

## Original Article

# Laboratory Evaluation of Synthetic Attractants for *Anopheles stephensi* Using High-Throughput Screening: A Step Towards Development of Mosquito Traps

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(Received 11 Nov 2024; accepted 22 May 2025)

## Abstract

**Background:** Diseases such as malaria are transmitted by *Anopheles* species, among which *Anopheles stephensi* is one of the most important malaria vectors in Iran. Reducing the transmission of mosquito-borne diseases depends on controlling the mosquito vector or minimizing human-vector contact. A promising method for control, surveillance, and monitoring involves using synthetic attractants in traps to target vectors. This study aims to determine the effective dose of octenol, isovaleric acid, lactic acid, hexanoic acid, isoamyl alcohol, myristic acid, and ammonium hydrogen bicarbonate using the high-throughput screening system (HTSS) device in the laboratory.

**Methods:** After rearing *An. stephensi* in the insectary, High-Throughput Screening System (HTSS) assay was used to obtain the 50% and 90% effective dose (ED) of the attractive compounds. Probit analysis was used to analyze the results and prepare the regression lines of ED<sub>50</sub> and ED<sub>90</sub>.

**Results:** This study showed that *An. stephensi* had the highest absorption to isoamyl alcohol (ED<sub>50</sub>= 0.57 mL/L, ED<sub>90</sub>= 1.04 mL/L), followed by isovaleric acid (ED<sub>50</sub>= 1.96 mL/L, ED<sub>90</sub>= 3.00 mL/L), myristic acid (ED<sub>50</sub>= 24.77 g/L, ED<sub>90</sub>= 47.08 g/L), octenol (ED<sub>50</sub>= 26.64 mL/L, ED<sub>90</sub>= 54.36 mL/L) and lactic acid (ED<sub>50</sub>= 54.98 mL/L, ED<sub>90</sub>= 132.9 mL/L), while hexanoic acid (ED<sub>50</sub>= 87.50 mL/L, ED<sub>90</sub>= 244.49 mL/L) per liter and ammonium hydrogen bicarbonate (ED<sub>50</sub>= 93.84 g/L, ED<sub>90</sub>= 234.01 g/L) showed the lowest absorption rate.

**Conclusion:** Our laboratory results identified isoamyl alcohol and isovaleric acid as highly effective attractants for *An. stephensi*. These compounds are strong candidates for inclusion in field-deployable traps after further validation.

**Keywords:** *Anopheles stephensi*; Mosquito attractive compounds; Effective dose; High-Throughput Screening

## Introduction

Malaria is still an important health problem in Iran (1). *Anopheles stephensi* is known as an important vector of urban malaria in the Middle East and the Indian subcontinent, and it is considered the main vector of malaria in southern Iran (2). Monitoring and controlling vectors are crucial in the fight against malaria. Humans have continually sought effective control methods to reduce the damage caused by mosquitoes. Methods such as chemical control, biological

control and environmental sanitation have been used so far to control and reduce the frequency of vectors. Factors such as climate change, travel and trade, resistance to insecticides and environmental pollution caused by insecticides, resistance to drugs, the lack of a highly effective vaccine and disruptions such as epidemics lead to the cessation or reversal of these control methods (3–6).

Successful malaria control depends on understanding the interactions between mosquitoes and humans (7). For this reason, new tools and methods are urgently needed to control the malaria vectors (8). Mosquito nets treated with insecticides have reduced human contact with vectors, but the emergence of insecticide resistance poses a significant threat to this approach (9). While repellents reduce human-vector contact by discouraging biting (10), an alternative strategy is to attract vectors to traps using synthetic compounds, thereby removing them from the environment (11). Moreover, with the improvement of trapping methods, many scientific and practical goals can be achieved, including local investigation of mosquito species and their population abundance, monitoring of invasive mosquito species, investigating the status of pathogens transmitted through vectors and predicting disease epidemics. The use of attractants could reduce the reliance on insecticides in the future (12). However, the effective use of attractants to control mosquitoes requires understanding the mechanisms that attract mosquitoes to humans (13).

Odors released by the host have signs that attract mosquitoes to feed on blood (14). More than 300 chemical compounds have been identified in human skin. Several of these compounds have been well demonstrated to play a role in mosquito-host interactions (15). As a result, nowadays, many volatile compounds, natural odor compounds, or extracts are used to trap different mosquito species (12). Host chemicals, including lactic acid, ammonia, octenol and specific amino acids, may serve as attractive cues for mosquitoes at close proximity to the host (16). For example, octenol, lactic acid, ammonia and hexanoic acid (17) are compounds known to be attractive to *An. stephensi* (15, 18). Isovaleric acid has also been effective as an attractant for *An. coluzzii* (19). The combination of lactic acid and ammonia (20) and other compounds, such as isoamyl alcohol (21), myristic acid (22) and ammonium hydroxide (20), has been used to attract *Aedes aegypti*.

Researchers have studied how blood-sucking mosquitoes respond to attractants using olfactory devices. The most widely used type of olfactory is the Y-tube, which provides airflow when female mosquitoes fly upwind toward the attractant source (16). However, the evidence indicates that these types of test devices cause the mosquitoes not to fly naturally due to the creation of artificial airflow and thus disrupt the mosquitoes' natural host-seeking behavior (23).

High-throughput screening system (HTSS) device can be mentioned as an alternative proposed method for testing attractant materials. This simple diffusion assay does not have the inconsistencies of artificial airflow. It is a simple and widely applicable method that does not require a complex laboratory setup (16). The HTSS protocol was originally designed to measure active synthetic or natural repellents: three chemical effects of toxicity and two primary behavioral avoidance responses (contact excitation and spatial repellency), not attractants. However, it was found that the HTSS assay, with only minor changes, could successfully optimize and screen the most effective lure candidates for each mosquito species (16, 24). By now, a few studies have been carried out using the HTSS on *An. stephensi*. This system can be used for other species, such as *Ae. aegypti* and *Ae. albopictus* in the laboratory and field. Therefore, this study aimed to utilize the HTSS to evaluate and determine the effective dose (ED<sub>50</sub> and ED<sub>90</sub>) of seven known candidate attractant compounds-octenol, isovaleric acid, lactic acid, hexanoic acid, isoamyl alcohol, myristic acid and ammonium hydrogen bicarbonate-for the malaria vector *An. stephensi*.

## Materials and Methods

### Rearing of *Anopheles stephensi*

To create a laboratory colony, we used the field strain *An. stephensi*, collected from Hormoodar Village, Bandar Abbas City, in the south of Iran. The collected samples were transferred to the mosquito insectary at the School of Pub-

lic Health, Tehran University of Medical Sciences and reared at a temperature of  $30 \pm 2$  °C, relative humidity of  $65 \pm 5\%$  and a light-to-dark period of 12 to 12 hours (25). After turning the larvae into pupae, they were separated and transferred to 30x30x30 cm cages, where they emerged as adults. An artificial blood-feeding device with whole human blood was used to feed female *An. stephensi* once every three days (26). The colony was fed with a 5–10% sucrose solution. *Anopheles* gravid, laying eggs in a clay bowl containing colorless water. Before hatching, the eggs were gently transferred to a tray containing 1500 ml of decolorized water. Fish meals were used as a special diet for *Anopheles* larvae. Three to five-day-old unfed female mosquitoes were starved for 12 hours before the testing.

### Preparing Chemical Attractants

The chemical compounds used in this study included lactic acid ( $C_3H_6O_3$ , brand: lactic acid Food Grade, manufacturer: Henan Jindan lactic acid Technology Co., Ltd., China), ammonium hydrogen carbonate ( $NH_4HCO_3$ , brand: ammonium bicarbonate, manufacturer: Suzhou Xiangyuan Special fine chemical Co., Ltd., China), hexanoic acid ( $C_6H_{12}O_2$ , brand: caproic acid, manufacturer: wuhan youji Industries Co., Ltd., China), octenol ( $C_8H_{16}O$ , brand: octenol mosquito attractant, manufacturer: Anhui Sealong Biotechnology Co., Ltd., China), isovaleric acid ( $C_5H_{10}O_2$ , brand: isovaleric acid, manufacturer: Wuhan Fortuna Chemical Co., Ltd., China), isoamyl alcohol ( $C_5H_{12}O$ , brand: isoamyl alcohol, manufacturer: Zhengzhou Meiya Chemical Products Co., Ltd., China) and myristic acid ( $C_{14}H_{28}O_2$ , brand: myristic acid 98%, manufacturer: Guangdong Guanghua Sci-Tech Co., Ltd., China). Serial concentrations with effective concentrations between 5 and 95% were prepared and tested in comparison with the positive control of ammonia and the negative control of ethanol. Each substance was tested separately and the attraction 50 and 90% effective doses ( $ED_{50}$  and  $ED_{90}$ ) were determined.

### High-throughput Screening System (HTSS)

The HTSS diffusion assay for evaluating attractants consists of four partitions: Each control or treatment cylinder (No. 1, Fig. 1) was made of a tube (external diameter 10.2 cm, thickness 0.6 cm) with a length of 14 cm. Each transparent cylinder and selection chamber (No. 2, Fig. 1) was made of tubes with the same outer diameter and thickness as the control and treatment cylinders, but with a length of 15.9 cm. In the middle of the length of the transparent cylinders, a hole is intended for the transfer and release of mosquitoes. The end caps (No. 3, Fig. 1) and connecting parts (No. 4, Fig. 1) are made of plexiglass. A circular port for the transfer of mosquitoes is located between the treatment control and the selection chamber (Fig. 1).

Tests started in August 2022 and finished in August 2023. Twenty female *An. stephensi* (3–5 days old), which had been starved for 12 hours, were released into the control chamber, which is clean and free of any compound. After 15 minutes of rest, they were allowed to move to the space between the control and the treatment. After 60 minutes, the mosquitoes that moved to the attractive treatment, and/or stayed in the selection chamber, or moved to the control, were caught and counted. To avoid visual bias towards mosquitoes, the experiments were performed in a dark room. The average temperature at the time of testing was 24 °C (range 23–26 °C) and the relative humidity (RH) averaged 47% (range 25–60%). Five replications were considered for each concentration to avoid errors in the test. In comparison, ammonia was used as a positive control and ethanol was used as a negative control in simultaneous tests.

### Data Analysis

Probit software was used to analyze the results, prepare the regression line, and calculate the  $ED_{50}$  and  $ED_{90}$ . The results were displayed using descriptive tables and graphs. Sta-

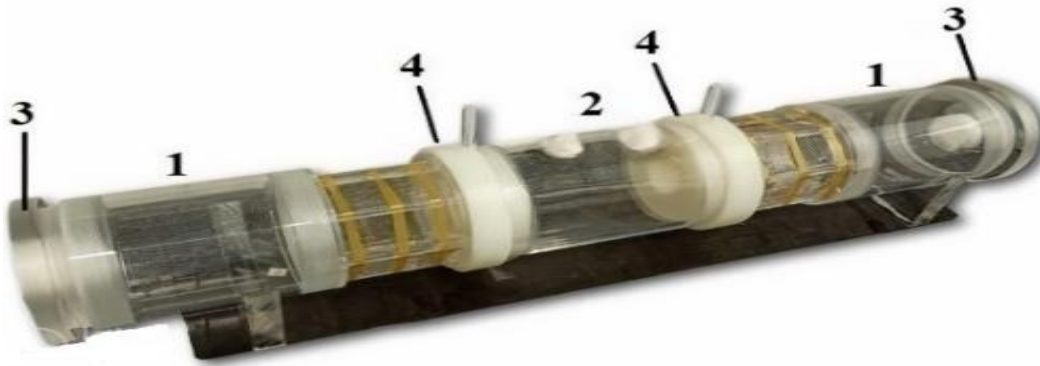
tistical analysis was based on 95% confidence intervals.

## Results

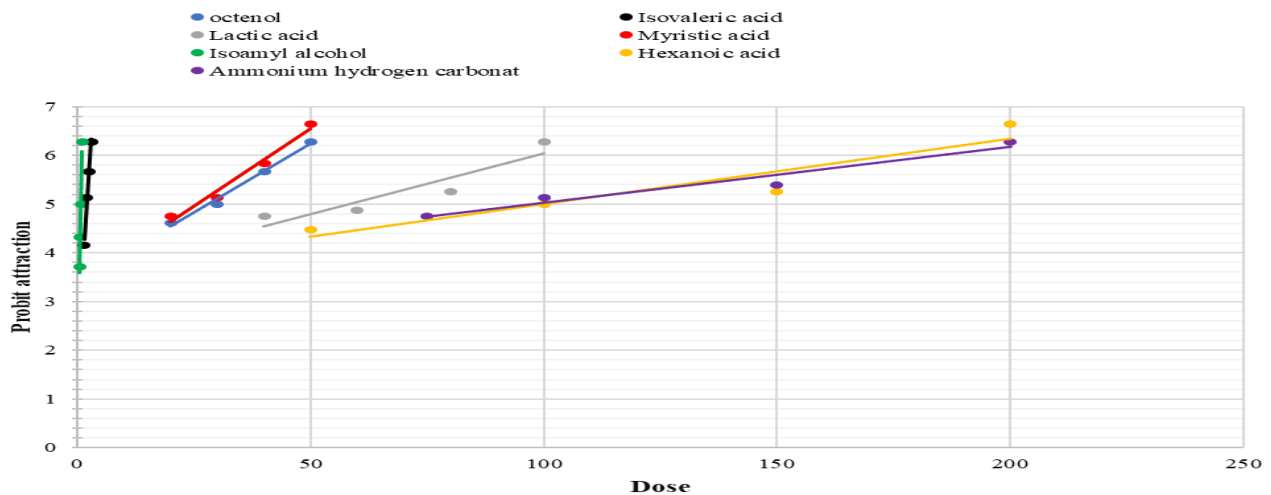
### HTSS Assay

The results for the attraction of *An. stephensi* to octenol, isovaleric acid, lactic acid, myristic acid, isoamyl alcohol, hexanoic acid, and ammonium hydrogen bicarbonate are presented in Tables 1 and Figures 2 and 3. Among the tested compounds, isoamyl alcohol ( $ED_{50}=0.57$  mL/L,  $ED_{90}=1.04$  mL/L) exhibited the

highest attraction activity for *An. stephensi*, followed by isovaleric acid ( $ED_{50}=1.96$  mL/L,  $ED_{90}=3.00$  mL/L), myristic acid ( $ED_{50}=24.77$  g/L,  $ED_{90}=47.08$  g/L), octenol ( $ED_{50}=26.64$  mL/L,  $ED_{90}=54.36$  mL/L) and lactic acid ( $ED_{50}=54.98$  mL/L,  $ED_{90}=132.9$  mL/L). The lowest attraction was observed for hexanoic acid ( $ED_{50}=87.50$  mL/L,  $ED_{90}=244.49$  mL/L) and ammonium hydrogen bicarbonate ( $ED_{50}=93.84$  g/L,  $ED_{90}=234.01$  g/L). The 95% confidence limits for the  $ED_{50}$  and  $ED_{90}$  values, along with the regression equations, are provided in Table 1.



**Fig. 1.** Components of the high-throughput behavioral screening device: (1) control or treatment cylinder, (2) selection chamber, (3) end caps and (4) connecting parts

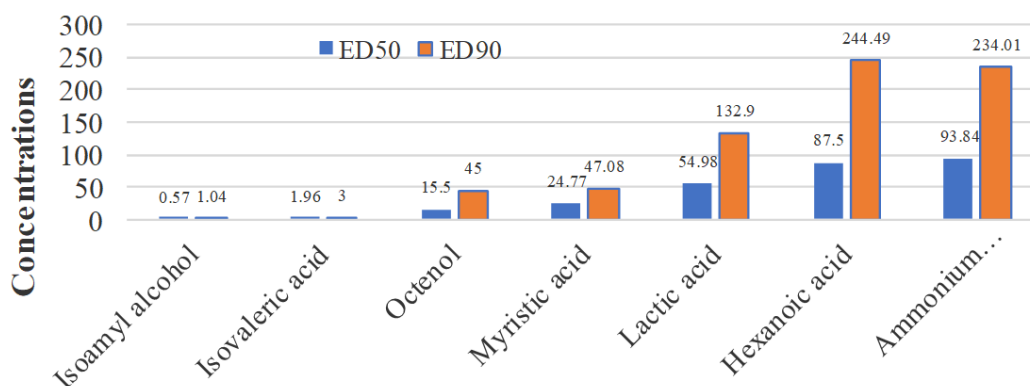


**Fig. 2.** Dose-response regression lines for the attraction of *Anopheles stephensi* to various test compounds. Concentrations are expressed in mL/L for all compounds except myristic acid and ammonium hydrogen bicarbonate, which are expressed in g/L

**Table 1.** Attraction activity of various compounds against adult female *Anopheles stephensi* (20 mosquitoes for each test), including regression parameters

| Attractive Compound             | Doses   | Attraction Rate | Regression equation    |
|---------------------------------|---------|-----------------|------------------------|
| Octenol*                        | Control | 0               | $Y = 0.0585x + 3.97$   |
|                                 | 10      | 35              |                        |
|                                 | 20      | 50              |                        |
|                                 | 30      | 80              |                        |
|                                 | 40      | 90              |                        |
| Isovaleric acid*                | Control | 0               | $Y = 1.38x + 2.205$    |
|                                 | 1.5     | 20              |                        |
|                                 | 2       | 55              |                        |
|                                 | 2.5     | 75              |                        |
|                                 | 3       | 90              |                        |
| Lactic acid*                    | Control | 0               | $Y = 0.0248x + 3.548$  |
|                                 | 40      | 40              |                        |
|                                 | 60      | 45              |                        |
|                                 | 80      | 60              |                        |
|                                 | 100     | 90              |                        |
| Myristic acid**                 | Control | 0               | $Y = 0.0638x + 3.357$  |
|                                 | 20      | 40              |                        |
|                                 | 30      | 55              |                        |
|                                 | 40      | 80              |                        |
|                                 | 50      | 95              |                        |
| Isoamyl alcohol*                | Control | 0               | $Y = 4.175x + 1.91$    |
|                                 | 0.4     | 25              |                        |
|                                 | 0.6     | 50              |                        |
|                                 | 0.8     | 75              |                        |
|                                 | 1       | 90              |                        |
| Hexanoic acid*                  | Control | 0               | $Y = 0.0135x + 3.66$   |
|                                 | 50      | 30              |                        |
|                                 | 100     | 50              |                        |
|                                 | 150     | 60              |                        |
|                                 | 200     | 95              |                        |
| Ammonium hydrogen bicarbonate** | Control | 0               | $Y = 0.0114x + 3.8881$ |
|                                 | 75      | 40              |                        |
|                                 | 100     | 55              |                        |
|                                 | 150     | 65              |                        |
|                                 | 200     | 90              |                        |

\*: mL/L, \*\*: g/L



**Fig. 3.** Effective Dose 50 and Effective Dose 50 values for the attraction of adult *Anopheles stephensi* to various test compounds. Concentrations are expressed in mL/L for all compounds except myristic acid and ammonium hydrogen bicarbonate, which are expressed in g/L

## Discussion

Various mosquito traps use olfactory or visual attractants to improve catching rates for research and control purposes (16, 24). Laboratory tools like olfactometers and wind tunnels help evaluate mosquito responses to these lures before larger field tests (16, 24, 27). However, these devices require precise conditions and are mainly used in advanced laboratories (24). This study measured the dose-response of *An. stephensi* to several attractive compounds using a simple passive device without mechanical airflow (16, 24). The results indicate that chemical compounds can be effectively screened using this method (16, 24). A high-throughput screening system (HTSS) device is a versatile tool that can assess multiple functions, such as contact repellency, toxicity, spatial repellency, and attraction of chemicals (24, 28, 29). Similar to previous research, this study confirmed that the HTSS test is a simple system useful for evaluating and optimizing chemical attractants in small-scale settings before scaling up to larger spaces (16, 24). The compounds were selected based on attraction studies and included lactic acid (22, 30, 31), octenol (22, 32), isovaleric acid (19, 33, 34), isoamyl alcohol (21), myristic acid (22), ammonium hydrogen bicarbonate (35) and hexanoic acid (36). The results of this study provide significant insights into the attraction of *An. stephensi* to different compounds. Among the tested compounds, isoamyl alcohol and isovaleric acid showed the highest attraction effect. For isoamyl alcohol, the effective dose that attracted 50% (ED<sub>50</sub>) of mosquitoes was 0.57 mL/L, and the 90% effective dose (ED<sub>90</sub>) was 1.04 mL/L. This high level of attractiveness suggests that isoamyl alcohol may play an important role in the ecological interactions of *An. stephensi* (37). Adding isoamyl alcohol to a standard composition significantly improved its attractiveness to *An. gambiae* (33). Isoamyl alcohol was significantly attractive to *An. gambiae* in laboratory and semi-field tests when tested separately (38). The at-

tractiveness of different fatty acids to different mosquito species was confirmed and emphasized that some aldehydes and alcohols tend to induce strong responses in *Anopheles* species (39). Volatile substances produced by human skin bacteria play a role in attracting mosquitoes (40). Isovaleric acid is one of these compounds and the bad smell produced in the armpits is due to it (41, 42). The relatively strong attraction observed with Isovaleric acid in our study (ED<sub>50</sub>=1.96 mL/L and ED<sub>90</sub>=3 mL/L) makes it a suitable candidate for further studies to understand its underlying mechanism in mosquito attraction. In research on Isovaleric acid, it was found that this compound is one of the key components in host-derived odors that successfully attract mosquitoes and that by increasing the relative abundance of volatile carboxylic acids butyric acid, isobutyric acid and isovaleric acid in total body odor, it increases *Anopheles* attraction (43). In a study that investigated the efficiency of known repellents and attractants against *Ae. albopictus* and *Culex quinquefasciatus*, *Cx. quinquefasciatus* was not sensitive to low doses of Isovaleric and attracted only when isovaleric reached higher doses of about 0.005 mg/ml (44). The results of the current study show an ED<sub>50</sub> of 1.96 for Isovaleric acid, supporting the idea that certain carboxylic acids play an important role in mosquito attraction. This consistency across studies reinforces that these volatile compounds are effective cues for host-seeking behavior in *Anopheles* species (40, 45, 46).

Octenol and myristic acid showed moderate attraction activities with higher ED<sub>50</sub> and ED<sub>90</sub> values (15.50, 45.00 and 24.77, 47.08 g/L, respectively). This suggests that while they have some efficacy as attractants, they are significantly less effective in this study than isoamyl alcohol and isovaleric acid. The difference in attraction levels can be attributed to the structural features of these fatty acids, which can affect their volatility and interaction with mos-

quito sensory receptors (40). In contrast to the moderate attraction observed for myristic acid and octenol in this study, previous research suggests that while these compounds can be attractive, it can be estimated that their effectiveness depends on the concentration, environmental conditions and differences in the mosquito genus or species (22). The effectiveness of octenol as a mosquito attractant was first demonstrated in 1989 (15). When used alone, octenol has been a good attractant for only a few species. This may mean that octenol or myristic acid merely acts as a synergist because when combined with CO<sub>2</sub> or other attractants, they increase mosquito catches in traps rather than alone (47). The findings of our study, where myristic acid and octenol show moderate attractiveness levels with higher ED<sub>90</sub> values, lend credence to this claim and suggest that more optimal conditions or combinations with other attractants may be necessary to increase their effectiveness.

In this study, lactic acid with ED<sub>50</sub> and ED<sub>90</sub> of about 55 mL/L and 132.90 mL/L, respectively, did not show a strong attraction effect. It has been shown that 1 g of lactic acid attracted 75–29% of mosquitoes in 3 minutes (30). In their study, Omrani et al. (48) observed that lactic acid alone was unable to attract *An. stephensi* at the doses they tested, and only the highest dose of lactic acid was used, i.e., 6 µg/min in combination with 90 or 410 ppm, CO<sub>2</sub> attracted it. It has also been reported that lactic acid alone is less attractive to *An. gambiae* than when combined with ammonia. Furthermore, even after lactic acid is removed from the blend of attractants derived from human skin, sweat remains attractive to mosquitoes (47). Another study observed that lactic acid alone can attract *Ae. aegypti*, but it was attractive for *An. gambiae* and *Ae. albopictus* when it was combined with CO<sub>2</sub> or ammonia and octenol, respectively (12). From these studies, it can be concluded that the compound of lactic acid alone is weak in attracting mosquitoes, especially Anopheline species. Per-

haps the main causes of these differences in the results are the level of the dose used, the difference in the system to the Olfactometer test, the duration of the tests and the time of its performance, the inherent differences in the sensitivity or preference of the olfactory receptor among mosquito species and geographical strains (48).

In contrast, hexanoic acid (ED<sub>50</sub>=87.50 mL/L, ED<sub>90</sub>=244.49 mL/L) and ammonium hydrogen bicarbonate (ED<sub>50</sub>=93.84 g/L, ED<sub>90</sub>=234.01 g/L) showed the lowest level of attraction. The significantly higher effective dose values for these compounds indicate their limited ability to attract *An. stephensi* under the conditions tested. This may reflect their chemical properties that do not match well with the sensory detection mechanisms used by this mosquito species or the specific potential ecological role these substances play. It seems that hexanoic acid alone is not a strong mosquito attractant, and it is synergistically effective in combination with other attractant materials. BioGents (BG) mixture consisting of lactic acid, hexanoic acid and ammonia in attracting *Ae. aegypti* (49) and *Ae. albopictus* (50, 51) is effective. Similarly, another study showed hexanoic acid in lower doses (10–6 g to 10–4 g) was not so attractive to *Ae. albopictus* and attracted 20% and 30% of mosquitoes at 10–3 g and 10–2 g, respectively (13, 52). In their study, Xie et al. (51) tested hexanoic acid and six other compounds to attract *Ae. albopictus* and among the five tested concentrations (10, 1, 0.1, 0.01 and 0.001%), hexanoic acid showed the most effect in the highest concentration (10%) in attracting this mosquito. In another study, Williams et al. (53) investigated host-seeking behavior and determined the response of different populations of *Ae. aegypti* to increase doses of lactic acid alone and in combination with ammonia or hexanoic acid, using a y-olfactometer. The combination of lactic acid with ammonia or hexanoic acid resulted in a significant increase in mosquito attraction, although not for all populations. Another study

aimed to improve odor baits for monitoring *Ae. aegypti* populations found that among carboxylic acids, hexanoic acid was more effective than the commercial lure BG. The traps containing a combination of hexanoic acid and carbon dioxide outperformed BG, despite BG already including hexanoic acid as one of its main components. The study also emphasized that the attractiveness of hexanoic acid depends on its release rate, with slower and isolated release proving more effective than faster or combined dispersion of lactic acid plus hexanoic acid (30 mL/min), significantly increased attractiveness compared to lactic acid alone or with Hexanoic acid at 0.3 or 3 mL/min. In the aforementioned study, hexanoic acid alone (0.3 mL/min) showed low or no attraction for *Ae. aegypti*. Some ammonium compounds can significantly attract mosquitoes, which means that the type or even species of mosquitoes can have a large effect on the effectiveness of these attractants (12). The low levels of attraction observed for ammonium hydrogen bicarbonate are striking when compared to studies showing varying effectiveness of ammonium compounds.

Ammonia hydrogen bicarbonate, which has the property of releasing ammonia, has been used as an attractant for other insects, including fruit flies (Diptera: Tephritidae) and blowflies (Diptera: Calliphoridae) in addition to mosquitoes (35). However, in the study of Kim et al. (54), traps with ammonium hydrogen bicarbonate compound collected more *Cx. pipiens* with averages of 26.3, 22.5 and 43.8% at 1000, 10000 and 20000 ppm, respectively, then control traps (54). Also, in a study by Kim et al. (35) investigating the attraction effect of ammonium hydrogen Bicarbonate for *Cx. pipiens*, they found that traps that emit 1000, 10,000 or 20,000 ppm of ammonium hydrogen bicarbonate increased the host-seeking behavior of *Cx. pipiens* compared to control traps. From these results, it can be concluded that the attractiveness of ammonium hydrogen bicarbonate is effective even in relatively high con-

centrations. The limited response of *An. stephensi* to these compounds may reflect inherent differences in olfactory receptor sensitivity or preference among mosquito species.

## Conclusions

This study demonstrates the varying levels of attractiveness of various compounds for *An. stephensi*, where Isoamyl alcohol and Isovaleric acid are known as the most effective attractants. The low effective dose of these compounds indicates a promising direction for improving mosquito trapping and control strategies. The effectiveness of this compound can be used in the development of targeted traps that use its attractant properties to reduce the mosquito population in endemic areas. On the other hand, the limited attraction observed with Lactic acid, Hexanoic acid and Ammonium Hydrogen Bicarbonate suggests that although they may contribute to overall olfactory cues, they are not sufficient as independent attractants. This highlights the need for a multifaceted approach in vector control strategies, where different attractants can be combined to create a synergistic effect that maximizes efficiency. Understanding the specific attraction mechanisms of these compounds can help design more effective traps and bait systems in field experiments. Based on this, future studies should investigate interactions between different attractants and environmental factors, as well as their performance in field or semi-field conditions. In addition, investigating the basic mechanisms of the process of receiving olfactory cues in mosquitoes can provide useful information about how these insects perceive different compounds, leading to the development of new attractants or repellents. Consequently, it is important to optimize the use of attractants such as isoamyl alcohol or isovaleric acid to help in vector control procedures and ultimately reduce malaria transmission. Continued research in this area is critical

to developing innovative solutions that can effectively combat the ongoing threat of malaria and other mosquito-borne diseases.

## Acknowledgements

We thank the staff of the mosquito insectary, Department of Vector Biology and Control of Diseases, School of Public Health, Tehran University of Medical Sciences, for their cooperation in breeding mosquitoes. This project has been financially supported by the Deputy of Research, Tehran University of Medical Sciences. Contract number is 1401-4-299-58724.

## Ethical considerations

This research has been registered with the ethics code IR.TUMS.SPH.REC.1400.309 in the Ethics Committee of the School of Public Health, Tehran University of Medical Sciences. Conflict of interest statement.

## Conflict of interest statement

The authors declare there is no conflict of interest.

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