Screening and Identification of the Botanical Attractant From a Chinese Tea for Aedes Albopictus Oviposition

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Research

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Abstract

Background: The use of oviposition attractants is a promising approach to control populations of mosquito vectors at the source; however, the knowledge on oviposition stimulation in *Aedes albopictus* is very limited. In recent years, tea bags have been proven to attract *Aedes aegypti* to lay eggs, but the sources of tea are wide and varied; moreover, the factors and mechanisms in tea bags that attract mosquitoes remain unclear.

Methods: The effects of three kinds of Chinese tea, nonfermented, fully fermented and postfermented, were comparatively evaluated for their ability to attract mosquito oviposition at dose of 5g/L, 1.5g/L and 0.5g/L, respectively. The attractants in the Liu-pao tea infusion were isolated with gas chromatography–mass spectrometry (GC-MS).

Results: Among the three types of fermented teas, the Guangxi Liu-pao tea infusion was a successful oviposition attractant for *Ae. albopictus* at all the dose tested, and the average OAIs of 5 g/L Liu-pao tea soaked for 7 d and 14 d were 0.73 and 0.67, respectively. Further, in one month of soaking 0.5g/L Liu-Pao tea, the average oviposition activity indexes (OAIs) for *Ae.aegypti* of soaking for 21 d and 28 d were 0.89 and 0.63, respectively. Functional analyses showed that the compound cedrol at 1 mg/L tested for *Ae. albopictus*, the average OAI was 0.38.

Conclusion: Liu-pao tea can effectively attract *Ae. albopictus* to lay eggs, and cedrol is one of the ingredients attracting *Aedes* mosquitoes to lay eggs. These results shed light on the development of *Aedes* mosquito oviposition attractants and will help to develop innovative monitoring and prevention strategies for *Aedes*-borne diseases.

Introduction

Gravid mosquitoes use chemosensory (olfactory, gustatory or both) cues to select a suitable oviposition site[1]. In nature, cues are usually complex and consist of plant infusions, microbes, immature mosquitoes and predators[2]. Different vector mosquitoes, such as *Anopheles*, *Culex* and *Aedes*, prefer different types of breeding habitats [3]. In the life cycle of mosquitoes, the oviposition site is essential. Mosquito activities are restricted in the aquatic stage, and the breeding ground cannot be changed in the larval/pupal stage. Therefore, gravid female mosquitoes will choose the most suitable oviposition site for their offspring. Sufficient nutrients, no predators and low levels of competition are characteristics of mosquito oviposition sites.

In recent years, many studies have shown that bacteria-derived chemicals in the water of the breeding site play a vital role in the choice of oviposition location[4–6]. Ponnusamy et al used (GC-MS) to analyze the volatiles in white oak leaves and found 16% nonanoic acid, 83% myristic acid and 1% mixture of methyl myristate to stimulate *Aedes aegypti* oviposition [7, 8]. Eneh et al demonstrated the importance of combining bacterial flora with physical and chemical conditions for the selection of mosquito oviposition sites. Moreover, the microbes separated from plant infusions (e.g., separated liquid of *B. subtilis* from oak leaf infusions) stimulate and attract mosquito oviposition[7, 9, 10].

Previous studies have shown that *Ae. albopictus* likes to lay eggs in small containers with plant leaves. However, the types of plants are usually different in different regions. Importantly, *Aedes* mosquitoes exhibit "skip oviposition" behavior, preferring to use artificial containers as oviposition sites[11]. These breeding sites are usually small and difficult to find, which makes them difficult to control. The preference of *Aedes* mosquitoes for container-type breeding grounds provides opportunities for using oviposition attractants to control gravid mosquitoes. The efficiency of the oviposition attractant is crucial. Commercially available attractive *Aedes* oviposition attractants are rare. Therefore, products that can attract *Ae. albopictus* to lay eggs are urgently needed. Furthermore, we also noted that teabags are readily available organic compounds that can improve *Ae. aegypti* oviposition[12]. However, there are a wide range of teabags, and their effects in attracting *Ae. albopictus* have not been tested. Moreover, other factors, such as the quality and fermentation cycle of tea[13] as well as the amount, most effective conditions (such as soaking time, etc.) and the diversity of microbial species[6] affecting the attractiveness of mosquito oviposition are worth exploring.

Materials And Methods

1. Mosquitoes

The *Ae. albopictus* was collected from Foshan, and *Ae. aegypti* was collected from Haikou, Hainan and was provided by the Center of Disease Prevention and Control (CDC), Guangdong Province, China. Mosquitoes were kept under controlled conditions with a light/dark ratio of 14:10 h at a mean temperature of 27 ± 1°C and a mean relative humidity (RH) of 75 ± 5%. The larvae were fed fish food, and the adults were given 10% sugar solution. After adult mosquitoes were fed for 3–5 d, they were fed to Kunming mice (4–6 weeks old), which were provided by the Laboratory Animal Center of Southern Medical University.

2. Main reagents

The details of each compound used in the experiments are shown in Table 1.

Table 1
Details of each compound used in the experiments

Name	CAS	Purity	Brand
Cedrol	77-53-2	≥ 98 %	Aladdin
Linalool oxide	60047-17-8	≥ 97 %	Aladdin

Jiangxi green tea was purchased from Ningdu Xiaobuyan Tea Industry Co., Ltd. Ningdu, Jiangxi Province, 2017. Tianlao black tea was purchased from Xinchang Caiyan Xiandao Tea Co., Ltd., Xinchang, Zhejiang

Province, 2016. Guangxi Liu-pao tea was purchased from Guangxi Wuzhou Tea Factory Co., Ltd. Cangwu Guangxi Province, 2016. All types of tea were used within the shelf life; for dark tea, the longer it was stored, the stronger its fragrance became.

We selected teas according to their different fermentation levels[14] as follows: nonfermented tea (Jiangxi green tea); fully fermented tea (Tianlao black tea); and postfermented tea (Guangxi Liu-pao tea).

3. Dual-choice oviposition bioassays

Two oviposition cups were placed in yurt nets (2.0 × 1.8 m) for 48 hours to induce mosquitoes to lay eggs, and the positions of the two cups were changed every other experiment. Each group of experiments was set up with 3 to 6 repetitions. Ten female gravid mosquitoes were released into the Mongolian yurt (2.0 m×1.8 m, Fig. 1), which allowed for free-flying mosquitoes. Mosquito oviposition cups were placed in the yurt for 48 h for oviposition selection. After removing the oviposition cups, the number of eggs laid by each group of mosquitoes was recorded, and the oviposition rate and oviposition activity index (OAI) [15] were calculated.

oviposition rate = Nt/ (Nt + Nc)

OAI = (Nt- Nc) / (Nt + Nc)

where Nt is the number of eggs laid in the treatment cup and Nc is the number of eggs laid in the control cup.

4. Screening the most attractive tea infusion

According to the degree of fermentation, nonfermented tea, fully fermented tea and postfermented tea were selected as candidates. First, 5 g nonfermented tea (Jiangxi green tea); fully fermented tea (Tianlao black tea); postfermented tea (Guangxi Liu-pao tea) (16.8 g of 1/ 4 dose plant infusion attracts mosquito oviposition [6]) was added to 1 L water, and about every 3 d the effect of 5 g/L was tested for its effect on oviposition selection; for Guangxi Liu-pao tea the doses of 5 g/L, 1.5 g/L and 0.5 g/L were also tested every 7 d .

5. Exploring the ingredients in Liu-pao tea that play a major role in Ae. albopictus **oviposition**

5.1: Effects of microbes in Liu-pao tea infusion in attracting mosquitoes to lay eggs

To determine the role of microbes in Liu-pao tea at different doses and time points, we used autoclaving to make sterile (microbe-free) infusions. Every 7 d we examined the doses of 0.5 g/L, 1.5 g/L and 5 g/L Liu-pao tea infusion with the following comparisons: autoclaved versus nonautoclaved, nonautoclaved versus blank control, autoclaved versus blank control, for attracting *Ae. albopictus* oviposition. Each experiment had 3–6 replicates, and the OAI was calculated.

5.2: GC-MS identification of volatiles from Liu-pao tea infusion

The samples resulted in the biggest difference in mosquito oviposition were selected; specifically, Liu-pao tea infusions at 1.5 g/L and 5 g/L were soaked for 14 d, and that at 0.5 g/L was soaked for 1 d. Each time point was repeated 3 times, so a total of 9 samples were used for GC-MS detection (instrument parameters are described in Table 2). The main aroma compounds detected were diluted to different concentrations versus the blank control to attract mosquitoes to lay eggs. We focused on cedrol concentrations from 0.01 mg to 10 mg and linalool oxide concentrations from 0.00001 mg to 0.01 mg.

Table 2
Instrument parameters

Project	Parameters
Incubation Temperature	60°C
Preheating Time	15 min
Incubation Time	30 min
Desorption Time	4 min
Front Inlet Mode	Splitless Mode
Front Inlet Septum Purge Flow	3 mL/min
Carrier Gas	Helium
Column	DB-Wax(30 m×250 μm×0.25 μm)
Column Flow	1 mL/min
Oven Temperature Ramp	40°C hold on 4 min, raised to 245°C at a rate of 5°C/min, hold on 5 min
Front Injection Temperature	250°C
Transfer Line Temperature	260°C
Ion Source Temperature	230°C
Quad Temperature	150°C
Electron Energy	-70 eV
Mass Range	m/z:20–500
Scan Mode	Scan
Solvent Delay	0 min

6. Data processing

The mass spectrum data obtained by GC-MS analysis were searched using ChromaTOF software (V 4.3x, LECO) and the NIST standard library. Through the comparison of mass spectrum data, the compounds with higher matching degrees were screened, and peak extraction, baseline correction, and solution were performed. Then, convolution, peak integration, peak alignment, mass spectrum matching, etc. was performed [16]

7. Statistical analysis

Microsoft Excel was used for data analysis. For oviposition experiments, if the oviposition rate was > 50% and OAI > 0, the experimental group attracted mosquito oviposition; otherwise, mosquito oviposition was repelled. When OAI > 0.3, the experimental group significantly attracted mosquito oviposition, and the greater the index was, the stronger the effect[5]. T tests were used to compare the differences between the experimental group and the control group in attracting mosquitoes to lay eggs.

Results

Screening results of tea infusions that attract mosquitoes to lay eggs

A preliminary egg-laying effect test was carried out on teas with different fermentation degrees (nonfermented, fully fermented and postfermented teas) and infusion times (approximately every 3 days) at 5 g/L. The results showed that postfermented tea at 3 d ($t = 6.87$, $p = 0.002$), 6 d ($t = 8.43$, $p = 0.001$), 9 d ($t = 16.77$, $p < 0.0001$), 12 d ($t = 9.64$, $p < 0.0001$) and 18 d ($t = 5.95$, $p = 0.004$) had statistically significant effects on mosquito-egg laying compared with the control ; the average OAIs at 3, 6, 9, 12, and 18 d were 0 .45, 0 .72, 0 .62, 0.35, 0.74, 0.36, and 0 .38, respectively, and at each time period the OAI was greater than 0.3 (Fig. 3).

For the other two types of tea, there were no significant differences between the experimental group and the control group in oviposition (Fig. 3). These data indicated that postfermented tea infusion is a good botanical infusion candidate for attracting *Ae. albopictus* oviposition.

Furthermore, we refined the research on the oviposition effect on *Ae. albopictus* by Liu-pao tea and reduce the concentrations of Liu-pao tea used for this purpose, that is, we examined the doses of Liu-pao tea at 5 g/L, 1.5 g/L and 0.5 g/L at the same time every 7 days. The results showed that, except for the 5 g/L Liu-pao tea soaked for 7 d ($T = 14.37$, $P < 0.0001$) and 14 d ($T = 13.65$, $P < 0.0001$) and 1.5 g/L Liu-pao tea soaked for 7 d ($T = 7.48$, $P < 0.0001$), 14 d ($T = 6.05$, $P = 0.004$) and 28 d ($T = 5.15$, $P = 0.0004$), there were significant differences between the experimental group and the control group in oviposition (Fig. 4). The average OAI except for those of the treatments with 0.5 g/L and 1.5 g/L soaked for 1d and 0.5 g/L and 1.5 g/L soaked for 21 d, were less than 0.3; the average OAIs of other doses soaked for 28 days at different time points were all greater than 0.3 (Fig. 4). It is worth noting that the average OAIs of 5 g/L Liu-pao tea soaked for 7 d and 14 d were 0.73 and 0.67, respectively, which were higher than the average OAIs of 1.5 g/L Liu-pao tea soaked for 7 d and 0.5 g/L soaked for 14 d. In summary, it can be seen that the higher the concentration of Liu-pao tea up to 5 g/L, the better the effect of attracting *Ae. albopictus* to lay eggs, and soaking the solution between 7 d and 14 d resulted in the best mosquito-attracting effect.

Furthermore, we tested the effect of soaking 0.5 g/L Liu-pao tea for 28 days on the egg laying of *Ae. aegypti* and found that compared with the control, soaking for 21 d and 28 d significantly attracted *Ae. aegypti* to lay eggs. The average OAIs were 0.89 ($T = 9.10$, $P = 0.0008$) and 0.63 ($T = 6.33$, $P = 0.003$), respectively. Compared with *Ae. albopictus*, the best soaking time for oviposition attraction in *Ae. aegypti* was obviously backward (Fig. 5).

Effects of microbes in Liu-pao tea infusion on attracting mosquitoes to lay eggs

Except for the results at 7 d, the average OAI was a slightly higher than 0.3 when comparing the preautoclaved and postautoclaved sterilization results, while at 15 and 21 d, the average OAI with postautoclaved sterilization was higher than the average OAI with the preautoclaved conditions (Fig. 6). Therefore, it can be preliminarily speculated that the bacteria in Liu-pao tea infusion may not be a key factor in attracting mosquitoes to lay eggs, and it may be the odor of the Liu-pao tea infusion or the odor produced by the microbes in it that attracts mosquitoes to lay eggs.

GC-MS identification of putative oviposition volatiles from Liu-pao tea infusion

GC-MS was used to analyze and identify the volatile components in the Liu-pao tea extract, combined with computer retrieval technology to identify the identified compounds, and a total of 223 peaks were separated (Fig. 7). Among them, there were 7 compounds with a relative content greater than 1% in the 0.5 g/L sample soaked for 1 d, 8 compounds with a relative content greater than 1% in the 5 g/L sample soaked for 7 d, and a relative content greater than 1% in the 5 g/L sample soaked for 14 d. There were 8 kinds of compounds, which were classified into carbon and oxygen compounds, alkanes, ketones, benzenes and nitrogen according to their chemical compositions. Carbon dioxide, octamethylcyclotetrasiloxane, toluene and nitrogen were all present in the samples at 3 time points. With further combined analysis of the aromatic substances of Liu-pao tea, it was found that the main aromatic substances linalool and cedrol were also present in the relevant samples. The ion chromatograms of these two substances in Liu-pao tea extracts after 7 days are shown in Fig. 8. The specific information and relative content of these two substances are shown in Table 3.

Table 3
Relative contents of aroma substances in GC-MS samples

Sample number	Linalool oxide (Linalool) %	Cedrol %
C 1–1	0 .0194	0. 0 073
C 1–2	0 .0212	0.0085
C 1–3	0.0336	0.0148
C 7 – 1	0.1465	0.0216
C 7 – 2	0.190	0.0335
C 7 – 3	0.3536	0.0389
C 14 – 1	0.0588	0.0279
C 14 – 2	0.0266	0.0133
C 14 – 3	0.0235	0.0135
NOTE: C1 was 0.5 g/L soaked for 1 d; C7 was 5 g/L soaked for 7 d; C14 was 5 g/L soaked for 14 d, l-3 correspond to 3 repeats.		

Effects of the main aroma compounds in Liu-pao tea on mosquito oviposition

We tested the effects of cedrol and linalool oxides on *Ae. albopictus*, and the results showed that when cedrol was added at 1 mg/L, the average OAI was 0.38, indicating that it has a significant effect in attracting *Ae. albopictus* to lay eggs. However, for each concentration of linalool oxide tested, we did not observe an obvious effect on *Ae. albopictus* oviposition (Fig. 9).

Discussion

Plant debris and microbes living in water are the food sources of most mosquito larvae [2], and food source is very important in mosquito oviposition site selection. Multiple experiments have proven that botanical infusions such as those of white oak, bermuda and bamboo[4–6], as well as the compounds isolated from these infusions[17, 18], stimulate or attract oviposition of one or more mosquito species. We screened the effects of unfermented tea, fully fermented tea, and postfermented tea on the oviposition of *Ae. albopictus* and found that Liu-pao postfermented tea had a significant effect on the oviposition of *Ae. albopictus*. Liu-pao tea infusion was the best tea infusion confirmed as an oviposition attractant for gravid *Ae. albopictus*.

Several studies have shown that microbes isolated from plant extracts (such as *Bacillus subtilis* isolated from oak leaf extracts) can stimulate and attract mosquitoes to lay eggs [7, 9, 10]. Furthermore, microbes are involved in the fermentation process of Liu-pao tea[19]. In this experiment, we found that *Ae. albopictus* oviposition preference increased with increasing soaking time of the Liu-pao tea infusion. This is consistent with other studies that found that the soaking fermentation cycle[13] will affect attractiveness. However, we could not distinguish whether the microbes themselves or the volatiles produced by the microbes and tea attracted mosquitoes to lay eggs. After autoclaving the Liu-pao tea infusion, we found that its effect in attracting *Ae. albopictus* to lay eggs did not decrease. Therefore, it is speculated that the release of chemical volatiles is associated with microbial activity, although it may not be the microbes themselves but possibly the metabolites of tea products that attract mosquitoes.

Furthermore, in this experiment, GC-MS was used to screen Liu-Pao tea extracts for substances with a relative content greater than 1%, and it was found that these substances were present in the samples at all time points. Previous studies have shown that cedrol is one of the main aroma components of Liu-pao tea[20]. After testing, it was found that this substance plays a role in attracting *Ae. albopictus* to lay eggs. This study proved for the first time that cedrol can clearly induce oviposition in *Ae. albopictus* at a concentration of 1 mg/L. Lindh et al also found that cedrol can induce *Anopheles gambiae* to lay eggs [21], and Eneh et al showed that cedrol is produced by fungi on the roots of grasses near mosquito breeding sites [22]. However, from the present results, we noted that the effect of cedrol in attracting *Ae. albopictus* to lay eggs was not as good as that of Liu-pao tea infusion.

Gravid mosquitoes are an important target for mosquito control and surveillance. Volatile oviposition stimulants will help selectively attract gravid mosquitoes. These findings will allow us to explore the combined effects of Liu-pao tea and *Bacillus thuringiensis* subsp. Israel or other insecticides help to exploit ‘attract and kill’ strategies against *Aedes* mosquitoes.

Conclusion

In the current study, we proved that Liu-pao tea has the effect of attracting *Ae. albopictus* to lay eggs and identified a compound from the tea, cedrol, that played a major role in attracting oviposition. These results pave the way to explore attract-and-kill strategies for *Aedes* mosquito control.

Abbreviations

GC-MS: gas chromatography–mass spectrometry;

OAI: oviposition activity index

Declarations

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Authors’ contributions

All authors contributed significantly to this study. XGC conceived the study and coordinated its implementation. XGC, JBG, XHZ and KW participated in the experimental design. LHX, TL, WQY, YGX and SYZ performed the experiments. LHX analyzed the data and drafted the manuscript which was critically revised by XGC. All authors read and approved the final manuscript.

Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

No specific permits were required for our experiment. This study did not involve endangered or protected species.

Informed consent

Informed consent was obtained from all co-authors of the study.

References

1. Takken W, Knols BG. Odor-mediated behavior of Afrotropical malaria mosquitoes. Annu Rev Entomol. 1999;44(1):131–57.
2. Afify A, Galizia CG. Chemosensory Cues for Mosquito Oviposition Site Selection. J Med Entomol. 2015;52(2):120–30. .doi:/10.1093/jme/tju024.
3. Muir LE, Thorne MJ, Kay BH. *Aedes aegypti* (Diptera: Culicidae) vision: spectral sensitivity and other perceptual parameters of the female eye. J Med Entomol. 1992;29(2):278–81.
4. McPhatter LP, Debboun M. Attractiveness of botanical infusions to ovipositing *Culex quinquefasciatus*, *Cx. nigripalpus*, and *Cx. erraticus* in San Antonio. Texas *J Am Mosq Control Assoc*. 2009;25(4):508–10.
5. Obenauer P, Allan S, Kaufman P. *Aedes albopictus* (Diptera: Culicidae) oviposition response to organic infusions from common flora of suburban Florida. J Vector Ecol. 2010;35(2):301–6.
6. Ponnusamy L, Wesson DM, Arellano C, Schal C, Apperson CS. Species composition of bacterial communities influences attraction of mosquitoes to experimental plant infusions. Microb Ecol. 2010;59(1):158–73.
7. Ponnusamy L, Xu N, Nojima S, Wesson DM, Schal C, Apperson CS. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. Proc Natl Acad Sci U S A. 2008;105(27):9262–7.
8. Ponnusamy L, Schal C, Wesson DM, Arellano C, Apperson CS. Oviposition responses of *Aedes* mosquitoes to bacterial isolates from attractive bamboo infusions. Parasit Vectors. 2015. 8:486.doi:/10.1186/s13071-015-1068-y.
9. Hazard E, Mayer M, Savage K. Attraction and oviposition stimulation of gravid female mosquitoes by bacteria isolated from hay infusions. Mosq News. 1967;27:133–6.
10. Trexler JD, Apperson CS, Zurek L, Gemeno C, Schal C, Kaufman M, Walker E, Watson DW, Wallace L. Role of bacteria in mediating the oviposition responses of *Aedes albopictus* (Diptera: Culicidae). J Med Entomol. 2003;40(6):841–8.
11. Maciel-de-Freitas R, Lourenço-de-Oliveira R. Does targeting key-containers effectively reduce *Aedes aegypti* population density? Trop Med Int Health. 2011;16(8):965–73.
12. Snetselaar J, Andriessen R, Suer RA, Osinga AJ, Knols BG, Farenhorst M. Development and evaluation of a novel contamination device that targets multiple life-stages of *Aedes aegypti*. Parasit Vectors. 2014;7:200. doi:/10.1186/1756-3305-7-200.
13. Ponnusamy L, Xu N, Böröczky K, Wesson DM, Ayyash LA, Schal C, Apperson CS. Oviposition responses of the mosquitoes *Aedes aegypti* and *Aedes albopictus* to experimental plant infusions in laboratory bioassays. J Chem Ecol. 2010;36(7):709–19.
14. Xin DD, Li DX, Zhang H. Chemical Changes of Different Kinds of Tea with the Processing. Food Res Dev. 2020;41(02):216–24.

15. Kramer WL, Mulla MS. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. *Environ Entomol* 1979, 8(6):1111–1117.
16. Kind T, Wohlgemuth G, Lee DY, Lu Y, Palazoglu M, Shahbaz S, Fiehn O. FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. Anal Chem. 2009;81(24):10038–48.
17. Mboera L, Mdira K, Salum F, Takken W, Pickett J. Influence of synthetic oviposition pheromone and volatiles from soakage pits and grass infusions upon oviposition site-selection of *Culex* mosquitoes in Tanzania. J Chem Ecol. 1999;25(8):1855–65.
18. Millar JG, Chaney JD, Mulla MS. Identification of oviposition attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusions. J Am Mosq Control Assoc. 1992;8(1):11–7.
19. Yang YZ, Wang Y, Li H, et al.. Microbial diversity of Guangxi Liubao and Chongqing Bowl teas [J]. Acta Tea Sinica. 2019;60(3):93–8.
20. Zheng PC, Liu PP, Wang SP, et alTeng JF, Lin ZL, Gong ZM. Comparative Analysis of the Aroma Components in Five Kinds of Dark Tea. Sci Technol Food Ind. 2018;39(22):82–6.
21. Lindh JM, Okal MN, Herrera-Varela M, Borg-Karlson A-K, Torto B, Lindsay SW, Fillinger U. Discovery of an oviposition attractant for gravid malaria vectors of the *Anopheles gambiae* species complex. Malar J. 2015;14(1):119.
22. Eneh LK, Saijo H, Borg-Karlson A-K, Lindh JM, Rajarao GK. Cedrol, a malaria mosquito oviposition attractant is produced by fungi isolated from rhizomes of the grass Cyperus rotundus. Malar J. 2016;15(1):478.

Figures

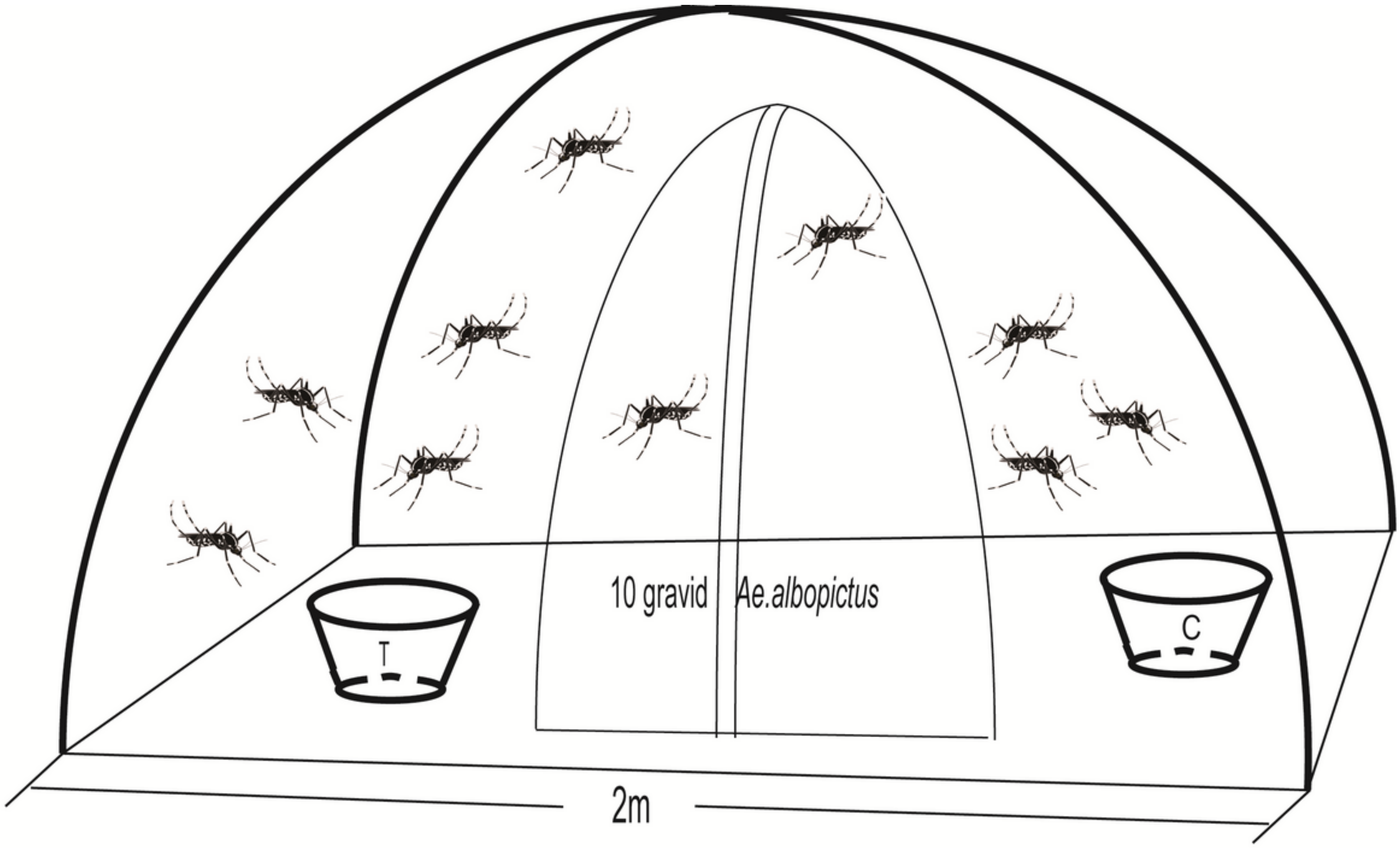

Figure 1

Pattern diagram of mosquito oviposition.

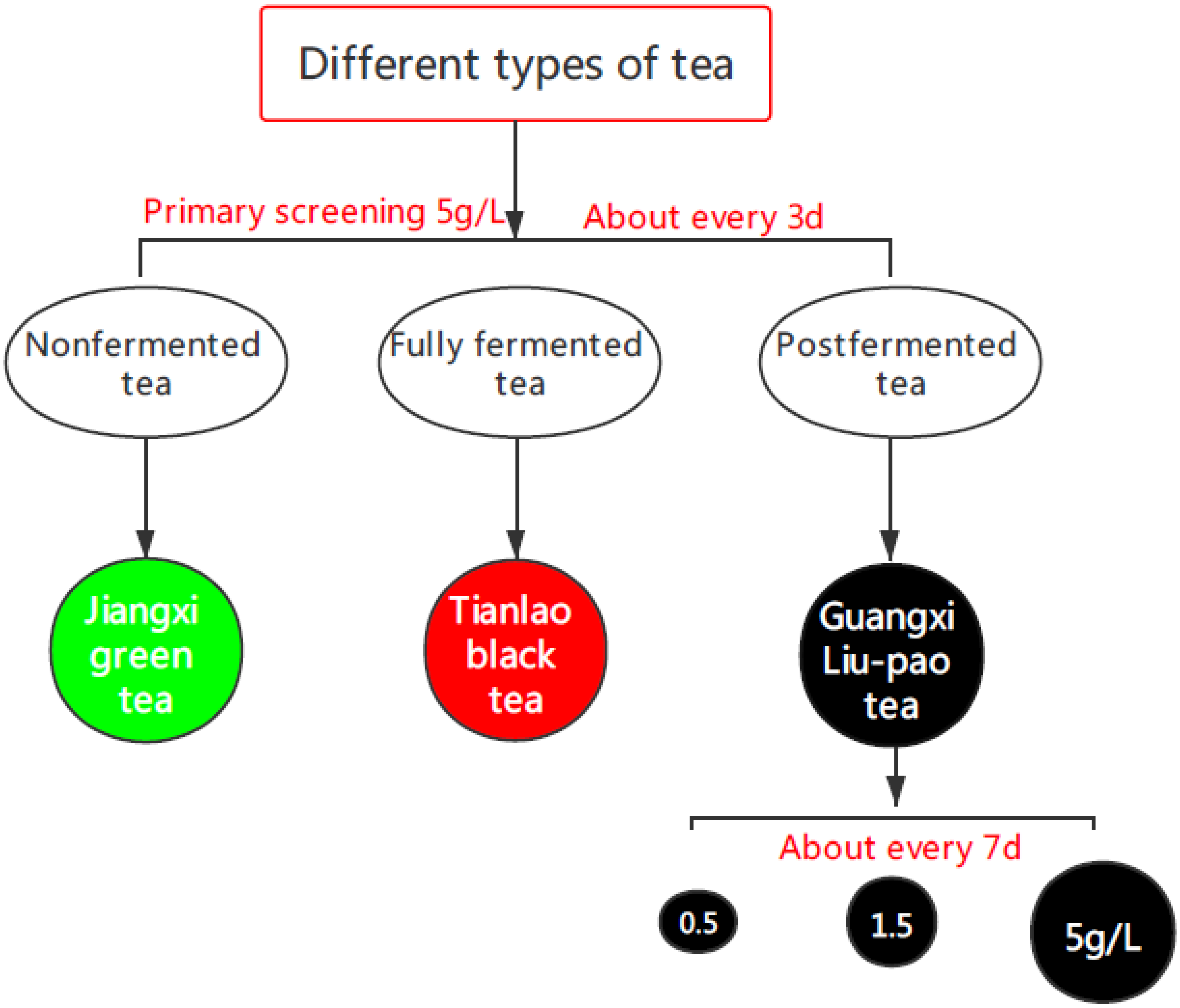

Figure 2

Flow chart of different types of teas used to attract Ae. albopictus to lay eggs.

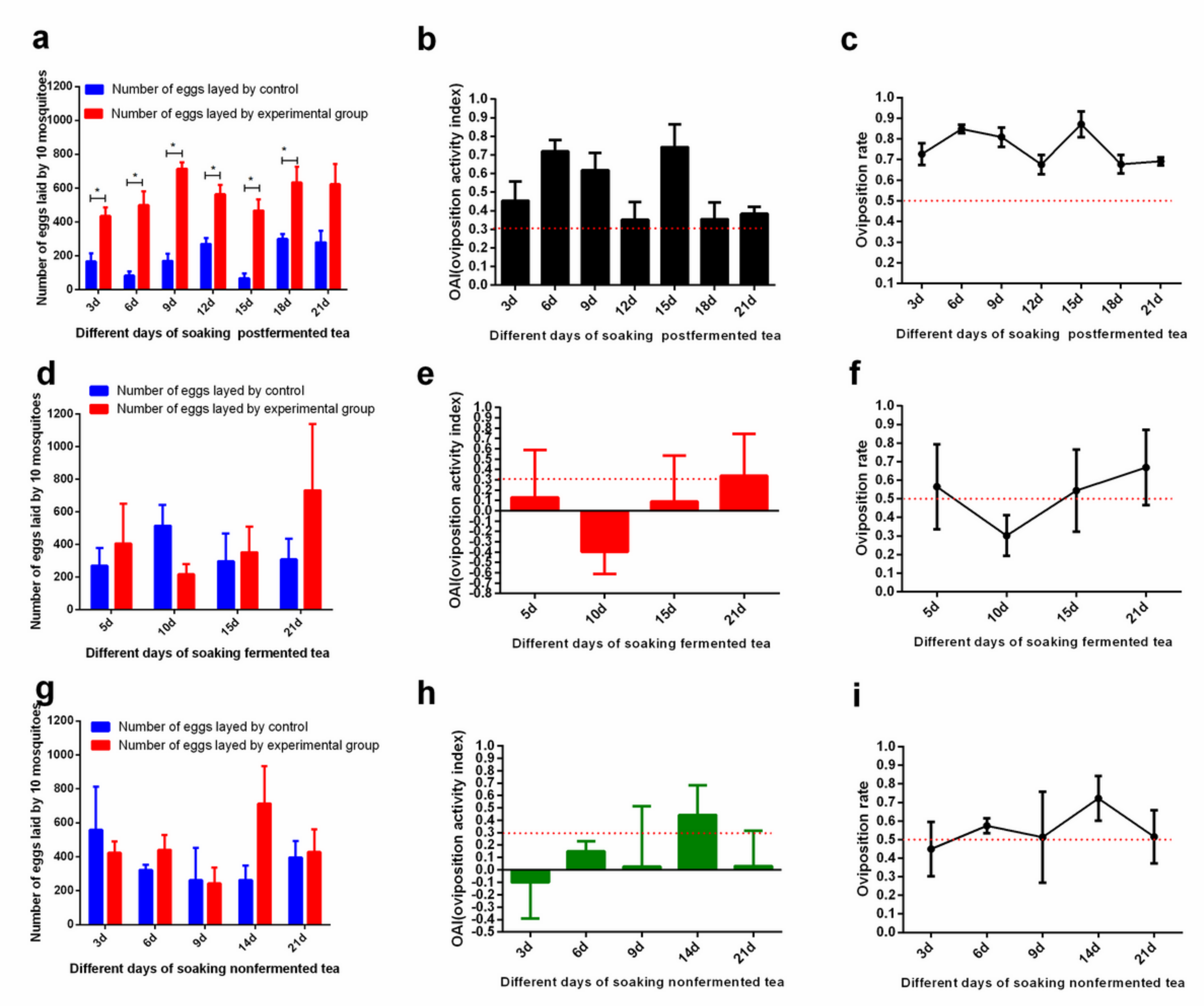

Figure 3

Effect of three kinds of fermented tea on attracting Ae. albopictus oviposition. Different days of soaking postfermented tea a.) Average number of eggs layed b.) OAI c.) Oviposition rate; Different days of soaking fermented tea d. Average number of eggs layed e.) OAI f.) Oviposition rate; Different days of soaking nonfermented tea g.) Average number of eggs layed h.) OAI i .) Oviposition rate. adg Bars represent the means ± SD, b- c,e – f, h-i Bars represent the means ± SE (n=3 - 6).

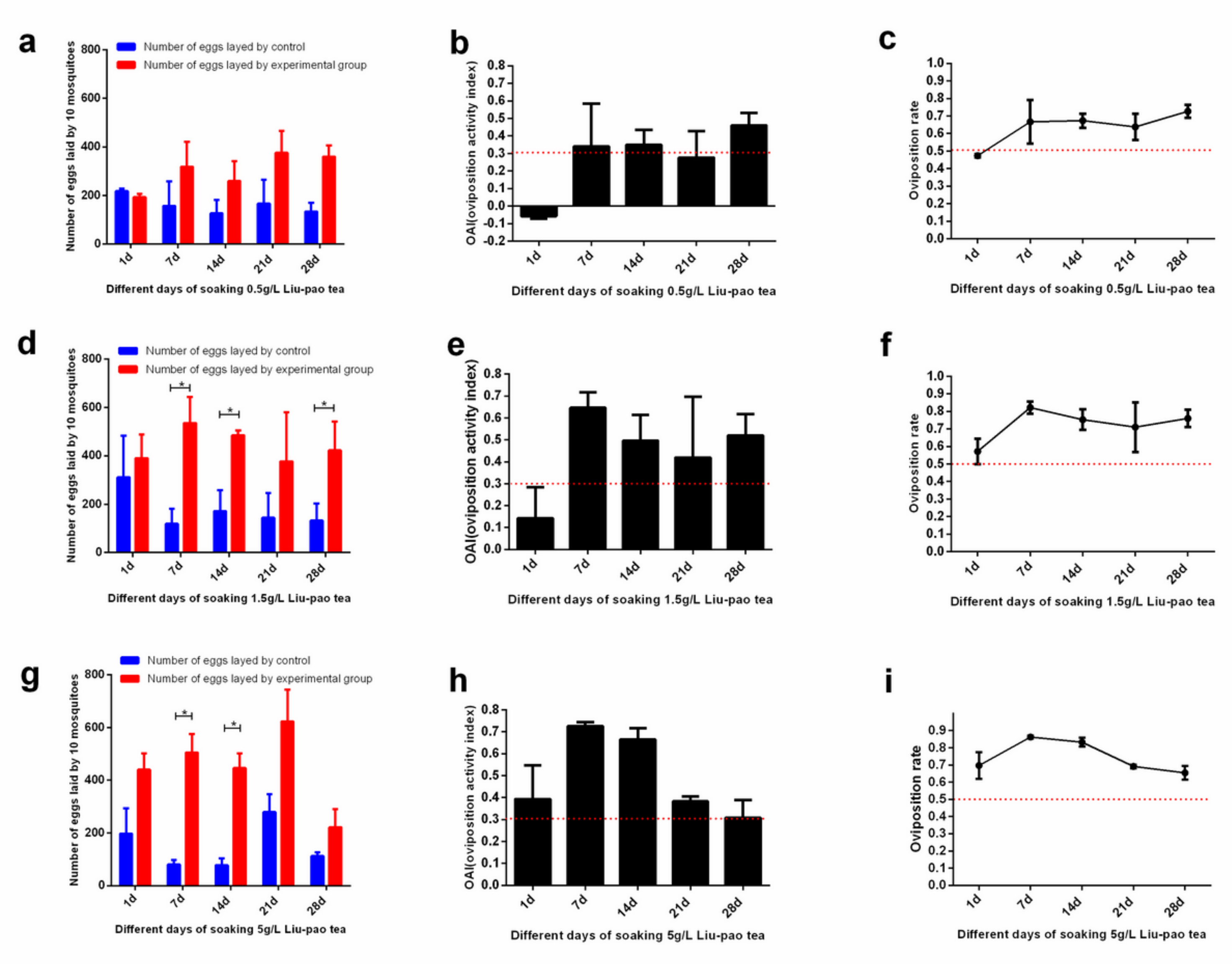

Figure 4

Effects of Liu-pao tea soaking at different doses on attracting Ae. albopictus oviposition within 28 days. a) The average number of eggs laid with 0.5 g/L b). The average number of eggs layed with 1.5 g/L c). The average number of eggs layed with 5 g/L d). The OAIs of different doses of Liu-pao tea. a-c. Bars represent the means ± SD, d. Bars represent the means ± SE (n = 3-6) * P <0.05 , ** P <0.01 , *** P <0.001.

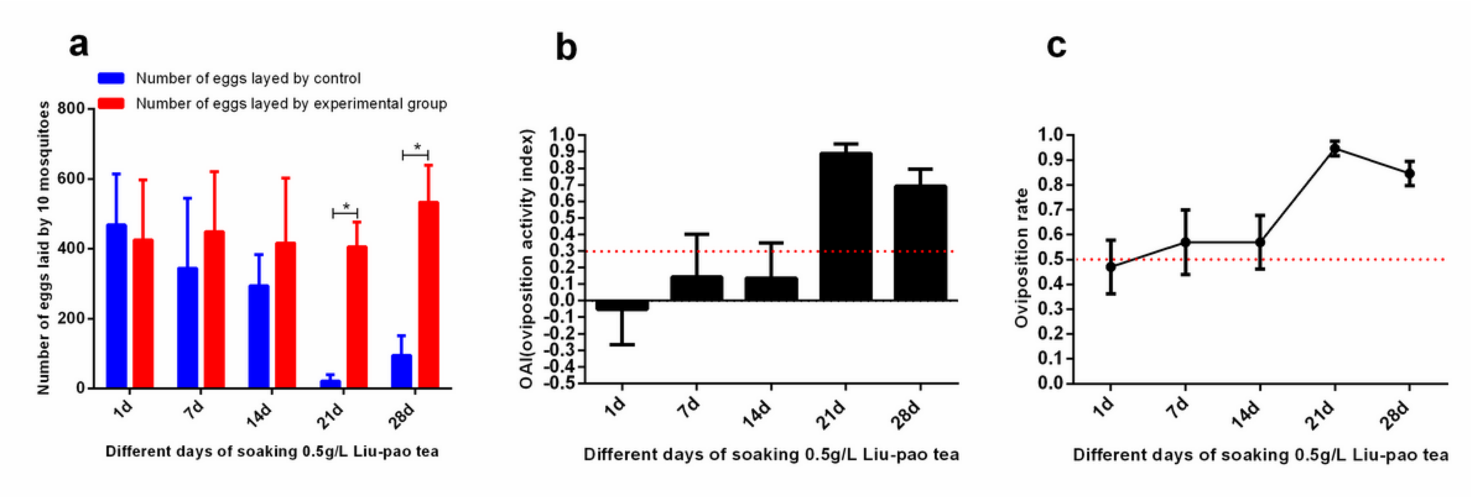

Figure 5

Effect of 0.5 g/L Liu-pao tea on Aedes aegypti oviposition within 28 days. A.) Average number of eggs layed b.) OAI c.) Oviposition rate; a. Bars represent the means ± SD, bc. Bars represent the means ± SE (n = 3-6) * P <0.05, ** P <0.01, *** P <0.001.

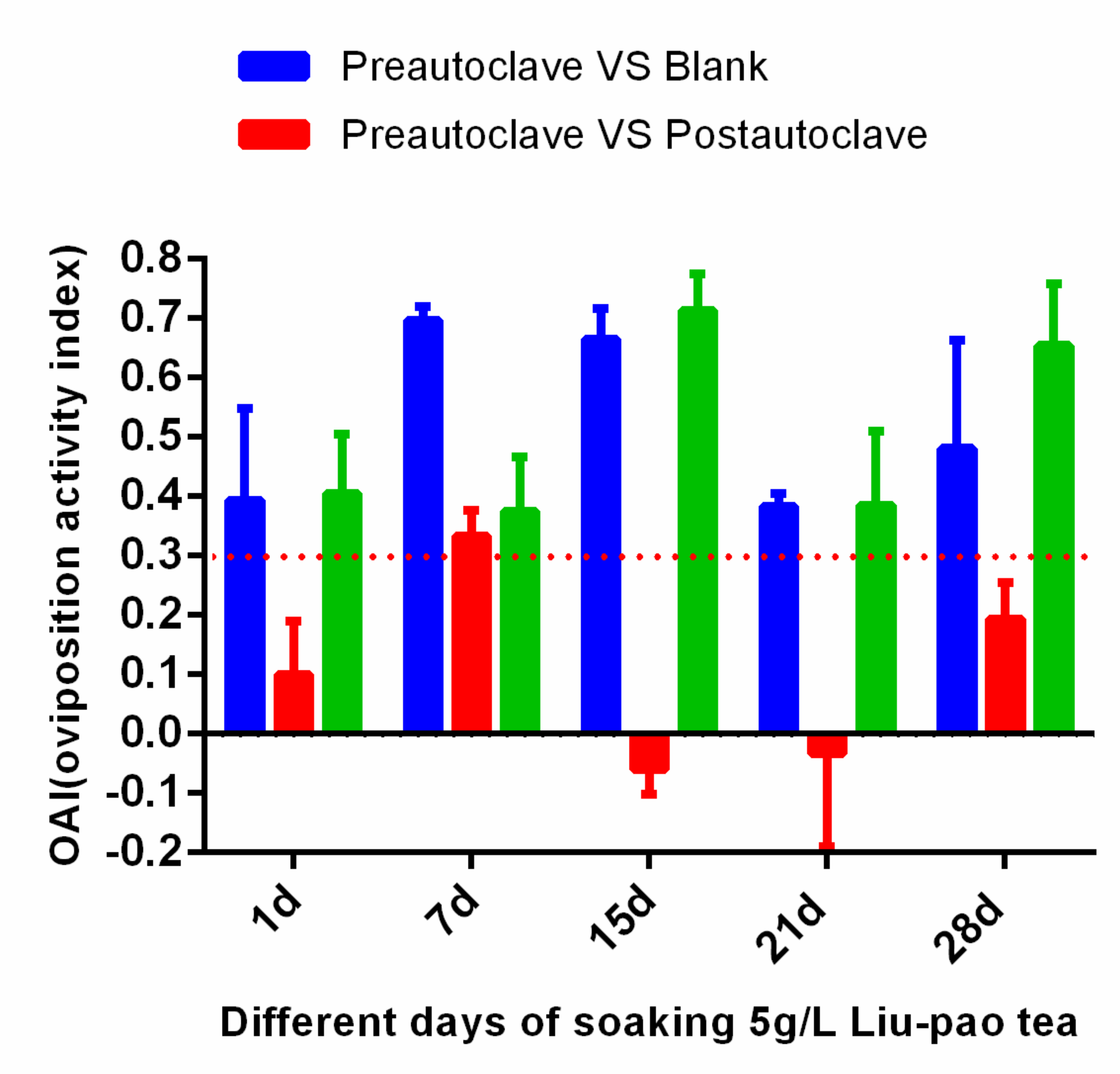

Figure 6

Effect of different days of Liu-pao tea soaking on attracting Ae. albopictus oviposition before and after autoclaving. Bars represent the means ± SE (n = 3-6).

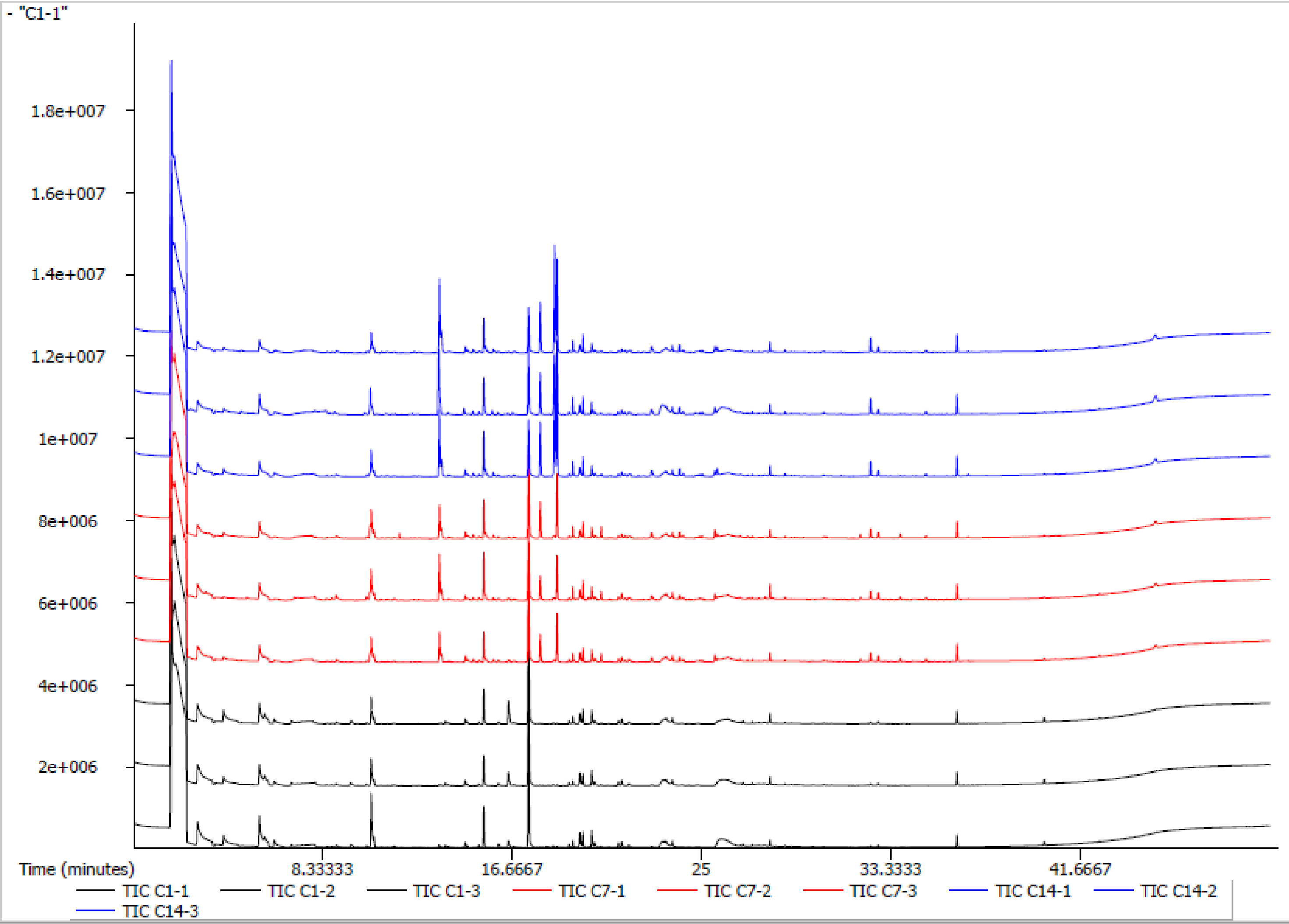

Figure 7

GC-MS detection of total ion chromatograms of Liu-pao tea extracts soaked for different days. C1 (1-3) was 0.5 g/L soaked for 1 d, C7 (1-3) was 5 g/L soaked for 7 d, C14 (1-3) was 5 g/L soaked for 14 d.

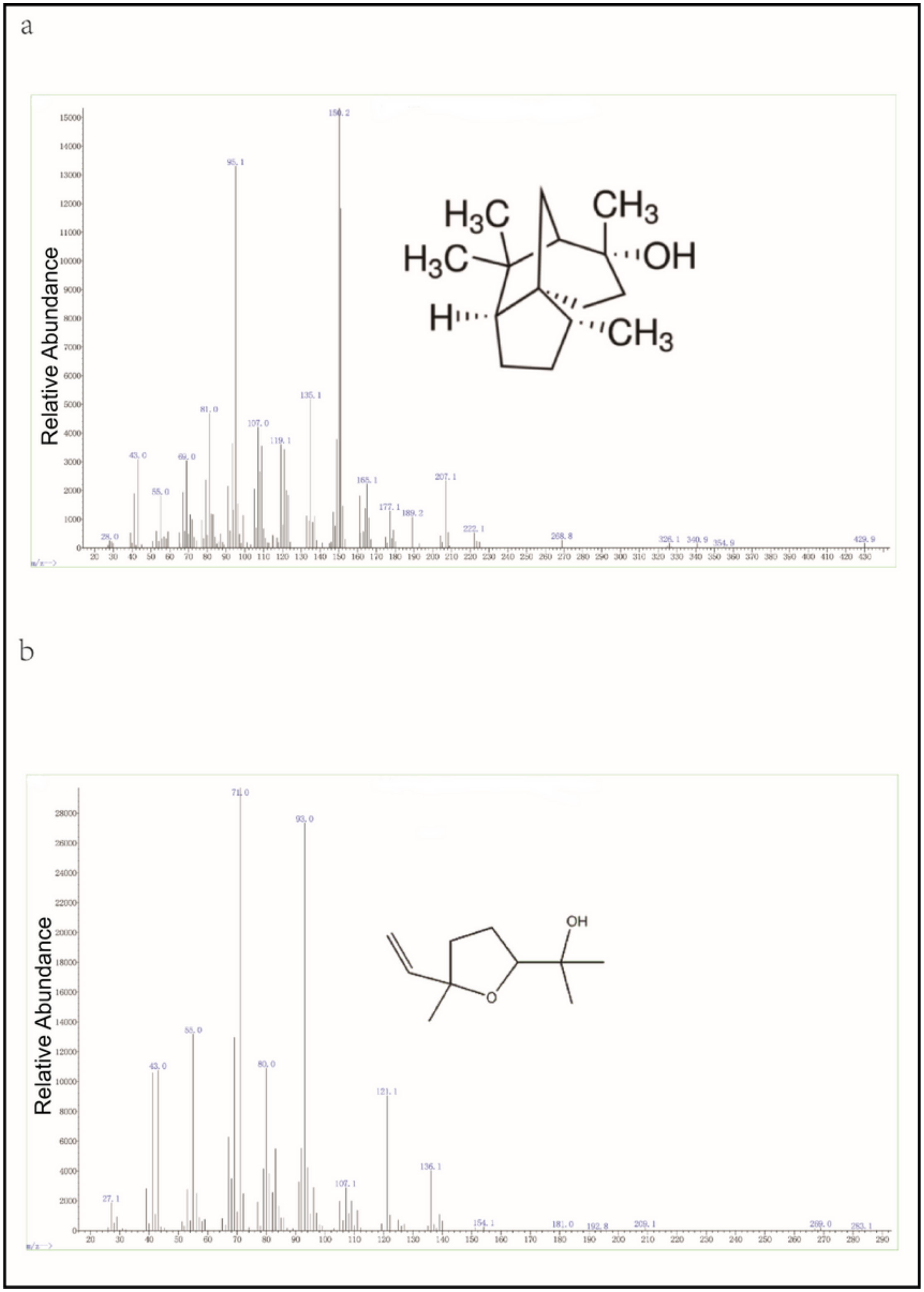

Figure 8

Ion chromatograms of cedrol (a) and linalool (b) in Liu-pao tea extracts after 7 days.

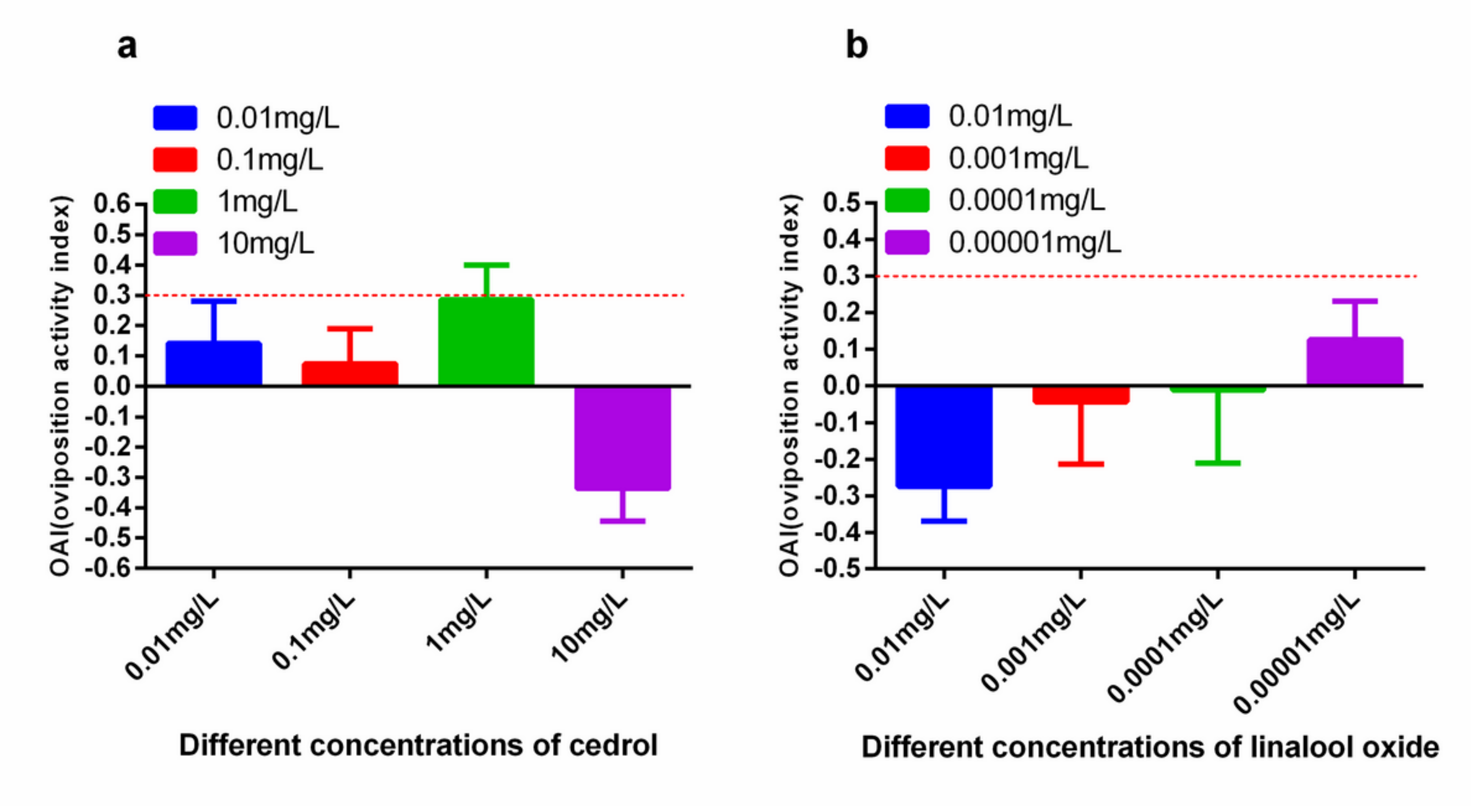

Figure 9

Effects of different concentrations of cedrol (a) and linalool oxide (b) on attracting Ae. albopictus oviposition. Bars represent the means ± SE (n = 3-6).

Supplementary Files

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- GraphicalAbstract.tif