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Short Communication

Reduction of mosquito biting pressure by timed-release 0.3% aerosolized geraniol

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ABSTRACT

We conducted a study to determine the degree of personal protection provided by the Terminix[®] ALLCLEAR[®] Mosquito Mister – Lantern Edition. This outdoor unit was operated to disperse an aerosolized aqueous 0.3% geraniol emulsion in timed-release intervals of 5.0, 7.5, and 10.0 min. Human volunteers participated in landing catch experiments to test the effect of geraniol sprayed at pre-set time intervals, at two distances: (1) 18 ft (5.49 m), the maximum effective distance claimed by the manufacturer, and (2) 9 ft (2.74 m), half the effective distance from the unit. When aerosolized geraniol was dispensed, reductions in biting pressure (landing, probing and biting mosquitoes) of *Culex pipiens* and *Aedes albopictus*, at all times and distances, were evident compared to dispensation of the water spray control. The degree of protection correlated well with the distance from the subject and the time interval of releases. The 5 min time interval mode reduced overall biting pressure by more than 90% at 9 ft (2.74 m) and 18 ft (5.49 m). Reduction of biting pressure in the 7.5 min mode was still well over 80% and even in the 10 min mode, overall protection was observed in the 10 min mode, at the periphery of the area the unit claims to protect (300 ft²), with a biting pressure reduction of approximately two-thirds.

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1. Introduction

The global threat of mosquito-borne diseases, such as West Nile Virus (WNV) and Malaria, and their associated morbidity and mortality highlights the need for effective insect repellents. It is therefore important to know which repellent products can be relied on to provide consistent and prolonged protection from bites. In recent years, botanical insect repellents have become increasingly popular as viable alternatives to synthetic chemical pest repellents (Wirtz et al., 1980; Omolo et al., 2004; Kaufman et al., 2010) because they reputedly pose little risk to the environment or human health. Specifically, many claims have been made regarding the repellent properties of citronella essential oil and various terpene alcohols such as geraniol and linalool (Choi et al., 2002; Barnard and Xue, 2004; Park et al., 2005).

Numerous "alternative" botanical products (Cox, 2005), many in the form of area repellents, have been marketed to consumers

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mainly to control nuisance mosquitoes. In developed countries, these products are widely used by consumers trying to rid themselves of mosquitoes and biting flies in back yards, but one of the concerns with outdoor use is that the repellent molecules may be diluted by airflow or driven away by change in wind direction.

Ideal area repellents need an optimal degree of volatility, making it possible for an effective vapor concentration to be maintained (Gerberg and Novak, 2007; Moore et al., 2007). Vapors should also repel multiple species of biting insects, cause no irritation to the skin or mucous membranes and be nontoxic to humans and animals. Botanical repellents in the form of candles, diffusers, and misters could potentially provide sufficient protection provided they fit the above criteria.

The purpose of this study was to determine the degree of personal protection afforded by a timed-released, water-based emulsion of 0.3% geraniol mist dispensed by a pressurized spray can in an area of high mosquito biting pressure in Israel. To ensure quality control, the current study adheres to the above-mentioned EPA guidelines, as well as to currently accepted standards for testing insect repellents (EPA, 1999; Govere and Durrheim, 2007; Barnard et al., 2007).

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E.E. Revay et al. / Acta Tropica xxx (2012) xxx-xxx

2. Materials and methods

2.1. Equipment

2

The apparatus tested in this study was the Terminix[®] ALLCLEAR[®] Mosquito Mister – Lantern Edition that releases aerosolized repellent at three pre-programmed intervals of 5.0, 7.5, or 10.0 min. The unit is also equipped with a remote control that allows the user to release repellent on demand if desired.

The aqueous 0.3% geraniol emulsion was contained in a pressurized spray can within the Mosquito Mister. The unit was charged and operated with a single spray can for each of the three timed-spray periods. The aerosol products were stored less than 1 month in the laboratory at room temperature (approx. $23 \,^{\circ}$ C) before they were tested.

2.2. Volunteers

Six volunteers, four male and two female professional entomologists/medics, provided their written informed consent to participate in this study. As part of the consent process, the participants were fully advised of the nature and purposes of the test and the possible health risks from exposure to chemicals and insect populations. They were required to avoid alcohol, caffeine, and fragrance products (e.g., perfume, cologne, hairspray, lotion, etc.) during the entire test period. For the tests, volunteers were seated in chairs, remaining as motionless as possible, facing towards the mister, with one arm extended at a 45° angle, resting on thighs, in front of them. The skin outside the test area (including the hand, the forearm, and bicep) was covered with regular clothes to protect from mosquito bites; volunteers wore long trousers and short-sleeved shirts. Since tests were conducted just after sunset, the volunteers and assistants were situated approximately 2.4 m from a 50-W garden lantern, which allowed them to identify and count landing mosquitoes.

2.3. Test site and weather conditions

Tests were performed on the northern Mediterranean coastal plain of Israel in suburban Haifa ($32^{\circ}48'56''$ North, $34^{\circ}59'21''$ East). The study took place in late November 2011 and experiments were conducted in the early evening from 18:00 to 21:00 local time (just after sunset). The test and the control sites were surrounded on all sides either by buildings or vegetation such that air movement was minimal. Weather conditions were consistent throughout the study with early evening temperatures ranging from 20 to $24^{\circ}C$ and periods with a few clouds. Smoke cartridges were placed at a distance of 100 m from the experimental site to verify airflow and were carefully monitored during the experiments. No unfavorable weather conditions were observed during the trial periods.

In accordance with United States Environmental Protection Agency (US EPA) recommendations (EPA, 1999), a site was selected that has a minimum biting pressure (1 bite/min) with at least two different mosquito genera and species. Common mosquitoes at the test and nearby control site are *Culex pipiens* and *Aedes albopictus*.

2.4. Experimental setup

To determine the area coverage of aerosol from the Mosquito Mister, the unit was mounted on a pole 1.6 m above the ground, and letter-sized overhead projector acetate sheets (Din A4 plastic sheets, 100 pieces) were mounted on wooden poles, 0.5 m above the ground, arranged in a $10 \text{ m} \times 10 \text{ m}$ square in front of the unit, at a density of one sheet per m². The unit was mounted at the edge of the square, halfway between the corners (5 m) and adjusted to spray towards the center of the square. After three sprays, the

sheets were recovered and examined with a $3 \times$ magnifying glass for droplet coverage. Sheets with 10 or more droplets were regarded as positive for coverage with the misting unit. The procedure was replicated six times.

We tested the repellency rate for each of the three programmable time intervals (5.0, 7.5 and 10.0 min) by situating volunteers at distances of 9 ft (2.74 m) and 18 ft (5.49 m) from the unit, resulting in six trials; n = 18 at 9 ft (2.74 m) and n = 18 trials at 18 ft (5.49 m) over a period of 3 consecutive days. Each evening, we tested one of the three programmable-release time intervals; landing rates were evaluated for five min, which enabled the group of volunteers to finish one trial in half hour.

On the first day, two spray can-equipped units were randomly assigned to the volunteers. The misters (with the assigned cans either active – filled with geraniol, or control – filled with water) were rotated on successive trial days through the two selected sites. The participants were not informed which mister was equipped with active material and which not. In every trial, all six volunteers rotated three times trough the treated station and three times through the control station resulting in18 counts at 9 ft (2.74 m) from the unit and 18 counts at 18 ft (5.49 m) from the unit, both at the testing site and at the control sites. The participants alternated back and forth between the treated and control areas.

All mosquitoes that landed were counted, even if they took flight immediately. Landing, probing and biting events (in time intervals of 5 min) on the arm were, counted, pooled, and recorded on data sheets by two assistants standing behind the volunteers at a distance of about 1 m.

3. Results

3.1. Mosquito composition

One day prior to the trials, we collected a base-line sample of 100 mosquitoes on human bait from18:00 to 21:00 h. The catch was composed of 68 *Cx. pipiens* and 32 *Ae. albopictus.* Both species could be easily and reliably identified on sight while landing.

3.2. Area coverage

Area coverage was considered positive for all acetate square sheets covered with 10 or more droplets. The average area coverage of the mister unit was calculated over six repetitions and was determined to be 52.33 m^2 (563.28 ft^2). The highest droplet densities were observed in a cone shaped area of 120° and towards the center of the $10 \text{ m} \times 10 \text{ m}$ square. Densities of 10 or more droplets were observed up to the most distant side of the square (10 m).

The product claims area coverage of $300 \, ft^2 \, (27.87 \, m^2)$ and accordingly we adjusted our experiments to the claims and tested for an area of 120° with a total area coverage $339.29 \, ft^2 \, (31.52 \, m^2)$. This is one-third (or 120°) of a full circle with a radius of 18 ft (5.49 m). We also decided to test efficacy at 9 ft (2.74 m) from the unit, as this is half the maximum distance reached by the spray. The area covered at this distance is $84.82 \, ft^2 \, (7.88 \, m^2)$.

3.3. Reduction of biting pressure

The degree of protection supplied by the Mosquito Mister correlates well with the distance from the subject and the time interval of releases (Tables 1 and 2). We tested the unit under fairly high biting pressure conditions (i.e., high rate of landing, probing and biting mosquitoes) and the 5 min time interval had an overall biting pressure reduction rate of more than 90% at 9 ft (2.74 m) and 18 ft (5.49 m) distances (Tables 1 and 2). In the 7.5 min interval mode, the overall biting pressure reduction was still well over 80% and even in the 10 min mode, overall protection was slightly above 80%

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E.E. Revay et al. / Acta Tropica xxx (2012) xxx-xxx

Table 1

Human landing catch results at a distance of 9 ft (2.74 m), where numbers represent pooled landing, probing and biting events over 3 trials. Decrease of biting pressure is presented as percentage reduction.

Mode	Cx. pipiens			Ae. albopictus			Total mosquitoes		
	Control	Treated	Reduction	Control	Treated	Reduction	Control	Treated	Reduction
5.0 min	152	1	99.34%	113	3	97.34%	265	4	98.49%
7.5 min	127	11	91.34%	77	9	88.31%	204	20	90.20%
10.0 min	214	34	84.11%	162	39	75.93%	376	73	80.11%

Table 2

Human landing catch results at a distance of 18 ft (5.49 m), where numbers represent pooled landing, probing and biting events over 3 trials. Decrease of biting pressure is presented as percentage reduction.

Mode	Cx. pipiens			Ae. albopictus			Total mosquitoes		
	Control	Treated	Reduction	Control	Treated	Reduction	Control	Treated	Reduction
5.0 min	157	8	94.99%	123	5	95.93%	280	13	95.53%
7.5 min	139	23	83.45%	89	18	79.78%	228	41	82.02%
10.0 min	252	79	68.65%	155	57	63.23%	407	136	66.59%

at a distance of 9 ft (Table 1). The lowest but still reasonable protection level was observed in the 10 min mode, at the periphery of the area the unit claims to protect, with a biting pressure reduction of two-thirds (Table 2).

4. Discussion

In this study, much care was taken to select areas with appropriate and stable weather conditions. It was especially important to work in an environment of no or minimal air movement. Under these conditions, the Mosquito Mister dispensing a 0.3% waterbased geraniol emulsion provided a steady and satisfactory level of protection from biting mosquitoes (Tables 1 and 2). The results show that with little or no air movement a person can be well protected as long as they are within the plume of the repellent mist.

Choice of active ingredient is an important factor in the performance of an area repellent system. Geraniol-based products are available commercially in several countries and in several forms. A plant-derived alcohol, geraniol is considered completely safe for use and appears on the US Food and Drug Administration (US FDA) Generally Regarded as Safe (GRAS) list and is classified by the US EPA as a minimum risk pesticide under section 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). It has been demonstrated to be effective in repelling mosquitoes and may even have insecticidal properties (Xue et al., 2003; Barnard and Xue, 2004; Kaufman et al., 2010). Moreover, geraniol has also been shown to be repellent to other insects such as houseflies, stable flies (Mann et al., 2010) and ticks (Weldon et al., 2011).

In previous experiments in Israel, 5% geraniol candles were about twice as effective as those with 5% linalool and were about 5 times as effective as 5% citronella candles in protecting a person from being bitten by mosquitoes when used indoors (Müller et al., 2008b). When used outdoors to protect volunteers in a high biting pressure environment, the same 5% geraniol candles reduced the mosquito pressure by an average of 56% over a distance of 1.0 m. In a low biting pressure environment, geraniol candles reduced the mosquito pressure by an average of 62% (Müller et al., 2008a). A similar study, also conducted in Israel, showed that geraniol (4-6 mg/h) released indoors by diffusers, reduced Cx. pipiens mosquitoes caught by nearby CDC light traps by 36% (Sirak-Wizeman et al., 2008). The Cx. pipiens mosquito carries WNV and was a key vector responsible for the New York outbreak in 1999, which rapidly swept across North America to the West Coast, north into southern Canada and south into the Caribbean and Latin America (Reisen and Brault, 2007).

Not surprisingly, protection of a human volunteer should be greater when they are situated closer to the source of the repellent, an assumption confirmed by Müller et al. (2008a), who found that increasing the distance between candle and subject to 2 m and 3 m, greatly reduced repellency. This trial shows that a single misting unit, under optimal conditions, can reduce mosquitoes at a distance of 9 ft (2.74 m) by as much as 99% when used at 5.0-min intervals and even at 10-min intervals, biting pressure reduction is still a comfortable 80%. When used at 18 ft (5.49 m) from the subject, biting pressure is still reduced by \sim 95% during 5.0-min intervals, by \sim 82% at 7.5-min intervals, and by \sim 67% at 10-min intervals. Currently accepted guidelines require candles, coils, vaporizing mats or other such products to provide at least a 50% repellency rate to make a reliable claim that the product repels mosquitoes (Govere and Durrheim, 2007). Under the conditions in this study, the Terminix[®] ALLCLEAR® Mosquito Mister is well in excess of the 50% reduction required. Moreover, based on the area coverage results, and using these time intervals, the maximum protection distance of the Mosquito Mister probably exceeds 18 ft with a coverage area well in excess of the 300 ft² claimed by the manufacturer. Keeping in mind the importance of the time interval in which the units mist to achieve a good protection level, the added function "mist now" allows the consumer to reduce the biting pressure according to his needs.

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4

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E.E. Revay et al. / Acta Tropica xxx (2012) xxx-xxx

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