



Original article

Bioassay studies on the reaction of *Aedes aegypti* & *Aedes albopictus* (Diptera: Culicidae) on different attractantsM.H. Nur Athen^a, C.D. Nazri^{a,c,d,*}, C. Siti Nazrina^{a,b}^a Centre of Environmental Health and Safety, Faculty of Health Sciences, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia^b Centre of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia^c Integrated of Mosquito Research Group (I-Merge), Faculty of Health Sciences, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia^d Malaysia Association Environmental Health (MAEH) Research Centre, 43300 Seri Kembangan, Selangor, Malaysia

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ABSTRACT

Background: The studies on mosquito attractants had been develop intensively in the recent years. However, the study on fruit peel extract as mosquito attractant was scarce, even though various fruits had demonstrated the ability to attract different types of mosquito species.

Objective: This study aims to determine the potential of *Carica papaya* (papaya) and *Ananas comosus* (pineapple) peel extracts to attract *Aedes albopictus* and *Aedes aegypti*.

Methods: The *Aedes* mosquitoes response to the fruit peel extracts were conducted in the no-choice and choice assay using modified olfactometer. The Preference Index (PI) in each assay was calculated and arc-sine transformed before conducting independent *t*-test to determine the significant different between the mean arc-sine transformed PI and the tested hypothesis mean PI.

Result: No choice assay indicate both *Aedes* species have significant attraction to the papaya and pineapple peel extracts ($p < 0.05$). In choice assay, *Ae. albopictus* is revealed to equally attracted to the papaya and pineapple peel extracts ($p > 0.05$) while *Ae. aegypti* is significantly attracted to the papaya peel extract ($p < 0.05$).

Conclusion: The study had identified that both fruit peel extracts able to attract *Aedes* mosquitoes with *Ae. albopictus* is equally attracted to papaya and pineapple peel extracts while *Ae. aegypti* is more attracted to the papaya peel extract than the pineapple peel extract.

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1. Introduction

Attractive toxic sugar bait (ATSB) is a method developed for the adult mosquito control which imply the “attract and kill” strategy. The ATSB commonly consist of attractant aromatic compound such as fruit juices and/or flower accents, feeding stimulant such as sugar, and oral insecticide such as boric acid. This method has been widely tested throughout the last few years and are highly effective in controlling mosquitoes (Fiorenzano et al., 2017). It has been

successfully applied against different types of mosquito species in laboratory and field setting, such as *Aedes aegypti* (Scott-fiorenzano et al., 2017), *Aedes albopictus* (Junnila et al., 2015), *Culex quinquefasciatus* (Qualls et al., 2016) and *Anopheles sergentii* (Revay et al., 2015). The selection of attractant use in ATSB is also crucial for effective ATSB implementation. In recent years, studies on mosquito preferences have been actively conducted using different materials. Some examples of mosquito attraction studies include mosquito attraction to host derived chemicals such as L-lactic and 1-octen-3-ol (Scott-fiorenzano et al., 2017), synthetic flora based attractants such as phenylacetaldehyde, linalool oxide, phenylethyl alcohol, and acetophenone (Fikrig et al., 2017), plant tissue, ripe fruits, seedpods, floral and extra floral (Sissoko et al., 2019).

On the other hand, papaya and pineapple are two examples of tropical fruits produced in Malaysia (Nor Azlina, 2014). Previous studies have shown that papaya attracts several mosquito species. For instance, the field study of Sissoko et al. (2019) has used plant baited glue net traps (GNTs) to study the attraction of *Aedes aegypti*

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in twenty different plants. The mosquitoes caught overnight in the GNTs were counted for attraction analysis. Afterwards, their result indicates papaya fruit was one of the plants that was able to attract male and female *Ae. aegypti* (Sissoko et al., 2019). Similar method was used by Malmgren (2015) to identify wild mosquitoes' attraction in six local plant species. Mosquito attraction in this study was determined by applying sticky glue traps surrounding the experimented plant and then calculate the number of mosquitoes on the trap. The study shows that papaya attracts *Culex* and *Anopheles* mosquito species (Malmgren, 2015). Moreover, Oriental fruit fly *Dacus dorsalis* H, melon fruit fly *Dacus cucurbitae* C, and Mediterranean fruit fly *Ceratitis capitata* also attracted to papaya (Ranjeet and Ramanuj, 2018). Additionally, study by Ranjeet and Ramanuj (2018) also indicates that pineapple attracts various pest such as fruit flies (*C. capitata*, *D. cucurbitae*, and *D. dorsalis*), pineapple caterpillar (*Thecla basilides*, *Metanastius ritchei*, *Batrachedra methesoni*, *Paradiophorus crenatus*) and pineapple mite (*Steneotarsonemus ananas*).

However, the use of fruit waste as mosquito attractant is limited despite the fact that studies on mosquito preferences were tested using various fruits. The fruit waste such as the peel is commonly discarded and not being fully utilized. Additionally, fruit peels are commonly studied for their antioxidant and antimicrobial potentials (Saraswaty et al., 2017). Therefore, the present study aims to determine adult *Ae. albopictus* and *Ae. aegypti* responses to papaya and pineapple peel extracts. The attraction study was conducted in modified olfactometer which allows mosquitoes to be exposed to maximum of two attractants at the same time. Hence, each mosquito species was subjected to no-choice and choice assay. In no-choice assay, the mosquitoes were exposed to papaya or pineapple peel extracts only while the choice assay exposed mosquitoes to papaya and pineapple peel extracts at the same time. The result obtained is used in assisting mosquito baiting and development of ATSB for mosquito control.

2. Materials and methods

2.1. Insect colony and maintenance

The laboratory strain of *Ae. aegypti* and *Ae. albopictus* eggs (F₁₈₆) came from the colony maintained in the insectarium of Vector Control and Research Unit (VCRU), Universiti Sains Malaysia. The *Aedes* eggs were submerged and hatched in a plastic container (40 × 40 × 5 cm) containing approximately 700 mL of dechlorinated water. The hatched larvae were fed with powdered chicken liver until pupation and the water was changed daily to avoid formation of scum and to maintain water volume (Fiorenzano et al., 2017). After that, plastic pipette was used to transfer pupae to smaller plastic container filled with dechlorinated water. This container was then transferred inside a mosquito cage (30 × 30 × 30 cm) covered with muslin netting and a cloth sleeve fitted at the front. Cotton soaked with 10% sucrose solution was placed in a universal bottle and placed inside the cage for the adult mosquitoes food source. The female adult mosquitoes laid eggs after mating process. Blood feed was given to 5–7 days old adult mosquito for four hours (Fiorenzano et al., 2017). Most species of mosquito's gut are still developing after 1–3 days of adult emergence thus the mosquitoes mainly feed from sugar sources during that time. Moreover, female mosquitoes preferentially lay their eggs on a variety of man-made breeding sites in the surroundings of human properties around 3–4 days (Maciel-de-Freitas et al., 2007). Therefore, blood feed was given after 5–7 days if adult emergences to increase the fecundity rate of female mosquitoes. Ovitrap was introduced into the cage after 48 h of the blood meal (Heil, 2011). The ovitrap is made from a plastic container half filled

with water and a cone shaped filter paper which is placed in the container to serve as oviposition site. The filter paper containing the eggs was air-dried and labelled as F₁₈₇ for *Ae. aegypti* and *Ae. albopictus*. The F₁₈₇ generation was hatched and reared to adult as described above. The insectarium environment was thermostatically controlled at 29 ± 3 °C with the relative humidity of 75 ± 10% and 12:12 h light: dark cycle throughout the experiment (Xue and Donald, 2003).

2.2. Papaya and pineapple peels extraction

(i) Sample preparation

Twenty fresh papaya and pineapple used in this study were obtained from CKS Supermarket, Telipok, Sabah. Both fruits were washed thoroughly to remove mud and debris that may be present on the surface of the fruits. The fruit flesh and peels were separated using a manual peeler for the papayas and knife for the pineapples. The peels were washed again to remove debris and flesh that are still present. After that, the papaya and pineapple peel stripes were cut into approximately 2 mm slices in length before being laid on separated aluminium trays. The tray with papaya peels were dried in a microwave (Panasonic® NN-ST25JB) for 5 min in medium microwave power while pineapple peels were microwaved for 10 min in medium power.

The papaya and pineapple peels have different time in the microwave due to the pineapple peels were thicker than the papaya peels. The drying process was important to avoid degradation (Sepúlveda et al., 2018). Dried peels were then blended into powder using a blender (Panasonic® MX-GM1011 H) for maximum contact of the dried sample to the extraction solvent. After being blended, the papaya peels have turned into powder whereas the pineapple peels still have some fibre remain intact. Lastly, the powdered peels were placed in an air-tight plastic bag before being stored in dry place until further use. In the end, a total of 59.305 g of dried papaya peels and 54.034 g of dried pineapple peels were obtained from the twenty fresh fruits. Therefore, this study found that one papaya fruit produced approximately 2.97 g of dried peel whereas one pineapple produced approximately 2.70 g of dried peel.

(ii) Fruit Peel Extraction

The peel extraction method was adapted and modified from Hu et al. (2017). The papaya peel extract was prepared by weighting 1 g of papaya peel and placed inside a 50 mL beaker (Bomex®). Since the substances contained in plants are more soluble in organic solvents such as ethanol, methanol and hexane, 80% ethanol was used in the present study. Therefore, 10 mL of 80% ethanol solution was added into the beaker. The beaker was then placed on a mechanical shaker (GFL Orbital Shaker 3017) for 30 min at 150 rpm to mix powdered papaya peel with the solvent. The mixture consist of papaya and ethanol was then transferred in 50 mL vial and centrifuged (Centurion Scientific K3 Series) at 3000 rpm for 10 min to recover supernatant. The first supernatant was transferred into 50 mL beaker and 2 mL of 80% ethanol was added into the vial then centrifuged at 3000 rpm for 10 min to collect the second supernatant. Previous step was repeated to obtain the third supernatant. After that, the collected supernatants were mixed evenly by manually shaking the beaker and then filtered through a 0.45 µm filter paper (Whatman®) to separate the powder residue before it is placed in a rotary evaporator. The rotary evaporator (Heidolph Hei-VAP Value) water bath was set at 70 °C to concentrate the extract. After the solvent was separated, the peel extract was filtered again to remove residue, placed in a glass bottle and refrigerated at –4 °C until further use. The pineapple peel extract was produced as described above.

2.3. Experimental design

(i) Olfactometer design

Olfaction is the major sensory modality involved in the resource searching behaviour of insects including vector mosquitoes (Diptera: Culicidae). To date, our current countrywide knowledge on the host-seeking behavior of mosquitoes is mainly confined to host preference, which has exclusively come from field studies. Olfactometer is a scientific tool which more naive aspects of man-vector can clarify contact under controlled and less biased conditions. Therefore a modified olfactometer was used to determine the response of sugar seeking mosquitoes towards papaya and pineapple peel extracts (Fig. 1). The olfactometer designed based on Afify et al. (2014) which is made from Plexiglas and consisted of a flight chamber attached to holding chamber at one side and two attraction bioassay chambers at the other end. The holding chamber was a tube (diameter: 4.4 cm, length: 12.5 cm) with a mesh cap on one end to enable airflow and a sliding gate connected to the flight chamber at the other end. This holding chamber is use to hold the female adult mosquitoes and allow them to acclimatised before being release to the flight chamber. Meanwhile, the flight chamber was a box (21 cm × 29.7 cm × 10.5 cm)

with three circular openings (diameter of circle: 4 cm). The purpose of the flight chamber was for the mosquitoes to decide on flying towards either one of the attraction bioassay chambers. The attraction bioassay chamber was a tube (diameter: 4.4 cm, length: 12.5 cm) with a sliding gate attached to flight chamber and a mesh cap at the other end to enable airflow. The inside of this chamber also has another mesh screen to prevent mosquito from reaching the experimented attractant and to avoid visual attraction. Additionally, the mosquitoes present inside the attraction bioassay chambers after the gate was closed were considered attracted to peel extracts. Before testing the attractants, a preliminary experiment whether mosquitoes would choose either side (test chamber) of the olfactometer when given the same attractant choice in both chamber (two fingers of an experiment).

(ii) Evaluation of Mosquitoes Response to Fruit Peel Extracts

The evaluation of mosquitoes response to fruit peel extracts was performed using two methods; (1) no-choice assay in which the mosquito species were offered only one attractant either “papaya peel extract” or “pineapple peel extract” with control (empty port) and (2) choice-assay in which two attractant were offered in

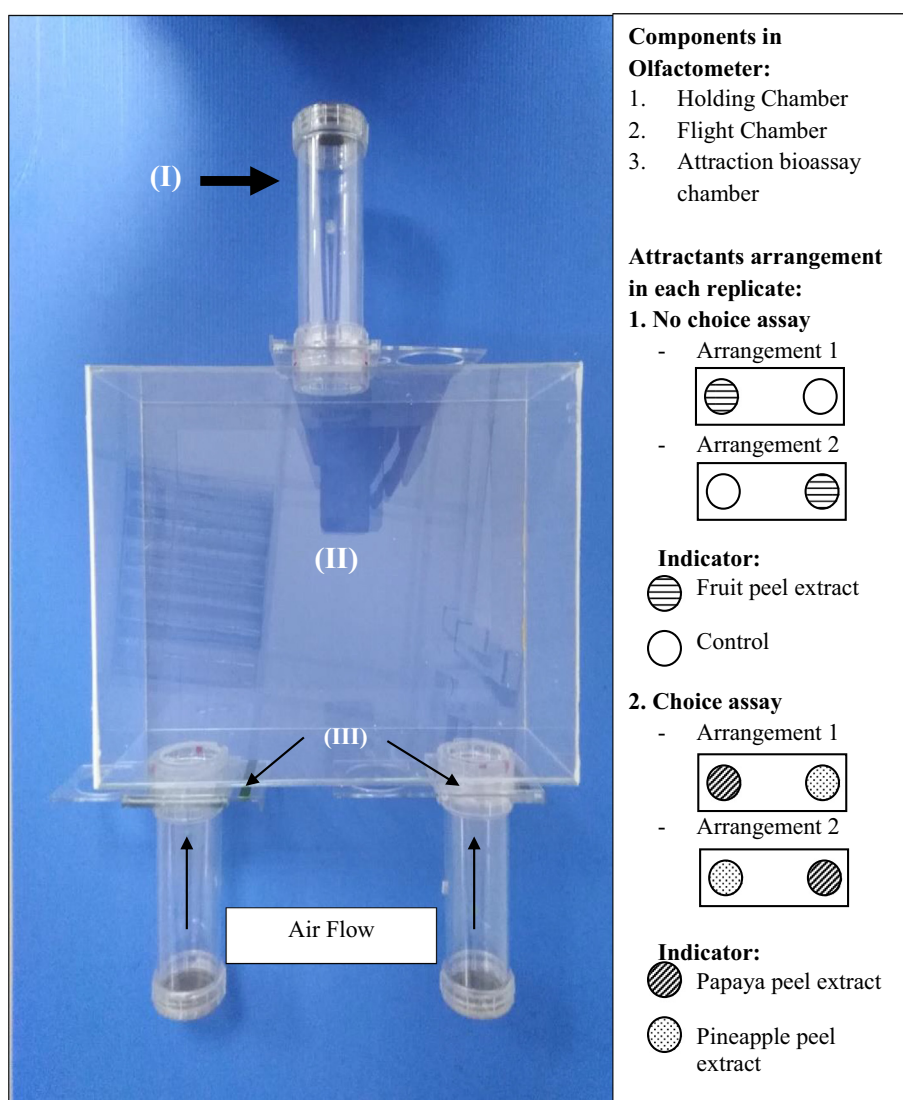


Fig. 1. The different parts of the modified olfactometer to determine the response of sugar seeking mosquitoes modified from Afify et al. (2014); (I) holding chamber; (II) flight chamber and (III) attraction bioassay chamber.

this test (papaya peel extract and pineapple peel extract). The no-choice assay was conducted in four replicates where each experiment was set up by aspirating twenty ($n = 20$) 5–7 days old female *Aedes* mosquitoes into a holding chamber and left for acclimatisation for 30 s before opening the gate to the flight chamber and the attraction bioassay chambers. At the same time in the attraction bioassay chamber, a cotton wool soaked with 5 mL of papaya peel or pineapple peel extract was placed in one of the chamber while the other chamber will be left empty (control). The empty chamber act as a negative control because the mosquitoes were not expected to have any response towards the chamber. The sliding gate for attraction bioassay chambers were opened and a fan was placed at the end of each mesh cap attraction bioassay chambers to allow the peel extract scent to flow into the flight chamber. Additionally, the fan was turned on throughout the experiment. After 30 s, the holding chamber gate was open to release the mosquitoes into the flight chamber. The attraction bioassay chamber gates were closed after 2 min and the number of mosquitoes on the mesh screen are counted and removed from the olfactometer. The test was repeated as described above using different attractant pairs stated in Table 1. In every test, the olfactometer was washed and dried before used again to remove lingering scent from the previous test. Additionally, the position of peel extracts and control in attraction bioassay chambers were rotated to prevent the mosquitoes from side bias, and new batch of mosquitoes unexposed to the attractants was used in every test.

2.4. Statistical analysis

The attraction tests for *Ae. albopictus* and *Ae. aegypti* were performed in four replicates. The Preference Index (PI), described by Afify et al. (2014), was used to indicate the response of gravid female mosquitoes to different attractants in olfactometer. The Preference Index was calculated as:

$$\text{Preference Index (PI)} = \frac{\text{Number of mosquitoes in the test chamber} - \text{Number of mosquitoes in the control chamber}}{\text{Number of mosquitoes in the test chamber} + \text{Number of mosquitoes in the control chamber}}$$

This PI gives values from -1 to $+1$, with 0 indicating neutral response, negative values indicate mosquito repellence while positive values indicate mosquito attraction. The PI was calculated for each replicate and arcsine transformed PI in IBM SPSS using the following command language to generate the desired transformation:

$2 * \text{ARSIN}(\text{SQRT}(\text{PI}))$

This data transformation is necessary so that data appear to more closely meet the assumptions of t -test statistical inference procedure. Then, the mean arcsine transformed Preference Index was

Table 1
Attraction pairing bioassay of *Aedes* mosquitoes.

Mosquito species	Attraction bioassay	Attraction pairs	
		Chamber I	Chamber II
<i>Aedes aegypti</i>	No-choice	Papaya peel extract	Empty port
		Pineapple peel extract	Empty port
	Choice	Papaya peel extract	Pineapple peel extract
<i>Aedes albopictus</i>	No-choice	Papaya peel extract	Empty port
		Pineapple peel extract	Empty port
	Choice	Papaya peel extract	Pineapple peel extract

tested using Levene's test for assumption of equal variance across the samples. On the other hand, the tested hypothesis in this study is each attractant is different from a 50:50 choice with mean PI is 0 . Therefore, the independent t -test was conducted to determine the significant difference between the mean arcsine transformed PI and the tested hypothesis mean PI ($PI = 0$). Additionally, equal variance across the samples was assumed when p values in Levene's test was more than 0.05 while the mean result considered significantly different when the p values in t -test was less than 0.05 . All analysis was conducted using IBM SPSS Version 21.

3. Result

3.1. Response of *Aedes* mosquito to fruit peel extracts in no choice assay

The analysis on *Ae. albopictus* and *Ae. aegypti* responses to the attractants in the no choice assay were conducted by calculating the Preference Index (PI), arcsine transformed the PI using the formula stated above and then analysed the data using the independent t -test analysis. The t -test was used to analyse the significant difference between the mean arcsine transformed PI and the mean tested hypothesis PI ($PI = 0$). Therefore, the statistical analysis for each mosquito species in the no-choice assay were elaborated down below.

In the no choice assay for *Ae. albopictus*, the PI values in the papaya peel extract indicate that the mosquitoes attracted to the peel extract in all replicates with the positive PI values of 0.27 , 0.14 , 0.67 and 0.20 (Fig. 2A). These four PI values were then arcsine transformed before compared to the mean PI of 50:50 choice using the t -test analysis. The Levene's test indicates that there was unequal variance across the samples with the p values was less than 0.05 ($F = 6.96$, $p = 0.04$). Further analysis using t -test shows that the mosquitoes were significantly attracted to the papaya peel

extract ($M = 0.32$, $SE_{\text{mean}} = 0.12$) with the p values is less than 0.05 ($t(3) = 4.66$, $p = 0.02$, $95\% \text{ CI} = 0.37, 1.98$). On the other hand, *Ae. albopictus* mosquitoes were also attracted to the pineapple peel extract in the no-choice assay test ($PI = 0.33, 0.33, 0.67$ and 1.00) (Fig. 2B). Afterwards, the Levene's test shows that there was unequal variance across the samples with the p value less than 0.05 ($F = 6.64$, $p = 0.04$) while the t -test indicates there was significant mosquitoes' attraction to the pineapple peel extract ($M = 1.88$, $SE_{\text{mean}} = 0.45$) with the p value was less than 0.05 ($t(3) = 4.17$, $p = 0.03$, $95\% \text{ CI} = 0.44, 3.31$). The overall result of no choice assay for the *Ae. albopictus* was illustrated in Fig. 2.

Meanwhile, the result in the papaya peel extract no-choice assay for *Ae. aegypti* indicates that the mosquitoes were attracted to the peel extract in all replicates (Fig. 3A). Then, the homogeneity test shows that there was unequal variance assumed across the samples with p values was less than 0.05 ($F = 7.98$, $p = 0.03$). Furthermore, there was significant difference between the mean arcsine transformed PI of the papaya peel extract ($M = 1.79$, $SE_{\text{mean}} = 0.46$) and the mean tested hypothesis PI ($t(6) = 2.30$, $p = 0.08$, $95\% \text{ CI} = -0.10, 3.07$). The analysis suggests that *Ae. aegypti* mosquitoes were attracted to the papaya peel extract.

On the other hand, the negative PI value in the first replicate for the pineapple peel no-choice assay displays the mosquitoes repellence to the peel extract. Subsequently, the last three trials show positive PI values which indicates that the mosquitoes were attracted to the pineapple peel extract (Fig. 3B). Moreover, the p

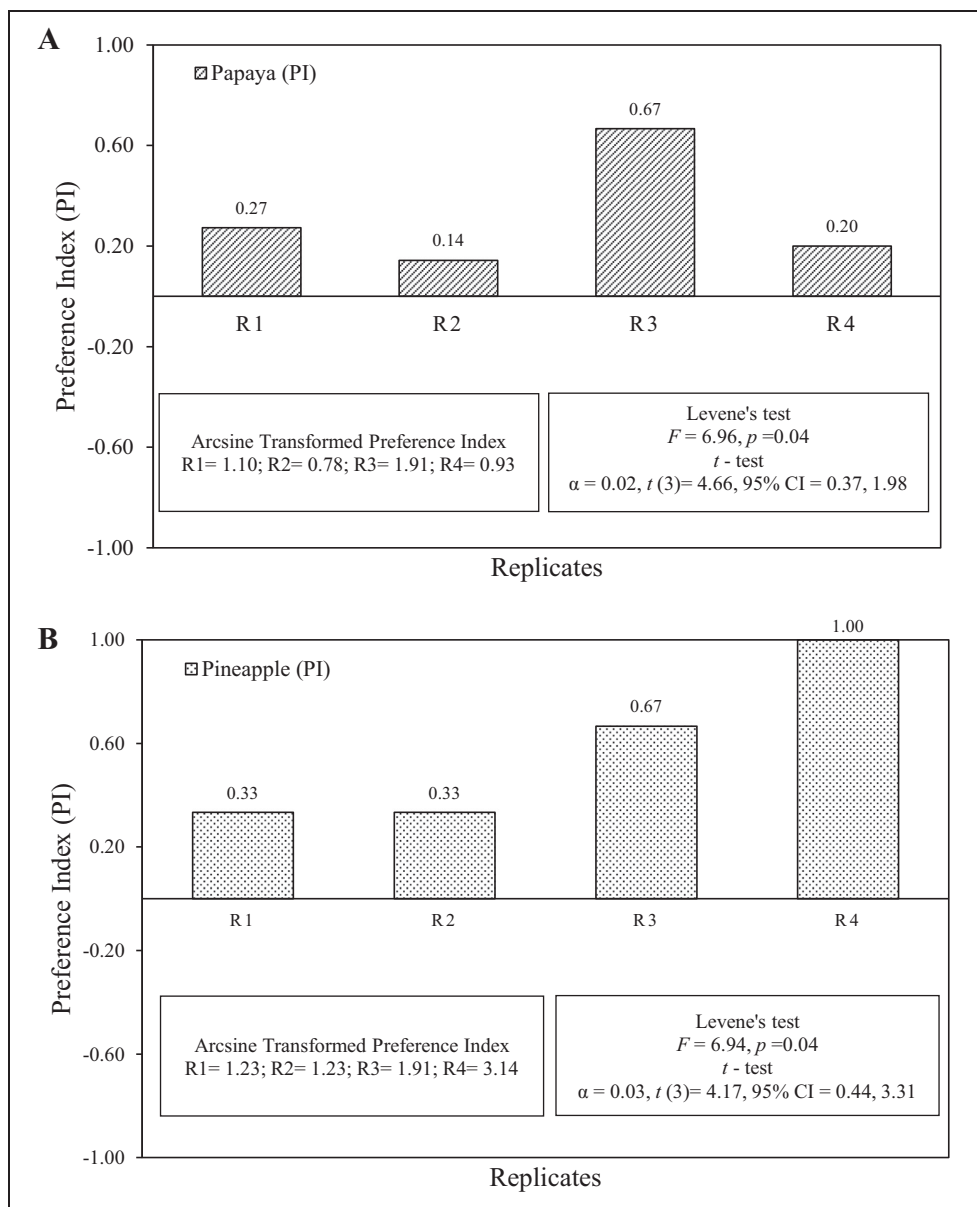


Fig. 2. The Preference Index (PI) of *Aedes albopictus* in non-choice assay. The bar graph shows the preference index in each trial for (A) the papaya peel extract assay (diagonal striped bar) and (B) the pineapple peel extract assay (dotted bar). The lower right box the value of arcsine transformed PI and the box in lower left shows the value of Levene's and t - test analysis. Notes: Positive PI values indicate mosquito's attraction.

values of Levene's test is less than 0.05 ($F = 157.42, p = 0.00$) thus equal variances not assumed across the samples. The t -test analysis shows that the *Ae. aegypti* was significantly attracted to pineapple peel extract ($M = 2.27, SE_{\text{mean}} = 0.51$) with the p value was higher than 0.05 ($t(3) = 4.48, p = 0.02, 95\% \text{ CI} = 0.66, 3.89$). The result of analysis above were presented in the Fig. 3 below.

In conclusion, *Ae. albopictus* and *Ae. aegypti* mosquitoes were often show positive PI values in the no-choice assay replicates for the papaya and pineapple peel extracts. The analysis on the arcsine transformed PI also indicates that the mosquitoes were significantly attracted to the peel extracts when presented individually. The result for no-choice assay above are display in Fig. 4.

3.2. Response of *Aedes* mosquito to fruit peel extracts in choice assay

In order to identify the *Aedes* mosquitoes preferences between the papaya and pineapple peel extracts, the choice assay was con-

ducted using the olfactometer. The assay was carried out by exposing the mosquitoes to both peel extracts simultaneously. *Ae. albopictus* mosquitoes were attracted to pineapple peel extract in first, third and fourth replicate of choice assay with positive PI values of 0.25, 0.20 and 0.33 respectively. Contrary to the result in the pineapple peel extract, the result in the papaya peel extract showed the mosquitoes repellence in the same replicates. Meanwhile, the *Aedes* mosquitoes show neutral response to both peel extracts in the second replicate. Afterwards, the PI values were arcsine transformed and analysed in Levene's and t - test. The homogeneity test shows that there was unequal variance across the samples, $p < 0.05$ ($F = 7.39, p = 0.04$). Moreover, there was no significant difference between the mean arcsine transformed PI ($M = 0.80, SE_{\text{mean}} = 0.27$) and the mean tested hypothesis PI ($t(3) = 2.92, p = 0.06, 95\% \text{ CI} = -0.07, 1.67$). Therefore, the analysis indicates that the chances of *Ae. albopictus* mosquitoes to choose between papaya peel and pineapple peel extracts when presented

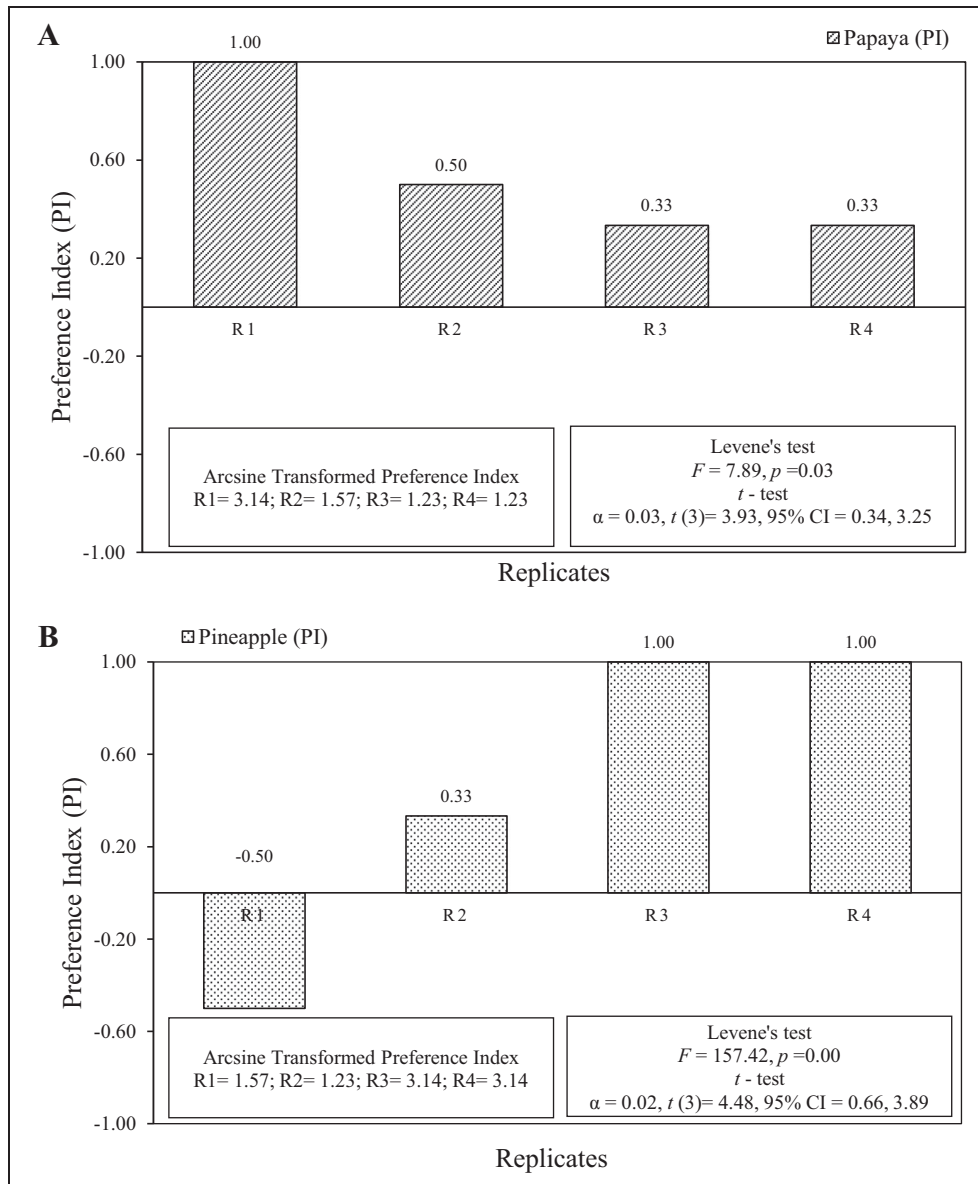


Fig. 3. The Preference Index (PI) of *Aedes aegypti* in non-choice assay. The bar graph shows the preference index value in each trial for (A) the papaya peel extract assay (diagonal striped bar) and (B) the pineapple peel extract assay (dotted bar). The lower right box the value of arcsine transformed PI and the box in lower left shows the value of Levene's and t - test analysis. Notes: Positive PI values indicate the mosquito's attraction and negative PI values indicate mosquito's repellence.

simultaneously were 50:50 ratio. In other words, *Ae. albopictus* mosquitoes were equally attracted to both peel extracts (Fig. 5A).

In the choice assay for *Ae. aegypti*, the papaya peel extract display positive PI values whereas the pineapple peel extract display negative PI values in every choice assay replicates (Fig. 5B). Further analysis using the Levene's test indicates that there was unequal variance across the arcsine transformed PI with $p < 0.05$ ($F = 6.89, p = 0.04$). On the other hand, there was significance difference between the arcsine transformed PI ($M = 1.93, SE_{\text{mean}} = 0.42$) and the mean 50:50 choice PI ($t(3) = 4.60, p = 0.02, 95\% \text{ CI} = 0.59, 3.26$). This shows that *Ae. aegypti* mosquitoes were significantly attracted to the papaya peel extract as compared to the pineapple peel extract when present simultaneously.

Therefore, the result of choice assay indicate that the *Ae. albopictus* was equally attracted to both experimental peel extracts (Mean PI = 0.80). Alternatively, *Ae. aegypti* more attracted to the papaya peel extracts (Mean PI = 1.93) (Fig. 6).

4. Discussion

Ae. albopictus and *Ae. aegypti* response to papaya and pineapple peel extracts were conducted in a modified olfactometer. The olfactometer allows the mosquitoes to be exposed to two attractants at the same time. Therefore, the response study was conducted in no-choice and choice assay. In no-choice assay, the mosquitoes were exposed to either papaya or pineapple peel extracts whereas in choice assay the mosquitoes were exposed to both fruit peel extracts simultaneously. In the end, the result analysis point out both *Aedes* species attracted to papaya and pineapple peel extracts when present alone. On the other hand, *Ae. albopictus* was equally attracted to both peel extracts unlike *Ae. aegypti* that was more attracted to papaya peel extract when the peel extracts were presented simultaneously.

Aedes mosquito attraction and sugar feeding behaviour to plant tissue, fruits, seed pods, flowering, no-flowering plants was

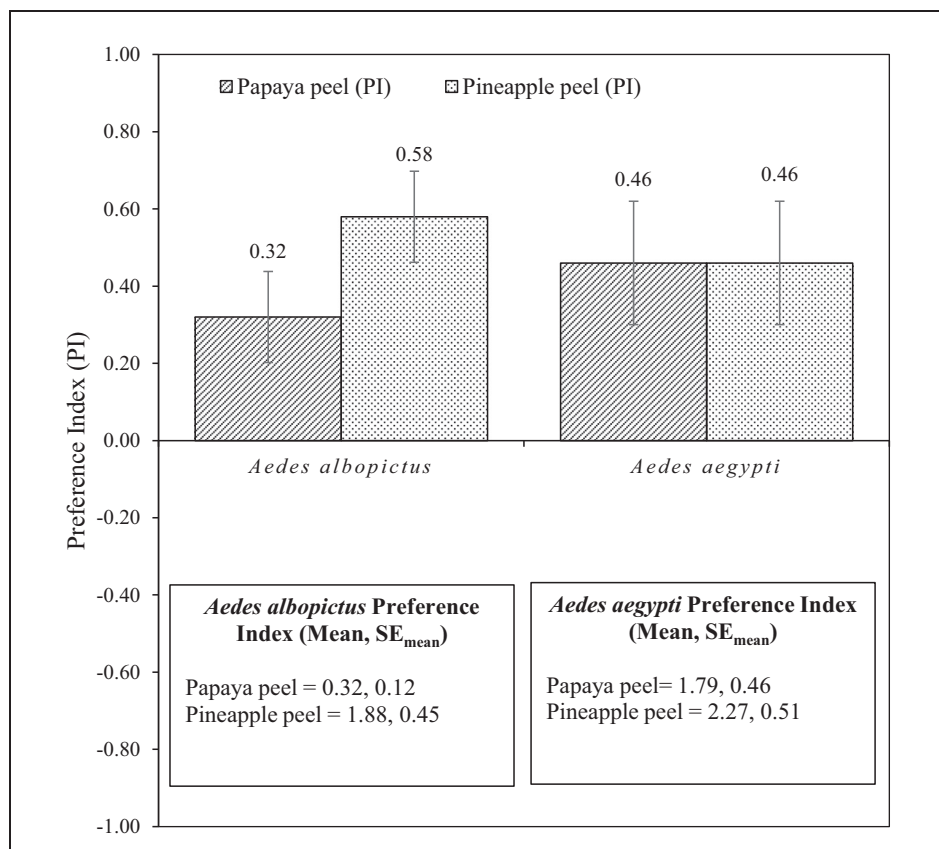


Fig. 4. The mean Preference Index (PI) of *Aedes albopictus* and *Aedes aegypti* in non-choice assay. The bar graph represent the mean preference index in each trial for the papaya peel extract assay (diagonal striped bar) and the pineapple peel extract assay (dotted bar). The lower right and left box show the values of mean PI and mean Standard Error (SE_{mean}) in *Aedes albopictus* and *Aedes aegypti* respectively. Notes: Positive PI values indicate mosquito's attraction.

observed in several studies such as Sissoko et al. (2019) and Müller et al. (2011). However, study on fruit peel extracts were able to attract mosquito is uncommon since fruit peels were usually extracted for their antioxidant and antimicrobial potential (Dabesors et al., 2017; Saraswaty et al., 2017; Ang et al., 2012). In the present study, adult *Ae. albopictus* is equally attracted to papaya and pineapple peel extracts when both are present simultaneously to this mosquito species. This may be due to the species preferences as an exophilic and exophagic mosquito. This mosquito species is attracted to various plants in the field and laboratory settings (Müller et al., 2011). In Müller et al. (2011) unpublished laboratory result, *Ae. albopictus* readily feed on all twenty eight tested sugar sources. Meanwhile, the field attraction part of Müller et al. (2011) study has shown *Ae. albopictus* is significantly attracted to four of six ornamental flowers, four of eleven wild flowers, damaged and fermenting *C. siliqua*, and all five of the tested fruits with the attraction index (mean number of mosquitoes attracted to the baits/mean number of mosquitoes attracted to the control) ranged from 2.5 to 50.0. Moreover, *Ae. albopictus* is attracted to various plants and actively forage for sugar sources because they need regular sugar meals for nutrition and energy as demonstrated in laboratory and field studies (Müller et al., 2011; Xue et al., 2010). Xue et al. (2010) had also stated that female *Ae. albopictus* would starve on blood diet alone as the blood meals only useful for eggs maturation. These mosquito species may be attracted to the papaya and pineapple peel extracts due to their need for regular intake of sugar meals. Therefore, they readily feed on any available sugar sources. However, the attraction may differ in the field setting with abundance of sugar sources.

On the other hand, *Ae. aegypti* is significantly attracted to papaya peel extract only. The low attraction of *Aedes aegypti* to

the fruit peel extracts in general may due to their endophilic preferences (Chadee, 2013; Perich et al., 2000). Female *Ae. aegypti* have been found ingesting blood in the absence of sugar availability for survival (Gary and Foster, 2001). Edman et al. (1992) study on *Ae. aegypti* at domestic settings in Thailand have shown that the blood-fed female mosquitoes survived well and rarely fed on sugar. Therefore, *Ae. aegypti* may be less dependent on sugar for survival compared to other mosquito species and seldom feed on sugar (Edman et al., 1992). However, this contradict with Qualls et al. (2016) fields study findings. Their study on *Ae. aegypti* in low and high vegetation habitats in Durán, Ecuador has demonstrated outdoor sugar feeding is a common behaviour in this mosquito species (Qualls et al., 2016). They suggested that *Ae. aegypti* is opportunistic in its sugar-feeding behaviour due to no significant difference in staining rate by plant type and impact of habitat in their study (Qualls et al., 2016). In the same study by Qualls et al. (2016), *Ae. aegypti* collected indoors have higher sugar stain than *Ae. aegypti* collected outdoors, which indicates that sugar-feeding behaviour occurs before *Ae. aegypti* enter houses. This notion was supported by Chadee et al. (2014) study which has shown both male and female *Ae. aegypti* exhibit diel sugar feeding periodicity prior to blood-feeding that generally takes places indoor for this species with significant evening peak for both sexes at 16:00 to 18:00.

Additionally, the laboratory and field studies by Sissoko et al. (2019) supported the attraction of *Ae. aegypti* to papaya peel extract. Their laboratory study on sugar feeding of sixty *Ae. aegypti* mosquitoes (30 male and 30 female) to potential sugar sources for twenty four hours in mosquito cages. The study result indicates that this mosquito species is attracted to *Acacia macrotachia*, *Carica papaya*, *Cucumis melo*, *Mangifera indica* and *Prosopis juliflora* due to more than 80% of mosquito population tested for presence of sugar

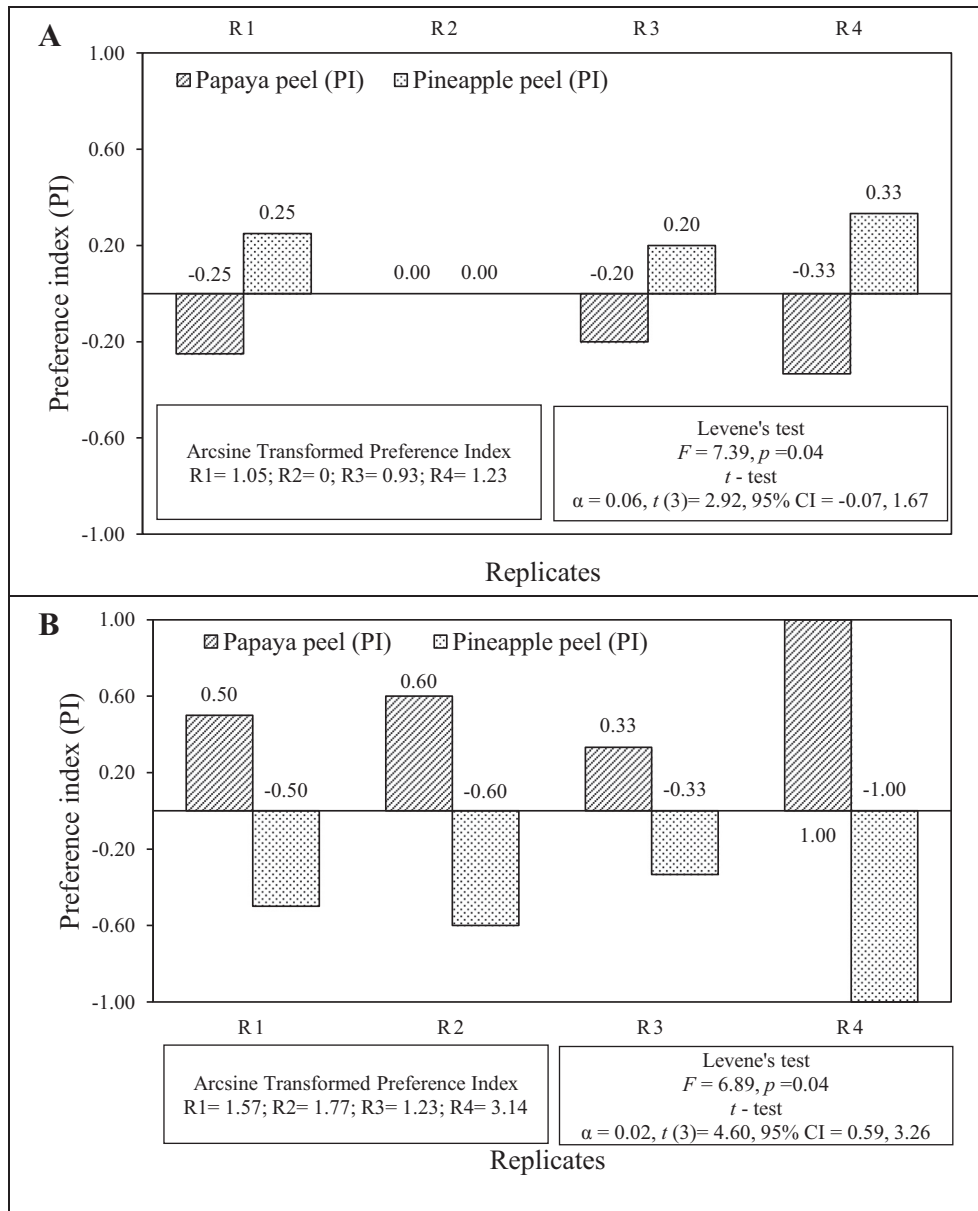


Fig. 5. Preference Index (PI) of *Aedes albopictus* (A) and *Aedes aegypti* (B) in choice assay. The bar graph represent preference index in each replicate for papaya peel extract (diagonal stripped bar) and pineapple peel extract (dotted bar). The lower right box the value of arcsine transformed PI and the box in lower left shows the value of Levene's and t -test analysis. Notes: Positive PI values indicate mosquito's attraction, 0 indicates no response and negative values indicate mosquito's repellence.

is sugar positive (Sissoko et al., 2019). In the field part of Sissoko et al. (2019) study, the female *Ae. aegypti* is significantly attracted to eleven out of twenty sugar sources while male was attracted to eight out of these. Papaya is one of the sugar sources which attracted both male and female *Ae. aegypti* with attraction index of 6.75 and 9.80 respectively.

In addition to the mosquitoes' feeding preferences, the volatile compounds emitted from plants also plays an important role in attracting mosquitoes. For example, *Culex pipiens* and *Ae. aegypti* demonstrated antennal responses to volatile compounds of Silene otite inflorescences comprising phenylethyl alcohol, phenylacetaldehyde, lilac aldehydes, (Z)-3-hexenyl acetate, linalool oxide, linalool, benzaldehyde, lilac alcohol, acetophenone, methyl salicylate and hexanal (Jhumur et al., 2007). Among those compounds, linalool oxide, linalool and hexenyl acetate prompted the strongest antennal response (Jhumur et al., 2008). Additionally, Nyasembe et al. (2012) demonstrated *Anopheles gambiae* attraction to plant

compounds such as hexanal, limonene, (Z)- and (E)-linalool farnesene. Otienoburu et al. (2012) study shown the attraction of *Culex pipiens* to phenylacetaldehyde, benzaldehyde and (E)-2-nonenal emitted from *Asclepias syriaca*. Nevertheless, some plant compounds have repellent effect. Deletre et al. (2013) have tested repellent effect of twenty plant extracts to *Anopheles gambiae*. They had identified lemongrass and coleus plant extracts caused significant repellent to *Anopheles gambiae* at all concentrations tested. Further phytochemical analysis found the major compound in lemongrass and coleus were citral (75%) and epoxyocimene (74.4%) respectively (Deletre et al., 2013).

Besides that, the phytochemical of papaya peel extract consist of terpenoids, tannins, alkaloids, saponins steroid, phenols, fixed oils and fats, phenolic and flavonoids compounds (Sarmad et al., 2018). Furthermore, papaya peel also contained cysteine proteases, papain, chymopapain, glycyldopeptidase, and caricain (Chaiwut et al., 2010). Meanwhile, the phytochemical analysis of pineapple

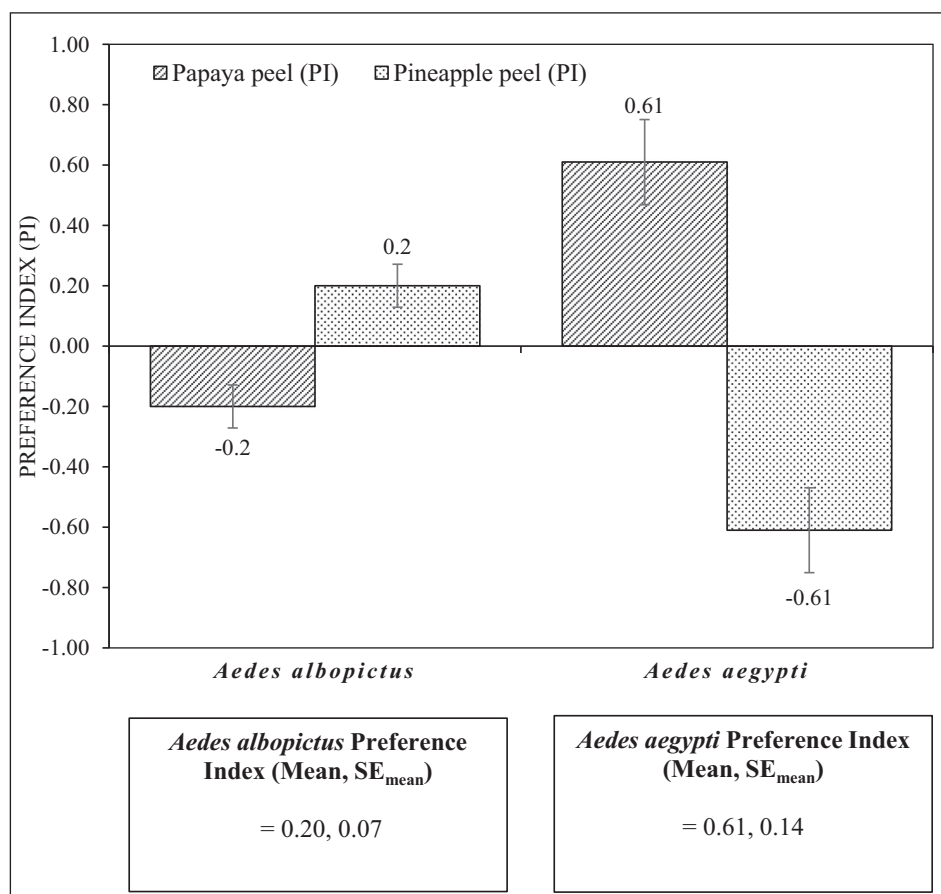


Fig. 6. The mean Preference Index (PI) of *Aedes albopictus* and *Aedes aegypti* in choice assay. The bar graph present the mean preference index and standard error (SE_{mean}) in the papaya peel extract (diagonal stripped bar) and the pineapple peel extract (dotted bar) for each mosquito species. The lower right and left boxes show mean PI and SE for *Aedes albopictus* and *Aedes aegypti* respectively. Notes: Positive PI values indicate mosquito's attraction and negative values indicate mosquito's repellence.

peel extract shows presence of oxalate, alkaloids, phytate, tannins, glycosides, phenolic and flavonoids compounds (Dabesors et al., 2017). There were identical compounds discovered in papaya and pineapple peel extracts. The detection of identical volatile compounds from different plant species in previous studies imply the qualitative and quantitative component of plant compounds that imparts specific sensory impression on insects rather than the presence of a certain individual compound (Najar-Rodriguez et al., 2010).

5. Conclusion

The result no choice assay implies *Aedes* mosquitoes are attracted to papaya and pineapple peel extracts when they were exposed to one peel at a time. However, the mosquitoes response in choice assay when they were exposed to fruit peel extracts simultaneously indicates *Ae. albopictus* is attracted to papaya and pineapple peel extracts while *Ae. aegypti* was significantly attracted to the papaya peel extract only. Therefore, further study on papaya and pineapple peel extracts to attract mosquitoes in field setting need to be conducted to establish the attraction in the environment with abundance of sugar sources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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