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ORIGINAL PAPER

# Development of a mosquito attractant blend of small molecules against host-seeking *Aedes aegypti*

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**Abstract** A mosquito's dependence on olfaction in the hunt for human host could be efficiently exploited to protect humans from mosquito bites. The present study is undertaken to make the most attractant compound blend for *Aedes aegypti* mosquitoes to lure them to traps. Eleven molecules (M1–M11) at different dilutions were screened for attractancy against non-blood-fed adult female mosquitoes in an olfactometer. The results showed that the attractancy was dependent on both the chemical nature of the molecule and the strength of the odor. Out of 11 molecules screened, 9 showed significant attractancy ($P < 0.05$) when tested individually. The attractancy was in the order of M11 > M7 > M6 > M10 > M9 > M3 > M2 > M1 > M4 with attractancy indices (AIs) 86.11, 55.93, 55.17, 54, 52.94, 52, 50, 43.64, and 32, respectively, at the optimum dilutions. Seven blends (I–VII) were made and were screened for attractancy against *Ae. aegypti*. All the blends showed significant attractancy ($P < 0.05$). The attractancy was in the order of blend VII > III > IV > I > VI > V > II with AIs 96.63, 89.19, 65, 57.89, 56.1, 47.13, and 44.44, respectively. Among the seven blends, blend VII with constituent molecules M6, M9, M10, and M11 is the most promising with an AI value of 96.63. This blend will be useful in luring the host-seeking mosquitoes to traps. The field efficacy of these attractant blends may be explored in the future.

**Keywords** Mosquito · Attractant · *Aedes aegypti* · Host seeking · Olfaction

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## Introduction

Mosquito-borne diseases such as malaria, dengue fever, yellow fever, encephalitis, and lymphatic filariasis (http://www.who.int) affect millions of people every year across the world. Vector-borne diseases account for over 17 % of all infectious diseases (http://www.who.int/mediacentre/factsheets/fs387/en/). The world's fastest growing vector-borne disease is dengue, and the incidence of dengue has increased 30-fold over the last 50 years (http://www.who.int/denguecontrol/control_strategies/en/) though malaria is causing maximum mortality among these. Dengue is transmitted by the bite of a female mosquito infected with one of the four dengue virus (DENV1, DENV2, DENV3, or DENV4) serotypes. Preventing or reducing dengue virus transmission depends entirely in controlling the mosquito vectors or interruption of human vector contact. Vector transmission is reduced through the use or combination of three methods: environmental management, chemical control, and biological control (http://www.who.int/denguecontrol /control_strategies/en/). Various factors such vector and pathogen resistance to insecticides and drugs, globalization of travel and trade, unplanned urbanization, and environmental challenges such as climate change are having a significant impact on disease transmission in recent years (Gubler 1998; http://www.who.int/mediacentre/factsheets/fs387/en/). There is an obvious need for development of alternative tools to complement existing mosquito control strategies.

Disease transmission is in part forced by the prerequisite of a vertebrate blood meal by female mosquitoes to finish their gonotrophic reproductive cycle (Pitts et al. 2004). Mosquitoes make use of their sense of smell to target a human host. Recent studies investigate the efficacy of protecting humans from attack by using odorant-targeting mosquito olfactory receptors (Potter 2014). Odor baits are expected to be tolerable to communities in disease-prevalent regions unlike other vector-control means (Grieco et al. 2007). A mosquito's reliance on olfaction for finding a human host could be efficiently exploited to safeguard humans from attack by targeting mosquito olfactory receptors.

*Aedes aegypti* is the vector of dengue, yellow fever, and chikungunya in tropical regions. Conventional surveillance method for *Ae. aegypti* population depends on labor-intensive methods such as the sampling of immature stages from breeding sites and the utilization of ovitraps that sample the gravid females. However, some of the indices obtained lack epidemiological understanding (Focks 2003). Sampling procedures for adult *Ae. aegypti* such as human biting catch and aspiration can be hazardous and lengthy for operators (Focks 2003). Hence, there is a need for developing competent sampling methods for adult *Ae. aegypti*.

Majority of the mosquito monitoring and surveillance programs include the monitoring of adults using trapping devices. Trapping adult host-seeking female mosquitoes is the most effective means of obtaining an accurate picture of the risk of infection in any specified area (Geier et al. 2006). Geier et al. (1999) has demonstrated that mosquitoes are able to orient upwind under continuous odor stimulation and that upwind flight is dependent upon plume structure in different ways for different host odor components. Methods for assessing attraction of *Ae. aegypti* in the laboratory are well established using Y-tube olfactometers (Geier and Boeckh 1999). Research in the area of chemical and sensory ecology of mosquito attractants at the University of Regensburg resulted in the design of a new lure and trapping systems (Bio-Gents sentinel trap) mimicking convection currents created by a human body, employing attractive visual cues, and releasing attractants over a large surface area (Geier et al. 2006). Englbrecht et al. (2015) have shown that sustained use and proper placement of efficient mosquito traps such as BG-Sentinel mosquito traps can significantly reduce *Aedes albopictus* biting pressure.

Olfactory signals are the important outer stimuli that have an impact on mosquito behaviors such as host-seeking (Takken and Knols 1999; Mukabana et al. 2002), oviposition (Seenivasagan et al. 2009; Sharma et al. 2008, 2009; Guha et al. 2012), and sugar-feeding (Takken 1991). Finding a suitable host is crucial for the reproduction of most hematophagous mosquito species, as proteins from the host's blood are essential for the development of the eggs. The host-seeking flight of a mosquito can generally be classified into long-, medium-, and short-range attraction. Long distance attraction is determined by odor stimuli, medium distance attraction by odors and carbon dioxide ($CO_2$), and attraction from a short distance by odors, $CO_2$, and non-olfactory cues, such as heat, body moisture, and visual signals (Takken 1991). Using their very sensitive olfactory organs, these mosquitoes can prefer more attractive persons over less attractive ones by identifying chemicals present in breath, sweat, and other skin emanations coming from the persons (Takken and Knols 1999; Mukabana et al. 2002). Single compounds as well as odor mixtures attract female mosquitoes to their hosts. Female *Ae. aegypti* is attracted by a complex attractant signal from human hosts comprising of $CO_2$, L-lactic acid, ammonia, and fatty acids (Kellog 1970; Geier et al. 1999; Bosch et al. 1999, 2000; Steib et al. 2001).

The present study is therefore undertaken to identify an attractant compound blend for *Ae. aegypti* mosquitoes. The roadmap for this is to study the response of unfed *Ae. aegypti* towards small synthetic molecules singly and in combination and thereby identification of an effective blend attracting host-seeking *Ae. aegypti* mosquitoes.

## Materials and methods

### Mosquitoes

Four- to five-day-old non-blood-fed female *Ae. aegypti* mosquitoes reared from larvae to adults at 27 °C, 70–80 % relative humidity and at a 12 h/12 h light/dark photoperiod maintained at the Vector Control Research Centre, Pondicherry were used for the study. All mosquitoes were ad libitum access to distilled water throughout the experiments.

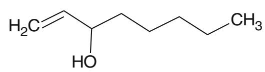
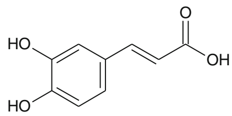
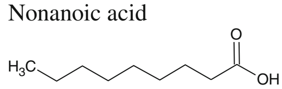
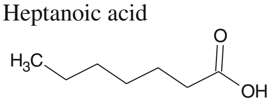
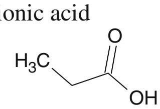
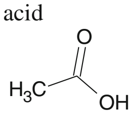
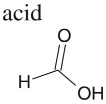
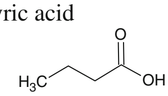
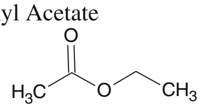
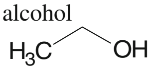
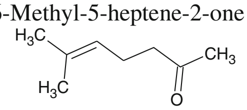
### Test materials

The list of synthetic molecules used for mosquito attractancy screening is given in Table 1. All chemicals with the highest purity grade available were purchased from either Sigma-Aldrich or Fluka (Sweden).

### Preparation of test solutions and odor delivery

Eleven odorant molecules were used for the present study. The selection of the odorants was based on previous description as host-volatile cues or associated with host selection of different mosquito species (Qiu et al. 2006). Test solutions were prepared by using either water or liquid paraffin at the following serial dilutions: 1/2, 1/10, 1/100, 1/1000, 1/10,000, and or 1/100,000 volume by volume depending on the solubility of the molecules. Liquid paraffin or mineral oil is a transparent, colorless, odorless, oily liquid composed of saturated hydrocarbons obtained from petroleum. Aliquots of 10 μl of each test sample were transferred on to a Whatman no. 1 filter paper (3 cm diameter) placed inside the test chamber. In the control chamber, similar filter paper with 10 μl of paraffin/water was used.

**Table 1** The response of the host-seeking *Ae. aegypti* to M1–M11 at different dilutions

| Test molecule | Concentration (dilution) | Mean no. of mosquitoes ± SE (*n*=4) | | *P* value |
|---|---|---|---|---|
| | | Test | Control | |
| M1 | 1/2 | 7.50 ± 2.02 | 12.50 ± 1.55 | 0.10 |
| 1-Octene-3-ol | 1/10 | 9.75 ± 2.17 | 6.50 ± 0.50 | 0.20 |
|  | 1/100 | 19.75 ± 4.17 | 7.75 ± 0.85 | 0.03 |
| | 1/1000 | 8.25 ± 0.95 | 4.75 ± 1.25 | 0.67 |
| | 1/10000 | 8.50 ± 1.50 | 7.25 ± 2.69 | 0.70 |
| M2 | 1/2 | 8.75 ± 1.65 | 4.75 ± 1.11 | 0.09 |
| Caffeic acid | 1/10 | 4.25 ± 1.65 | 2.25 ± 0.75 | 0.31 |
|  | 1/100 | 6.00 ± 1.08 | 2.50 ± 0.29 | 0.02 |
| | 1/1000 | 6.00 ± 1.22 | 2.00 ± 1.08 | 0.002 |
| | 1/10000 | 3.75 ± 0.75 | 1.75 ± 0.48 | 0.07 |
| M3 | 1/2 | 2.75 ± 0.25 | 4.75 ± 0.48 | 0.01 |
| Nonanoic acid | 1/10 | 2.75 ± 0.85 | 3.75 ± 0.85 | 0.44 |
|  | 1/100 | 3.00 ± 0.41 | 1.75 ± 0.85 | 0.24 |
| | 1/1000 | 4.75 ± 0.48 | 1.5 ± 0.29 | 0.001 |
| | 1/10000 | 3.00 ± 1.68 | 2.75 ± 0.48 | 0.89 |
| M4 | 1/2 | 8.25 ± 2.78 | 5.25 ± 1.80 | 0.40 |
| Heptanoic acid | 1/10 | 10.0 ± 3.14 | 12.25 ± 2.95 | 0.62 |
|  | 1/100 | 5.00 ± 1.47 | 7.75 ± 1.65 | 0.26 |
| | 1/1000 | 8.25 ± 1.03 | 4.25 ± 1.25 | 0.05 |
| | 1/10000 | 2.25 ± 0.48 | 3.75 ± 0.95 | 0.21 |
| M5 | 1/2 | 2.00 ± 1.08 | 3.25 ± 1.60 | 0.54 |
| Propionic acid | 1/10 | 3.50 ± 0.29 | 6.50 ± 0.87 | 0.17 |
|  | 1/100 | 3.25 ± 0.63 | 6.75 ± 1.25 | 0.05 |
| | 1/1000 | 5.00 ± 1.08 | 5.50 ± 0.65 | 0.71 |
| | 1/10000 | 6.00 ± 2.27 | 6.25 ± 2.29 | 0.94 |
| M6 | 1/2 | 7.25 ± 1.70 | 5.25 ± 0.75 | 0.32 |
| Acetic acid | 1/10 | 7.25 ± 0.75 | 8.25 ± 1.65 | 0.60 |
|  | 1/100 | 8.75 ± 1.18 | 5.25 ± 1.70 | 0.14 |
| | 1/1000 | 6.50 ± 2.72 | 3.25 ± 0.85 | 0.30 |
| | 1/10000 | 7.00 ± 1.41 | 3.00 ± 0.41 | 0.04 |
| | 1/100000 | 11.25 ± 1.70 | 2.25 ± 0.85 | 0.002 |
| M7 | 1/2 | 4.75 ± 0.63 | 5.25 ± 2.14 | 0.83 |
| Formic acid | 1/10 | 4.25 ± 2.02 | 3.50 ± 1.19 | 0.76 |
|  | 1/100 | 9.50 ± 2.10 | 4.75 ± 1.25 | 0.10 |
| | 1/1000 | 5.25 ± 1.18 | 2.50 ± 0.50 | 0.08 |
| | 1/10000 | 11.50 ± 2.10 | 3.25 ± 0.63 | 0.01 |
| | 1/100000 | 7.50 ± 1.55 | 3.00 ± 1.41 | 0.07 |
| M8 | 1/2 | 5.00 ± 2.35 | 4.00 ± 1.58 | 0.74 |
| Butyric acid | 1/10 | 3.50 ± 0.87 | 5.75 ± 1.11 | 0.16 |
|  | 1/100 | 4.25 ± 0.48 | 2.25 ± 0.63 | 0.05 |
| | 1/1000 | 3.25 ± 1.03 | 5.25 ± 0.85 | 0.19 |
| | 1/10000 | 3.00 ± 1.68 | 4.00 ± 1.22 | 0.59 |
| M9 | 1/2 | 3.75 ± 1.49 | 2.5 ± 1.55 | 0.58 |
| Ethyl Acetate | 1/10 | 3.25 ± 0.75 | 1.0 ± 0.41 | 0.04 |
|  | 1/100 | 4.50 ± 1.32 | 3.0 ± 1.41 | 0.47 |
| | 1/1000 | 7.00 ± 2.68 | 3.5 ± 2.18 | 0.35 |
| | 1/10000 | 5.50 ± 0.87 | 2.50 ± 0.96 | 0.06 |
| M10 | 1/2 | 3.75 ± 1.55 | 3.5 ± 2.87 | 0.94 |
| Ethyl alcohol | 1/10 | 10.25 ± 5.95 | 4.75 ± 3.09 | 0.44 |
|  | 1/100 | 4.50 ± 0.96 | 2.0 ± 0.41 | 0.05 |
| | 1/1000 | 19.25 ± 5.31 | 5.75 ± 1.65 | 0.05 |
| | 1/10000 | 3.50 ± 0.65 | 1.5 ± 0.65 | 0.07 |
| M11 | 1/2 | 4.75 ± 0.85 | 3.5 ± 4.19 | 0.54 |
| 6-Methyl-5-heptene-2-one | 1/10 | 7.5 ± 2.02 | 2.25 ± 0.95 | 0.21 |
|  | 1/100 | 9.25 ± 3.97 | 2.0 ± 0.71 | 0.02 |
| | 1/1000 | 6.5 ± 3.23 | 1.25 ± 0.95 | 0.11 |
| | 1/10000 | 16.75 ± 8.71 | 1.25 ± 0.63 | 0.05 |

### Laboratory bioassay for mosquito attractancy

Experiments were performed in an indigenous olfactometer as reported earlier (Nisha et al. 2013). Briefly, the olfactometer consists of a mosquito cage/flight chamber (30 × 30 × 30 cm) fitted with two glass tubes of 60 cm in length. Each glass tube has two ends, one broad end with a diameter of 6 cm connected to the flight chamber and another narrow end of 1.5 cm diameter inserted into a mosquito-collecting container. Each container has two holes, one for inserting the glass tube and another for inserting a silicon tube through which humidity-controlled air enters into the container supplied through a small air pump. Test materials were placed in the test container, and the control was with base solvent without test material. The air carrying the odor enters into the long tube and finally reaches the flight chamber. For each experiment, 50 female *Ae. aegypti* mosquitoes of 4–5 days old, which had not received a blood meal, were introduced into the flight chamber using a manual aspirator with access to distilled water from damp cotton wool. The experiments of 60 min duration were carried out at daytime since *Ae. aegypti* is a diurnal mosquito. The mosquitoes that had trapped in the control and the test containers were noted after anesthetization with diethyl ether. The left-out mosquitoes in the flight chamber were discarded by manual aspirator. Each experiment was done with a new set of mosquitoes, clean trapping containers, and new stimuli. Care was taken to avoid contamination of the equipment with human volatiles by using hand gloves. Each molecule was tested singly at different concentrations and combinations. Each experiment was repeated four times at different days.

The attractancy index (AI) (Pascual-Villalobs and Robledo 1998; Khalequzzaman et al. 2002) was calculated as

$$100 \times (T-C)/(T+C) = \text{Rating (attractancy)}$$

where $T$ is the number of mosquitoes in the test and $C$ is the number of mosquitoes in the control. The materials were then classified (Beroza and Green 1963) based on the attractancy rating 1–15 (class I), 16–33 (class II), and 34–100 (class III). A zero value shows no choice for control or test, and a negative value indicated repellent nature.

### Preparation of blend of small molecules for mosquito attractancy testing

Small molecules were selected based on their mosquito attractancy index falling more than 50 and prepared the blend with the concentrations which exhibited maximum attractancy index. Seven blends (I–VII) were prepared and tested for mosquito attractancy as described above.

### Statistical analysis

The means were compared by independent samples $t$ test using SPSS 16.0, the Statistical Package of Social Sciences for windows. This was done by clicking Analyze → Compare Means → Independent-Samples T - Test and then providing variables to be compared and a grouping variable with specific values specified. $P$ value <0.05 was taken as "statistically significant."

## Results and discussion

A total of 11 small synthetic molecules (M1–11) with molecular weight less than 500 as listed in Table 1 were screened at different dilutions against unfed adult *Ae. aegypti* mosquitoes for attraction and compared simultaneously with control, and the results are given in Table 1. For each molecule, the attractancy index was calculated to find out the dilution at which maximum attractancy response was shown by the non-blood-fed *Ae. aegypti* mosquitoes and the results are given in Fig. 1. A total of seven blends of mosquito-attractant molecules were tested, and the results are given in Fig. 2. All the blends showed significant ($P < 0.05$) mosquito attractancy.

The comparison of the mean values of 1-Octene-3-ol (M1) and control using $t$ test showed that at 1/100 the $P$ value is significant with a value of 0.03. At all the other dilutions, the value of $P$ was >0.05. The attractancy indices for M1 at different dilutions are shown in Fig. 1. The attractancy index values were −25, 20, 43.64, 26.92, and 7.94 respectively at 1/2, 1/10, 1/100, 1/1000, and 1/10,000 dilutions of M1. At higher concentration, M1 exhibited repellency with an AI value of −25. As the concentration was reduced, the attractancy of M1 was increased and at 1/100 dilution it reached the peak value 43.64 and on further dilution the AI value showed a decreasing trend. M1 is falling into class III attractant as the AI value is >33.

The comparison of the mean values of the caffeic acid (M2) and control using $t$ test showed that at 1/100 and 1/1000 the $P$ value is significant with a value of 0.02 and 0.002, respectively. At all the other dilutions, the $P$ value was >0.05. The AI values were 29.63, 30.77, 41.18, 50, and 36.36 respectively at 1/2, 1/10, 1/100, 1/1000, and 1/10,000 dilutions of M2. The mosquito attractancy showed an increasing tendency on dilution up to 1/1000 and then showed a decreasing trend. Maximum attractancy was observed at 1/1000 dilution with an AI value of 50 and hence, M2 is falling into class III attractant.

The comparison of the mean values of nonanoic acid (M3) and control using $t$ test showed that at 1/2 and 1/1000 the $P$ value is significant with a value of 0.01 and 0.001, respectively. The AI values were −26.67, −15.38, 26.32, 52, and 4.35 respectively at 1/2, 1/10, 1/100, 1/1000, and 1/10,000

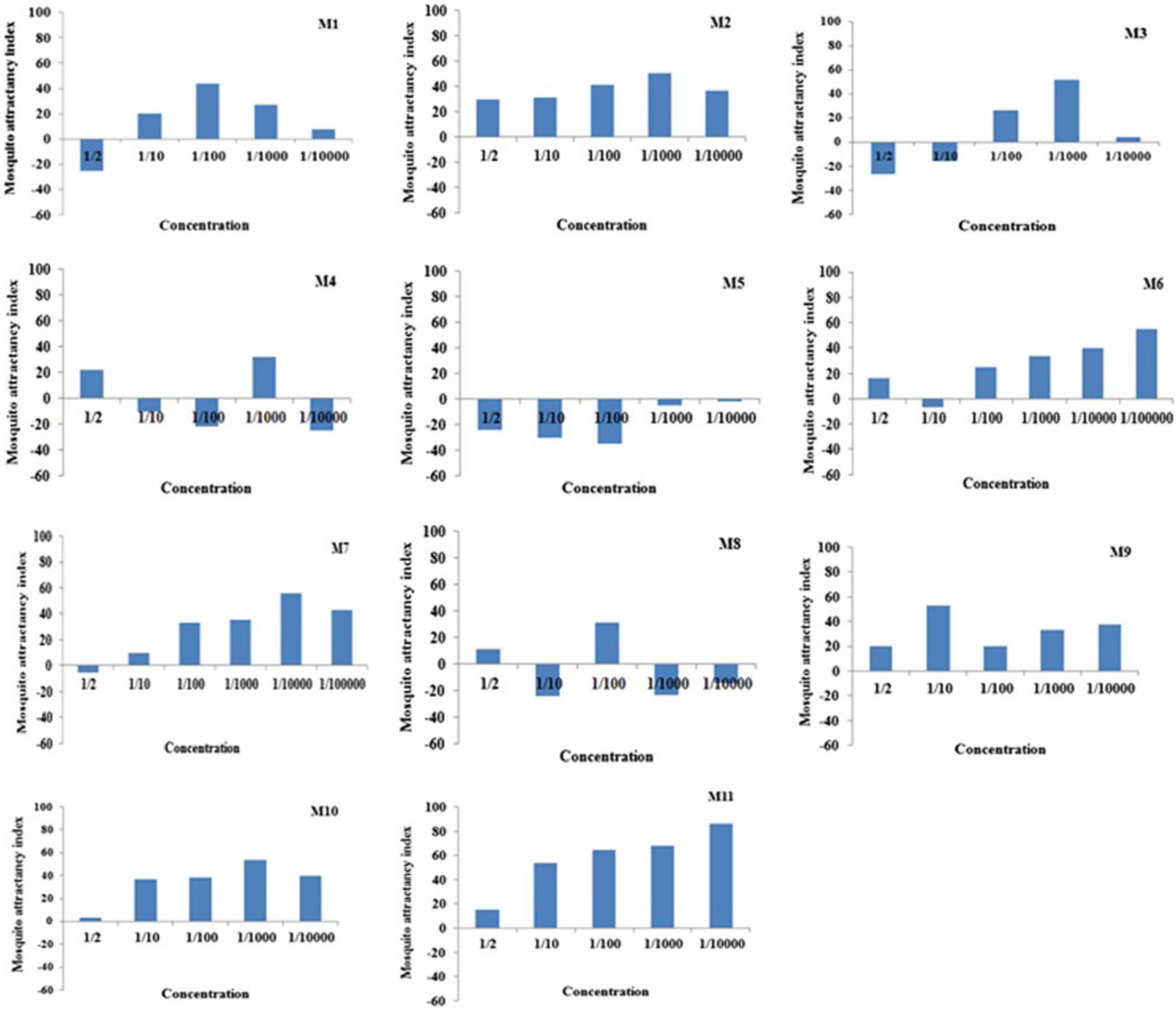



**Fig. 1** Mosquito attractancy indices for different dilutions of M1–M11

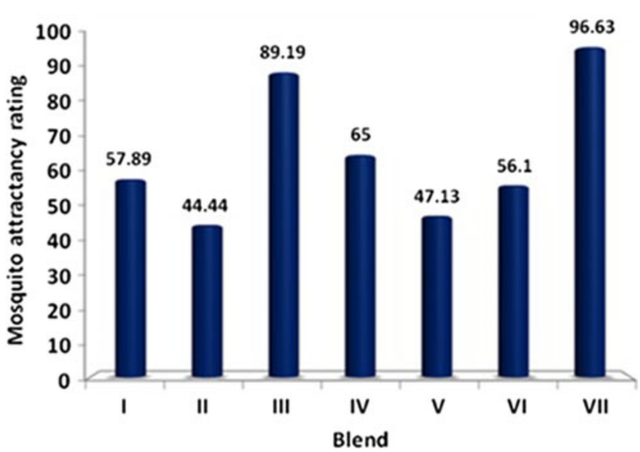



**Fig. 2** Mosquito attractancy of different blends

dilutions of M3. At higher concentrations, M3 exhibited repellency with an AI value of −26.67 followed by −15.38. As the concentration was reduced, the attractancy of M3 was increased and at 1/1000 dilution it reached the peak value of 52 and on further dilution the AI values were decreased. For M3, the maximum attractancy was observed at 1/1000 dilution with an AI value of 52 and M3 is falling into class III attractant.

The comparison of the mean values of heptanoic acid (M4) and control using $t$ test showed that at 1/1000 the $P$ value is significant with a value of 0.05. At all the other dilutions, the $P$ value was >0.05. The attractancy index values were 22.22, −10.11, −21.57, 32, and −25 respectively at five dilutions of M4. Maximum attractancy was observed at 1/1000 dilution with an AI value of 32 and hence M4 is falling into class II attractant.

The comparison of the mean values of propionic acid (M5) and control using *t* test showed that at 1/100 the *P* value is significant with a value of 0.05. The attractancy index values were −23.81, −30, −35, −4.76, and −2.04 respectively at 1/2, 1/10, 1/100, 1/1000, and 1/10,000 dilutions of M5. The negative values obtained for AI at all test concentrations show that M5 is not attracting the non-blood-fed female *Ae. aegypti*, instead showing repellency.

The response of non-blood-fed *Ae. aegypti* mosquitoes to acetic acid (M6) at six dilutions viz., 1/2, 1/10, 1/100, 1/1000, 1/10,000, and 1/100,000 are given in Table 1. The comparison of the mean values of the test and control using *t* test showed that at 1/10,000 and 1/100,000 the *P* value is significant with a value of 0.04 and 0.002, respectively. At all the other dilutions, the *P* value was >0.05. The attractancy index values were 16, −6.45, 25, 33.33, 40, and 55.17 respectively at six dilutions of M6. The response of mosquitoes to M6 was found to be concentration dependent except for 1/10 dilution. Maximum AI was observed at 1/100,000 dilution with a value of 55.17. M6 is falling into class III attractant.

The comparison of the mean values of formic acid (M7) and control using *t* test showed that at 1/10,000 the *P* value is significant with a value of 0.01. At all the other dilutions, *P* value was >0.05. The attractancy index values were −5.0, 9.68, 33.33, 35.48, 55.93, and 42.86 respectively at 1/2, 1/10, 1/100, 1/1000, 1/10,000, and 1/100,000 dilutions of M7. At higher concentration, M7 exhibited repellency with an AI value of −5. As the concentration was reduced, the attractancy of M7 was increased and at 1/10,000 dilution it reached the peak value 55.93 and on further dilution the AI value showed a decreasing trend. Hence, for M7, the maximum attractancy was observed at 1/10,000 dilution with an AI value of 55.93 and M7 is falling into class III attractant.

The attractancy index values for butyric acid (M8) were 11.11, −24.32, 30.77, −23.53, and −14.29 respectively at 1/2, 1/10, 1/100, 1/1000, and 1/10,000 dilutions of M8. M8 gives inconsistent results against *Ae. aegypti*. It seems that this compound has no influence on the behavior of the mosquitoes.

The comparison of the mean values of ethyl acetate (M9) and control using *t* test showed that at 1/10 the *P* value is significant with a value of 0.04. The attractancy index values were 20, 52.94, 20, 33.33, and 37.5 respectively at 1/2, 1/10, 1/100, 1/1000, and 1/10,000 dilutions of M9. Maximum attractancy was observed at 1/10 dilution with an AI value of 52.94, and hence, M9 is falling into class III attractant.

The comparison of the mean values of ethyl alcohol (M10) and control using *t* test showed that at 1/100 and 1/1000 the *P* value is significant (0.05) for both dilutions and for all the other dilutions $P > 0.05$. The attractancy index values were 3.45, 36.67, 38.46, 53.85, and 40 respectively at 1/2, 1/10, 1/100, 1/1000, and 1/10,000 dilutions of M10. At higher concentration, M10 exhibited very low attractancy index with an AI value of 3.45. As the concentration was reduced, the attractancy of M10 was increased and at 1/1000 dilution it reached the peak value 53.85 and on further dilution the AI value showed a decreasing trend. Maximum attractancy was observed at 1/1000 dilution with an AI value of 53.85, and hence, M10 is falling into class III attractant.

The comparison of the mean values of 6-methyl-5-heptene-2-one (M11) and control using *t* test showed that at 1/100 and 1/1000 the *P* value is significant (0.02 and 0.05, respectively) for both dilutions and for all the other dilutions $P > 0.05$. The attractancy index values were 15.2, 53.8, 64.4, 67.7, and 86.1 respectively at 1/2, 1/10, 1/100, 1/1000, and 1/10,000 dilutions of M11. Maximum attractancy was observed at 1/10, 000 dilution with an AI value of 86.1, and hence, M11 is falling into class III attractant.

A total of seven mosquito-attractant blends (I–VII) were prepared with the optimum dilutions of the molecules falling in class III and AI-attractancy index >50. The molecules used in the blends were nonanoic acid (M3), acetic acid (M6), formic acid (M7), ethyl acetate (M9), ethyl alcohol (M10), and 6-methyl-5-heptene-2-one (M11). The response of the non-blood-fed adult female *Ae. aegypti* to the blends are shown in Table 2. The comparison of the mean values of the test and control using *t* test showed that *P* value was <0.05 in all the cases showing statistical significance.

The attractancy indices for the blends are shown in Fig. 2. Blend I containing five promising attractant molecules (M3 + M6 + M7 + M9 + M10) showed an attractancy index of 57.89. Blend II containing M6 + M7 + M9 + M10 showed an AI of 44.44. Blend III containing M6 + M9 + M10 showed an AI of 89.19. In the case of blend IV containing M6 + M7, the observed AI was 65. Blends V (M6 + M9) and VI (M6 + M10) showed AI values 47.13 and 56.1, respectively. Blend VII containing M6 + M9 + M10 + M11 showed an AI of 96.63. Among the seven blends screened for mosquito attractancy, blend VII showed highest attractancy with an AI value of 96.63 followed by III, IV, I, VI, V, and II.

**Table 2** The response of the host-seeking *Ae. aegypti* to attractant blends

| Test blend | Mean no of mosquitoes ± SE ($n = 4$) | | *P* value |
|---|---|---|---|
| | Test | Control | |
| I | 3.75 ± 0.48 | 1.00 ± 0.41 | 0.005 |
| II | 9.75 ± 2.17 | 3.75 ± 0.95 | 0.05 |
| III | 8.75 ± 0.25 | 0.50 ± 0.29 | 0.00 |
| IV | 8.25 ± 1.55 | 1.75 ± 0.85 | 0.01 |
| V | 16.00 ± 3.67 | 5.80 ± 1.93 | 0.05 |
| VI | 16.00 ± 2.48 | 4.50 ± 1.04 | 0.005 |
| VII | 29.25 ± 3.5 | 0.5 ± 0.29 | 0.03 |

Carboxylic acids are general lipid constituents of mammalian skin and emanations (Bernier et al. 2002) and many are well-documented as attractants. Short-chained carboxylic acids were attractive for *Ae. aegypti* in combination with L-lactic acid (Bosch et al. 2000). Aliphatic carboxylic acids have been reported as attractive for *Anopheles gambiae* (Knols et al. 1997) and *Ae. aegypti* (Carlson et al. 1973) and elicit electrophysiological responses (Meijerink and van Loon 1999). This study also has included seven carboxylic acids viz., formic acid, acetic acid, propionic acid, butyric acid, heptanoic acid, nonanoic acid, and caffeic acid and studied the response of the host-seeking *Ae. aegypti* mosquitoes to different dilutions of them. Among these acids, acetic acid and formic acid showed promising attractancy while propionic acid exhibited repellency and other acids showed moderate attractancy when tested individually.

Alcohol ingestion has attracted more mosquitoes; however, sweat production or skin temperature after ethyl alcohol ingestion does not attract mosquitoes but the attraction rather might be due to the presence of unknown chemical substances on the skin after ethyl alcohol ingestion (Shirai et al. 2002). 1-Octen-3-ol is a volatile component of bovine (Hall et al. 1984) and human breath (Bernier et al. 2000). Its potential role as an attractant has been known for different hematophagous insect species, such as mosquitoes (Essen et al. 1994; Nisha et al. 2013) and tsetse flies (Hall et al. 1984). This study showed that non-blood-fed mosquitoes were attracted to 1-octene-3-ol and ethyl alcohol.

The use of blends of 2 or 3 attractants often increases trap collections more than the use of blend 1 attractant alone (Gillies 1980; Bernier et al. 2003). This study also observed the efficacy of blends in attracting the mosquitoes than the individual molecules or in other words, the blends or combination of compounds show clear evidence for synergism in attraction. The ability of *Ae. aegypti* to get better host source localization via multimodal amalgamation of sensory cues could work to boost the probability that the female mosquito gets a blood meal, a behavior correlated to mosquito reproduction. Such kind of synergistic tactics to direct sensory perception may finally yield a strong strategy to deter mosquitoes from finding humans (Tauxe et al. 2013; Turner et al. 2011; Jones et al. 2011).

The current study reports influence of 11 small synthetic molecules on the day-biting vector mosquito *Ae. aegypti* at different concentrations. The attractancy of the mosquitoes to the attractant molecules was dependent on both the nature of the molecule and the strength of the odor (concentration) as evidenced by the different attractancy indices at different dilutions of the molecules. Our analysis of concentration-dependent mosquito responses surely demonstrates that mosquito response to odors is significantly modulated by odor concentrations. This finding is not surprising and is entirely consistent with related studies on odor-coding intensities in *Drosophila* (Wang et al. 2003; Hallem and Carlson 2006) and mammals (Ma and Shepherd 2000). Out of eleven, eight molecules showed significant attractancy ($P<0.05$) to unfed *Ae. aegypti* adult mosquitoes when tested individually. The attractancy was found to be in the order of 6-methyl-5-heptene-2-one (M11) > formic acid (M7) > acetic acid (M6) > ethyl alcohol (M10) > ethyl acetate (M9) > nonanoic acid (M3) > caffeic acid (M2) > 1-octene-3-ol (M1) > heptanoic acid (M4) with attractive indices 86.1, 55.93, 55.17, 53.85, 52.94, 52, 50, 43.64, and 30.6 respectively at the optimum dilutions. Propionic acid (M5) showed a negative value for AI at all dilutions tested showing the repellent nature, and butyric acid (M8) showed inconsistent results.

This work reveals the importance of selected chemicals in blends of odors affecting mosquito behavior, similar to pheromone blends in moths and bark beetles (El-Sayed et al. 1999). Seven attractant blends were prepared from six molecules showing AI value of more than 50 and screened for attractancy against non-blood-fed *Ae. aegypti* mosquitoes. All the blends showed significant attractancy ($P<0.05$) to unfed *Ae. aegypti* adult mosquitoes. The attractancy was found to be in the order of blend VII > blend III > blend IV > blend I > blend VI > blend V > blend II with attractive indices 96.63, 89.19, 65, 57.89, 56.1, 47.13, and 44.44, respectively. Blend VII, blend III, blend IV, blend I, and blend VI showed promising attractancy to non-blood-fed *Ae. aegypti* mosquitoes compared to the attractancy shown by the constituents when tested individually. Among the seven blends, blend VII with constituent molecules viz., 6-methyl-5-heptene-2-one (M11), acetic acid (M6), ethyl acetate (M9), and ethyl alcohol (M10) is the most promising with an AI value of 96.63.

A total of 11 molecules and seven blends were studied for mosquito attractancy against host-seeking adult female *Ae. aegypti* mosquitoes. Six molecules and five blends were showing mosquito attractancy index of more than 50. The most promising, blend VII (6-methyl-5-heptene-2-one, acetic acid, ethyl acetate, and ethyl alcohol), showed an AI value of 96.63. Hence, this study has identified a promising mosquito-attractant blend. This attractant blend may have potential as trap lure components to enhance collection of mosquitoes in attractant-baited surveillance traps. Artificial feeding systems for mosquitoes are important for establishment and maintenance of colonies in the absence of vertebrate hosts and for inoculation in disease transmission studies. Field efficacy of these attractant blends may be explored in the future.

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