

Efficacy of Some Wearable Devices Compared with Spray-On Insect Repellents for the Yellow Fever Mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae)

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Abstract

The current Zika health crisis in the Americas has created an intense interest in mosquito control methods and products. Mosquito vectors of Zika are of the genus *Aedes*, mainly the yellow fever mosquito, *Aedes aegypti* L. The use of repellents to alter mosquito host seeking behavior is an effective method for the prevention of mosquito-borne diseases. A large number of different spray-on repellents and wearable repellent devices are commercially available. The efficacies of many repellents are unknown. This study focuses on the efficacy of eleven different repellents in reducing the number of *Ae. aegypti* female mosquitoes attracted to human bait. We performed attraction-inhibition assays using a taxis cage in a wind tunnel setting. One person was placed upwind of the taxis cage and the mosquito movement towards or away from the person was recorded. The person was treated with various spray-on repellents or equipped with different mosquito repellent devices. We found that the spray-on repellents containing N,N-Diethyl-meta-toluamide and p-menthane-3,8-diol had the highest efficacy in repelling mosquitoes compared to repellents with other ingredients. From the five wearable devices that we tested, only the one that releases Metofluthrin significantly reduced the numbers of attracted mosquitoes. The citronella candle had no effect. We conclude that many of the products that we tested that were marketed as repellents do not reduce mosquito attraction to humans.

Key words: repellent, *Aedes*, yellow fever mosquito, DEET, taxis cage

Aedes aegypti is the principal vector for Zika, chikungunya, yellow fever, and dengue, worldwide and responsible for historic and ongoing outbreaks of these arboviral diseases (Gubler 2004). The geographical distribution of *Ae. aegypti* has expanded significantly over the last two decades (Jansen and Beebe 2010). This species can be found in many regions across the world, including the Americas. This species has successfully established populations across the United States southern border (Jansen and Beebe 2010, Joy et al. 2012). Zika virus is the latest of these arboviruses to invade the Americas (Fauci and Morens 2016). It has established itself in Brazil and spread explosively from there (Saiz et al. 2016). In June of 2016, the first autochthonous transmission within the United States was reported from Miami Dade County in Florida. Zika infection causes mild symptoms in about 18% of the infected patients, but has been linked to debilitating developmental defects in infants (Mlakar et al. 2016, Rubin et al. 2016).

The impending threat of Zika outbreaks in North America has created a massive public interest in mosquito control products and there are thousands of products currently commercially available. A search on www.amazon.com using the keywords “mosquito repellent” gained 13,333 hits on 16 August 2016. Putative repellents and mosquito control devices are presented to the consumer in several different ways including: spray-on repellents in pressurized spray bottles or diffusers, creams, repellent or insecticide-treated clothing, area repellents like candles and live plants that are rich in essential oils. There is also an abundance of wearable devices that are available, including: bracelets, sonic devices, clothing, and skin patches (Debboun and Strickman 2013).

There have been several studies that have shown an association between the use of personal protection products and a reduction in mosquito bites and disease incidence (Debboun and Strickman 2013). For example, permethrin-treated military clothing has shown

to be effective in significantly reducing mosquito bites in the covered regions (Schreck and Kline 1989). Application of the repellent DEET to ankles and feet of the population in an African village significantly reduced the incidence of malaria (Durrheim and Govere 2002). Repellent soaps that contain DEET and permethrin have been shown to successfully reduce malaria infections (Kroeger et al. 1997, Kroeger et al., 1999). Permethrin treated clothing has also been shown to reduce malaria incidence (Rowland et al. 1999). A double blind study concluded that the use of oil of lemon eucalyptus as repellent helps preventing malaria infections (Hill et al. 2007, Maia and Moore 2011).

The efficacy of different spray-on repellents on various species of mosquitoes has been tested in numerous studies (Fradin and Day 2002). We have shown in a previous study using a Y-tube olfactometer that the spray-on repellents DEET and oil of lemon eucalyptus (active ingredient p-menthane-3,8-diol [PMD]) are effective in repelling *Ae. aegypti* and *Aedes albopictus* (Rodriguez et al. 2015). The transdermal Vitamin B₁ patch did not show any significant reduction in attraction as compared to the control. Other wearable devices such as OFF! Clip-on (Johnson & Son, Inc., Racine, WI) and Terminix ALLCLEAR Sidekick (Universal Pest Solutions, LLC, Dallas, TX) have been tested and shown to reduce biting pressure in both *Aedes* and *Culex* mosquitoes (Revay et al. 2013). As a general trend, we could confirm that repellents based on essential oils often have shorter repellency effects compared with DEET and PMD-based products (Patel et al. 2012, Rodriguez et al. 2015).

The World Health Organization has several suggestions for testing spatial repellents (WHO 2013). These tests can be conducted in several environments including: the laboratory, semi-field tests, and field studies. Some common laboratory tests to determine attraction and inhibition include: in vitro blood feeding systems like the arm-in-a-cage test, alternative choice systems like the Y-tube, olfactometer, tests or a taxis cage. Taxis cages can be used in both a lab setting and in field work (Lorenz et al. 2013).

In this study, we used a taxis cage inside a wind tunnel to evaluate the changes in overall attraction of *Ae. aegypti* to a human subject using various repellent products. We tested five spray-on repellents, five wearable devices, and one candle.

Materials and Methods

Mosquito Culture

Ae. aegypti ROCK strain were attained from the Malaria Research and Reference Reagent Resource Center (ATCC 2015). Mosquito rearing procedures described in (Marquardt 2004), were followed using an artificial blood feeding system. Approximately 500 mosquito larvae were reared per 33 × 38 cm plastic developing pan filled with ~2 liters of deionized water. The pans with larvae were incubated in an insect chamber at 27°C and given dry cat food pellets ad libitum. Pupae were collected in cups and placed into a BugDorm-1 Insect Rearing Cage (30 × 30 × 30 cm, BugDorm Store, Taichung, Taiwan). The cage was placed in an insectary that was maintained at 27°C and 80% humidity. The photoperiod within the insectary was 14:10 (L:D) h. The adult mosquitoes were given access to 20% sucrose solution, ad libitum.

Approximately 24 h prior to the experiment, the mosquitoes were placed outside the insectary to acclimatize to room temperature and low humidity that exists at the wind tunnel facility. In addition, the mosquitoes were sugar-starved for 24 h prior to the experiment, but given deionized water to maintain hydration. The mosquitoes were not anesthetized prior to the tests. In preliminary

tests, we found that anesthetizing mosquitoes with temperature or CO₂ alters their behavior for several hours. The female *Ae. aegypti* mosquitoes used in this experiment were between 1.5 and 2 weeks old. Mosquitoes were only used one time and then discarded after each test.

Institutional Review Board Approval

The New Mexico State University Institutional Review Board approved this study. Title: "Efficacy of different insect repellents", study no. 11505A.

Repellents

Repellent devices and sprays were predominantly acquired through Amazon, some were purchased locally in Las Cruces, NM. Table 1 illustrates all of the details provided by each label, including: active ingredients, type of repellent, manufacturer, estimated protection time. The repellents were presented the following way: The sonic device was attached to the chest of the bait person; for the bracelets, one was attached to each wrist of the test person; the OFF! Clip-on device was attached at the leg of the test person; the aerosol sprays were sprayed on a person for 15 s each side (~5 ml was applied to each side of the body); the pump sprays were applied 25 sprays per side (~5 ml were applied to each side of the body); the citronella candle was placed downwind next to the test person.

Taxis Cage

The taxis cage used in this study is a custom-built alternative choice system that was built similarly to models in (Lorenz et al. 2013) with modifications (Fig. 1A). It consists of three interconnected chambers. The middle chamber is separated from the outer chambers with a funnel made from mosquito screen with an opening of 5 cm diameter in the middle. Access between the cages can be opened or closed with a Plexiglas barrier that can be moved via a pulley system. This system allows the segregation of chambers, which allows for an analysis on the proportion of mosquitoes that move towards an attractant in a given amount of time.

Wind Tunnel

The experiment was done in the NMSU low-speed wind tunnel (Fig. 1B). This open return wind tunnel has a test section dimension of 1.2 m(W) × 1.2 m(H) × 14.6 m(L). Flow is induced with a fan installed downstream the test section. Flow speed can be continuously set from 0 to 35 m/s. The intake of the wind tunnel is 4 × 4 m to ensure the turbulence intensity in the test section is <1%. For all experiments a constant air current of 2 m/s was maintained.

Mosquito Attractants

All experiments were performed using two volunteers that served as attractants. The volunteers were instructed to not bathe or use personal hygiene products <15 h prior to the experiment.

Attraction-Inhibition Assays

For the mosquito attraction-inhibition testing, the taxis cage was placed on a roller board inside the wind tunnel (Fig. 1A). Figure 1C illustrates the overall experimental design. Approximately 50–125 female mosquitoes were aspirated into a collection vial and immediately placed in the middle chamber of the taxis cage. The mosquitoes were allowed to acclimate for 2 min prior to each evaluation. The subject was sealed in the wind tunnel 1 or 3 m upwind of the taxis cage. The trap doors of the taxis cage were then opened and the

Table 1. Product Name, product type, active ingredients, manufacturer/distributor/seller, and estimated time of protection

Product name	Product type	Active ingredient(s)	Manufacturer/ distributor/sold by	Estimated protection time ^a
OFF! Clip-on	Wearable Device (fogger)	Metofluthrin (31.2%)	Sold by S.C. Johnson & Son, Inc., Racine, WI, USA	12 h
Personal Sonic Mosquito Repeller	Wearable Device (speaker)	N/A	PIC Corporation, Linden, NJ, USA	50 h
Mosquitavert	Wearable Device (bracelet)	Geraniol	Body Harmonic, LLC., Portland, OR, USA	N/A
Mosquito-NO!	Wearable Device (bracelet)	Geraniol Oil (30%) (extract from citronella, lavender and peppermint)	Mosquito-NO!	10 d
Invisaband	Wearable Device (bracelet)	Geraniol Oil (30%)	Invisaband	120 h
Cutter Lemon Eucalyptus	Spray-On	Oil of lemon eucalyptus (30%) ~ 65% PMD	Spectrum, Division of United Industries Corporation, St. Louis, MO, USA	6 h
Kids Herbal Armor	Spray-On	Oil of Soybean (11.5%) Oil of Citronella (10%) Oil of Peppermint (2.0%) Oil of Cedar (1.5%) Oil of Lemongrass (1.0%) Oil of Geranium (.05%)	Tender Corporation, Littleton, NH, USA	10 h
Avon Skin-So-Soft Bug Guard Plus Picaridin	Spray-On	Picaridin (10%)	Avon Products, Inc. Distr. New York, NY, USA	6 h
Repel Sportsmen Max Formula	Spray-On	DEET (40%)	WPC Brands Inc.	N/A
Ben's Tick & Insect Repellent	Spray-On	DEET (98%)	Tender Corporation, Littleton, NH USA	10 h
Cutter Citro Guard	Area repellent (candle)	Oil of Citronella (3%)	Spectrum, Division of United Industries Corporation, St Louis, MO, USA	40 h

^a Based on reapplication suggestions and/or estimated protection times provided on the label of the product.

Table 2. Average attraction rates of *Ae. aegypti* mosquitoes in the taxis cage at 1 m

Treatments (N = 4)	Average attraction rate (±SE)
Positive control	88.82% (±1.36%)
Negative control	17.43% (±1.14%) ***
OFF! Clip-on	27.14% (±6.04%) ***
Mosquito-NO!	84.00% (±1.52%) ns
Invisaband	87.54% (±2.24%) ns
Personal Sonic Mosquito Repeller	91.17% (±2.97%) ns
Mosquitavert	91.31% (±2.56%) ns
Cutter Lemon Eucalyptus	29.62% (±6.31%)***
Ben's Tick & Insect Repellent	33.70% (±4.06%)***
Kids Herbal Armor	59.66% (±4.89%)***
Repel Sportsmen Max Formula	68.55% (±6.42%)***
Avon Skin-So-Soft Bug Guard Plus Picaridin	78.65% (±6.00%)**
Cutter Citro Guard	90.95% (±1.96%) ns

P values: $P < 0.05^*$; $P < 0.01^{**}$; $P < 0.001^{***}$; not significant (ns); N, number of replicates.

mosquitoes were allowed to roam for 15 min. After 15 min, the trap doors were closed and the mosquitoes were counted based on their location within the taxis cage. Before every repellent evaluation, control tests were executed to determine overall attraction the mosquitoes had to the volunteers on this particular day. If a minimum threshold of an 80% attraction rate to the volunteer was not achieved for the control experiments, the experiments were rescheduled to another day. The sprays and devices were put on the volunteer immediately before the 15-min test. The positive control consisted of a volunteer without repellents. A negative control was performed to determine the movement of the mosquitoes without a volunteer stimulus. The total number of mosquitoes that flew

towards the attractant was used to calculate the total attraction of mosquitoes after a 15-min period.

Product efficacy was tested at two different distances between the volunteer and the taxis cage setup, 1 and 3 m. These distances were arbitrarily chosen in order to detect delusion and homogenization effects of the active ingredients released by the devices or sprays tested.

Two volunteers were used as attractants in this study. Each volunteer was prescreened in preliminary tests to have a >80% attraction rate in our positive control setup. Each volunteer was tested twice with each treatment/time point for a total of four replicates. Each volunteer was subjected to only one treatment each day.

Statistical Methods

To evaluate the efficacy of each repellent, one-way analysis of variance was used. The independent variable was the rank-transformed ratio of the number of mosquitoes that were moved toward the attractant versus the total number of mosquitoes in the test.

Results

Controls

Both positive and negative controls were performed to determine the overall movement of mosquitoes. The negative control consisted of the taxis cage without the volunteer present. The overall attractant rate for the negative control at 1 and 3 m were $17.43 \pm 1.72\%$ and $13.18 \pm 2.97\%$, respectively (Table 2 and 3). The positive control consisted of the taxis cage with a volunteer less any repellents. At a distance of 1 and 3 m, the positive control attractant rates were $88.82 \pm 1.36\%$ and $91.25 \pm 1.24\%$, respectively.

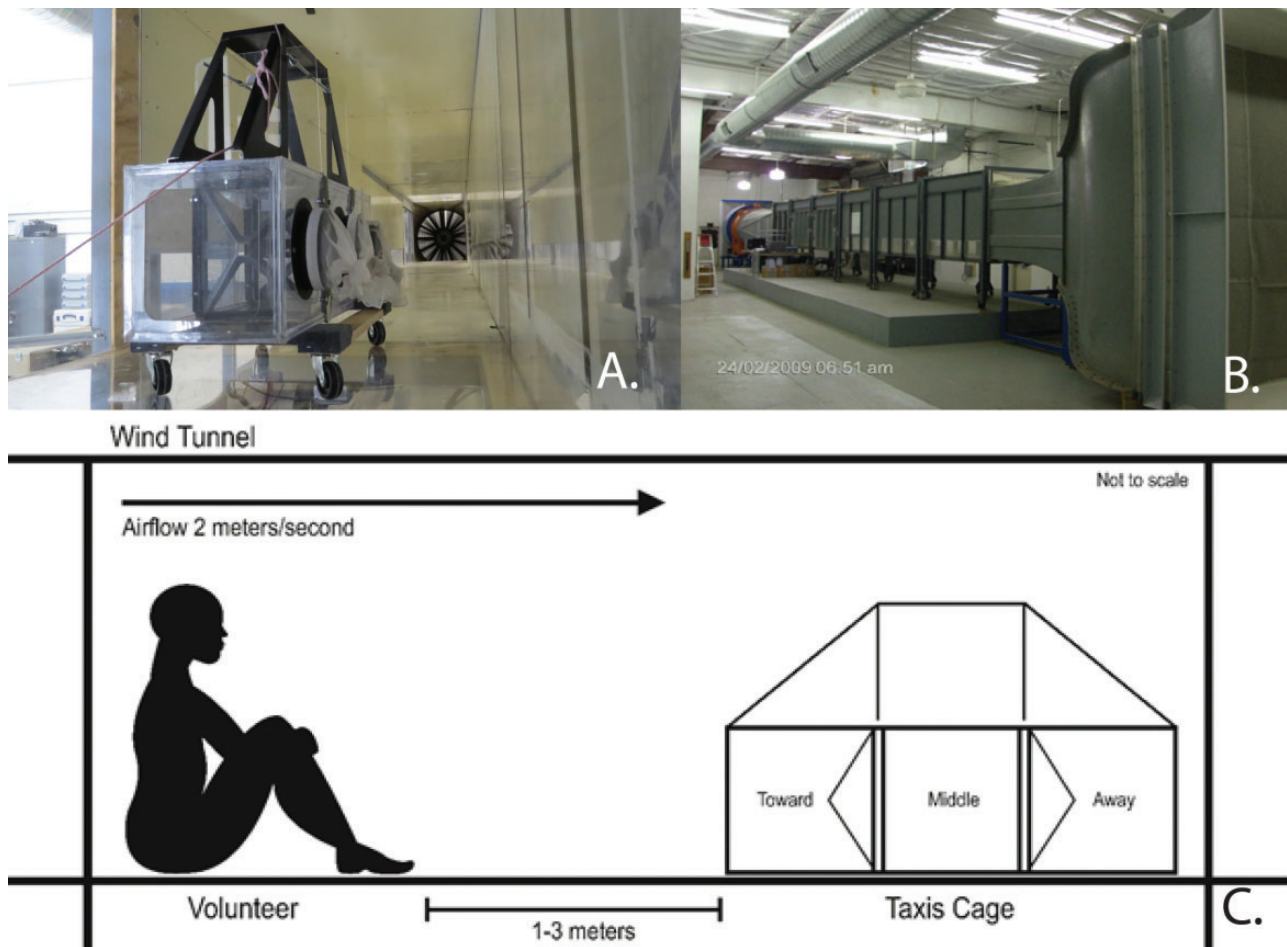


Fig. 1. Wind tunnel/taxis cage experimental design.

Table 3. Average attraction rates of *Ae. aegypti* mosquitoes in the taxis cage at 3 m

Treatments (N = 4)	Average attraction rate (\pm SE)
Positive control	91.25% (\pm 1.24%)
Negative control	13.18% (\pm 2.97%)*
OFF! Clip-on	46.89% (\pm 2.99%)*
Cutter Lemon Eucalyptus	51.64% (\pm 4.70%)*
Ben's Tick & Insect Repellent	23.46% (\pm 4.42%)*
Kids Herbal Armor	73.31% (\pm 0.71%)*

P Values: $P < 0.05^*$; $P < 0.01^{**}$; $P < 0.001^{***}$; not significant (ns); N, number of replicates.

Attraction-Inhibition at a 1-m Distance

Table 1 shows the results of the attraction-inhibition assays performed at a 1 m distance (\sim 3.3 ft.). From the five wearable devices tested in this study, only the Off! Clip-on device resulted in a significant decrease in the number of mosquitoes that moved towards the volunteer. The products containing 30% PMD (Oil of lemon eucalyptus) or 98% DEET produced equally strong reductions in attraction. The citronella candle did not significantly reduce mosquito attraction.

Attraction-Inhibition at a 3-m Distance

We then tested one device and three sprays that had been successful in the previous experiment at a distance of 3 m. The results of the

experiments when the human attractant was placed 3 m (\sim 9.8 ft) upwind of the taxis cage are shown in Table 3. With the exception of the DEET product, the attraction rates increased in all tests.

Supplementary Material 1 [online only] shows the statistical differences between all treatments at 1 and 3 m.

Discussion

Adult mosquitoes use their sense of olfaction to locate hosts (Takken 1991, Zwiebel and Takken, 2004, Galizia and Rossler 2010). Specific chemical cues and odorants produced by the vertebrate host's skin or the bacterial community living on it as well as CO₂ and other chemicals exhaled while breathing trigger host-seeking behavior over longer or shorter ranges (Zwiebel and Takken 2004, Galizia and Rossler 2010). The majority of insect repellents function by reducing overall attraction of the blood-seeking female mosquito to the human host.

The recurrent outbreaks of arboviruses like dengue, chikungunya, and lately Zika in Americas has created a large market for a variety of mosquito repellent and control products (Ioos et al. 2014, Karwowski et al. 2016). However, it has become apparent that not all repellents and/or repellent devices actually reduce mosquito attraction and that in many cases the claims made by the vendors of these products are exaggerated or simply false (Fradin and Day 2002; Fuss et al. 2007, 2013; Hill et al. 2007; Fuss and Ray 2009; Rodriguez et al. 2015).

Wind tunnels have been used to study insect olfactory orientation behavior towards pheromones, hosts, or other cues (Visser 1976, Miller and Roelofs 1978, Elzen et al. 1986, Tumlinson et al. 1989). Mosquito host location has been studied using wind tunnel setups (Gillies 1980, Eiras and Jepson 1991) as well as the effects of insecticides (Mount et al. 1976). The mosquito taxis cage/wind tunnel assay we developed for this series of experiments measures the variation in attraction of female mosquitoes to a host and can be used to test this variation at different distances. In our opinion, this setup resembles a natural setting better than the standard “arm-in-cage” test or olfactometers. It is also superior to field testing because using the wind tunnel allows standardizing the air flow from the bait person towards the cage and allows the constant movement of odors and CO₂ plumes from the bait person towards and through the taxis cage. This setup creates a strong attraction to properly prepared *Ae. aegypti* females. On average about 90% of all mosquitoes in our tests ended up moving towards the bait within 15 min, which is much higher and faster than the averages measured in a comparable study with a similar setup with a tunnel minus regulated air flow (Lorenz et al. 2013).

The Personal Sonic Mosquito Repeller (PIC Corporation, Linden, NJ) we tested had no effect on the overall mosquito attraction to the human subject. This confirms the results of a previous study that tested similar devices and found that mosquitoes are unperturbed by them (Foster and Lutes 1985) as well as a field study that did not find any significant effect on mosquito landings when using a sonic device (Revay et al. 2013). Interestingly, one study reported an increase in *Ae. aegypti* biting activity when such devices were switched on (Andrade and Cabrini 2010). We are not aware of any scientific study showing that mosquitoes can be repelled by sound waves and therefore we consider these devices as the modern equivalent of snake oil.

None of the bracelets we tested caused any significant reduction in mosquito attraction. Although the active ingredients in some bracelets may be mosquito repellents, we hypothesize that the concentrations that are emitted by all of the bracelets that we tested were too low to have an effect. Based on our results, we conclude that these bracelets in general do not offer adequate protection from mosquito bites.

The only wearable device that reduced mosquito attraction rates was the OFF! Clip-on Metofluthrin nebulizer. Mosquito attraction rates were reduced close to that of the negative control. OFF! Clip-on killed 100% of the mosquitoes in each replicate. In field tests, this device has been shown to reduce the number of bites that test persons experienced from *Ae. albopictus* and *Aedes taeniorhynchus* mosquitoes by 70 and 79%, respectively (Xue et al. 2012). Another field study found similar results (Revay et al. 2013). From all devices we tested, OFF! Clip-on was the only wearable device that had an effect in our study.

The results of our study show clearly that DEET and PMD are very effective mosquito repellents when used in sufficient concentration, while the other active ingredients had lower efficacy. This finding confirms the findings of several other studies that found DEET and PMD the most effective and longest lasting mosquito repellents currently available (Fradin and Day 2002, Carroll and Loye 2006). The Kids Herbal Armor product, which contains a mixture of essential oils as active ingredients, performed equally well as a 40% DEET solution and better than a 10% picaridin solution.

The citronella candle combined with a human subject attracted slightly more mosquitoes than the human bait person alone;

however, this difference was not statistically significant. We found no indication that such candles repel *Ae. aegypti* females.

By moving the taxis cage away from the human subject, we anticipated to better mix and homogenize attractants released by the test person with the various repellents we tested. Since the wind tunnel is a closed system we don't expect a large dilution effect between the two distances we tested. Interestingly, there was an increase in attraction with all products with the exception of the 98% DEET solution. We hypothesize that the repellent properties seem to dissipate more with better mixing of these products with the attractants released by the test person.

We have developed a novel testing setup for mosquito repellents using a taxis cage within a wind tunnel. This setup allows the control of a critical variable—wind speed—that cannot be controlled in a field setting or in many other semi-field test setups. This setup allowed us to collect highly significant data on the effects of various repellents. Due to the high reproducibility of our test results, we can detect even minute repellency effects that cannot be detected in other setups.

We found that a sonic device, three bracelets, and a citronella candle had no repellency effect on *Ae. aegypti* females in our test setup. Also, we found that all the spray-on repellents that we tested had a repellency effect. It is important to note that even though we found repellents in our study with significant reductions in attraction as compared to the control, some of the attraction rates are rather high. For optimal protection, a consumer should select the products that have the most significant reduction in attraction. At a time where vector-borne diseases like Zika is a real threat, the most egregious danger to the consumer is the false comfort that some repellents give them protection against *Ae. aegypti* when they actually offer none. Even if a product offers a slight reduction in attraction as compared to the control, there is still a high probability of mosquito-human interaction; especially in areas with higher populations of *Ae. aegypti* mosquitoes.

It has to be noted that the series of experiments that we performed focused on the efficacy of wearable devices and spray-on repellents against *Ae. aegypti* females. Further studies are necessary to explore the efficacy of these interventions on repelling other mosquito species.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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