Control of phlebotomine sandflies in confined spaces using diffusible repellents and insecticides

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Roy Faiman
Laboratory of Malaria and Vector Research

Amer Al-Jawabreh
LRU-Jericho

Alon Warburg
Hebrew University of Jerusalem

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Control of phlebotomine sandflies in confined spaces using diffusible repellents and insecticides

M. SIRAK-WIZEMAN[1], R. FAIMAN[1], A. AL-JAWABREH[2] and A. WARBURG[1]

[1]Department of Parasitology, Kuvin Center for the Study of Tropical and Infectious Diseases, Hebrew University-Hadassah Medical School, Jerusalem, Israel and [2]Leishmaniasis Research Unit-Jericho, Palestine. Al-Quds University affiliated

Abstract. The control of phlebotomine sandflies (Diptera: Psychodidae), the vectors of leishmaniasis, is directed mostly against adults as larvae develop in unknown or inaccessible habitats. In the current study we tested geraniol, a natural plant-derived product, as a space repellent and the synthetic pyrethroid prallethrin as a diffusible insecticide. Geraniol was dispersed in the air using diffusers with an electric fan and prallethrin was evaporated using electrically heated evaporators. Both substances were tested in inhabited bedrooms and in tents. Geraniol failed to effect significant reductions in the numbers of either *Phlebotomus papatasi* Scopoli in rooms or *Phlebotomus sergenti* Parrot in tents. In laboratory experiments, geraniol proved ineffective in preventing sandflies from feeding. By contrast, prallethrin was highly effective in reducing the number of sandflies in rooms as well as in tents. Exposure of sandflies to prallethrin in laboratory experiments caused 97% mortality rates. Both prallethrin and, to a lesser extent, geraniol reduced the number of *Culex* mosquitoes captured in tents. Electric liquid-vaporizers with 1.5% prallethrin are highly effective in protecting people from sandfly bites in confined spaces and may be useful in combating cutaneous leishmaniasis.

Key words. *Phlebotomus papatasi*, *Phlebotomus sergenti*, geraniol, insecticide control, mosquitoes, prallethrin, sandflies, Israel, Jericho.

Introduction

Phlebotomine sandflies transmit leishmaniases, a group of diseases that currently threaten 350 million people in 88 countries (Desjeux, 2001). The World Health Organization (WHO) estimates that over 2.3 million new leishmaniasis cases occur each year and that at least 12 million people are presently infected worldwide (WHO, 2007).

Cutaneous leishmaniasis (CL) in the Middle East is caused by two *Leishmania* (Trypanosomatidae: Kinetoplastida) species: *Leishmania major* Yakimoff & Schokhor and *Leishmania tropica* Wright. The most common reservoir species of *L. major* is the sand rat, *Psammomys obesus* Cretzschmar, and its vector is the sandfly *Phlebotomus papatasi* Scopoli (Schlein *et al.*, 1984; Wasserberg *et al.*, 2002). *Leishmania tropica*, transmitted by *Phlebotomus sergenti*, is the causative agent of anthroponotic urban CL from Turkey, which extends throughout Syria, Iraq and Afghanistan to Pakistan and India (Douba *et al.*, 1997; Tayeh *et al.*, 1997; Rowland *et al*, 1999; Reithinger *et al.*, 2003). Recent studies in Israel demonstrate that *L. tropica* can also exist as a zoonosis with a reservoir in rock hyraxes (*Procavia capensis* Pallas) (Jacobson *et al.*, 2003; Svobodova *et al.*, 2006).

Phlebotomus papatasi is highly anthropophilic (tends to bite humans) as well as endophilic (bites indoors), whereas the vector of zoonotic *L. tropica* in Israel, *P. sergenti*, is rather exophilic and largely refrains from indoor activity (Schnur *et al.*, 2004). Conversely, *P. sergenti* in urban CL centres, such as Sanliurfa in Turkey and Kabul in Afghanistan, are highly endophilic (Killick-Kendrick *et al.*, 1995; Volf *et al.*, 2002). Thus, people living in endemic areas in the Middle East are at constant risk of contracting CL, both outside and inside their homes.

Sandfly larvae, unlike larvae of mosquitoes, are not aquatic and for that reason may be found in large numbers, even in arid deserts. Indeed, phlebotomine sandflies are amongst the most prevalent blood-sucking insects affecting humans in deserts and semi-desert regions of North Africa, the Middle East and

Correspondence: Alon Warburg, Department of Parasitology, Kuvin Center for the Study of Tropical and Infectious Diseases, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel. Tel.: + 1 972 2 675 7080; Fax: + 1 972 2 675 7425; E-mail: warburg@cc.huji.ac.il

parts of Asia. An average of 25–30 sandflies were collected in houses situated close to the periphery of a village in the Judean Desert east of Jerusalem (Schnur *et al.*, 2004). According to one report from Tallil Air Force Base in Iraq, up to 1000 sandflies per trap per night were collected in CDC miniature light traps placed in the base and about 10 flies per trap were collected inside tents (Burkett *et al.*, 2007; Coleman *et al.*, 2007; Dalton, 2008).

The control of phlebotomine sandflies is notoriously problematic because the breeding sites of their immature stages are mostly unknown and usually inaccessible. Therefore, most control efforts are aimed at adults. Depending on application techniques, timing and target species, sandflies are known to be highly susceptible to insecticides (Alexander *et al.*, 1995a; Alexander & Maroli, 2003; Wilamowski & Pener, 2003; Orshan *et al.*, 2006) and in certain areas effective control has been achieved as a side-effect of malaria control programmes (Vioukov, 1987). Residual formulations of DDT have also been used expressly to control sandflies (Hertig & Fisher, 1945; Hertig & Fairchild, 1948; Hertig, 1949). The synthetic pyrethroid deltamethrin has been used against sandflies in Bolivia (Le Pont *et al.*, 1989) and Brazil (Bermudez *et al.*, 1991; Marcondes & Nascimento, 1993). In other countries where sandfly vectors are endophilic, control of leishmaniasis has traditionally been based on residual insecticide spraying in houses, with significant effectiveness (Alexander *et al.*, 1995a; Vieira & Coelho, 1998; Alten *et al.*, 2003). Other studies have tested the efficacy of insecticide-impregnated textiles, such as curtains, bednets or bed covers, with varying degrees of success (Alexander *et al.*, 1995a, 1995b; Basimike & Mutinga, 1995; Elnaiem *et al.*, 1999; Kroeger *et al.*, 2002; Courtenay *et al.*, 2007). Environmental modification, involving the total eradication of rodents, destruction of burrow systems and spraying of herbicides to kill their food plants, has been demonstrably effective in controlling CL caused by *L. major* in foci in the Asian republics of the former USSR and in Tunisia (Vioukov, 1987; WHO, 1990). Several studies have shown that use of insecticide-impregnated collars on domestic dogs can reduce the numbers of biting sandflies and lower the incidence of infantile visceral leishmaniasis (Maroli *et al.*, 2001; Gavgani *et al.*, 2002; Maroli & Khoury, 2004). An extensive review of sandfly control methods and approaches has been published recently (Alexander & Maroli, 2003).

Geraniol ($C_{10}H_{18}O$), a plant-derived alcohol, is considered completely safe for use and appears on the US Environmental Protection Agency's (EPA) list of exempt substances. Geraniol has been demonstrated to be effective in repelling mosquitoes and apparently possesses insecticidal properties (Wirtz *et al.*, 1980; Xue *et al.*, 2003; Barnard & Xue, 2004; Omolo *et al.*, 2004). Geraniol-based products are available commercially in several countries. In the current study we tested whether geraniol prevents sandflies from entering confined spaces and whether it inhibits their biting (i.e. whether it is space-repellent).

Prallethrin ($C_{19}H_{24}O_3$) is a synthetic, non-carcinogenic pyrethroid categorized by the EPA as being moderately hazardous (WHO, 2004). Prallethrin is licensed for indoor use against mosquitoes and houseflies in most European countries, the U.S.A. and Japan (Matsunaga *et al.*, 1987). There have also been sporadic reports demonstrating the efficacy of prallethrin against sandflies (Kishore *et al.*, 2006). In the current study we evaluated the efficacy of prallethrin as a diffusible insecticide against sandflies biting inside rooms and tents.

Materials and methods

Study sites

The town of Ma'ale Adumim (population 32 000) is situated approximately 10 km east of Jerusalem in the Judean Desert (31°47′ N, 35°18′ E, altitude 400 m above sea level [a.s.l.]). Annual rainfall averages 200–400 mm, summers are hot and winters are mild (Goldreich, 1998). The study was conducted in peripheral houses of a northern neighbourhood built on a ridge flanked by gorges. The gorges are inhabited by rock hyraxes (*Procavia capensis*), the probable reservoir hosts of *Leishmania tropica* (Svobodova *et al.*, 2006), which cause CL in residents of that neighbourhood (A. Warburg, unpublished data, 2004). A preliminary survey using mouth aspirators identified bedrooms consistently infested with sandflies.

Additional studies were performed in the Palestinian town of Jericho, situated in the Central Jordan Valley some 15 km north of the Dead Sea (31°51′ N, 35°27′ E, at − 300 m a.s.l.). The climate of Jericho is extremely hot and rainfall averages 150–200 mm annually. The houses selected for the study were in the neighbourhoods of the Aqbat Jabr refugee camp, Wadi Quelt, Jericho City and the village of Duke.

Collection of sandflies

Nocturnally active sandflies were collected using CDC traps (J. W. Hock, Gainesville, FL), baited or un-baited with CO_2. Sandflies resting in rooms were collected using a mouth aspirator (Alexander, 2000). Aspirator collections of resting sandflies were always conducted by the same two-person team using flashlights during the late afternoons. Heads and terminalia of representative samples of sandflies were mounted and identified to species level using taxonomic keys (Artemiev, 1980; Lewis, 1982).

Geraniol experiments

Geraniol diffusers (model MZ-102; Intermatic, Inc., Spring Grove, IL) in experimental bedrooms and tents were connected to mains electricity supplies via a timer switch set to operate between 19.00 hours and 07.00 hours. The geraniol containers (FASST Products, New York, NY) were replaced every 72 h according to the manufacturer's instructions.

In the town of Ma'ale Adumim, three houses comprising seven rooms were selected for the experimental group and four houses comprising 11 rooms for the control group. Baseline levels were determined by collecting sandflies in all rooms in the late afternoons, using aspirators. Data were collected on six evenings over a period of 5 weeks. Thereafter, geraniol diffusers were introduced into the experimental rooms and sandflies were collected during a further nine visits over an additional 8 weeks. For experiments conducted in tents, four two-man tents (2.10 × 1.65 × 1.10 m, volume ~1.5 m^3) were placed in the gardens of the houses in which the experiments described above were conducted. The tents were placed approximately 15 m apart and 10 m from the houses. Each tent was fitted with a controlled gas outlet (500 mL/min) attached to a compressed CO_2 cylinder and five CDC light traps were suspended inside the tent with their openings ~50 cm above ground. A geraniol diffuser was placed on the ground in the centre of the experimental tents ~60 cm diagonally from the opening of the traps. Each experiment comprised two experimental tents and two control tents. The experiment was designed as a 2 × 2 Latin square, repeated four times and the locations of the experimental and control tents were alternated each time. *Culex pipiens* (L.) (Diptera: Culicidae) mosquitoes captured in traps inside tents were also counted.

Geraniol was also tested in the Palestinian town of Jericho in bedrooms where sandfly numbers were markedly higher than those recorded in Ma'ale Adumim. Experimental and control rooms were selected in the same house, and sandflies were monitored in rooms with CDC light traps instead of mouth aspirators over 6 nights.

A laboratory bioassay was used to test the effects of geraniol on the blood-feeding behaviour of wild-caught *P. papatasi*. The sandflies were kept in the insectary (75–80% RH, 26 ± 1 °C with access to 30% sugar solution) for a 24-h acclimatization period, confined in cylindrical mesh cages (30 cm diameter, 15 cm high), with 25–30 sandflies per cage. Cages were placed in experimental rooms (30–35 m^3), either 25 cm or 250 cm from the geraniol diffuser. After 60 min, anaesthetized chickens were placed underneath the cages in contact with the mesh and flies were allowed to feed on the exposed skin under the wing for 30 min. To determine engorgement status, flies were anaesthetized using CO_2 and females were scored as fed or unfed.

Prallethrin

Thermal evaporators designed for indoor use (Vape Magic™, Gauber Inc., Bologne; Italy) were operated through timer switches nightly at 20.00–04.00 hours. Liquid 1.5% prallethrin refill bottles were replaced as necessary every 20–25 nights. Study locations consisted of five control rooms in two houses and 13 experimental rooms in four houses. Baseline data were collected over 6 weeks (nine bi-weekly collections). Liquid 1.5% prallethrin evaporators were introduced to the experimental rooms and plugged into mains electricity via timer switches. The experiment was conducted for an additional 4 weeks with collections on 7 nights.

Experiments in tents were conducted as described for geraniol above, except that prallethrin evaporators replaced geraniol diffusers. Each experiment comprised two experimental tents and two control tents and the experiment was repeated four times with the locations of the experimental and control tents changed each time. *Culex pipiens* mosquitoes captured in traps inside tents were also counted.

Statistical methods

Arithmetic means were compared by Wilcoxon signed rank test; Wilcoxon–Mann–Whitney *U*-test was used, disregarding assumptions of homogeneity of variance or distribution, when datasets were not normally distributed. In order to control for putative effects of site, night or treatment, three-way ANOVA test was used to analyse the 2 × 2 Latin square design in tent experiments. The effects of geraniol on the percentages of blood-feeding flies and those of prallethrin on sandfly mortality were analysed by logistic regression (Adams, 2002; Dytham, 2003). All statistical analyses were carried out in SPSS Version 13 (SPSS Inc., Chicago, IL), and Microsoft® Office EXCEL 2003.

Results

The experiments described in the following sections were conducted during the summer seasons of 2006–07. In all, 974 sandflies were collected by aspirator inside bedrooms and 254 were identified. Of these, 250 (98.4%) were *P. papatasi* (77.3% females, 22.7% males) and four (1.6%) were *P. sergenti* (all female). Inverse proportions of species were collected in tents using CO_2-baited CDC traps; of the 3857 sandflies collected, 122 were dissected, mounted and identified. Of these, 90.2% were *P. sergenti*, 4.9% were *P. papatasi* and the rest were *Phlebotomus syriacus*, *Phlebotomus tobbi* and *Sergentomyia* spp.

Geraniol in bedrooms

A preliminary survey of houses in the Mitzpeh Nevo neighbourhood of Ma'ale Adumim using mouth aspirators identified bedrooms that were consistently infested with sandflies. There were fluctuations in the numbers of sandflies captured throughout the experiment. There was no significant difference between the numbers of sandflies collected in experimental rooms and those collected in control bedrooms after the introduction of geraniol diffusers into experimental rooms ($P > 0.05$, Wilcoxon–Mann–Whitney rank test; Fig. 1A). Residents of the houses who slept in experimental rooms did not feel any reduction in biting burden and did not report any improvement.

In Jericho, where experimental and control rooms were selected in the same house, there was also no significant difference between experimental bedrooms in which geraniol diffusers operated for 12 h every night and control rooms without any intervention ($P > 0.05$, Wilcoxon signed rank test; Fig. 2).

Geraniol in two-man tents

In the two-man tents placed in the gardens of the experimental houses in Ma'ale Adumim, the geraniol diffuser had no effect on the numbers of sandflies caught in CDC traps; there was no significant difference between experimental and control tents (Fig. 3A; $P > 0.05$, $F = 0.02$, d.f. $= 1$, three-way ANOVA). Effects of tent position and night were significant ($P < 0.05$,

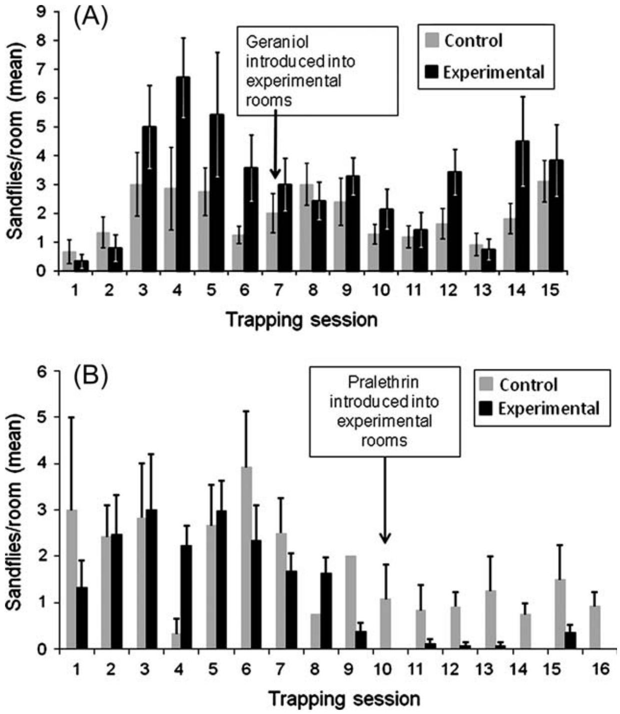

Fig. 1. Mean catch of sandflies (± standard error) in occupied bedrooms in Ma'ale Adumim. (A) Mean catch in rooms treated with geraniol as a space repellent. MZ-102 geraniol diffusers were introduced into seven experimental rooms and sandflies were collected from these and 11 control rooms weekly for six pre-treatment collections and nine post-treatment collections. No demonstrable effect was noted ($P > 0.05$, Mann–Whitney rank test). (B) Mean catch in rooms treated with prallethrin as a diffusible insecticide against sandflies. Evaporators with liquid prallethrin (1.5%) were introduced into 13 experimental bedrooms and sandflies were collected from these and five control rooms bi-weekly for nine pre-treatment collections and seven post-treatment collections. The difference between experimental and control rooms was highly significant ($P < 0.01$, Mann–Whitney rank test). See text for details.

$F = 6.56$, d.f. $= 3$, and $P < 0.01$, $F = 14.57$, d.f. $= 3$, respectively). A similar experiment was conducted using only incandescent light without CO_2. Numbers of sandflies attracted to the tents were much lower, but there was no demonstrable effect of geraniol (Fig. 3B; $F = 0.32$, d.f. $= 1$, $P > 0.05$, three-way ANOVA).

Effect of geraniol on blood-feeding of sandflies

Sandflies collected in experimental bedrooms and those collected in control bedrooms were visually scored to determine their blood-feeding rates. Surprisingly, flies collected in control rooms were less frequently engorged than flies collected in experimental rooms where they were exposed to geraniol vapour throughout the night (78.7% [$n = 61$] and 90.5% [$n = 84$], respectively). To test this further, a bioassay was conducted in the laboratory with wild-caught *P. papatasi*. The percentage of flies

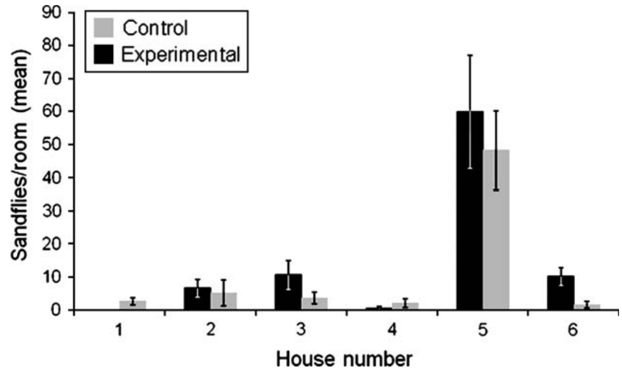

Fig. 2. Mean catch of sandflies (± standard error) in occupied bedrooms in Jericho. Geraniol was tested as a space repellent against sandflies. MZ-102 geraniol diffusers were introduced into experimental rooms and CDC light traps were placed 30–50 cm above them. Sandflies were collected by CDC light traps during 6 nights over a period of 5 weeks. No demonstrable effect was noted ($P > 0.05$, Wilcoxon signed rank test). See text for details.

that had taken a bloodmeal in cages placed 25 cm from the diffuser was somewhat reduced compared with the control (42 ± 3% and 54 ± 3.2%, respectively). However, flies in cages placed farther away (250 cm) from the diffuser fed more readily than those in control rooms (70 ± 8.1% and 54 ± 3.2%, respectively). Neither difference was statistically significant ($P > 0.05$, logistic regression).

Geraniol rate of evaporation and composition

The rate of evaporation of geraniol from the cartridge was measured by the continuous operation of four MZ-102 diffusers in a closed room (19 ± 1 °C). Two diffusers were operated at the manufacturer's recommended voltage of 3 V and two at 6 V. The geraniol cartridges were weighed before the beginning of the experiment and every 24 h during the experiment. The weight of the cartridge with the dry sponge was deducted from every measurement. Rates of evaporation were steady throughout the 30 days of the experiment, averaging 4.5 mg/h and 6.1 mg/h at 3 V and 6 V, respectively. The initial weight of the geraniol was about 14.5 g and > 10 g was left in the cartridge, even after 1 month of continuous operation. Analysis of the contents of the cartridge by gas chromatography/mass spectroscopy (GC/MS) verified the contents as > 99% geraniol.

Prallethrin in bedrooms

Experiments were conducted in the neighbourhood of Ma'ale Adumim in which the experiments with geraniol had been conducted previously, albeit not in the same houses. Following the introduction of prallethrin, the number of sandflies in experimental rooms dropped by ~95%. Although absolute numbers in the control rooms also dropped as a result of seasonal fluctuations, the difference between experimental and control rooms

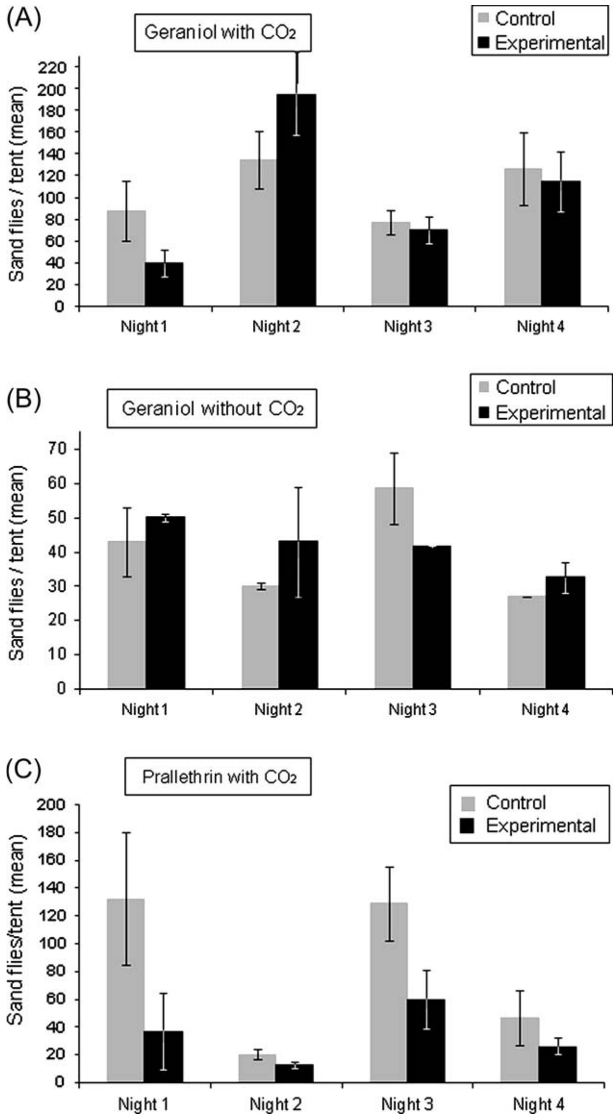

Fig. 3. Mean catch of sandflies (± standard error) per tent in two-man tents. (A) Tents with geraniol diffusers as a space repellent, with CO_2. (B) Tents with geraniol diffusers using light alone to attract flies to the tents. No demonstrable effect was noted ($P > 0.05$, three-way ANOVA). (C) Tents with prallethrin evaporators using CO_2 to attract flies to the tents. A highly significant decrease was noted (three-way ANOVA, $P < 0.01$). Five CDC light traps were used in each tent. See text for details.

remained highly significant (Fig. 1B; $P < 0.05$, Mann–Whitney rank test). Importantly, the residents of experimental bedrooms reported a significant improvement, with no sandfly bites.

Prallethrin in two-man tents

The experimental setup comprised four tents placed in gardens with five CO_2-baited CDC light traps as for the geraniol experiment, except that a prallethrin evaporator was placed on the ground in the centre of the experimental tents. Results showed a highly significant 59% decrease in numbers of sandflies in experimental compared with control tents (Fig. 3C; three-way ANOVA, $P < 0.01$, $F = 20.23$, d.f. = 1). Here again the effects of tent position and experimental night were highly significant ($P < 0.05$, $F = 7.72$, d.f. = 3 and $P < 0.01$, $F = 12.29$, d.f. = 3, respectively).

Effect of prallethrin on mortality of sandflies

The mortality of sandflies trapped in experimental tents was 97%, compared with 49.5% in sandflies from control tents, a highly significant difference (logistic regression, $P < 0.001$). Mortality was measured in the laboratory approximately 2–3 h after collecting the traps.

The mortality rates of sandflies exposed to prallethrin were also evaluated in the laboratory. The prallethrin evaporators were switched on for 30 min in a small room and the wild-caught sandflies, confined in mesh cages, were placed 250 cm from the evaporators. Sandflies were exposed to prallethrin vapour for 60 min. Thereafter, flies were maintained in the insectary (at 80–90% RH, 27 ± 1 °C), with access to 30% sugar solution. Mortality was scored 24 h later. The experiment was repeated three times. Results demonstrated a significant insecticidal effect, with sandfly mortality rates reaching 93.8 ± 6.2% in the experimental groups, compared with 23.1 ± 2.4% in the control groups (logistic regression, $P < 0.03$).

Effect of geraniol and prallethrin on Culex mosquitoes in tents

While conducting the experiments in tents, *Culex* spp. mosquitoes were also collected and counted. Interestingly, a significant reduction (39%) in the numbers of mosquitoes in tents with geraniol was noted compared with numbers in control tents (Fig. 4A; three-way ANOVA, $P < 0.05$, $F = 8.51$, d.f. = 1). A much more significant reduction was noted when 1.5% prallethrin was vaporized in the experimental tents; a highly significant decrease in numbers of mosquitoes (86.7%) in experimental tents was noted compared with numbers in control tents (Fig. 4B; three-way ANOVA, $P < 0.001$, $F = 55.06$, d.f. = 1). Almost all (>95%) the mosquitoes in traps placed in the experimental tents were dead by morning when traps were retrieved.

Discussion

The experiments reported in this study were designed to test the effects of geraniol and prallethrin on sandfly activity in enclosed spaces such as bedrooms and tents under semi-natural conditions. Unfortunately, experiments in inhabited homes are subject to many confounding factors. People may open or shut their windows, use insecticidal sprays, operate air conditioners, take vacations or simply disallow samplers to enter on a particular evening. Moreover, sandflies resting in rooms frequently select the darkest, hardest-to-find spots (e.g. behind cupboards or framed pictures), making it difficult to obtain adequate samples. Despite these limitations, we chose to work in bedrooms

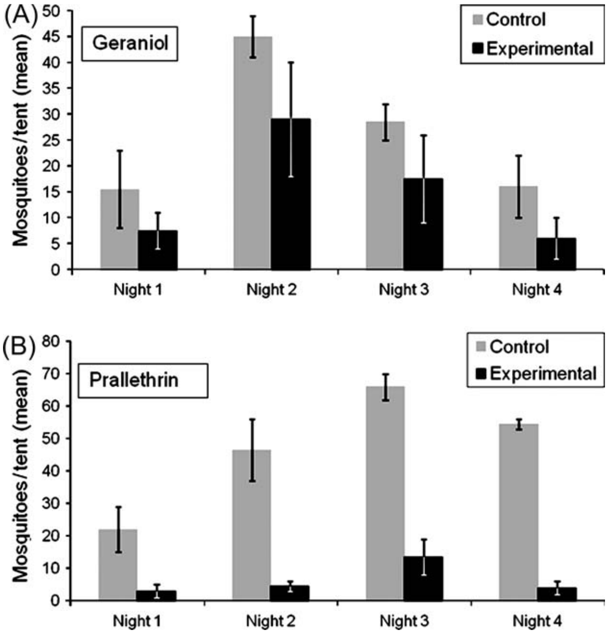

Fig. 4. Effect of geraniol and prallethrin on the presence of *Culex* mosquitoes in tents. (A) Mean catch of mosquitoes (± standard error) collected in tents treated with geraniol were significantly lower than the numbers collected in control tents (three-way ANOVA, $P < 0.05$). (B) Prallethrin caused a highly significant reduction in the number of mosquitoes captured in experimental tents compared with control tents (three-way ANOVA, $P < 0.001$).

because their conditions were exactly those we were intending to study and thus results were immediately relevant. Geraniol was selected because it is a safe natural product that can be used as a topical repellent (Wirtz *et al.*, 1980; Xue *et al.*, 2003; Barnard & Xue, 2004; Omolo *et al.*, 2004). When results using geraniol as a space repellent in rooms proved unsatisfactory (Fig. 1A), we decided to validate the methodology using prallethrin, a pyrethroid with proven efficacy against different household pests, including sandflies (Kishore *et al.*, 2006).

Experiments in tents were undertaken the following sandfly season, as the numbers of flies in bedrooms were lower than initially anticipated and after it became clear that the efficiency of geraniol diffusers was unsatisfactory in bedrooms. Therefore, in order to obtain larger numbers of sandflies and achieve higher concentrations of geraniol, small tents were selected and sandflies were sampled using CDC traps placed very close (<40 cm) to the geraniol diffusers. An added benefit to the experiments in tents was that a different sandfly species could be tested; *P. papatasi* were common in bedrooms, but mostly *P. sergenti* were found in tents.

Overall, geraniol was shown to be unsatisfactory as a space repellent against sandflies in bedrooms and in tents under four different experimental conditions: in occupied bedrooms with low numbers of *P. papatasi* sandflies in Ma'ale Adumim (Fig. 1A); in occupied bedrooms infested with high numbers of *P. papatasi* sandflies in Jericho (Fig. 2), and in tents infested with large numbers of *P. sergenti* sandflies, with or without CO_2 as an attractant (Fig. 3A, B). Moreover, at lower concentrations, geraniol even appeared to increase the rates of sandfly engorgement, although not significantly so. The geraniol evaporation rates provided by the MZ-102 diffusers were rather low (4–6 mg/h of geraniol, depending on voltage) and may have contributed to the failure to repel sandflies in rooms. However, even when using the MZ-102 diffusers in small tents with CDC light traps right next to them, sandflies were not repelled (Fig. 3A–C), whereas, under the same conditions, a significant reduction of 39% was noted in numbers of *Culex* mosquitoes (Fig. 4A). Moreover, experiments in the laboratory showed that sandflies readily took bloodmeals even when they were placed only 25 cm from a diffuser.

In apparent conflict with our results, recently published studies have described high rates of efficiency of geraniol in protecting human volunteers from sandfly bites. In these studies, a 5% geraniol candle burning 1 m from a volunteer sitting outside reduced mosquito bites by 56% and sandfly bites by 62% (Muller *et al.*, 2008a). Similarly, 5% geraniol candles burning indoors provided 85.4% protection against mosquitoes and 79.7% protection against sandfly bites (Muller *et al.*, 2008b). The experimental setup of the experiments described was different from ours. The burning of candles causes air currents and diffusion by heat and may somehow enhance the repellency of geraniol towards sandflies. We used cold diffusers. Furthermore, our experiments were conducted for entire nights (~10 h), whereas those of Muller *et al.* (2008a, 2008b) lasted only 1–3 hours. In any case, the conflicting results warrant further studies comparing diffusers and candles under a variety of experimental setups in order to elucidate the usefulness of this essential oil for combating sandflies and the diseases they transmit.

Most published reports on the use of diffusible pyrethroids as space repellents/insecticides are based on the responses of mosquitoes (Fales, 1968; Chadwick, 1970; Chadwick & Lord, 1977; Yap *et al.*, 1990; Amalraj *et al.*, 1992, 1996; Mosha, 1992). However, several studies on sandflies in which repellent effects were not distinguishable from insecticidal effects have been published (Alten *et al.*, 2003; Kishore *et al.*, 2006). In our study, prallethrin provided highly satisfactory results, effecting reductions of 95% and 59% in *P. papatasi* in bedrooms and *P. sergenti* in tents, respectively. Moreover, exposure of sandflies to prallethrin resulted in 94% mortality. Prallethrin was also effective against *Culex* in tents (Fig. 4B). The calculated rate of evaporation of 1.5% prallethrin solution was approximately 135 μL/h, emitting ~2 mg/h of the active ingredient. This rate is comparable with that calculated for pure geraniol above, confirming the much higher efficacy of prallethrin.

In conclusion, prallethrin emitted by thermal electric vaporizers was shown to be effective in reducing sandfly biting inside rooms and in reducing the numbers of sandflies in tents. Thus, diffusible prallethrin can be used inside closed spaces as a means for controlling sandflies and reducing the risk of leishmaniasis in endemic areas. As with any pest control strategy, usage of prallethrin should form only one approach within a more general, integrated management strategy to curtail biting sandflies.

Acknowledgements

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