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# Palm wines as potential *Aedes* mosquito repellant

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**Abstract**

Globally, *Aedes* mosquitoes cause morbidity and mortality of dengue, yellow fever and other arboviral infections. There is no effective vaccine for *Aedes* transmitted diseases so mosquito control remains the mainstay for their control. Semiochemicals play significant role in modulating insect behavior so are utilized to lure mosquitoes to their destruction or to repel them to halt infection transmission. Palm wines are potent source of semiochemicals but their effect on *Aedes* mosquitoes in our locality is not well understood. This study was undertaken to ascertain whether palm wine could impact on mosquito inflections. *Aedes* larvae were collected and bred in the laboratory to adulthood. Female mosquitoes were selected and tested for their reactions to two categories of palm wine – the up-palm and down-palm wines. An olfactometer was fabricated and applied to find how *Aedes* mosquitoes reacted in it when subjected to odours from the palm wines within 5 minutes. Data obtained were prepared and one way analysis of variance was used to compare means. Only 3% of mosquitoes reached the up-wine arm on day 2. However, when both wines were tested, 2.78 ± 2.78% of mosquitoes reached the down-palm wine terminal. Both wines repelled mosquitoes consistently, confirmed by their refusal to seek any of the palm wine odours. Repellence increased as days passed: initially upstream mosquitoes ranged 36.36 – 60% at the beginning, declining to 3.3 – 6.36% on the $8^{th}$ day; whereas downstream ranged from 40 – 63.63% at the beginning to reach 93.63 – 100% on the $8^{th}$ day. Palm wines semiochemicals repelled *Aedes* mosquitoes. Further testing may be required before utilization in formulated repellents for public use.

**Key words:** *Aedes,* palm wine, mosquitoes, semiochemical, olfactometer, repellent
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## INTRODUCTION

*Aedes* mosquitoes are associated with humans in the tropics but have been known to gain incursion all over the world owing to international trade. Their morphology and characteristics are described elsewhere (Soni *et al.,* 2018; Soares *et al.,* 2022). The insect had been incriminated in morbidity and mortality of significant number of people in different parts of the world who had suffered from any of West Nile, Japanese encephalitis, chikungunya, dengue, yellow fever, Zika and other pyrexia of unknown origin (Paixão *et al.,* 2018). There is therefore an overwhelming need to find a stable and long-lasting solution to address the problem of these mosquitoes. It is estimated that 5.2 billion people could be exposed to dengue, vectored by *Aedes* by year 2085 (Hales *et al*., 2002). Death attributed to yellow fever and other arthropod borne viral infections add to the enormity of annual death rate (Toma *et al.,* 2011). Currently, there are no effective vaccine for many important anthropogenic *Aedes* transmitted diseases like Zika and dengue (Muktar *et al.,* 2016). This predicament leaves mosquito control as the most potent way of controlling or preventing these diseases.

Chemical based signals influence insect behavior. These chemicals are sensed by insects because they are equipped to monitor such stimuli. Organic compounds that can influence insects to react to them are referred to as semiochemicals. Mosquitoes perceive semiochemicals from the air with their olfactory receptors. Many other behaviours like host location and oviposition site's location are mediated by volatile semiochemicals (Himidan *et al.,* 2013). Taking advantage of these behaviour, countermeasures against mosquitoes such as luring to kill or repel them are explored owing to semiochemical exploration (Yu *et al.,* 2015). In our locality, palm wine could be a cheap source of semiochemicals and had been reported to be attractive to *Anopheles* mosquitoes (Ugwu and Onwuzurike, 2018). Palm wines come from palm saps which are allowed to ferment to some extent. The sap is collected daily by wine tappers. In effect, what they obtain is palm sap that may age over approximately 24 hours. So even though they may be referred to as "fresh" palm wine, they contain some quantity of fermented products (Obahiagbon, 2012). The production of fermentation product may be continuous until microorganisms responsible gain attrition (Ukwuru and Awah, 2013). This means that the first line products could be acted upon to produce other fermentation products so that the initial product may in turn become the substrate for the next. So far, there had not been any report from our locality of the effect of palm wine over time on *Aedes* mosquitoes. This work sought to determine whether two categories of palm wines, up-palm and/or down-palm wine's attractiveness to *Aedes* mosquitoes would increase/decrease with time.

## MATERIALS AND METHODS

**Fabrication of olfactometer**: This was fabricated like other Y type olfactometer (Yu *et al.,* 2015; Ugwu and Onwuzuruike, 2018) using see-through plastic materials. The procedures were carried out in laboratories of South East Zonal Biotechnology Centre and the Department of Zoology and Environmental Biology, both departments located in the University of Nigeria Nsukka, Nigeria. The Y olfactometer was constructed as shown in Figure 1. The two upper arms (may be referred to arm or terminal) were where the test and control substances were introduced and were joined to the stem. The two arms were supplied with air from an air pump (Corning 850, Corning Science Products, Corning Limited, Essex, AO9 2DX, England) with controlled air flow (air pressure of 1 kg/cm$^2$ at 6 litres/minute) with a y tube and through the sample such that bubbles generated kept the fluid tested well stirred. This process generated volatiles which were pushed through the respective arm/terminal. Both arms were rejoined at the confluence to exit through the stem. The stem was longer of the three sections. It contained the release chamber located midway between the upper stem (upstream) and lower stem (downstream) part of the stem. The release chamber consisted of a rotatable cylinder placed in another fixed cylinder. The sides of the internal cylinder consisted of fabric net. The internal and external cylinders had some part cut away such that as the internal cylinder was rotated, the opened sections on both cylinders aligned to create space between 0 – 135,$^0$ depending on the degree of rotation. This chamber was for introduction of mosquitoes to the olfactometer via a tube when the compound cylinder was completely closed. Mosquitoes were equilibrated for some time (5 minutes) in the chamber before the cylinder was opened to release them.

**Collection and breeding of *Aedes* mosquitoes**: Mosquito larvae were collected from egbaite (Ugwu and Onwuzuruike, 2018) from Obollo Afor and Onuiyi, Nsukka. Breeding procedures were undertaken in above named laboratories. Floating characteristics were employed to isolate *Aedes* larvae ((Ugwu and Onwuzuruike, 2018)). Breeding to adulthood and feeding with 10 % glucose followed standard methods in net-covered buckets (WHO, 2003). Female mosquitoes were sorted using mouth-parts characteristics to separate them from males (Ugwu and Onwuzuruike, 2018). Collected adult female mosquitoes were left at room temperature and fed until when required

**Collection of palm sap/wine**

Fresh palm wine obtained from *Elaeis guineensis* were bought from Obollo Afor Market. Two categories of palm wine were collected: up-wine and down-wine ((Ugwu and Onwuzuruike, 2018)). These samples were allowed to remain in their respective plastic narrow-necked container covered with net at room temperature during the duration of the experiment to allow fermentation continue naturally by their yeast content (Ukwuru and Awah, 3013). A hundred ml amount from each category was used for each testing cycle.

**Experimental procedure**

The olfactometer was used to test for the response of the mosquitoes to the odours from the palm wines. 10 – 12 adult female mosquitoes were introduced into the release chamber with the aid of an aspirator/sucking tube. Mosquitoes were allowed to acclimatize for five minutes in air. Hereafter, the odours were introduced. After 30 seconds, the mosquitoes were released from the chamber by rotating the mesh screen (internal cylinder) thereby allowing them to escape from the chamber and make their choices: they were free to move toward the odour so that when they arrived at the confluence between the arms, they would make one more decision: to move to any one of the arms depending on how they respond to the preferred odour. The alternative response was their freedom to move away from the odour by moving downstream. Thereafter, the number of mosquitoes were counted and categorized according to the part of the olfactometer they rested at the end of 5 minutes. The experiment was carried out daily for 8 days as follows: control (water) and up-wine; water and down-palm wine; and up-palm wine against down-palm wine. Each test was triplicated. The olfatometer was rinsed in water and allowed to dry at 40 $^{0}$ C after each experiment. Each set of mosquitoes was used once and were killed by drowning.

**Data analysis**

Data obtained were initially processed with Microsoft Office Excel. Later, SPSS Version 16.0 was used for further analysis and one way ANOVA was used to compare the means of variables and the computation of level of significance.

**RESULTS**

**Time course of attractiveness of *Aedes* mosquitoes to palm wine**

*Aedes* mosquitoes were not attracted to up-palm wine and down-palm wine for the duration of the study. Rather they were repelled. The intensity of repulsion increased as the palm wines aged. The increased repulsion was marked by the mosquitoes moving more into the down section of the stem in the olfactometer.

**Attractiveness of *Aedes* mosquitoes to up-palm wine versus water**

Table 1 shows the attraction of the mosquitoes to up-wine versus water. Their preferences are shown by the number of mosquitoes seen in up or downstream arm of the stem and the water or palm wine arms of the olfactometer. Some negligible level of attraction to this category of wine to mosquitoes was only evident on day 2 with a mean of $3.03 \pm 3.03$. An equal number of mosquitoes was attracted to water on day 4. Mosquitoes spent their time in either the upstream or downstream section of the stem of the olfactometer. The number of mosquitoes in the upstream section decreased as the duration of exposure increased from days 1 to 8. Significantly different number of mosquitoes were present in the upstream arm on days 1, 2, and 3 compared to days 4, 5, 6, 7 and 8 ($p < 0.05$). The pattern appeared to have reversed in the downstream arm. The mosquitoes that were repelled from the upstream arm moved downstream, making the mean number of mosquitoes in the downstream arm to increase as the days of exposure increased.

**Attraction of *Aedes* mosquitoes to down-palm ine versus water**

Table 2 shows data obtained. Like the up-palm wine, mosquitoes were repelled. The intensity of repulsion was greater in the down-palm as $0.00 \pm 00$ mosquitoes were attracted to the palm wine terminal and water terminal showing that they were not attracted to this category of wine. All mosquitoes released from the chamber spent their time in the up or down sections of the stem.

A mean of 60% and 40% visited upper arm and lower arm of the stem respectively on the first day. This proportion inverted as time progressed. Finally, only 3.33 ± 3.33 and 96.67 ± 3.33 spent their time in the upper arm and lower arms of the stem respectively on the 8$^{th}$ day.

**Attraction of *Aedes* mosquitoes to up-palm wine versus down-palm wine**

Table 3 shows dater obtained. The response of *Aedes* mosquitoes in the olfactometer when both categories of wine were tested simultaneously was characterized by repulsion as mosquitoes, they did not move into either of the terminals but preferred to stay in the upstream and downstream section of the stem. No mosquitoes were seen in the up-wine arm whereas 2.78 ± 2.78 mean percentage of mosquitoes entered the down-palm wine terminal while 97 % remained in either section of the stem. On the first day, 36.36 ± 5.25 and 63.63 ± 5.8 percent of mosquitoes were respectively found in the up-stream and down-stream sections of the stem. These palm wine maintained their repulsiveness to mosquitoes as the days progressed though the level seen in up-wine was higher. Repellence increased as days passed: initially upstream mosquitoes ranged 36.36 – 60% at the beginning,

Table 1: Attraction of mosquitoes to up-palm wine and water from day 1 - 8.

| Duration (Days) | Mean number of mosquitoes introduced | Mean visiting mosquitoes (%) | | | |
|---|---|---|---|---|---|
| | | Palm wine terminal | Water terminal | Stem | |
| | | | | Upstream | Downstream |
| 1 | 10.00 ± 0.00 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 5.67 ± 0.33 (56.67 ± 3.33)$^{a}$ | 4.33 ± 0.33 (43.33 ± 3.30)$^{e}$ |
| 2 | 10.67 ± 0.33 | 0.33 ± 0.33 (3.03 ± 3.03) | 0.00 ± 0.00 (0.00 ± 0.00) | 6.33 ± 0.67 (59.09 ± 4.55)$^{a}$ | 4.00 ± 0.58 (37.88 ± 6.60)$^{e}$ |
| 3 | 9.67 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 5.33 ± 0.33 (55.19 ± 2.89)$^{a}$ | 4.33 ± 0.33 (44.81 ± 2.89)$^{e}$ |
| 4 | 10.33 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.33 ± 0.33 (3.03 ± 3.03) | 4.00 ± 0.57 (39.09 ± 6.58)$^{b}$ | 6.00 ± 0.58 (57.88 ± 4.08)$^{d}$ |
| 5 | 10.67 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 3.33 ± 0.33 (31.21 ± 2.69)$^{bc}$ | 7.33 ± 0.33 (68.79 ± 2.69)$^{cd}$ |
| 6 | 10.67 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 2.33 ± 0.33 (21.82 ± 2.77)$^{cd}$ | 8.33 ± 0.33 (78.18 ± 2.78)$^{bc}$ |
| 7 | 10.67 ± 0.67 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 1.33 ± 0.33 (12.22 ± 0.33)$^{de}$ | 9.33 ± 0.33 (87.78 ± 2.22)$^{ab}$ |
| 8 | 10.33 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 0.67 ± 0.33 (6.36 ± 3.19)$^{e}$ | 9.67 ± 0.33 (93.63 ± 3.19)$^{a}$ |

Values as mean ± standard error. Values with different alphabets superscript along a column were significantly different ($p < 0.05$).

Table 2: Attraction of mosquitoes to down-palm wine and water from days 1- 8 of exposure

| Duration (Days) | Mean number of mosquitoes introduced | Mean visiting mosquitoes (%) | | | |
|---|---|---|---|---|---|
| | | Palm wine terminal | Water terminal | Stem | |
| | | | | upstream | Downstream |
| **1** | 10.00 ± 0.00 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 6.00 ± 0.00 (60.00 ± 0.00)[a] | 4.00 ± 0.00 (40.00 ± 0.00)[f] |
| **2** | 10.33 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 5.00 ± 0.00 (48.48 ± 1.52)[b] | 5.33 ± 0.33 (51. 52 ± 1.52)[e] |
| **3** | 10.00 ± 0.00 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 4.00 ± 0.58 (40.00 ± 5.78)[bc] | 6.00 ± 0.58 (6.00 ± 5.77)[de] |
| **4** | 11.00 ± 0.58 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 3.33 ± 0.33 (30.20 ± 1.75)[cd] | 7.67 ± 0.33 (69.79 ± 1.75)[cd] |
| **5** | 11.00 ± 0.58 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 2.33 ± 0.33 (21.06 ± 2.04)[de] | 8.67 ± 0.33 (78.94 ± 2.04)[bc] |
| **6** | 10.67 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 1.33 ± 0.33 (12.42 ± 2.89)[ef] | 9.33 ± 0.33 (87.58 ± 2.89)[ab] |
| **7** | 11.33 ± 1.16 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 1.00 ± 0.58 (8.89 ± 4.84)[f] | 10.33 ± 0.88 (91.11 ± 4.84)[a] |
| **8** | 10.00 ± 0.00 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 0.33 ± 0.33 (3.33 ± 3.33)[f] | 9.67 ± 0.33 (96.67 ± 3.33)[a] |

Values as mean ± standard error. Values with different alphabets superscript along a column were significantly different ($p < 0.05$).

Table 3: Attraction of mosquitoes to up-palm wine and down-palm wine from day 1 - 8 of exposure

| Duration (Days) | Mean number of mosquitoes introduced | Mean visiting mosquitoes (%) | | | |
|---|---|---|---|---|---|
| | | Up-palm wine terminal | Down-palm wine terminal | Stem | |
| | | | | upstream | downstream |
| **1** | 11.00 ± 0.00 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 4.00 ± 0.58 (36.36 ± 5.25)[a] | 7.00 ± 0.58 (63.63 ± 5.80)[e] |
| **2** | 10.67 ± 0.67 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.33 ± 0.33 (2.78 ± 2.78) | 4.00 ± 0.58 (37.78 ± 6.19)[a] | 6.33 ± 0.67 (59.44 ± 5.80)[f] |
| **3** | 10.33 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 3.00 ± 0.00 (29.09 ± 0.91)[b] | 7.33 ± 0.33 (70.90 ± 0.91) |
| **4** | 10.33 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 2.33 ± 0.33 (2.73 ± 3.67)[e] | 8.00 ± 0.58 (77.27 ± 3.67)[d] |
| **5** | 10.33 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 1.67 ± 0.33 (16.06 ± 3.08)[bc] | 8.67 ± 0.33 (83.94 ± 3.08)[bc] |
| **6** | 10.33 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 0.67 ± 0.33 (6.36 ± 3.19)[d] | 9.76 ± 0.33 (93.63 ± 3.19)[ab] |
| **7** | 11.00 ± 0.58 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 11.00 ± 0.58 (100.00 ± 0.00)[a] |
| **8** | 10.33 ± 0.33 | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 0.00 ± 0.00 (0.00 ± 0.00) | 10.33 ± 0.33 (100.00 ± 0.00)[a] |

Values as mean ± standard error. Values with different alphabets superscript along a column were significantly different ($p < 0.05$).

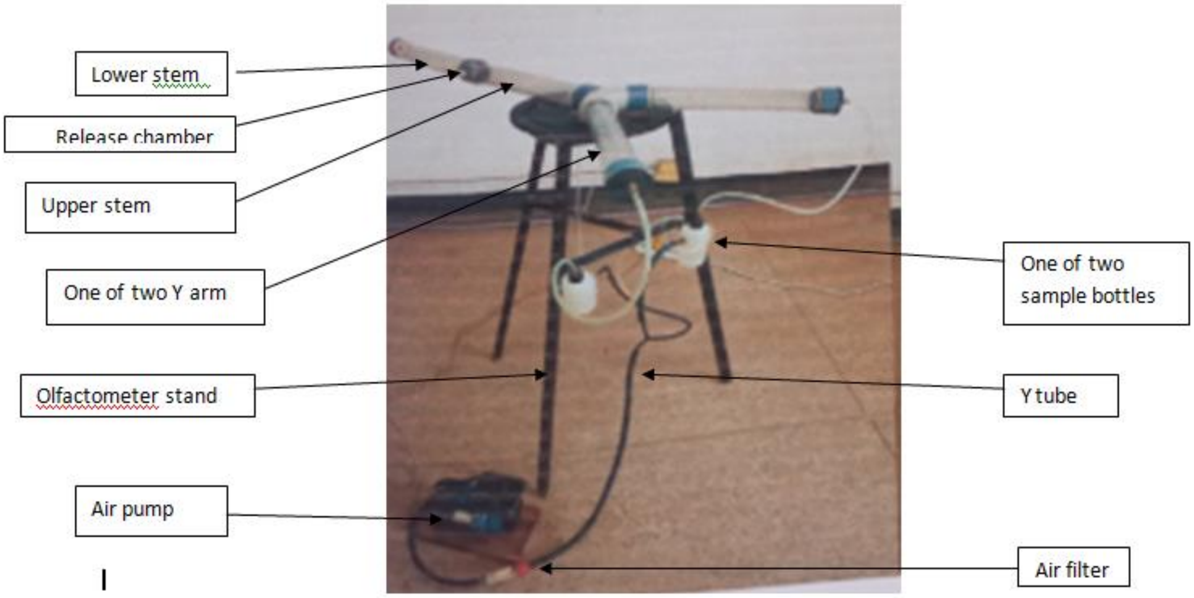


Figure 1: The olfactometer applied for the experiments showing important parts.

declining to 3.3 – 6.36% on the 8th day; whereas downstream ranged from 40 – 63.63% at the beginning to 93.63 – 100% on the 8th day (Tables 1, 2 and 3).

## DISCUSSION

We used two categories of palm wines to determine if *Aedes* mosquitoes would be attracted to them. The result apparently indicated that *Aedes* from the locality avoided palm wines. This appears to negate the response of flies generally to palm wine (Ugwu and Onwuzuruike, 2018). The use of plant products by man to provide protection against biting insects and against a variety of insect-borne diseases is well documented in literature. The different mosquito vectors exhibit contrasting responses to different chemicals and odours. This in turn leads to a variation in the effectiveness of different methods in controlling local mosquito population; hence there is need for integrated sets of control methods adapted to local settings which can be provided at minimal cost that are readily accessible to local people. One such key method may be use of locally available plants and plant products that have traditionally been used to deter mosquitoes (Dekker *et al.,* 2011). Synthetic repellents are usually too expensive for people living in rural areas especially in the tropics. The desire to motivate natives to participate in mosquito control with local materials formed the basis of this investigation.

Previous studies indicated that anopheles mosquitoes were attracted to palm wine because during their fermentation, carbon dioxide, a noteworthy attractant usually evolved (Ugwu and Onwuzuruike, 2018). Mweresa *et al.,* (2014) observed that majority (53.3%) of *Anopheles gambiae* released were attracted to carbon dioxide and subsequently trapped. Apparently, carbon dioxide and other volatiles produced by fermentation (Ukwuru and Awah, 2013) may be used to lure anopheles specie. Hoel *et al.,* (2007) reported that lactic acid and octanol in the presence of carbon dioxide attracted more mosquitoes than mosquito magnet trap. In similar dual-port olfactometer studies, Bernier *et al.,* (2003) studied lactic acid and acetone blend compared with lactic acid and carbon dioxide blends. They noted that attraction was observed in both terminals. So, it might have been expected that mosquitoes would have been attracted reasonably to the up-palm/down-palm wine and their combination in this study. This shortcoming is clearly shown in Tables 1, 2 and 3. This strange behavior may be dependent on the species and genetic difference/diversity prevalent in this locality. It has been observed that different species have different behavioural and olfactory responses (Obahiagbon, 2012). Geographical location may also affect mosquitoes' preferences. They may also be influenced by the variable nature of palm wines (Ugwu and Onwuzuruike, 2018). Our results suggest that the volatiles in the batch of palm wine used in this study may have suppressed/countered the known capacity of carbon dioxide to attract mosquitoes. This unusual finding may be an indication of genetic change or the unlikely event that the palm wines may have been compromised as unscrupulous tappers and traders are known to use sundry products to modify palm wines sold in the market.

*Aedes* mosquito repellence in this study were certainly due to palm wine volatiles. This observation is explained by the intensification of the production of volatiles which increased as time went on (Obahiagbon, 2012; Ukwuru and Awah 2013). The impetus in this observation (more mosquitoes moving to downstream of the stem) may be due to additive effects of volatile metabolites we noted marginally greater potency of repellency when the two categories of palm wine were combined (Table 3). In the final experiment where up-palm and down-palm wines were applied, both showed this repulsive property to *Aedes* mosquitoes and their effect seemed to be additive. Increased repellence of mosquitoes by palm wines with time may be reflected in the increased fermentation occurring in palm wines that produces more chemical compounds such as alcohol that may be responsible for increased repellence with time. From these findings, palm wine could be used as a standard where other substances that have related effects could be compared. In Aedes control therefore, palm wine could be used to calculate preference index which could be useful in grading effectiveness of odour-based responses for repellents (Yu *et al*., 2015).

## CONCLUSION

Though previous report on *Anopheles* was opposite to our finding, we could be reasonably safe to state that as far as *Aedes* mosquitoes from our locality is concerned, palm wine exerts a repulsive effect on them. Therefore, palm wine may be depended upon to source cheap repellents to control *Aedes* mosquitoes as well as associated infections. The potentials of palm wine may never be exhausted. Products from this could be incorporated into creams along with other known repellents to provide people who work in the open fields such as farmers better protection from *Aedes* and related vectors known to feed exophargously. However, further research must be done to identify the active ingredients that so powerfully suppressed the effects of carbon dioxide which ought to have attracted *Aedes* mosquitoes.


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## Author contribution

UFSO designed the study, constructed the olfactometer and edited the paper. OPC sourced the wild mosquitoes for the experiments and did the data analysis. Both authors contributed in writing all sections of this paper and conducting tests on mosquitoes and approved the final version of paper.

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