropical Medicine 2011; 941-947.
18. Malathi P, Vasugi SR. Evaluation of mosquito larvicidal effect of Carica Papaya against Aedes Aegypti. International Journal of Mosquito Research 2015; 2(3), 21-24.
19. Henny S, Arsunan AA. Hasanuddin I. Potential test of papaya leaf and seed extract (Carica papaya) as larvicides against Anapholes mosquito larvae motality. Sp in Jayapura, Papua Indonesia. International Journal of Scientific and Research Publications 2014; 4(6), 1-8.
20. Syed Ali M, Ravikumar S, Beula JM. Spatial and temporal distribution of mosquito larvicidal compounds in mangroves. Asian Pacific Journal of Tropical Disease 2012; 2(5), 401-404.
21. Kassim NFA, Webb CE, Russell RC. Is the expression of autogeny by Culex molestus Forskal (Diptera: Culicidae) influenced by larval nutrition or by adult mating, sugar feeding, or blood feeding. Journal of Vector Ecology 2014; 37, 162-71.
22. Pitts RJ. A blood-free protein meal supporting oogenesis in the Asian tiger mosquito, Aedes albopictus (Skuse). Journal of Insect Physiology 2014; (64), 1–6.
23. Serьlvedaa L, Romanна A, Aguilarb CN, Teixeiraa J. Valorization of pineapple waste for the extraction of bioactive compounds and glycosides using autohydrolysis. Innovative Food Science and Emerging Technologies 2018; 47, 38-45.
24. Barbosa DS, Rodrigues MMS, Silva AAE. Evaluation of attractive toxic sugar baits (ATSB) agianst Aedes aegypti (Diptera: culicidae) in laboratory. Tropical Biomedicine 2019; 36(2), 578-586.
25. Nur Athen MH, Nazri CD, Siti Nazrina C. Bioassay studies on the reaction of Aedes aegypti & Aedes albopictus (Diptera: Culicidae) on different attractant. Saudi Journal of Biological Sciences 2020; Advance online application. https://doi.org/10.1016/j.sjbs.2020.06.016
26. Nasir S, Batool M, Hussain S, Nasir I, Hafeez F. Debboun M. (2015). Bioactivity of Oils from Medicinal Plants against Immature Stages of Dengue Mosquito Aedes aegypti (Diptera: Culicidae). International Journal of Agriculture and Biology 2015; 17, 843-847.
27. Traore MM, Junnila A, Revay EE, Kravchenko VD, Lathi A, Fiorenzano JM, Quall WA, Kliene DL, Schlein Y, Beier JC, Xue R. Muller GC. Control of adult and larval Aedes albopictus with attractive toxic sugar baits (active ingredient: cinnamon-sesame oil) in Northeastern Florida. Journal of the Florida Mosquito Control Association 2019; 66, 20-26.
28. Xue R, Donald RB. Boric acid bait kills adult mosquitoes (Diptera: Culicidae). Journal of Economic Entomology 2003; 96(5), 1559-1562.