Field Evaluation of an Area Repellent System (Thermacell) Against *Phlebotomus papatasi* (Diptera: Psychodidae) and *Ochlerotatus caspius* (Diptera: Culicidae) in Sanlıurfa Province, Turkey

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ABSTRACT A field evaluation of a new area repellent system, Thermacell Mosquito Repellent (TMR, *cis-trans* allethrin), was conducted against phlebotomine sand flies and mosquitoes in Cunpolat village, Sanlıurfa Province, in southeastern Anatolia, Turkey, an area historically endemic for cutaneous leishmaniasis and high sand fly populations. The objective of this study was to determine the efficacy, duration of protection, and spatial characteristics of protection (downwind distance from point of release), of the TMR. Twelve adult volunteers (nine in the treatment and three controls) made collections from ankle to knee for 25 min every hour from 2100 to 0300 on six consecutive nights in August 2002. Treatment consisted of placing a TMR unit at the middle of the village and then placing human bait collectors at 2.3, 4.6, and 7.6 m away from the repellent unit. Results from the field tests showed highly significant protection provided by the TMR from attack by *Phlebotomus papatasi* (Scopoli) ($P < 0.001$) and *Ochlerotatus caspius* (Pallas) ($P < 0.001$) for up to 4 h postapplication. In the six nights that tests were done, a total of 949 sand flies and 1095 mosquitoes were collected from the untreated control sites. Only 86 sand flies and 83 mosquitoes were collected at all distances from the places treated with TMR. These results indicated that reduction in sand fly and mosquito biting rates in treated groups ranged from 87.5 to 97.7% (mean protection 92%) and 90.2–97.4% (mean protection 93%), respectively. The percentage reduction values were maintained above 90.0% for 6 h of the assessment period. Overall, the number of bites by the cutaneous leishmaniasis vector *Ph. papatasi* and also *Oc. caspius* was reduced >11-fold and 13-fold, respectively, by the TMR.

KEY WORDS *Phlebotomus papatasi*, *Ochlerotatus caspius*, *cis*/*trans* allethrin, area repellent, Thermacell

TURKEY IS THE LAST country in the temperate climate zone on the edge of the European continent in which certain vector-borne diseases are prevalent at endemic and occasionally epidemic proportions. Both visceral and cutaneous leishmaniasis are important vector-borne diseases in Turkey. The parasite is transmitted by the phlebotomine sand flies, *Phlebotomus papatasi* (Scopoli) and *Phlebotomus sergenti* (Parrot), especially in southeastern region of Anatolia (Volf et al. 2002). Sanlıurfa is the most important province of the region in terms of not only its vector species but also nuisance mosquitoes. Certain characteristics of the province, such as favorable climatic conditions, microhabitats, and also large-scale agricultural activities, allow for large population outbreaks of nuisance mosquito species. Thirty-eight of 50 mosquito species known in Turkey are known to occur in this province (Ramsdale et al. 2001). *Ochlerotatus caspius* (Pallas), which breeds in the cotton fields in the province, is a major nuisance species.

Control of sand flies and nuisance mosquitoes in Turkey is often a by-product of malaria control and specific measures against these insects are usually directed by the staff of antimalaria programs (Anonymous 1990). Insecticides and their conventional application methods usually play an important role in controlling sand flies and mosquitoes, especially in domestic and peridomestic situations and where people congregate in rural areas. Recently, the programs that have been used for malaria control over the last

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40 yr have encountered a number of serious problems such as low community participation, lack of trained manpower, and reservation among the people about the effects of spraying (Alten and Caglar 2002).

Today, alternative sustainable technologies usable by the people themselves are necessary for the control of vector-borne disease. Protection from arthropod bites is best achieved by avoiding infested habitats, wearing protective clothing, and using insect repellent (Harbach et al. 1990, Curtis 1992, Fradin 2001a, b). In many circumstances, applying repellent to the skin or to the space may be the only feasible way to protect against insect bites (Gupta and Rutledge 1991, Perich et al.1995, Rutledge et al. 1996, Govere et al. 2001). Given that a single bite from an infected arthropod can result in transmission of the pathogen to the individual bitten, it is important to know which repellent products can be relied on to provide predictable and prolonged protection from bites (Fradin and Day 2002). Personal protection by repellent is also an inexpensive and practical means of reducing the biting of phlebotomine sand flies, mosquitoes, and other arthropods, thus aiding in reducing the level of discomfort and/or the preventing of arthropod-borne disease transmission (Harbach et al.1990, Gupta and Rutledge 1994). Despite the broad spectrum effectiveness and improvements in traditional topical repellents, the threat of arthropod-borne disease is so grave as to demand continuing efforts to develop and evaluate innovative repellent systems. Space repellent, also referred to as area repellents, are generally applied to a limited area and are designed to reduce or eliminate arthropod biting in the treated area (Wirtz et al. 1981). Field evaluations of arthropod space repellents against natural populations (Wirtz et al. 1981, Rutledge et al. 1991) are required because the behavioral response of "wild populations" of insects has been found to differ from laboratory-reared populations.

The objectives of this study were to determine efficacy, duration of protection, and spatial characteristics of protection (downwind distance from point of release) compared with an untreated control of a space repellent system, Thermacell Mosquito Repellent (TMR) tested against the phlebotomine sand fly *Ph. papatasi* and the nuisance mosquito species *Oc. caspius* in the village of Cunpolat in Sanlıurfa Province, southeastern Anatolia of Turkey.

Materials and Methods

Study Area. The study was conducted in the locality of Cunpolat village (population: 480 people), latitude 36°49′ N, longitude 38°55′ E at an altitude 310 m, in Sanlıurfa Province, SE Anatolia, Turkey. The village is located in the Harran plain, which is known as the primary cotton production area in Turkey. This area has had a historical problem with malaria and cutaneous leishmaniasis, and high populations of phlebotomine sand flies and anopheline mosquitoes. In addition, people living in this area have been bothered by annoying attacks by culicinae mosquitoes such as *Oc. caspius* in both the daytime and nighttime.

Space Repellent System. The Thermacell Mosquito Repellent (TMR) (Schawbêl, Bedford, MA) system consists of a butane-fueled generator that produces heat on a metal surface. An inert pad impregnated with 513 mg of *cis*/*trans* allethrin insecticide is placed on the heating surface before starting the unit. The butane Thermacell that powers the TMR system is identical to cells used in portable hair curlers and other small heating units.

Study Design and Test Methods. A pilot study was conducted in the beginning to determine the optimum downwind coverage of the space repellent plume and for determining the location of the volenteers at the specified distances in 1–2 August 2002. At the same time, the landing rate was determined to standardize the collection time of the target insects at different time intervals.

The test involved three replicates of treatment and one untreated control with each replicate group 30 m apart. Tests were done for six nights, 3–8 August 2002. Twelve subjects (nine in the treatment places and three controls) were used in each experimental day. Treatment consisted of placing an area repellent system (TMR) in the middle of the village close to cotton fields and then placing human bait collectors at 2.3, 4.6, and 7.6 m away from the repellent unit (distance zero). The distance of the volunteer from the point of application (distance zero) of the space repellent was assigned randomly, based on a random number list which was assigned to subjects (S_1–S_{12}) the first day to obviate any bias caused by individual differences among test subjects and rotated on the subsequent days. In similar design, a TMR unit with a untreated pad was set at the untreated control place, which was ≈200 m apart from treated sites. At the beginning of each test, the volunteers grouped and received their assigned position in the test area. The volunteers then moved as a group into the test area, each picking up a set of six prelabeled capture cartons. To initiate a test, TMR units were turned on 15 min before first sampling time (time zero) and placed on the ground (distance zero). Efficacy was determined by human landing collections which consisted of the three persons at the above described distance from the units aspirating from the legs (ankle to knee) and arms all sand flies/mosquitoes landing on them. Starting time was selected previously to incorporate peak landing activity of the sand fly and mosquito species present in the study area. Collections were made for 25 min every hour for 6 h from 2100 to 0300, the major host-seeking activity period for *Ph. papatasi* and *Oc. caspius* in the study area. The allethrin-treated pads were changed at 4 h and the butane cartridge at 6 h. One principal investigator (PI) not included in the test operated an electric timer with alarm. A flash light was used to locate landing insects, as needed. At the end of each test period, the test participants were withdrawn from the test area and prepared for the next test period. All sand flies and mosquitoes collected were placed into labeled polyethylene glass and later killed and returned to the laboratory to be identified to species and numerated. Weather observations (air temperature,

Table 1. Average values of temperature, RH, and wind speed between 3 and 8 August 2002

	Temperature (°C)	RH (%)	Wind speed (m/s)	Direction
Mean	26.9 ± 1.7	34.5 ± 5.1	2.8 ± 0.4	SW
Minimum	22.3	19.8	2	SW
Maximum	32.2	50.5	4	SW

relative humidity, wind speed, and direction) were recorded every hour during the study period.

Significance of possible sources of error (among the various distances in the treatment area compared with untreated area from point of release of repellent) was assessed by one-way analysis of variance (ANOVA) (Zar 1996). A P value of <0.05 was considered statistically significant.

Results and Discussion

The average temperature (°C), RH (%), and wind speed (m/s) values for the 6-d test period are shown in Table 1. No significant differences were found among these values between treatment places or between days [ANOVA; temperature/RH/wind speed: $(F_{0.05(3.92)})$ F = 2.00/ $(F_{0.05(3.116)})$ F = 0.18/ $(F_{0.05\ (3.92)})$ F = 0.48]. Wind speed values measured at the study area were minimal (2–4 m/s) but enough to establish down wind drift from repellent release.

Results from the field tests showed highly significant protection was provided by the area repellent system (TMR) from *Ph. papatasi* and also *Oc. caspius* attack (Table 2). There were highly significant differences in number of both insects collected between sites treated with the TMR and the untreated control site for all comparisons (*Ph. papatasi*, F = 364.90, P < 0.001; *Oc. caspius*, F = 150.38, P < 0.001). On the six consecutive nights in August 2002 that tests were done, a total of 949 *Ph. papatasi* and 1,095 *Oc. caspius* females were collected from the untreated control sites. In contrast, collections from all three sites treated with the TMR yielded a total of only 86 *Ph. papatasi* and 83 *Oc. caspius* females biting subjects at all distances. These results indicated that an average reduction in sand fly and mosquito biting rates in the treated sites was 92 and 93%, respectively.

Results in Table 2 indicated that repellent efficacy of the TMR against *Ph. papatasi* and *Oc. caspius* in the treated sites ranged from 87.5 to 97.7% and 90.2 to 97.4%, respectively, depending on distances. However, highly significant differences were found among reduction rates of target organisms in different distances (*Ph. papatasi*, F = 41.91, P < 0.001; *Oc. caspius*, F = 19.13, P < 0.001; Table 2). The highest protection rate form the TMR was found at 2.3 m against *Ph. papatasi* (97.7%) and *Oc. caspius* (97.4%). Multiple factors play a part in determining how effective any repellent will be. These factors include the species of the biting organisms, biochemical attractiveness of biting arthropods, and the ambient temperature, RH, and wind speed (Muirhead-Thomson 1951, Maibach et al. 1966, Golenda et al. 1999, Fradin 2001a, b). Although the distance for performance of the area repellent system is an important factor, results from this study showed that the repellent activity of the TMR, even at 7.6 m, gave significant protection against *Ph. papatasi* (87.5%) and *Oc. caspius* (90.2%). The protection period and protection rates of the area repellent system in accordance with different time intervals are shown in Fig. 1.

According to Fradin and Day (2002), the density of organisms in the immediate surroundings, their activity level and periods are other important factors determining the protection rate of any repellent. In a prior study (Alten and Caglar 2001), the major host-seeking activity period for *Ph. papatasi* and *Oc. caspius* was found to be 2200–2400 and 2100–2300 h, respectively, in Sanlıurfa Province. In this study, there was no statistically significant difference between different time intervals of the study period (0–6 h) in terms of number of collected insects in the treated groups (*Ph. papatasi*, $F_{0.05}$, 2.17; F = 0.76; *Oc. caspius*, $F_{0.05}$, 2.17; F = 0.66). However, there were highly significant differences in the number of insects collected in the untreated control groups (*Ph. papatasi*, F = 8.33, P < 0.001; *Oc. caspius*, F = 42.39, P < 0.001). Neither *Ph. papatasi* nor *Oc. caspius* appeared to be more active at the first all nocturnal phase of the study period. In contrast to the findings of Fradin and Day (2002), the differences mentioned above had no negative effect on protection ability of the area repellent system between different collection times (Fig. 1). The TMR unit provided similar protection for the different time periods after application against both insects (*Ph. papatasi*, $F_{0.05}$, 2.17; F = 0.61; *Oc. caspius*, $F_{0.05}$, 2.17; F = 0.66). When the results were evaluated independent

Table 2. Number of *Ph. papatasi* and *Oc. caspius* females collected from human landing collections and repellent efficacy of the area repellent system (Thermacell) against both species

	Distance (meters)	*Phlebotomus papatasi*				*Ochlerotetus casplus*			
		4-h period		6-h period		4-h period		6-h period	
		No. of adults	Protection rate (%)	No. of adults	Protection rate (%)	No. of adults	Protection rate (%)	No. of adults	Protection rate (%)
Treatment	2.3	4	97.7	6	97.7	4	97.1	8	97.4
	4.6	19	92.5	24	92.6	20	92.5	27	93
	7.6	44	87.5	56	87.5	37	89.3	48	90.2
Control	2.3	208		257		259		297	
	4.6	243		299		297		355	
	7.6	302		393		364		443	

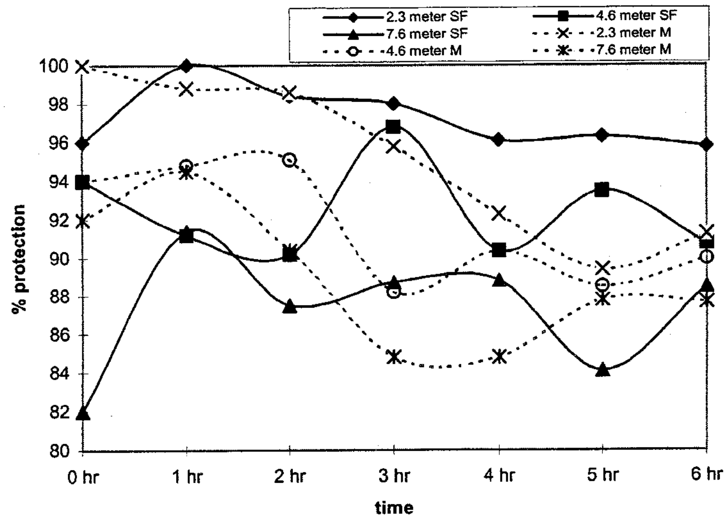

Fig. 1. Protection from bites as it relates to time after treatment with the area repellent system when tested against *Ph. papatasi* (SF) and *Oc. caspius* (M). ◆, 2.3 m SF; ■, 4.6 m SF; ▲, 7.6 m SF; x, 2.3 mM; ●, 4.6 mM; *, 7.6 mM.

from the distance factors, the protection rates were found at the range of 88.5–94% (the highest rate at the third hour after application) for *Ph. papatasi* and 88.4–94.6% (the highest rate between 0–1 h after application) for *Oc. caspius* in terms of collection times. Between time intervals, the highest protection rate (100%) was determined for the first hour for *Ph. papatasi* and at 0 to 1-h interval at 2.3 m (Fig. 1). The reduction in sand fly and mosquito biting rates in treated groups ranged from 82 to 100% and 84.8 to 100%, respectively, at different collection times depending on distances. The area repellent system (TMR) provided complete protection with no landing or biting against both insects during the first hour post-treatment. Throughout the next 4 h of test period, the TMR provided high repellency against both insects.

In conclusion, the results of our study show that the TMR gives significant levels of protection against phlebotomine sand flies and mosquitoes when used in the described application methods in conditions of southeastern Anatolia region of Turkey. Overall, the number of bites by the cutaneous leishmaniasis vector *Ph. papatasi* and also *Oc. caspius* were reduced >11-fold and 13-fold, respectively, with the TMR. Development of more efficacious repellents and/or self-protection methods against blood-feeding arthropod disease vectors is an important and challenging task (Klun and Debboun 2000). From this point of view, the TMR system is one potentially good alternative protection against arthropod bites that might transmit disease in southeastern part and also tourist regions of Turkey.

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