

# Fabrication of an olfactometer for mosquito behavioural studies

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## Abstract

**Background & objectives:** Olfaction is the major sensory modality involved in the resource searching behaviour of insects including vector mosquitoes (Diptera: Culicidae). To date, our current country-wide knowledge on the host-seeking behaviour of Iranian mosquitoes is mainly confined to host preference which has exclusively come from field studies. Olfactometer is a scientific tool by which more naive aspects of man-vector contact can be clarified under controlled and less biased conditions.

**Methods:** The wind tunnel and stimulus delivery system was constructed from acrylic materials based on previously introduced models with some modifications. Air supply and required light were ensured by a powerful compressor and incandescent bulbs, respectively. Desired level of temperature was maintained by controllable heating radiators. For humidity production a unique in-built piezo system was devised in the course of the air flow. Fine regulators facilitated the continuous generation of the humidity at a preset level.

**Results:** Titanium tetrachloride smoke plus monitoring of the wind speed revealed that the flow of air was proper and invariable. A desired level of humidity and temperature could be set up in just 10 and 15–45 min, respectively. These physical parameters varied only  $\pm 2\%$  (humidity) and  $\pm 0.15^\circ\text{C}$  (temperature) in a typical 20 min duration.

**Conclusion:** The first sophisticated olfactometer in the field of medical entomology in Iran is reported here. Fast set up and stability of physical parameters are its salient features. It is expected that with the aid of this olfactometer further information on the physiological principles of the host-seeking behaviour of mosquitoes become available soon.

**Key words** Host-seeking; Iran; mosquito; olfactometer; olfactory behaviour

## Introduction

Olfaction is the major sensory modality involved in resource-searching behaviour of insects<sup>1</sup>. In mosquitoes, it is mainly exploited in host-seeking and finding a suitable place for oviposition. It is conceivable that the natural survival of arthropod-borne disease agents through periodic travelling between vectors

and potential hosts is also tightly bound to the success of these olfaction-based activities; a process frequently called as coevolution.

By far, field studies have revealed that each mosquito species has its own host preference<sup>2,3</sup>. Very often, this specificity goes beyond and extends to the biting sites, too<sup>4,5</sup>. However, we still do not clearly

know the basis of this specialization. We also do not know well how mosquitoes find and approach their victims from a distance under the influence of different environmental conditions. On the other hand, field information is likely to be complicated by the availability and accessibility of hosts. Olfactometers are devised to unravel some of these yet little answered questions under controlled and less biased conditions. Application of the olfactometers in the field of entomology starts with research on insects of agricultural importance<sup>6</sup>. Later, it was extended to the insects of medical importance including vector mosquitoes<sup>7,8</sup>.

Different types of olfactometers were developed to study host-seeking principles in mosquitoes, while some were primarily simple<sup>9,10</sup>, others were rather complex<sup>11–14</sup>. But, not all evolved olfactometers were novel. At times, they were just modifications of previously introduced ones<sup>15–19</sup>. Most olfactometers, either horizontal or vertical, designed for experimentation on close range orientation of mosquitoes<sup>20–22</sup>, and just few ones dealt with long range orientation aspects<sup>23</sup>. This came to the subject once it was realized that host-seeking is rather an integrated chain of consecutive behaviours and not merely a single phenomenon<sup>24</sup>. On the other hand, in addition to attractive responses, repulsive reactions of mosquitoes were also taken into account<sup>25,26</sup>. Apart from selected research targets, advent of new technologies and at the same time keeping economy, various strategies in assembling the construct or simulation of physical parameters, and finally ingenuity and creativity of the researcher, each played role in introduction of new olfactometers to science.

Whatever type, generally, the most invariable part in all olfactometers is the wind-tunnel with a minimum of two choices in which a mosquito recognizes and responds to an odor. Such responses could be in the form of attraction, repulsion or indifference. Sometimes, there are facilities for provision and control of physical parameters like wind speed, temperature, humidity and light. In advance olfactometers, special devices for stimulus delivery or video-cameras for recording mosquitoes' flight path might also be present.

Despite of good political commitment and spending considerable amount of resources on malaria, it is still a source of Iranian Health System concern<sup>27</sup>. There are also records of some other mosquito-borne pathogens such as West Nile and Sindbis viruses and diseases like dirofilariasis and setariasis<sup>28–30</sup>. It is not unlikely that all or most of the responsible local mosquito vectors are among 64 yet reported species in Iran<sup>31</sup>. Since man-vector contact is a key element in the epidemiology of these diseases, getting better insight on its physiological principles which undoubtedly improves planning more efficient control measures encouraged us to construct our own olfactometer capable of being used in different settings but as stable as possible.

## Material & Methods

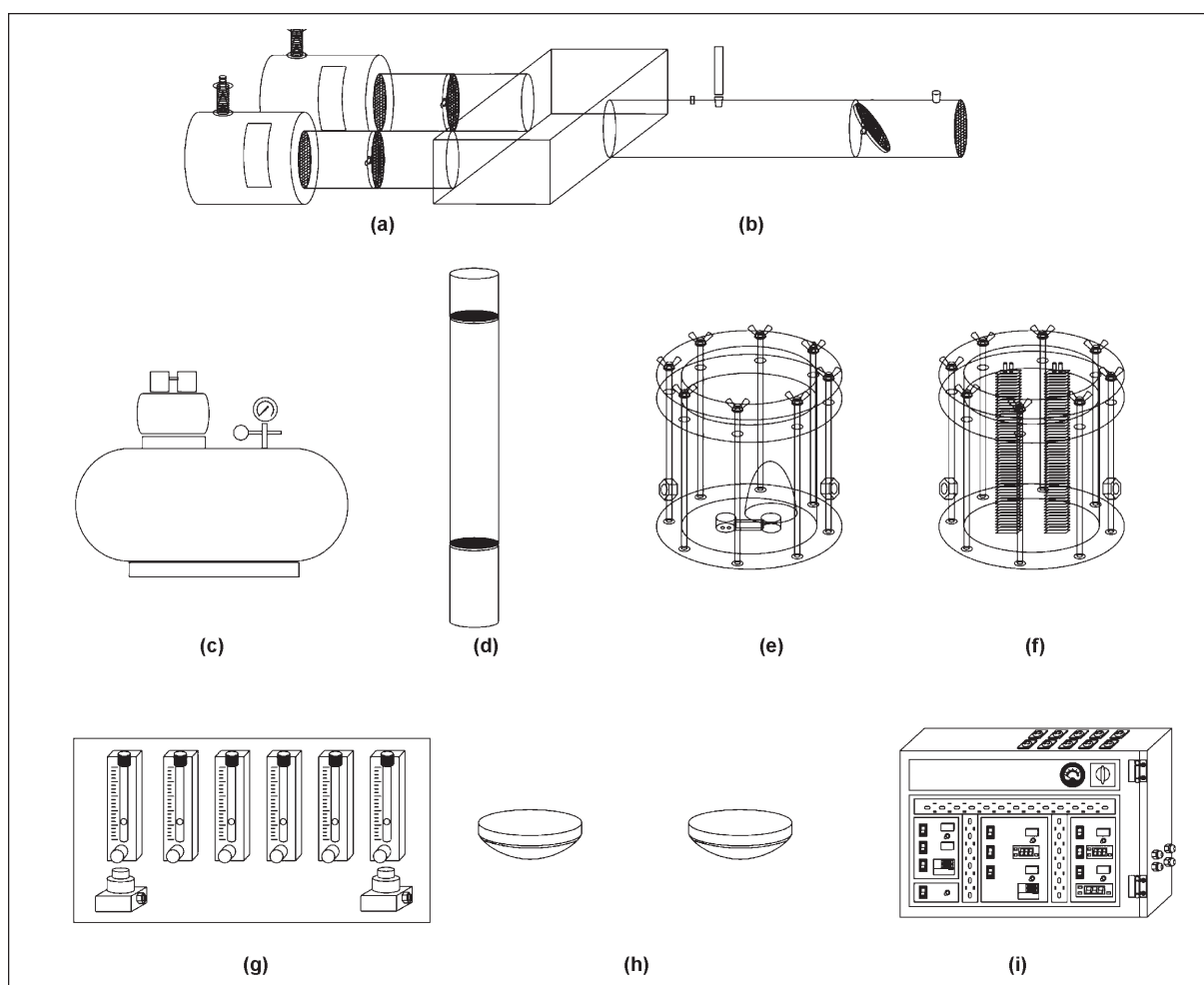
### Design of the olfactometer

*Wind tunnel:* The wind tunnel (125 cm, length) placed on a table is the main part of the olfactometer in which mosquitoes upwind. It has been made of acrylic tubes (9 cm inner diam, 3 mm wall thickness) and sheets (45×30×15 cm, as the central box) based on previously published work<sup>19</sup> (Fig. 1b). Its transparency suits careful observation of the position of mosquitoes which is necessary for counting and possibly tracking flight path in experiments. For each experiment, a small cylindrical acrylic cage (15×9 cm) containing known number of pre-conditioned mosquitoes can be connected to the downwind part of the wind tunnel. At the upwind side, there are two parallel arms (30×9 cm) each equipped with a rotary screen at the middle to entrap attracted mosquitoes. The rear ends of these arms are fused with two large acrylic stimulus chambers. Two displaceable white shields (30×130 cm) can be fixed in the supportive aluminium frame of the wind tunnel to prevent unintended optical stimulation of mosquitoes during experiments.

*Stimulus delivery system:* There is one stimulus chamber (20×15 cm) at the entrance of each air stream branch into the wind tunnel (Fig. 1a). A slit window

(5×15 cm, half circle) with a sliding door makes it feasible to insert the odor source of test inside. Adjacent to this window, at the top, there is a small unit (5×1.2 cm) called cartridge system. It is wired outwardly by circles of a heating coil. A small piece of filter paper can be impregnated with a test material and placed inside of a used clean dental glass cartridge, through which a controlled air stream can flow. Heating coils make faster release of the chemical stimulus into the treatment arm, if needed. Another possible route of stimulus delivery is the injection of a gaseous material by piercing a rubber septum over a small hole (3 mm) on the wall of incoming air stream just before the entrance into either arm.

*Physical parameters:* Pressurized air from an oil free air compressor (Fig. 1c) passes through a fortified tower of activated charcoal filter (50×9 cm, with 2 extra 10×9 and 15×9 cm parts at the top and bottom, respectively) (Fig. 1d) and finds its way to humidifying (Fig. 1e) and heating (Fig. 1f) chambers (28×35 cm) to get warm and humid, respectively. Removable doors of these acrylic chambers confer easy accessibility to their interior. Inside of each chamber, a speed controllable fan homogenizes humid or warmed air before departure. A piezo system (consisting of two independent subunits) generates puffs of cool mists in the humidity chamber. The power of piezo unit can be manually tuned up to such



*Fig. 1:* Different parts of the olfactometer: (a) Stimulus delivery chambers with cartridge units at the top; (b) Wind tunnel; (c) Oil free air compressor; (d) Activated charcoal filter; (e) Humidifying chamber with a high speed fan at the top and an intramural piezo system at the bottom; (f) Heating chamber with a high speed fan and two powerful radiators; (g) Gas flow control panel; (h) Over table incandescent bulbs; and (i) Electrical control panel.

a low level that continuously generate optimum amount of mists for a given humidity set point. An internal small conical reservoir (500 ml) supplies deionized water to this unit. In the heating chamber, two parallel electrical radiators (750 W each) warm up incoming humid air. Several serial manometers ensure fine regulation of air speed in the wind tunnel at a desired level. Anywhere after the activated charcoal filter, air flows in PVC pipelines (1.6 cm inner diam). The main air stream bifurcates just prior to the entrance into the wind tunnel where it sends an individual branch into either arm. Two over table 25 W incandescent bulbs (Fig. 1h) behind an opaque acrylic sheet scatter preset light on the wind tunnel at 80 cm height.

*Control panels:* Switches, automatic controllers and electronic monitors of humidifying and heating systems with feedbacks from sensitive sensors are positioned on the electrical control panel (Fig. 1i). Controllers of the heating coils in the cartridge unit, and light system are located on this panel, too. For more convenience, fine manometers and flowmeters are placed on a separate panel called gas flow control panel (Fig. 1g).

*Function:* Flow and turbulence of the air in the wind tunnel were checked first by titanium tetrachloride. This chemical agent reacts with humid air and produces a relatively dense white to yellowish smoke. In order to investigate stability of the physical parameters in the olfactometer outputs of electronic measurement devices for wind speed (hot wire anemometer, YK-2004 AH, Lutron®), temperature and humidity (High-Precision Miniature Humidity/Temperature Transmitter, EE07 Series, Elektronik®) and light (Lightmeter, LX-105, Lutron®) were sent into a computer and related data logged for 5 min with relevant software (SW-U801-Win, Lutron®; and E2-Interface Evaluation Software, E+E Elektronik®). More confidence on the stability of the temperature and humidity came from recordings in a 20 min period.

## Results

Titanium tetrachloride smoke revealed no back flow

or abnormal turbulence of air in the wind tunnel at 0.2 to 0.4 m/s wind speed. The synchronous logging of wind speed, light, humidity and temperature has been depicted for 5 min. This time interval is quiet more than what is normally required for a single experiment which is 5 min at most. Wind speed has no variation during such a typical period (Fig. 2a). Light intensity varies only 1 lux (Fig. 2b). Variations of temperature and relative humidity are less than 0.1°C and 1%, respectively (Figs. 2c and 2d). Even, these parameters vary only  $\pm 0.15^\circ\text{C}$  (temp.) and  $\pm 2\%$  (humidity) during a longer 20 min period (Figs. 3a and 3b). Generally, the system temperature stabilizes between 15 and 45 min depending on proximity of the desired set point to the laboratory temperature value. This time span is around 10 min for a preset humidity level (data are not shown here).

## Discussion

There is no report on olfactometric studies in Iran other than in the field of agricultural entomology and all so far used olfactometers are simple with minimal facilities for simulation of environmental conditions, if any<sup>32–34</sup>. Therefore, our apparatus is the first sophisticated dual-choice olfactometer in Iran which appears to be enough accurate to be used for research on olfaction-based behaviours of mosquitoes. Fast set up and stability of physical parameters at a desired level are its salient features.

Constructing an olfactometer starts with decisions on building the main part, i.e. the wind tunnel. Feinsod and Spielman<sup>35</sup> argued that the geometry of air current in moving-air olfactometers is hard to control and introduced their prototype single port olfactometer working with passive diffusion of chemostimulants in still air. Dogan and Rossingol<sup>25</sup> admitted this argument and introduced another olfactometer and called it a repellometer. Nonetheless, as the flying insects cannot recognize wind direction without referring to the silhouettes of environmental objects<sup>36,37</sup>, moving-air olfactometers are still adhered to and quiet more prevalently used than still-air olfactometers. Also, it is not impossible to do experi-

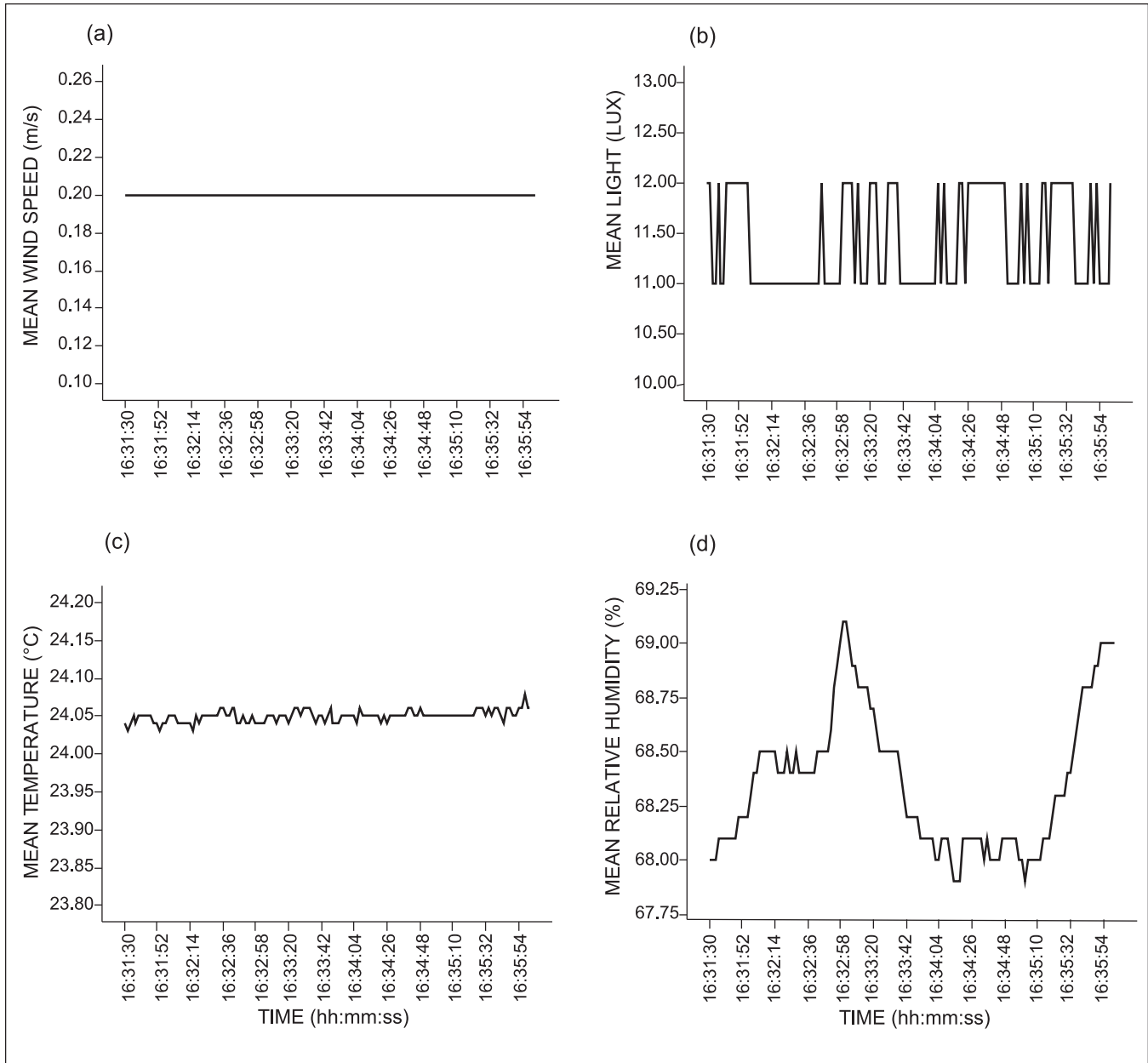


Fig. 2: Monitoring of physical parameters in a typical 5 min interval in the olfactometer: (a) Wind speed; (b) Light intensity; (c) Temperature; and (d) Humidity.

ment on repellent materials in such type of olfactometers<sup>38</sup>. Based on these concepts we preferred to find our construct as a moving-air olfactometer. In general, the designed and constructed wind tunnel in this study looks like what is once described by Geier and Boeckh<sup>11</sup> and Geier *et al*<sup>19</sup>. The same type has also recently used elsewhere<sup>39,40</sup>. An outstanding feature of this type is the rapidity of yielding reproducible results in a short space of time. Wind tunnel in

the original version is a Y-shape dual-choice chamber which its trifurcation part has been replaced with a simple box in the next version. Since this modification provides more realistic choices for mosquitoes we designed our wind tunnel on the same basis. However, according to what was available to us we used larger diameter acrylic tubes (9 cm inner diam, 3 mm thickness). Despite of the fact that this change may reduce undesirable wall effect on mosquitoes, it costs



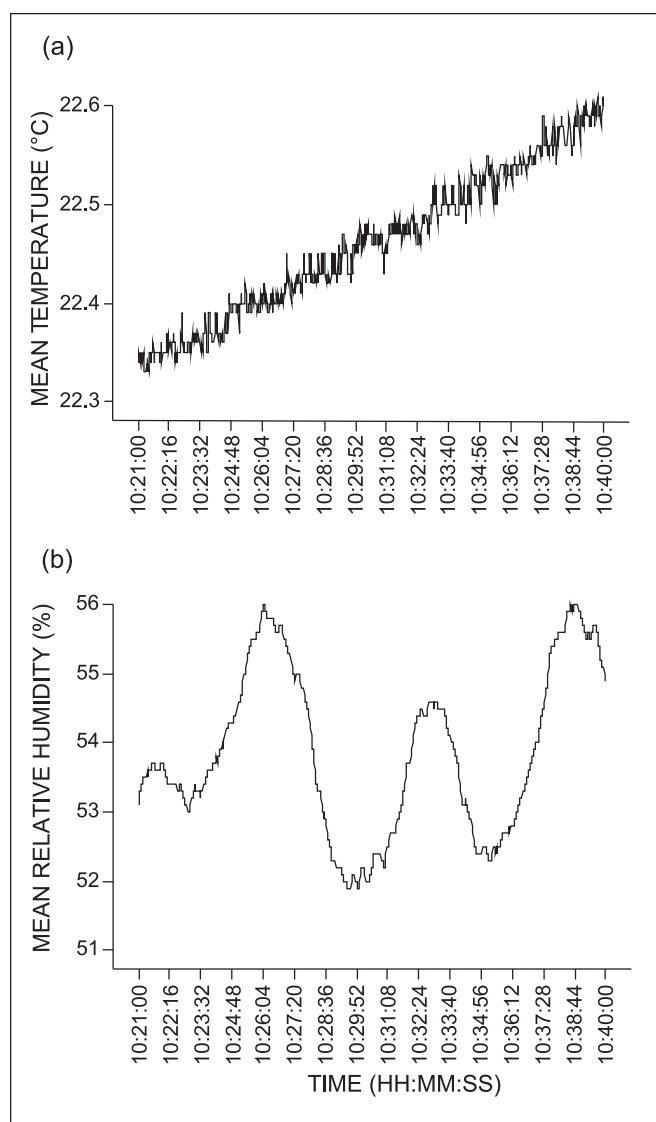


Fig. 3: Monitoring of physical parameters in a typical 20 min interval in the olfactometer: (a) Temperature; and (b) Humidity.

up to 60% lower concentration of a given chemostimulant in the wind tunnel compared with the original model. Therefore, cautions should be taken into account while comparing results of this olfactometer with others. The importance of wall effect has also been stressed by Kellogg and Wright<sup>10</sup>.

Air can be pulled or pushed or both in a moving-air olfactometer. In general, it is harder to establish a clean uncontaminated air current with two latter approaches. Geier and Boeckh<sup>11</sup> and Geier *et al*<sup>19</sup> used building's pressurized air system in their olfactome-

ter. We recruited a powerful air compressor in the next room, instead. Whatever the supply system, air has to be adequately cleaned before by means of a filter. This is usually a tower of activated charcoal. Dekker *et al*<sup>41</sup> and Geier *et al*<sup>42</sup> documented how structure of the odor plume influence on the upwind responses of mosquitoes in behavioural bioassays. We admittedly provisioned facilities for generating homogeneous, filamentous or turbulent plumes in the stimulus chamber of our olfactometer.

It has been shown that mosquitoes are able to recognize very small changes in the temperature and relative humidity levels of an air current<sup>43,44</sup>. Therefore, in order to increase the reproducibility of the results enough care must be taken of the stability of temperature and humidity in behavioral bioassays. Perhaps, this can be assured by the ability to keep these physical parameters with minimal variation for a relatively long time. Different approaches have been adopted by researchers for the provision of temperature and humidity in the olfactometers. A few did their works in climate controlled rooms. This is also likely for those who have not clearly pointed out how they have ensured physical conditions in their olfactometers.

In olfactometers with possibility of temperature adjustment, electrical energy is almost always employed as the source of heat. It is used either directly in the form of coils or indirectly by warming loops of main air stream inside of a water bath. We preferred the former strategy as it speeds up moving from one temperature set point to another one. In the most of yet reported olfactometers the variability of temperature is  $\geq \pm 0.5^\circ\text{C}$ . This value is only  $\pm 0.15^\circ\text{C}$  in this olfactometer for a relatively long typical 20 min. This provides a much stable condition for behavioral bioassays on mosquitoes.

Gas washing bottles have been popularly used for humidity production in olfactometers<sup>9,40,45</sup>. In this technique more humidity is generated by using warmer water. Despite of its simplicity, however, humidity regulation is rather a time consuming task and the resulted set point drift is also wide. Price *et*

*et al.*<sup>46</sup> ingeniously employed a water sprayer for humidity production. They also hired an advance system for tuning humidity level by adding required dry air into the humidified air stream. Variation of humidity level in their system reported to be  $< \pm 2\%$  during a 6 min time interval. Geier and Boeckh<sup>11</sup> and Geier *et al.*<sup>19</sup> combined both techniques, i.e. warm gas washing bottle for humidity production and the empirical addition of dry air to humid air current to make a lower humidity level. However, oscillation of humidity level was  $\pm 5\%$ . In our system we used particulate water droplets, too. But these are so much finer and are produced by a high frequency generator coupled with a piezo unit. Since this system recruits mechanical rather than thermal energy, it does not make the humid air warmer. In fact, the outgoing air is cooler than the incoming air. However, the generated mists might be still vaporized anyway. This is accomplished by two high speed fans in serially connected humidifying and heating chambers and the action of heating radiators within the heating chamber. In comparison with other yet mentioned studies the variation of humidity in our system ( $\pm 2\%$ ) is either less or more stable for a longer duration (at least 20 min). In any case, whatever technology is used, the laboratory temperature has to be set at a certain point to prevent condensation in the wind tunnel.

### Conclusion

First sophisticated Iranian olfactometer for research on the host-seeking behavior of mosquitoes is reported here. Fast set up and precise stability of physical parameters makes it an exclusive apparatus. It is expected that with the aid of this olfactometer further information on physiological principles of the host-seeking behaviour of mosquito vectors become available soon.

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