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Research Article

New Bioassay to Evaluate Repellency and Attractively of Chemical Products against Adults Mosquitoes *Aedes albopictus* and *Culex quinquefasciatus*

Tovo Mbolatiana Andrianjafy[1,2]*, Lala Harivelo Ravaomanarivo[1,2], Maonja Rakotondramanga[1], Voahangy Vestalys Ramanandraibe[1], Patrick Mavingui1,[3,4], and Marc Lemaire[1]*

[1]*Laboratory International Associate, ICBMS, CNRS, UMR 5246, University of Antananarivo-Lyon 1, Madagascar*
[2]*Department of Entomology, University of Antananarivo, Madagascar*
[3]*UMR PIMIT, INSERM 1187, CNRS 9192, IRD 249, University of La Réunion, Plateforme de Recherche CYROI, Saint-Denis, La Réunion*
[4]*Ecologie Microbienne, UMR CNRS 5557, USC INRA 1364, VetAgro Sup, FR41 Bio Environnement et Santé, University of Lyon 1, Villeurbanne, France*

***Corresponding authors**

Tovo Andrianjafy, Laboratory International Associate, University of Antananarivo, Madagascar, Tel: 261 33 04 530 00; Email: amthta@gmail.com

Marc Lemaire, ICBMS, CNRS, UMR 5246, Université Claude Bernard Lyon 1,43 Bd du 11 Novembre 1918, Bat CPE 69622 Villeurbanne, France, Tel: 34-06-258-21; Email: marc.lemaire.chimie@univ-lyon1.fr

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Abstract

Mosquito-borne diseases (MBD) are responsible for millions of people at risk worldwide. To control mosquito-vectors populations, an application of repellent and attractant is becoming a promising alternative but necessitates efficient bioassay methods. The purpose of this study was to estimate the efficiency of known repellents and attractants against mosquitoes *Aedes albopictus* and *Culex quinquefasciatus* (Diptera: Culicidae) using a simpler device that is comprised of a large cage (1.5m × 1m × 1m), a release cage and two bottle traps cylindrical (r=4cm, h=22cm). The whole device was placed in an experimentation room where physical conditions were noted regularly. The results showed that the bioassay is effective in measuring the targeted behavioral response. Significant sensitivities of the two species tested to low doses of compounds were observed. The method presented has many advantages including cost and reproducibility. It will allow testing new molecules in countries with limited resources.

ABBREVIATIONS

MBD: Mosquito-Borne Diseases; r: rayon; h: higher

INTRODUCTION

Actually mosquito-borne diseases (MBD) are a major problem in public health in the world and especially in Africa [1] *Aedes albopictus* and *Culex quinquefasciatus* are the main vectors of this MBD in many countries [2-4]. In recent decades, these two species have been involved in outbreaks of arbo viruses, including chikungunya and rift valley fevers, in Madagascar and neighboring islands [5-7]. The distribution and abundance of these diseases are strongly influenced by the presence of humans and the level of poverty [8]. Climate, ecological and socio-economic changes are events that provoke an increase in the density and spreading of the mosquito

Vectors with concomitant expansion of MBD in almost all the continents but the Antarctica. Controlling these vectors is now an important challenge for human and animal health, for the South as well for the North.

Current methods to fight against vectors are mainly based on eliminating larvae breeding sites and the reduction of adult mosquitoes by chemical insecticides and/or biological agents. Although many insecticides and biocides have proven their efficacy, collateral effects are observed on non-targeted

species and the ecosystems. In addition, their effectiveness is increasingly faced with the appearance of resistant mosquitoes. Therefore the development of alternative methods is necessary and encouraged worldwide. The use of repellents (to limit host-vectors contact) or of attractants (to reduce the populations of vectors by employing specific traps) seems to be two promising alternatives and cleaners strategies than insecticides' application for the limitation of MBD [1,9]. However, one of the major difficulties for the development of these strategies lies in the assessment of repellent or attractant properties of many natural or synthetic molecules that may be tested.

Several methods have already been used to evaluate the efficiency of repellent and attractant compounds on populations of mosquitoes. The most known are the assays performed directly on volunteers to repellents [10], or using a Y-olfactometer (push and pull) [11]. All these methods have produced valuable results but they also have limitations including ethical issue, high cost of equipment, and difficulties in achieving reproducible tests, time-consuming procedure, and finally remote systems conditions "in natura". The objective of this work was to evaluate the efficiency of known repellent and attractant compounds using a new simple method on mosquito's populations. Four products were tested with two attractant products: 1-octen-3-ol [12] and Isovaleric acid [13, 14] and two repellents: N, N-diethyl-3-methylbenzamide (DEET) [15] and 1-methyl-propyl 2- (2-hydroxyethyl) -1-piperidinecarboxylate (Picaridin) [16].

MATERIALS AND METHODS

Mosquito collection and breeding

The species *Aedes albopictus* and *Culex quinquefasciatus* widely present in Madagascar [17] were chosen in this study. Specimens necessary to the tests were collected in the peridomestic breeding sites of mosquitoes: Ankatso, Androhibe, Tsimbazaza, and channels discharge sewage Ampefiloha. All sites cited are located around of the capital Antananarivo, Madagascar. The larvae was directly collected in plastic bottles and stored in collection bottles filled with breeding site water. Collected specimens were placed inside coolers, and then brought back to the insectary of the Laboratory International Associate (LIA) located in Ampasapito Campus, Antananarivo.

Mosquito breeding was conducted in a room size (3m × 3.5m × 3.5m) where the temperature of was maintained at 25°C ± 3 with the relative humidity of 70% ± 3 and a natural photoperiod of 12h: 12h. Adult males and females were kept together in a cage Gauze (35cm × 35cm × 35cm). They were fed a 6% sucrose solution.

For *Aedes albopictus*, to produce next generations, four days after their emergences the adult females were fed on a rabbit who was put inside the breeding-cage; this meal took two hours and was done every three days. Females laid eggs in ovitraps containing a humid absorbent paper placed into the cage. Paper bearing eggs were collected every three days and placed in tanks of water for hatching. The larvae were fed with powdered dog

biscuits rich in Tetramin; pupae were collected in bowls (35cm × 12cm × 12cm) and placed in emergence cages. The 5 to 12 day-old females destined for the test were collected and separated from all remaining adults that were used as parental strains for future generations. In order to maintain experiments close to "in natura" conditions only three generations of mosquitoes were bred and tested before new collection campaign.

For *Culex quinquefasciatus,* the larvae collected from the field were reared in bowls filled with cottage water. They were fed with Tetramin until pupal stage, and then cotton soaked in 6% sucrose solution was suspended in the breeding cage for emerging adults. All females of 5 to 12 day-old were collected for the test. For each further test a new collection campaign of larvae was performed and all the following steps were done as above.

The Products Tested In this study, two repellent products were tested: DEET: N, N-diethyl-3 - methylbenzamide (C_{12} H_{17} NO); Picaridin: 1-methyl-propyl 2 - (2-hydroxyethyl) -1-piperidinecarboxylate (C_{12} H_{23} NO_3). The two repellents are produced by Bayer Chemical Company in Germany. Different doses of each repellent were prepared as follows: 0.125, 0.25, 0.5 and 1.2 mg/mL (w/v ethanol) for DEET, and 0.11, 0.22, 0.44, 0.88 and 1.76mg/mL (w/v ethanol) for Picaridin. Kairomone such as Octenol: 1-octen-3-ol (C_8 H_{16} O) by Aldrich Company (05284-25G); Isovaleric acid: 3-methyl butanoic acid (C_5 H_{10} O_2) produced by Tokyo Chemical Industry (TCI-MO182) were also tested. Different doses of each attractant were prepared as follows: 0.0059, 0.0084, 0.0127, 0.025, 0.10, and 0.41mg/mL (w/v of ethanol) for Octenol and 0.0006, 0.0013, 0.002,

0.003, 0.006, 0.013 and 0.057 mg/mL (w/v of ethanol) for Isovaleric acid. For all products, 100µL of solution of various concentrations were used for each assay.

Experimental design and test procedure

The tests were carried out in parallel in two experimentation rooms (3.5m × 2m × 2m). Each room composed: a heater for maintaining the temperature, filled bowls of water for maintaining the relative humidity, a large cage (1.5m x 1m x 1m) in which was placed a release cage hosting mosquitoes (35cm x 35cm x 35cm), one baited-trap and one control-trap (Figure 1).

The Traps are commercialized transparent plastic bottles of 1.5 liters, cut at 1/3 of the length from the superior extremity. For each trap, the cut portion will serves as a cover in the form of funnel and is placed at the top of the bottle. The whole is wrapped in black plastic bag. In each trap, we introduced 100mL of 6% sucrose solution to increase the activity of mosquitoes during the test, and a strip of filter paper 1.5cm wide and 17cm long having deposited thereon 100µL of ethanol solution corresponding to different doses of the tested products. A same quantity of pure ethanol was used as a control. The filter paper was not immerged in the sucrose solution. Traps were changed for every test.

A bowl containing the following mixture was used as a source of CO_2 to increase the activity of mosquito: 100mL of water previously boiled and then cooled, 1.83g of yeast (*Saccharomyces cerevisiae*) and 8.33g of white sugar [18]. During a test, the CO_2 source is placed in the middle of the two traps (control and test) outside the large cage. Indeed, it has been shown that the existence of a low dose of CO_2 in the atmosphere has a Positive effect on mosquito activity [19,20].

The integrity of the body (legs and wings) and physiological

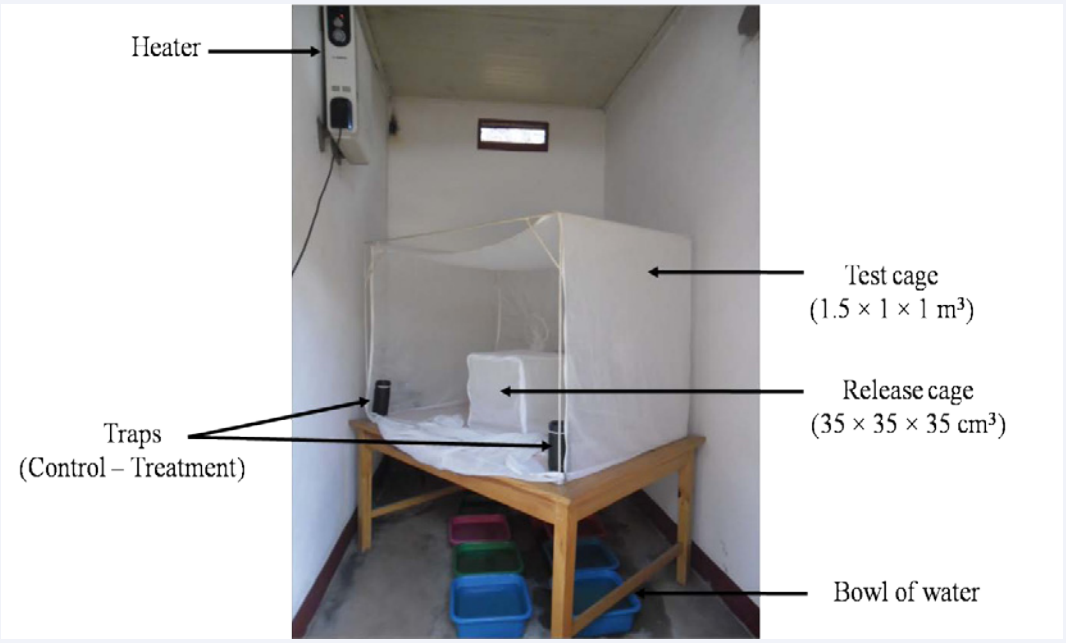

Figure 1 Experimental device with all components: test cage, heater, release cage, traps, and bowls of water (sub-l ateral view with an angle of 25°).

state of the individual were chosen as selection criteria for tested mosquitoes. 25 female mosquitoes aged of 5-12 days, were previously fasted for two hours in the release cage inside the large cage. After these hours, the two traps (control and test product) were placed inside the large cage, on two opposite sides. The test started at 9:00 pm for the two species by slowly opening the release cage to let the mosquitoes fly out. The total test time was 24 hours. Mosquito behavior response was Monitored at the end of this time-period by recording the number of mosquitoes present in baited trap, control trap, release cage and in the large cage. After this count, the traps were removed, the room was ventilated, nets were changed, and they were washed after each concentration. Eight replicates were carried out for each dose to be tested. After each assay, the location of the test and control trap was reversed, and the mosquitoes were replaced by new individuals. Blank tests are conducted regularly to ensure the proper conduct of trials in each experimental room. It's consisted of using only ethanol in the two traps during a test.

Data analysis

The activity of mosquitoes for the blank tests was taken as reference measurement. Mosquitoes that remained in the large or in the release cages are considered inactive during a test of a given product for a period of 24 hours, thus only mosquitoes that are moved and that were in the one or the other two traps (control-test) are considered for measuring the effect of the product. The results were expressed as follows:

Activity index (AI) corresponds to the percentage of mosquitoes that entered the traps: (P) + (T) compared to all the mosquitoes used for the test: (P) + (T) + (G). Only the tests for which the Activity index was higher than 30% were considered for the analysis.

$$AI\ \% = (P+T)/(P+T+G)*100$$

Repulsion index (RI) is the percentage of the difference observed in the number of mosquitoes in the control trap (T) and the test baited-trap (P) divided by the total sum of mosquitoes in both traps.

$$RI\ \% = (T-P)/(P+T)*100$$

Kairomone index (KI) represents the attractiveness of the tested product and is the percentage of the difference observed in the number of mosquitoes the test baited-trap (P) and control trap (T) divided by the total sum of mosquitoes in both traps. An index equal to zero implies that there is neither attractiveness nor repellency observed: there are so many mosquitoes in both the baited-trap and in the control trap. They are expressed by the formula below:

$$KI\ \% = (P-T)/(P+T)*100$$

RI: repulsion index, KI: Kairomone index (attractiveness of the tested product), P: number of mosquitoes caught in the attractive or repellent trap, T: number of mosquitoes caught in the control trap, G: number of mosquitoes stayed in the release and large cages.

All data were analyzed using the R software (Studio version 3.0.3). The *t-test* for independent samples of the averages was used for the four compounds. Then analyses of variance by *ANOVA* were done to sort out the variations between the different doses of every product. The means of two groups (treatment and control) were analyzed by *t-test* for paired samples and *Kruskal-Wallis test* for sorting the differences between treatments Groups at $P < 0.05$ for these analyzes, the confidence interval was estimated at 95%. So we took the value *P*-value = 0.05 as arbitrary of the observed values (*t*= value observed in *t test; F*= value observed in *ANOVA; H*= value observed in *Kruskal-Wallis test).* The evaluation of the reliability of the interpretations was also performed by study of the standard deviations (± SD) and the standard errors (± SE) that was illustrated in the tables and graphs.

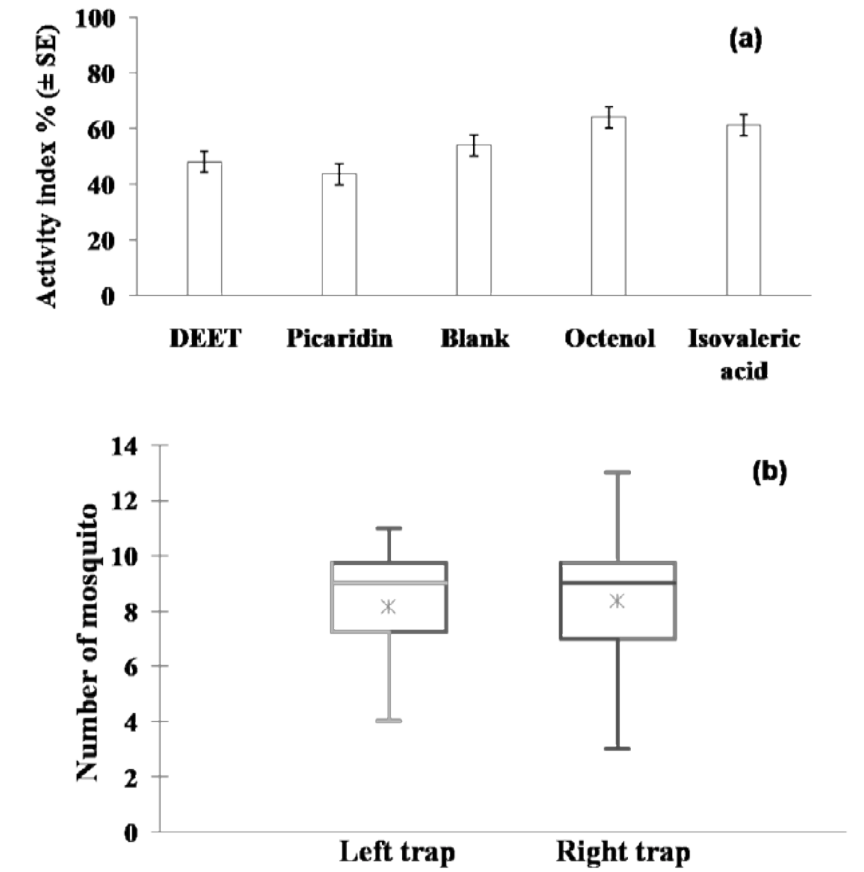

Figure 2 Results of blank t ests showing: (a) activity index (mean % ± SE) of mosquitoes report as repellents (DEE T and Picaridin) and as attractants (Octenol and Isovaleric acid); (b) box plots of number of mosquito in the left and right traps.

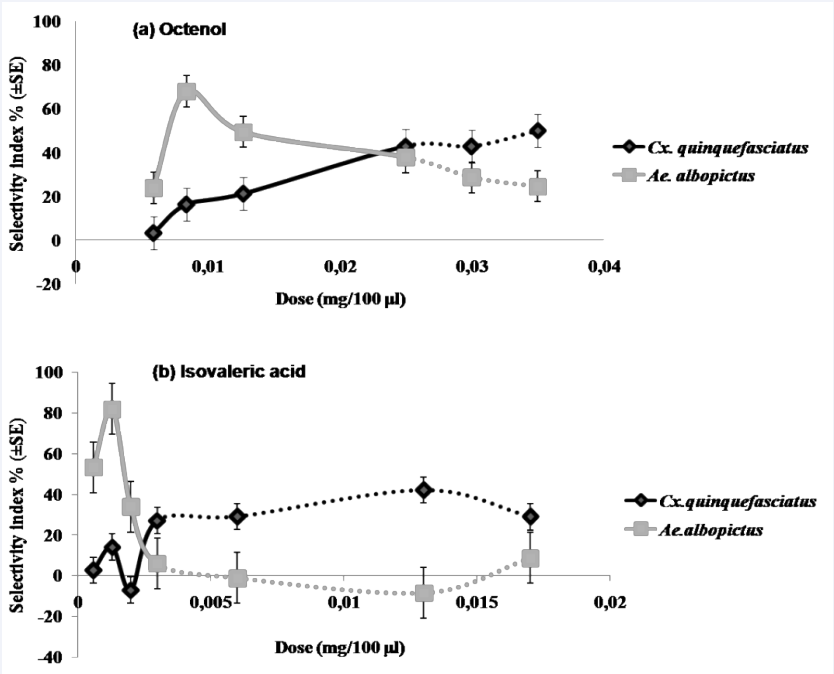

Figure 3 Kairomone index (mean % ± SE) according to the doses of attra ctants products for the two mosquito species *Aedes albopictus* and *Culex quinquefasciat us*: (a) Octenol, (b) Isovaleric acid. The das hed lines corresponded to the same kairom one effect for an interval doses.

RESULTS AND DISCUSSION

A total of 400 tests were conducted corresponding to 160 with repellents (DEET and Picaridin), 208 with attractants (Octenol and Isovaleric acid), and 32 with blank tests. The tests were performed on 9,200 individuals. In general, changes in the activity index are between 45-70%. Very few percentages of activity below 30% were recorded about 2 out of 100 tests. For the blank test, an average of 54% activity was recorded. The activity of mosquitoes observed in presence of the attractant has been higher than observed in presence of the repellent and the blank test. ($AI_{attractants}$=62.5% >AI_{blank}=54.4% >$AI_{repellent}$= 45.5%). Between repellents (DEET and Picaridin), the activity index of mosquitoes shows no significant differences (t= 1.91, P= 0.092). Similar results for both attractants (Isovaleric acid and Octenol) with (t= 1.145, P= 0.27) (Figure 2a). The results showed that there is no significant difference between the number of mosquito recorded in the right and the left traps during the blank tests (Figure 2b).

Effectiveness of compounds to attract mosquitoes

For *Aedes albopictus,* significant differences (H= 12.75, P= 0.026) between the mean number of mosquitoes recorded in the baited-trap were observed for the different doses of Octenol. The dose 0.0084mg/mL was the most attractant P <0.001 (Table 1). For this dose, the average number of mosquitoes trapped in the presence of Octenol was highest (46.6 ± 4%) in comparison to the lower number in the control trap (9.3 ± 6%). It corresponds to a maximum dose of Octenol to attract a large number of *Aedes albopictus* individuals with kairomone index KI=67%. A progressive decrease in the attractant effect is recorded for higher doses. In the case of *Culex quinquefasciatus,* significant differences (H= 24.93; P < 0.001) between the mean number of mosquitoes recorded in the baited-trap were also observed for the different doses of Octenol. The dose 0.41mg/mL was the most significant with P <0.001 (Table 2). For this dose, the average number of mosquitoes identified in the attractive trap was highest (48 ± 10%) and lowest in the control trap (16.4 ± 9.1%). It corresponds to a maximum dose of Octenol to attract a high number of *Culex quinquefasciatus* individuals and a kairomone index KI=50%. Below this dose, a lower attractive effect is recorded.

The variation of kairomone index for Octenol was different between the two mosquito species (Figure 3a). For *Aedes albopictus,* a maximum attractant effect was obtained for 0.0084mg/mL dose with kairomone index of 70%, and then the effect gradually decreases at higher doses. For *Culex quinquefasciatus,* a gradual increase was observed with a plateau reached at the dose 0.025mg/mL which corresponded also to a maximum attractant effect with a kairomone index of about 40%. At 0.025mg/mL dose a crossing point was observed in both species (KI =40%).

Significant differences (H= 20.39, P= 0.0023) were observed between the mean numbers of *Aedes albopictus* individuals recorded in the baited-trap for the different doses of Isovaleric acid. The dose 0.0013mg/mL was the most attractant (P <0.001) (Table 3), and thus corresponded to an optimal dose of Isovaleric acid to attract a large number of mosquitoes. Similarly significant (H= 18.67; P= 0.004) Isovaleric acid dose-dependent kairomone was also seen for *Culex quinquefasciatus*. The most attractant dose (P <0.001) was about 0.013mg/mL (Table 4) and corresponded to an optimal dose of Isovaleric acid to attract a large number of individuals.

The variation of kairomone index for Isovaleric acid was different between the two mosquito species (Figure 3b). For

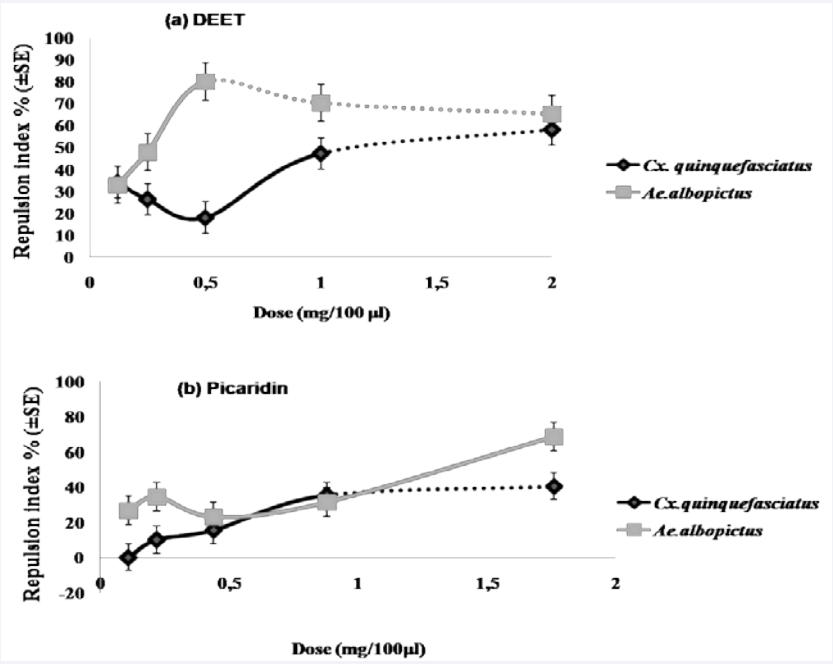

Figure 4 Repulsion index (m ean % ± SE) according to the doses of rep ellents products for the two mosquito species *Aedes albopictus* and *Culex quinquefasciatus*: (a) DEET, (b) Picaridin. The dashed lines represent the plateau effect for an interval doses.

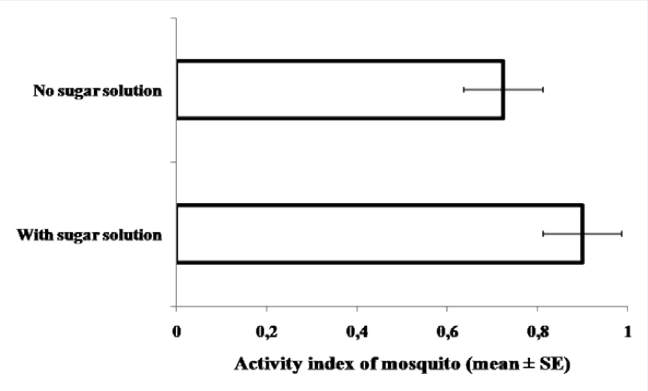

Figure 5 Effect of the presence of sugar solution on the activity index o f mosquito (*Aedes albopictus*) during the test.

Table 1: Comparing *Aedes albopictus* recorded (mean % ± SD) in the control and treatment traps by *t test* with replicates (n=8) and the total number of mosquitoes tested (N=200) and percentage number of kairomone index for different doses of Octenol.

Doses (mg/mL)	Control	Treatment	*t*	*P*-values	KI	T°C/ HR% *
0.0059	22 ± 5.4	38 ± 15.5	-2.1	0.084	23.81	21/50
0.0084	9.3 ± 6	46.6 ± 4.8	-11.6	<0.001	67.98	22.3/48
0.0127	18 ± 7.4	51.3 ± 9.2	-7.2	<0.001	49.43	21.6/50.3
0.025	19.3 ± 3.9	43.3 ± 6.8	-6.4	<0.001	37.87	22.3/46
0.1	25.3 ± 7.8	44.6 ± 5.8	-5.1	0.003	28.64	23/45.3
0.41	34.6 ± 4.1	28 ± 12.8	1.03	0.34	24.64	21.6/49

KI refers to percentage of kairomone index. P-values in bold type were considered significant p < 0.05 *average temperature and relative humidity in the test room for each dose.

Table 2: Comparing *Culex quinquefasciatus* recorded (mean % ± SD) in the control and treatment traps by *t test* with replicates (n=8) and the total number of mosquitoes tested (N=200) and percentage number of kairomone index for different doses of Octenol.

Doses (mg/mL)	Control	Treatment	*t*	*P*-values	KI	T°C/ HR% *
0.0059	26.6 ± 7.7	28.4 ± 7.04	-0.49	0.63	3.26	24.2/67.7
0.0084	20.4 ± 12.8	30.6 ± 16.9	-1.12	0.29	16.35	25.35/60
0.0127	16 ± 16.8	19.4 ± 9.6	-0.4	0.69	21.19	25.4/60.5
0.025	19 ± 12.7	47.4 ± 14.2	-3.15	0.016	42.94	24/64.2
0.1	16 ± 8.8	39 ± 9.2	-4.35	0.003	42.86	22/63
0.41	16.4 ± 9.1	48 ± 10	-6.47	<0.001	50.13	24/64

KI refers to percentage of kairomone index. P-values in bold type were considered significant p < 0.05 *average temperature and relative humidity in the test room for each dose.

Table 3: Comparing *Aedes albopictus* recorded (mean % ± SD) in the control and treatment traps by *t test* with replicates (n=8) and the total number of mosquitoes tested (N=200) and percentage number of kairomone index for different doses of Isovaleric acid.

Doses(mg/mL)	Control	Treatment	*t*	*P*-values	KI	T°C/ HR% *
0.0006	13.3 ± 7.4	42.6 ± 7.8	-5.5	0.0027	53.21	23/60
0.0013	5.3 ± 4.1	52 ± 7.5	-13.22	<0.001	81.86	22.4/57
0.002	22 ± 12.5	40.6 ± 4.6	-2.94	0.032	33.78	22.4/58
0.003	26.6 ± 8.6	26.6 ± 8.6	-0.48	0.64	5.89	21.7/55
0.006	30 ± 12.8	30 ± 12.8	0.25	0.8	-1.25	22.2/60
0.013	36 ± 5.6	36 ± 5.6	1.19	0.28	-8.51	24/53.5
0.057	26.6 ± 14.6	26.6 ± 14.6	-0.89	0.41	8.67	23.4/50

KI refers to percentage of kairomone index. P-values in bold type were considered significant p < 0.05 *average temperature and relative humidity in the test room for each dose.

Aedes albopictus, maximum attractant effect was obtained for 0.0013mg/mL dose with kairomone index of 80%, and then the effect gradually decreases at higher doses. For *Culex quinquefasciatus,* a gradual increase was observed with a plateau reached at the dose 0.003mg/mL which corresponded also to a maximum attractant effect with kairomone of the order of 30 to 40%. At 0.002mg/mL dose, we had the same attractive effect for both species (KI= 16.5%).

Effectiveness of Compounds to Repel Mosquitoes

Significant differences (H= 9.53; P= 0.049) were observed between the mean numbers of *Aedes albopictus* individuals recorded in the baited-trap for the different doses of DEET. The dose 0.5mg/mL was the most repellent $P < 0.001$ (Table 5), and thus corresponded to an optimal dose of DEET to repel a large number of mosquitoes. For *Culex quinquefasciatus,* significant differences (H= 16.03; P= 0.003) were also observed for the different doses of DEET. The doses 1mg/mL and 2mg/mL were the most repellent $P<0.001$ (Table 6), and corresponded therefore to an optimal dose of DEET to have a repulsion index superior to 50% on the two species.

The variation of repulsion index for DEET was different between the two mosquito species (Figure 4a). For *Aedes albopictus*, maximum repellent effect was obtained for 0.5mg/mL dose with a repulsion of 80%, and then the effect reached a plateau at higher dose. For *Culex quinquefasciatus*, a gradual increase effect was observed with a plateau reached at the dose1mg/mL which corresponded also to a maximum repellent effect with repulsion of the order of 50 to 60%. At 2mg/mL dose, we had the same repellent effect for both species (RI= 58%).

No significant differences (H= 2.41, P= 0.66) were observed between the mean numbers of *Aedes albopictus* individuals recorded in the baited-trap for the different doses of Picaridin until the 0.88mg/mL dose. The dose 1.76 mg/mL was the most repellent dose P <0.001 (Table 7) with mean number of mosquito (test) = 11.3 ± 5.3% and (control) = 59.3 ± 6.8%, and corresponded to an optimal dose of Picaridin to repel a large number of mosquitoes. For *Culex quinquefasciatus,* significant differences (H= 14.65; P= 0.005) were observed between the mean numbers of *Culex quinquefasciatus* individuals recorded in the baited-trap for the different doses of Picaridin. The dose 1.76mg/mL was the most repellent P <0.001 (Table 8), and thus corresponded to an optimal dose of Picaridin to repel a large number of mosquitoes.

The variation of repulsion index for Picaridin was different between the two mosquito species (Figure 4b). For *Aedes albopictus,* there is a small repellent effect of around 30% between the doses 0.11 and 0.88mg/mL. A maximum repellent effect was obtained for 1.76mg/mL dose with a repulsion of 70%. For *Culex quinquefasciatus* a gradual increase was observed with a plateau reached at the dose 0.88mg/mL which corresponded also to a maximum repellent effect with repulsion of the order of 35 to 40%. At 0.88mg/mL dose, we had the same repellent effect was observed for both species (RI = 35%).

The results of blank tests without attractant or repellent have shown that over 54.4% of tested individuals were active. Rests of individuals are supposed non response but not insensible against products. In natural environment, many conditions explain this activity behavioral response such as abiotic factors: temperature, humidity, photoperiod and luminosity which are known to influence mosquito behavior [21]. The tests conducted during the rainy season with conditions such as the presence of a storm were found to be disturbing

For mosquito activity. Generally, the changes of the ambient conditions decreased activity of mosquitoes and even make them inactive for hours. As expected, we observed that mosquito activity was closely linked to changes of temperature, as a drop in temperature within the room causes a decrease in activity of the mosquitoes during the bioassay. But as reported by Barnard in 2005, their contribution to experimental error can be minimized by random selection of test subjects, the use of appropriate sample sizes in bioassays and by Recognizing and avoiding pseudo replication [22].

The source of CO_2 was found to play important role about activation of mosquitoes during the tests. Previous studies have shown that CO_2 plays an important role in the activation of mosquitoes [20,23], rather than acting as a real attractant [19,24,25]. In our assays, results obtained without CO_2 have shown a significant lower activity of both *Culex quinquefasciatus* and *Aedes albopictus.* The results presented in this article are obtained in the presence of CO_2 source. The same effect was observed in the presence of sucrose solution 6%, the activity of mosquitoes was significant higher than with water alone respectively 0.90 ± 0.08 and 0.72 ± 0.05 (t= 3.57; P= 0.012) (Figure 5). It may be explain by the attractively propriety of the sucrose solution.

Table 4: Comparing *Culex quinquefasciatus* recorded (mean % ± SD) in the control and treatment traps by *t test* with replicates (n=8) and the total number of mosquitoes tested (N=200) and percentage number of kairomone index for different doses of Isovaleric acid.

Doses(mg/mL)	Control	Treatment	*t*	*P*-values	KI	T°C/ HR% *
0.0006	31 ± 8.4	33 ± 10.4	-0.35	0.73	2.6	25/54.3
0.0013	22.6 ± 7	30.6 ± 10	-1.69	0.15	14	24.3/53
0.002	34 ± 22.3	27.4 ± 17.6	-0.54	0.6	-7.1	24.7/50
0.003	23 ± 7.6	43 ± 16.6	-2.88	0.02	27	24.8/47.3
0.006	23 ± 7.6	42 ± 7.6	-4.86	0.002	29	23/63
0.013	19 ± 4.6	46.4 ± 6.3	-10.31	<0.001	42	25/52
0.057	24 ± 14.6	43 ± 6.3	-5.94	0.001	28.9	25.6/53

KI refers to percentage of kairomone index. P-values in bold type were considered significant $p < 0.05$ *average temperature and relative humidity in the test room for each dose.

Table 5: Comparing *Aedes albopictus* recorded (mean % ± SD) in the control and treatment traps by *t test* with replicates (n=8) and the total number of mosquitoes tested (N=200) and percentage number of repulsion index for different doses of DEET.

Doses (mg/mL)	Control	Treatment	*t*	*P*-values	RI	T°C/ HR% *
0.125	34 ± 9.7	17.3 ± 7.4	3.02	0.029	32.86	24.3/50
0.25	38 ± 15.3	14.6 ± 14.2	2.26	0.072	47.99	23.7/48.2
0.5	41.3 ± 11.2	4.6 ± 4.6	8.05	<0.001	80.26	24/56
1	40.6 ± 9.9	6.6 ± 6	5.58	0.0025	70.44	24.6/66
2	53.3 ± 36.8	10 ± 6.5	3.4	0.019	65.33	24.7/60.8

RI refers to percentage of repulsion index. P-values in bold type were considered significant P < 0.05 *Average temperature and relative humidity in the test room for each dose.

Table 6: Comparing *Culex quinquefasciatus* recorded (mean % ± SD) in the control and treatment traps by *t test* with replicates (n=8) and the total number of mosquitoes tested (N=200) and percentage number of repulsion index for different doses of DEET.

Doses (mg/mL)	Control	Treatment	*t*	*P*-values	RI	T°C/HR% *
0.125	42 ± 16.4	19 ± 9	2.89	0.023	32.9	24.6/64.9
0.25	39.5 ± 10.5	23 ± 7.3	4.5	0.003	26.37	25.7/61.6
0.5	40.5 ± 8.4	27.5 ± 2.5	4.2	0.004	18.14	25.3/62
1	50.5 ± 6.02	18.5 ± 6.3	12.7	<0.001	47.39	24.5/61.4
2	50 ± 3.7	13.5 ± 6	12.2	<0.001	58.28	23.6/57.7

RI refers to percentage of repulsion index. P-values in bold type were considered significant P < 0.05 *Average temperature and relative humidity in the test room for each dose.

Table 7: Comparing *Aedes albopictus* recorded (mean % ± SD) in the control and treatment traps by *t test* with replicates (n=8) and the total number of mosquitoes tested (N=200) and percentage number of repulsion index for different doses of Picaridin.

Doses (mg/mL)	Control	Treatment	t	P-values	RI	T°C/HR% *
0.11	24.6 ± 15.8	16.6 ± 17	0.62	0.56	26.78	23.3/50
0.22	32 ± 5.6	16 ± 6.6	4.14	0.009	34.6	25.3/49.4
0.44	27.3 ± 11.1	16.6 ± 9.2	1.38	0.22	23.31	22.9/56
0.88	24 ± 5	12.6 ± 3.9	9.22	<0.001	31.54	24.6/53.3
1.76	59.3 ± 6.8	11.3 ± 5.3	20.78	<0.001	68.85	23.4/66

RI refers to percentage of repulsion index. P-values in bold type were considered significant P < 0.05 *Average temperature and relative humidity in the test room for each dose.

Table 8: Comparing *Culex quinquefasciatus* recorded (mean % ± SD) in the control and treatment traps by *t test* with replicates (n=8) and the total number of mosquitoes tested (N=200) and percentage number of repulsion index for different doses of Picaridin.

Doses (mg/mL)	Control	Treatment	t	P-values	RI	T°C/HR% *
0.11	33 ± 5.1	33.5 ± 9	-0.17	0.86	0.3	25.4/55.4
0.22	34.5 ± 9	28 ± 7.7	1.87	0.1	10.23	24.4/55.5
0.44	42.5 ± 8.5	31 ± 5.9	4.7	0.002	15.57	25.4/50.8
0.88	49.5 ± 9.5	23.5 ± 6.9	5.07	0.001	35.38	24.6/50.4
1.76	48.5 ± 4.9	20.5 ± 2.5	18.52	<0.001	40.58	25.8/50.8

RI refers to percentage of repulsion index. P-values in bold type were considered significant P < 0.05 *Average temperature and relative humidity in the test room for each dose.

In our results, we found that mosquito activity was higher for attractants (Octenol and Isovaleric acid) compared to repellents and the controls. This stronger reactivity of the mosquito to the attractant can be explained by the natural host seeking behavior of adult's females. Using attractant molecules, fasting mosquitoes behave like in the presence of their host and increase their activity [18].

Concerning the variable responses observed for the same product, temperature, humidity and wind speed are among the factor that could influence the effectiveness of the tested products [26]. The volatility of the product depends on the temperature and wind speed. The more temperature is raised or that wind is strong, the more volatility of the products is raised and their short efficiency length. In our case, variations of temperature could occur following storms or electricity cuts. On the other hand, the speed of wind and the volatility of the products cannot be controlled because the rooms are naturally submitted to the action of wind closely like in natural. Nevertheless taking into

account the large number of tests used here the replicate of the results showed clearly that the chemical structures of either kairomones or repellents were the more important parameters in our method. Moreover repellency and attractiveness effects observed during our experiments are obtained in conditions closer to the "natural environment" compared to the other methods.

The dose of the product is another important factor that determines its effectiveness. The results showed that in the presence of very low dose of the two attractants (Octenol: 0.0059 to 0.10mg/mL; Isovaleric acid: 0.0006 to 0.003mg/mL) the kairomone index was high on *Aedes albopictus*, while high doses (on the order of 0.41mg/mL for Octenol and from 0.006mg/mL for Isovaleric acid) resulted in the opposite repellent effect. In 1991, Kline and colleagues found similar results on *Culex salinarius* [27], as they demonstrated that increasing the dose of Octenol caused a reduction in the number of mosquitoes captured .In this experiment, a high attractant effect was observed only when in synergy with CO_2 [28]. However different behavior was observed for the species *Culex quinquefasciatus* who appeared to be insensitive to low doses of attractive compounds, but beginning to be attracted only when both attractant compounds, Octenol and Isovaleric, reached higher doses of about 0.25 and 0.005mg/mL respectively. When comparing the maximum attractant effect of the attractant compounds towards the two mosquito species, *Aedes albopictus* was found far more sensitive than to *Culex quinquefasciatus.* This can be explained by the ecological and evolutionary trajectories of the two species. *Culex quinquefasciatus* is inhabitant of highly polluted environments (eg. stagnant water, sewers) suggesting that it Olfactory organ evolved in the presence of several high doses of chemical stimuli of these habitats, from its larval stage to adulthood. This selective pressure has possibly selected for populations that became insensitive to low doses of these kairomones. On the contrary, *Aedes albopictus* that generally requires more stable environments, less polluted in terms of chemicals both during the lower stage at the imago stage for example: temporary water puddles, tree holes [29], has developed a sensory system sensitive to lower doses.

According to our results, DEET and Picaridin caused evident repellent effects on both *Aedes albopictus* and *Culex quinquefasciatus* even at low dose (< 1mg/mL). Previous studies have shown similar effectiveness of these two repellent compounds on *Aedes aegypti* and *Anopheles gambiae* [10]. Using our test, DEET has a higher repellent effect than the Picaridin at the same dose of the products on *Aedes albopictus*. It confirms the place of DEET as being the leader standard of the repellent [30,31]. The significant differences between the effects of five doses of DEET and Picaridin show the existence of an optimal dose for which a maximum repellent effect was observed for each product which is respectively 0.5 and 1.76mg/mL. This demonstrates that the sensitivity of the two mosquito species to the two repellents is different. At the same dose of product, *Aedes albopictus* was more sensitive than *Culex quinquefasciatus.* Similar in the results of attractants compounds, this can also be explained by the difference in the bio-ecology of these two species.

In this study, a newly experimental device system that can be useful for evaluation of mosquito attractants and repellents were demonstrated. Biological and environmental parameters were optimized as possible close by in natural conditions for effective analysis of attractants and repellents against the two species *Aedes albopictus and Culex quinquefasciatus* female adults.

CONCLUSION

In conclusion, during our tests, the two species tested react at low doses of both repellents and attractants. However, at the same dose of the compounds tested *Aedes albopictus* was more sensitive than *Culex quinquefasciatus*. Optimal doses of the tested compounds on the two species have been identified, for which the attractant effect was maximum whereas the repellent effect reached a plateau. In our bioassay, these doses correspond respectively to 0.0084mg/mL Octenol and 0.5mg/mL DEET for attractant and repellent effects with *Aedes albopictus* respectively and to 0.0025mg/mL Octenol and 1mg/mL DEET with *Culex quinquefasciatus*. These results are close to those obtained by other authors using other methods eg: DEET, ED_{90}= 20.8 mg/cm^2 in direct tests on volunteers and for Octenol, a range of concentration 0.1-100mg/L for mosquito traps in field attracted *Culex* and *Aedes* [11], indicating the efficiency of our device and method to measure the sensitivity of *Aedes albopictus* and *Culex quinquefasciatus* to repellents and attractants. This approach seems much more advantageous compared to other techniques in terms of reproducibility, easy and especially cost that is affordable. This method will allow us to test new natural or synthetic products that can be used in the control of host-vector contact.

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REFERENCES

1. Rhodain F. Problèmes posés par l'expansion d'*Aedes albopictus*. Bull Soc Path Exotic. 1996; 89: 137-141.
2. Cancrini G, Scaramozzino P, Gabrielli S, Di Paolo M, Toma L, Romi R. *Aedes albopictus* and *Culex pipiens* implicated as natural vectors of *Dirofilaria repens* in central Italy. J Med Entomol. 2007; 44: 1064-1066.
3. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. Microbes Infect. 2009; 11: 1177-1185.
4. Schwartz O, Albert ML. Biology and pathogenesis of chikungunya virus. Nat Rev Microbiol. 2010; 8: 491-500.
5. Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med. 2006; 3: 263.
6. Roger M, Beral M, Licciardi S, Soulé M, Faharoudine A, Foray C, et al. Evidence for circulation of the rift valley fever virus among livestock in the union of Comoros. PLoS Negl Trop Dis. 2014; 8: 3045.

7. Tantely LM, Boyer S, Fontenille D. A review of mosquitoes associated with Rift Valley fever virus in Madagascar. Am J Trop Med and Hyg. 2015; 92: 722-729.

8. Mendonca FAC, Silva KFS, Santos K K, Ribeiro KAL, Sant Ana AEG. Activities of some Brazilian plants against larvae of the mosquito *Aedes aegypti*. Fitoterapia. 2005; 76, 629-636.

9. Vershult NO, Mbadi PA, Kiss GB, Mukabana WR, Van Loon JJ, Takken W, et al. Improvement of a synthetic lure for *Anopheles gambiae* using compounds produced by human skin microbiota. Malaria J. 2011; 8: 10-28.

10. Badolo A, Ilboudo-Sanogo E, Ouédraogo AP, Constantini C. Evaluation of the sensitivity of *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes to two insect repellents: DEET and KBR 3023. Trop Med Int Health. 2004; 9: 330-334.

11. Zhengyan W, Jianchu M, Shimiao Z. Laboratory and Field Evaluations of Potential Human Host Odors for *Aedes albopictus* Skuse (Diptera: Culicidae). J Agric Urban Entomol. 2006; 23: 57-64.

12. Takken W, Kline DL. Carbon dioxide and 1-octen-3-ol as mosquito attractants. J Am Mosq Control Assoc.1989; 5: 311-316.

13. Leyden JJ, Kenneth J, Erhard H, Labows JN, Kligman AM. The Microbiology of the Human Axilla and Its Relationship to Axillary Odor. J Invest Dermatol. 1981; 77: 413-416.

14. Zeng X, Leyden L, Lawley H, Sawano K, Nohara I, Preti G. Analysis of characteristic odors from human male axillae. J Chem Ecol. 1991; 17: 1469-1492.

15. Mccabe ET, Barthel WF, Gertler SI, Hall SA. Insect repellents, III. N,N-diethylamides. J Org Chem.1954; 19: 493-498.

16. Moore SJ, Debboun M. History of insect repellents in Insect repellents: Principles, Methods and Use, ed. CRC Press. 2006; 3-29.

17. Zouache K, Raharimalala FN, Raquin V, Tran-Van V, Raveloson LH, Ravelonandro P, et al. Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar. FEMS Microbiol Ecol. 2011; 75: 377-389.

18. Smallegange RC, Schmied WH, Van Roey KJ, Vershult NO, Spitzen J. Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria mosquito *Anopheles gambiae*. Malar J. 2010; 9: 292.

19. Gouck HK, Mcgovern TP, Beroza M. Chemicals tested as space repellents against yellow-fever mosquitoes. Esters. J Econ Entomol. 1967; 60: 1587-1590.

20. Gillies MT. The role of carbon dioxide in host-finding by mosquitoes (Diptera:Culicidae). Rev Bul Entomol Research. 1980; 70: 525-532.

21. Frances SB, Klein TA, Hildebrandt DW, Burge R, Noigamol C, Eikarat N, et al. Laboratory and field evaluation of DEET, CIC-4, and AI3-37220 against *Anopheles dirus* (Diptera: Culicidae) in Thailand. J Med Entomol. 1996; 33:511-515.

22. Barnard DR. Biological assay methods for mosquito repellents. J Am Mosq Control Assoc. 2005; 2: 12-16.

23. Kröber T, Kessler S, Frei J, Bourquin M, Guerin PM. An *in vitro* assay for testing mosquito repellents employing a warm body and carbon dioxide as a behavioral activator. J Am Mosq Control Assoc. 2010; 26: 381-386.

24. Spitzen J, Smallegange RC, Takken W. Effect of human odors and positioning of CO_2 release point on trap catches of the malaria mosquito *Anopheles gambiae* sensu stricto in an olfactomètre. Physiol Entomol. 2008; 33: 116-122.

25. Mboera LEG, Takken W. Carbon dioxide chemotropism in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes. Rev Med Vet Entomol. 1997; 85: 355-368.

26. Barnard DR, Xue RD. Laboratory evaluation of mosquito repellents against *Aedes albopictus*, *Culex nigripalpus*, and *Ochlerotatus triseriatus* (Diptera: Culicidae). J Med Entomol. 2004; 41: 726-730.

27. Kline DL, Dame DA, Meisch MV. Evaluation of 1-octen-3-ol and carbon dioxide as attractants for mosquitoes associated with irrigated rice fields in Arkansas. J Am Mosq Control Assoc. 1991; 7: 165-169.

28. Cilek JE, Hallmon CF, Johnson R. Semi-field comparison of BG lure, Nonanal and Octen-3-ol to attract adult mosquitoes in Northwestern Florida. J Am Mosq Control Assoc. 2011; 27: 393-397.

29. Delatte H, Dehecq J, Thiria J, Domerg C, Paupy C, Fontenille D. Geographic distribution and developmental sites of *Aedes albopictus* (Diptera: Culicidae) during a Chikungunya epidemic event. Vector-Borne Zoonotic Dis. 2008; 8: 25-34.

30. Curtis CF, Lines JD, Ijumba J, Callaghan A, Hill N, Karimzad MA. The relative efficacy of repellents against mosquito vectors of disease. Med Vet Entomol. 1987; 1: 109-19.

31. Debboun M, Frances SP, Strickman D. Insect repellents: principles, methods, and uses. Boca Raton, FL: CRC Press. 2007.

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