PALM WINES AS POTENT ATTRACTANT TO *ANOPHELES* MOSQUITOES

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ABSTRACT

Control of Anopheles vector is effective in halting malaria. Entrapping them by deceit with semiochemicals they must respond to could be sustainable control approach but poverty and other impediments prevent use of such luring strategy in Sub Saharan Africa. Elaeis guineensis could be alternative accessible and cheap unexplored source of semiochemicals for mosquitoes control. We evaluated the response of Anopheles mosquitoes to categories of palm wine. Anopheles larvae were selected from mosquito juveniles collected from egbaite. At emergence, female mosquitoes were subjected to a 2-way olfactometer to evaluate their responses to odours from 4 categories of palm wines. Fresh up-palm wine attracted 50.00 ± 5.77% of mosquitoes, its sub-category, ebacha up-wine, attracted 45 %. Fresh down-wine attracted 70 % of vectors as against 68.33 % of ebacha down-wine. The foregoing categories compared to the control attracted significantly more mosquitoes (respectively, p = 0.007; p = 0.005; p = 0.002; p = 0.001). Comparison of fresh up-wine with fresh down-wine showed that significantly more mosquitoes visited down-wine (t = - 5.060, p = 0.007). Ebacha up-wine and ebacha down-wine comparison revealed that significantly more mosquitoes visited ebacha down-wine (t = - 4.950, p = 0.008). The preference indices calculated for the 4 categories of palm wine ranged from 57.27 ± 14.90 to 85.98 ± 7.73. Palm wines are attractive to Anopheles mosquitoes. Down-wine was more effective attractant. Up wine lost its activity faster than down-wine. Palm wines could sustainably be employed in malaria vector control programmes.

Keywords: Malaria/mosquito control, *Anopheles*, *Elaeis guineensis*, Semiochemicals, Palm wine, 2-way olfactometer

INTRODUCTION

In 2016, WHO (2017) estimated that 216 million cases of malaria occurred and resulted in 445,000 deaths globally. WHO reported that 80% of deaths were in countries of sub-Saharan Africa apparently endemic to *Plasmodium falciparum*, a protozoan parasite infection which accounted for 99 % of cases. The disease is spread via the bite of infected female mosquitoes of the genus *Anopheles* (Walshe *et al.*, 2017). *Plasmodium falciparum* is widespread in Africa, while *P. vivax, P. ovale*, and *P. malariae* infections are less common and constrained in nature (Roucher *et al.,* 2014; Howes *et al.,* 2015). Progress made in malaria control recently has prompted increasing global exchange of ideas to engender malaria elimination and eradication with promising new tools to control vector among others (Hemingway *et al.,* 2016). Vector control is effective in halting malaria transmission (Killeen *et al*., 2014) hence it is one of the four basic technical control elements. However, WHO (2017) acknowledges the fact of increasing vector resistance to prevailing insecticides. Another very effective option in vector control is by entrapping them by deceit with semiochemicals which they must respond to believing they are being guided in their quest to meet their basic biological needs.

Semiochemicals are natural compounds that convey chemical messages. Insects depend on them for within/between species communication. They are detected directly from the air by insects with their olfactory receptors, located in most insects, in sensilla hairs on the antennae. These odours provoke the insect both in the response of antennal neuron and behavior such as host aversion (Vinauger*et al*., 2018). Other mosquito behaviours like search for nectar, host location and oviposition are mediated by volatile semiochemicals in the environment including host odours (Sumba *et al*., 2007; Verhulst *et al*., 2010; Himeidan *et al*., 2013; Smallegange *et al*., 2013; Yu *et al*., 2015). Such sites could be laced with attractants or stimulants for mass trapping/monitoring insects or possibly in combination with insecticides to control disease vectors such as gravid mosquito population (Himeidan *et al.,* 2013; Matowo *et al*, 2013). Several semiochemical substances have been used in previous studies to lure and kill mosquitoes. Yu *et al.* (2015) used benzaldehyde, benzyl alcohol, acetophenone, etc. Smallegange *et al.* (2010) listed ammonia, lactic acid, and aliphatic carboxylic acids, while Scott-Fiorenzano *et al.* (2017) used L-lactic and 1-octen-3-ol. In-house mosquitoes are said to have decreased considerably due to insecticides and other control measures, but attaining the same feat outdoors have proven difficult because of exophagic/exophilic mosquitoes and lack of appropriate baits (Jhumur *et al.,* 2007; Chaccour *et al*., 2010; Nyasembe *et al*., 2014). According to Verhulst *et al.* (2011), identification of volatiles released by human skin bacteria led to the discovery of compounds that have impact on the host-seeking behaviour of *A. gambiae.* They further showed that 3-methyl-1-butanol may be used to increase mosquito trap catches, whereas 2-phenylethanol has potential as a spatial repellent and that the two compounds could be applied in push-pull strategies to reduce mosquito numbers in malaria endemic areas. This beautiful approach would have been most suited for those most hit by scourge of mosquito/malaria – Sub Saharan Africa. For poverty stricken people and natives of the region to participate in such mosquito control campaigns, there must be readily available and affordable answer. Unfortunately, most times, people in these areas are not even aware of these chemicals and therefore do not ask for them from traders in local markets. A likely way out to this problem is sourcing for semiochemicals from local materials within such endemic areas. The extract of oil palm trees (*Elaeis guineensis*) appears well disposed to meeting such needs. They are common in West Africa and elsewhere located 10 degrees north or south of equator (Obahiagbon, 2012). This plant is the most popular indigenous tree in Southern Nigeria and the economic base of South Eastern Nigeria. Consequently, extracting its economic benefits engage most adults.

Wines obtained from *Elaeis guineensis* are enriched with peculiar odour that are known to attract *Musca domestica, Anthrophila* species and a host of other members of the Class Insecta relatives of mosquitoes. Lasekan *et al*. (2009) using high resolution gas chromatography-olfactometry and mass spectrometry, obtained 24 odour-active compounds from palm wine. Fresh up-wine, the fluid collected by tapping the young inflorescence at the growing end of the tree is one category of palm wine. Another category, *ekpo,* down-wine, is collected by felling the palm tree which is allowed to stay for some days, and thereafter, cutting an oblong hole into the leafy part of the trunk. Early morning palm wine is sugary and contains small concentration of alcohol (Karamoko *et al*., 2012; Santiago-Urbina *et al*., 2013). This makes it a very attractive healthy beverage for all and especially children during morning hours. It is generally good for consumptions because it does not contain additives (Chandrasekhar *et al.,* 2012). Palm wine is a cloudy whitish unstable cocktail with initial sweet pleasant taste and brief shelf life. The wine is consumed in a variety of flavors varying from sweet to vinegary (Azam *et al.,* 2003). Both types of wines are therefore common because they are cheaply obtainable. In most villages and local markets, they are bought during early morning hours directly from wine tappers and from retailers/traders in later part of the day and far into the night. Palm wines that were not consumed after the first day are called *ebacha* and are also sold at giveaway prices. Natives use palm wines for purposes such as entertainments, compounding of medicines (Obahiagbon, 2012) and a host of other cultural practices.

Biochemically, palm wine constitutes of hydrocarbons, sugars, amino acids/proteins, organic acids, lipids, terpenes, alcohols, minerals, trace elements and numerous phytonutrients which play significant roles in human health (Uzochukwu *et al.*, 1994; Lasekan *et al.*, 2009; Obahiagbon, 2012). The principal components responsible for the odour of palm wine are higher alcohols, esters, acids, aldehydes and ketones (Santiago-Urbina and Ruiz-Teran, 2014). In addition, palm wine is a nutritionally rich medium for the growth of many microorganisms among which are yeast species. Different species of yeast can be found in palm wine (Stringini *et al.*, 2009; Ukwuru and Awah, 2013). Its increasing alcoholic credentials when allowed to remain in ambient temperature is because of the fermentation of its sugary components which ultimately generate more volatile compounds among which are the usual carbon dioxide and alcoholic products. The foregoing proves that palm wine is rich in arrays of biochemical compounds of which semiochemicals are inclusive. No study exist that evaluated mosquitoes' response to palm wine odourants. In this study, we evaluated the response of *Anopheles* mosquitoes, the principal malaria vector, to categories of palm wine.

MATERIALS AND METHODS

Collection of Mosquitoes and Selection of Anopheles Species: Mosquito larvae were collected from *egbaite* (collection of clay-pots water reservoir within a compound and deposited into a basin) (Ugwu, 2011; 2015). The larvae fed continuously on microorganisms, algae and other organic matter obtained at the same time from the habitat they were found in. A few larvae (5 – 8) were placed on a beaker containing clear water. Larval floating behaviour was used to select only *Anopheles* larvae (WHO, 2003). Selected mosquito larvae were put in another transparent mesh covered plastic bucket so that when hatched they will not escape. Thereafter, emergent females were selected and placed in another similar container with the aid of sucking tube where they were fed on a constant diet of 10 % glucose solution pending time of use (Chaccour *et al.*, 2010).

Construction of a 2-Way Olfactometer: The type used was like that of Yu *et al.* (2015) with some small modifications. The olfactometer was fabricated from extruded polyvinyl chloride (PVC) square pipes, sides 5 cm, consisting of a u shaped base and a top cover. This cover fitted snuggly with pressure such that leakage of air was impossible. Sections of the top were cut off, replaced with transparent plastic materials and sealed with adhesive such that air leakage was also impossible. This permitted direct observation of test organisms in the olfactometer. Each of the two arms of the olfactometer measured 32 cm and was at right angles to each other. Taking bearing from the release section, the longest part (the stem) actually had two sections: the downstream and the upstream sections. The upstream end of the stem was funnel shaped, narrowed to open a square shaped hole with side 1.5 cm. The downstream/upstream sections measured 45 cm from the release chamber. When released, mosquitoes were free to move away from the release chamber either downstream or upstream.

The release chamber located midway on the stem consisted of two cylinders: the outer and inner. The former which had one end closed was fixed through a hole which passed from one side of the stem to the other but resting inside at the base. Section of this cylinder which matched the 4.8 cm internal side of the stem was cut off. The inside cylinder similarly had one end closed and consisted of screening mesh which could be rotated while inside the external cylinder. The section which approximated the cut section of the external cylinder was similarly cut off. The chamber was closed by rotating the inner cylinder so that the mesh screen was uppermost. To open the chamber, the open side of the inner cylinder was rotated uppermost. The stem and each of the two arms were detachable. These segments were united with the aid of metal brackets and silicon rubber which were applied to seal spaces to ensure the joints were airtight. Air was delivered to the olfactometer from a Corning 850 air compressor (Corning Science Products, Corning Limited, Halstead, Essex, CO92DX, England) with air pressure of 1 kg/cm squared at 6 litres per minute. This was facilitated through rubber tubing connected as shown in Figure 1 to as well deliver the odour from the palm wine to the olfactometer.

Categories of Palm Wine: Early morning palm wines – fresh *up-wine* and *ekpo,* fresh *down-wine* were bought directly from palm wine tappers at Obollo Afor market. Test on them provided data for the major categories. Both were left at room temperature for 24 hours to become *ebacha,* thus becoming the two sub-categories. The four categories of palm wine were used in the study. Each category was tested with water as control, then the 2 major categories were compared to one another and the two sub-categories were similarly compared.

Test for Mosquito Response to Palm Wine Odours: In respective container, 100 ml of water (control) and test sample were placed and connected as shown in Figure 1. With the aid of a sucking tube, 20 female *Anopheles* mosquitoes were placed in the release chamber. They were allowed 10 minutes to acclimatize (Yu *et al*., 2015). Thereafter, the connections to the control and palm wine were coupled. Mosquitoes in the release chamber were released 30 seconds later by rotating away the mesh screen thereby allowing them to react to the stimuli.

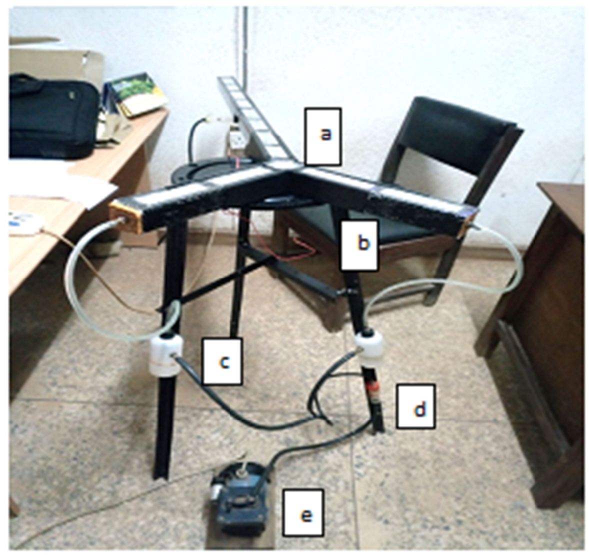

Figure I: Set up showing the connection to the 2-way olfactometer: a, 2-way olfactometer; b, metal stand; c, test odour/control container; d, air filter; e, air pump

Mosquitoes were required to respond to odour of test in a y-maze olfactometer where the insects had to fly upwind and to make a choice between one arm bringing the test odour (palm wine) and a control arm bearing only the control. The numbers of mosquitoes in the olfactometer arms were counted 5 minutes later. Thereafter, all mosquitoes in the olfactometer were removed by aspiration. After each trial, the olfactometer was disassembled, washed with water and allowed to dry at room temperature before the next test. The tests were replicated 3 times for each category.

Data Analysis: The response pattern of mosquitoes to each category of palm wine was collected and analyzed. Comparisons of variables were done using *t*-test. Level of significance was set at 5 % probability level. Computing the preference index (PI) of the categories of palm wine was done to assess the attractiveness observed in the dual choice olfactometer. According to Yu *et al.* (2015), the following formula was employed for the calculation: PI = Number of *Anopheles* attracted to test – number of *Anopheles* attracted to control ÷ number of *Anopheles* attracted to test + number of *Anopheles* attracted to control x 100.

A positive value shows favourable response by mosquitoes to odorant while a negative value indicates the contrary. Charts were prepared in Microsoft Office Excel (Microsoft Incorporated, Redmond, USA).

RESULTS

The pooled results of the first three experiments involving fresh palm wines and *up-wine* against *down-wine* are shown in Figure 2.

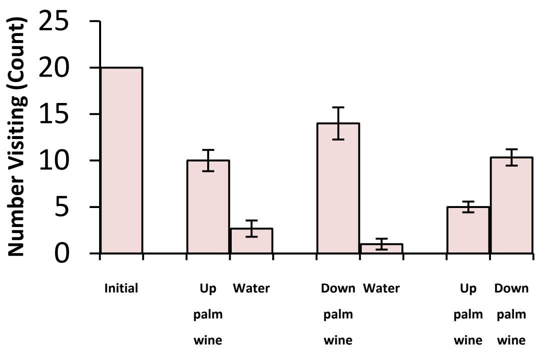

Figure 2: Responses of *Anopheles* mosquitoes to fresh palm wines. The bars denote the mean and SE of mosquitoes responding to respective odour

Mosquitoes that visited fresh *up-wine* were 50.00 ± 5.77 % of the number of mosquitoes at the start whereas only 13.33 ± 4.41 % visited water instead of the fresh *up-wine*. The number of mosquitoes that visited neither the fresh up wine or water was 7.33 ± 0.33 (i.e. 36.67 ± 1.67%. There was a significant difference in the number of mosquitoes that visited either the palm wine or water (t = 5.047, p = 0.007). Down wine performed better as the number of mosquitoes that visited it as against those that visited water was significantly more different (t = 7.120, p = 0.002) and only about 1 % visited water while 29 % visited neither; all the rest (70 %) visited *down-wine*. The set up matching *down-wine* with *up-wine* confirmed the supremacy of down wine as 51.67 % visited down wine but only 25 % responded to up wine where 23.33 % visited neither the palm wine nor the down wine. Analysis of the data indicated that there was a significant difference in mosquitoes' responses to the two grades of palm wine (t = - 5.060, p = 0.007).

The two categories of *ebacha* tested against control as well as the responses when the two were compared against each other are shown in Figure 3.

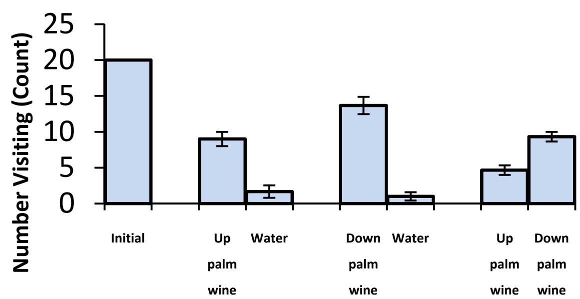

Figure 3: Responses of *Anopheles* mosquitoes to *ebacha* palm wines. The bars denote the mean and SE of mosquitoes responding to respective odour

The percentage of insect visiting *up-wine ebacha* reclined to 45 % with 8.33 % visiting water instead. Therefore, 46.67 % of mosquitoes were not attracted to this category. However, there was still a significant difference when compared to control (t = 5.500, p = 0.005). When *ebacha down-wine* was evaluated, it was discovered that 68.33 % of insect visited this category whereas only one percent visited water while 26.67 % did not visit either the control or the test. There was still a highly significant difference between the attraction to the palm wine with respect to water (t = 9.500, p = 0.001). The last set up involving the comparison of responses to *ebacha up-wine/down-wine* indicated that, consistently, the latter appeared to have double attractive potential in attractiveness to *Anopheles* (46.67 %) than the former (23.33 %). Thirty percent of *Anopheles* were not attracted to either of *ebacha up-wine/down-wine* although there was a significant difference in attractiveness between the two categories (t = - 4.950, p = 0.008).

Table 1 presents the preference indices of the two categories of fresh and *ebacha* wines confirming that all the categories of palm wines used for the study attracted mosquitoes.

Table 1: Preference indexes of categories of palm wine

Category of palm wine	Number of *Anopheles* attracted to test	Number of *Anopheles* attracted to control	Preference Index	*p*-value
Fresh up wine	10.00 ± 1.15	2.67 ± 0.88	57.27 ± 14.90	0.007
Fresh down wine	14.00 ± 1.73	1.00 ± 0.58	85.30 ± 8.91	0.002
Ebacha up wine	9.00 ± 1.00	1.67 ± 0.88	73.50 ± 13.76	0.005
Ebacha down wine	13.67 ± 1.20	1.00 ± 0.58	85.98 ± 7.73	0.001

DISCUSSION

In this study, we were able to show that *Anopheles* mosquitoes responded positively to palm wine odours. The response in definite terms connotes that in a given population of *Anopheles* vectors, between 45 – 70 % would be attracted; with *up-wine* occupying the lower rungs and *down-wine* accountable for the upper limit. This vital information could make a great difference in the way mosquitoes are controlled in regions where *Elaeis guineensis* occur and where there is skilled manpower to extract the wine. In countries like Nigeria, importation of manufactured goods is chaotic, it would be impossible for the general population to rely on the use of volatile chemicals that are not readily available as a means for mosquito control. When such vulnerable populations are denied access to mosquito control, their health, economic activities, quiet night rest, etc. are compromised. Thus it would be a great relief for such people to discover that, the war they were incapable of taking active part in for ages, they can now not only fight back but could aspire to winning the battle against mosquitoes/malaria because the required tool is now readily at their disposal. This would engender their psychological uplift and improve the positive disposition of the average Sub-Saharan African who had been brought up to expect aids from donors from developed countries to face such public health challenges.

One other problem encountered in mosquito control is sustainability of an intervention measure. This occurs because resources employed are limited and substances like insecticides are costly to procure. However, if there is sufficient resources, replenishment can be considered to sustain vector control. The fact on ground is that poverty in Sub Saharan Africa is palpable as mosquito/malaria had been associated with poverty (Worrall *et al*., 2005). With insecticide based intervention, issues of sustainability is compounded by the fact that the activities of active ingredients in these compounds progressively decline and expire. Another twist to the imbroglio is that vectors acquire resistance such that their vulnerability to such substances at initial stages could be very high only to wane as time goes on as a result their innate biological potentials such as avoidance, arrest, gene acquisitions, etc. Palm wine is not likely to induce resistance in any way because it is a food source to insects generally and odour guide to them. Since every animal must feed to stay alive, the question of acquiring resistance to palm wine odours is not feasible. Therefore, its use fits in conveniently in the regions where they are found because it is very cheap, available and accessible throughout the year. It is therefore the promising sustainable mosquito-luring intervention measure to beat in Sub Saharan Africa and elsewhere. The performances observed suggest that palm wines contain potent ingredients that could be isolated, synthesized industrially and employed in entomological exigencies.

The difference between *up-wine* and *down-wine* unveiled in this study is intriguing. The disparity is palpable by the way men react to *down-wine* with disdain. Consequently, *up-wine* is much more valued costing between 4 – 6 times that of *down -wine*. Men generally prefer the *up-wine* on account of the stimulant potentials – its effects are felt in a few minutes after consumption if taken by noon or later. This property definitely is due to its higher alcoholic content which increases when allowed to ferment. Time and high ambient temperature appear to enhance its alcoholic conversion while cold temperatures retard the process. Unlike *up-wine*, *down-wine* hardly intoxicates. Natives know it by its intense sugary taste long after tapping. It can retain the sugary taste even up to the third day at room temperature. The implication of the foregoing is that though both grades of palm wine are affordable, *down-wine* would be preferable as a mosquito control tool. Second, observing the two samples of wines, one would note the stronger effervescence signifying obvious evolution of gases that may include carbon dioxide. This reaction is far more avid in *up-wine*, a fact most tappers concurred with. Therefore, *down-wine* with lower gas evolution suggests that its higher preference may not dominantly depend on carbon dioxide generation potential. Following from this premise, thirdly, it appears that the alcoholic content of palm wine does not as well play an overriding role in attracting mosquitoes. That is to say other contents, probably linked to the sugar content might be responsible for the greater effectiveness of *down-wine* as mosquito attractant. Fourthly, based on sweeter taste of *down-wine*, one might have expected it to have higher alcoholic potentials. Since this is not the case, one could safely conclude that it may have other components which retard sugar degradation into alcohol; or alternatively, its sweetness may be due to other non-sugar content such as octenol, aldehydes, etc. that are not easily broken down (Lasekan *et al*., 2009). Lastly, Stringini *et al.* (2009) indicated that there is a large number and diverse yeasts and other micro-organism such as lactic acid bacteria and acetic acid bacteria present during tapping of palm wine. Since *down-wine* is collected from palm trees already at ground level, it ought to have richer microbial population diversity thereby engendering greater number of volatiles attractive to mosquitoes as observed in this study.

One of the objectives of semiochemical experimentation is to arrive at a formulation that will command complete attraction to a given vector population. Though the two grades of palm wine could not achieve that target in this study, it nevertheless made a generous pass mark, attaining up to 70 % when *down-wine* was used alone and making a gain of 5 % when both are used simultaneously. This is a likely indicator that the two grades of palm wines have very little additive or synergistic effects. Further, it suggests that fewer semiochemicals may be required to combine with *down-wine* to achieve wholesome attraction. That is, one or two chemicals may be required to make up for the 30 % of *Anopheles* mosquitoes that were not attracted. This scenario opens further avenue to find suitable local volatiles that may have additive or synergistic effects with palm wine. It may be plausible to suggest that combinations involving other volatiles unrelated to palm wines such as carbon dioxide, sugar and alcohol may prove useful since those are already contained in our test materials.

A conceivable problem of palm wine use as attractant is that, based on organoleptic qualities, differences exist among palm wine depending on source. Thus, it is easy to discriminate palm wine from Obollo Eke, Obollo Afor/Enugu Ezike and Ukehe (towns within Nsukka zone) by taste. Palm wine from Obollo Eke area are noted to have a peculiar taste and rather watery, characteristics adduced to clayish soil unlike those from Ukehe, Obollo Afor/Enugu Ezike areas that are sandy soils. In addition to soil types, ambient temperature and human factors could greatly modulate qualities of palm wines. Verhulst *et al.* (2010) did provide evidence that the skin flora in man determine the odour that is attractive to infected mosquito vectors. It is likely this reasoning may obtain in palm wine biodegradation where emanating odours depend on player microorganism present at point of tapping. The implication is that strengths and weaknesses of palm wine could be inconsistent with respect to mosquito attraction. There is the need for further clarification in this regard by researches.

Conclusion: Availability of *Elaeis guineensis* in regions where mosquitoes abound appear to be a disguised blessing in that its products could be developed to sustain mosquito/malaria control. This study found that its fermented sap, palm wine, is attractive to malaria vector. The two broad categories, fresh *down-wine* and fresh *up-wine*, command attractiveness that can be used to lure up to 75 % of *Anopheles* species. Moreover, their sub-categories, *ebacha down-wine* and *up wine* reveal that the former minimally lost its attractive potential while the later lost up to 5 %. The implication is that *ebacha up-wine* could trend faster towards repellence while *ebacha down-wine* could retain its attractiveness to mosquitoes for longer periods. It follows that the two broad groups of palm wine could be further developed to ultimately yield products that could be employed in efficient pull-push approach to vector control. Therefore, further testing to identify key components is recommended. Then, in Sub Saharan Africa where dearth of state of the art technology is the norm, use of palm wine in raw forms to set mosquito traps is advisable while it is also expedient to further test palm wines to expose fully its secondary and tertiary products which might also improve our knowledge and enhance development of better push-pull affordable and sustainable mosquito control systems.

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