Journal of the American Mosquito Control Association, 17(1):23–27, 2001

SUSCEPTIBILITY OF SAND FLIES TO SELECTED INSECTICIDES IN NORTH AFRICA AND THE MIDDLE EAST[1,2]

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ABSTRACT. The purpose of this study was to determine the baseline susceptibility of 4 species of phlebotomine sand flies from North Africa and the Middle East to various insecticides. Susceptibility was determined using the World Health Organization test kits for measuring resistance in mosquitoes exposed to insecticide-impregnated papers. Fifty, 90, and 99% lethal doses were calculated for bendiocarb, cyfluthrin, DDT, malathion, permethrin, and resmethrin on *Phlebotomus bergeroti, P. langeroni, P. papatasi,* and *P. sergenti.* The least toxic insecticide to all species was DDT, followed by malathion and permethrin in order of increasing toxicity. Cyfluthrin was the most toxic to *P. langeroni* and *P. papatasi,* followed by resmethrin and bendiocarb in order of decreasing toxicity. Resmethrin exhibited the highest toxicity to *P. bergeroti* followed by cyfluthrin and bendiocarb, whereas bendiocarb was most toxic to *P. sergenti,* followed by cyfluthrin and resmethrin in order of decreasing toxicity. An attempt was made to obtain data for deltamethrin, but close response data were insufficient to determine regression lines for this insecticide on these species. However, analysis of preliminary data indicated that deltamethrin is highly toxic to these sand flies.

KEY WORDS *Phlebotomus,* insecticides, bioassay, toxicity, lethal dose

INTRODUCTION

In North Africa and the Middle East, sand flies of the genus *Phlebotomus* are important vectors of cutaneous leishmaniasis caused by *Leishmania major* and *L. tropica* (Killick-Kendrick 1990). Members of the Multinational Force and Observers, a peace-keeping force located in the Sinai region of eastern Egypt, have suffered well-documented outbreaks of this disease (Mansour et al. 1989, Fryauff et al. 1993). In addition, a before and after serosurvey for U.S. military personnel assigned to this force in 1994–95 (N = 308) showed a prevalence rate for sand fly fever virus (Naples ~ 43.8% and Sicilian ~ 3.8%) (Graham, unpublished data). In recent years, a visceralizing form of *L. tropica* emerged in the Persian Gulf, with cases occurring among U.S. military personnel serving in Desert Storm and Desert Shield (U.S. Department of Defense 1991, Magill et al. 1993).

To date, insecticides remain the primary tool for controlling sand fly populations. However, in general, relatively little information is available on insecticide susceptibility or resistance for sand flies. For *Phlebotomus papatasi* (Scopoli), the majority of the recorded insecticide susceptibility studies have been with DDT. The 1st was conducted on sand flies from Egypt and the Sudan. These populations were found to be susceptible to both DDT and dieldrin (Schmidt and Schmidt 1969). The 1st report of DDT, as well as dieldrin, resistance in the populations of *P. papatasi* and *P. argentipes* (Annandale and Brunetti) occurred during a kala-azar outbreak in North Bihar State (India) in 1978 and was based on World Health Organization (WHO) susceptibility testing (Kaul et al. 1978). Rahman et al. (1982) reported DDT resistance in areas around Delhi (India) and Mukhopadhyay et al. (1987) and Saxena (1987) found similar resistance patterns among *P. papatasi* in different areas, Bihar State (India). These latter 2 reports of resistance were based on control failures using DDT as a residual spray to control sand flies during another kala-azar outbreak. As recent as 1993, DDT- and dieldrin-resistant *P. papatasi* were discovered in the Panchahal District of Gujarat State (India) (Thapar et al. 1993). In the New World, Mazzarri et al. (1997) reported that *Lutzomyia longipalpis* (Lutz and Neiva) sand flies in Venezuela showed low levels of resistance for fenitrothion, pirimiphos methyl, and permethrin, whereas deltamethrin, lambdacyhalothrin, propoxur, and malathion were still effective. In Brazil, DeSilans et al. (1998), testing only cypermethrin, observed that it was effective against sand flies.

Insecticide susceptibility and resistance tests conducted on *P. papatasi,* or other phlebotomines endemic to the North Africa and Middle East regions, are very few. Penner and Wilamovsky (1987) found that *P. papatasi* specimens collected from the Jordan Valley of Israel were susceptible to both DDT and permethrin. One study conducted in the Islamic Republic of Iran in 1988 found *P. papatasi* suscep-

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Table 1. Response of *Phlebotomus* spp. sand flies to bendiocarb.[1]

Species	*n*	Slope ± SE	χ^2	LD_{50} mg AI/ml (95% CI)	LD_{90} mg AI/ml (95% CI)	LD_{99} mg AI/ml (95% CI)
P. bergeroti	604	4.60 ± 0.45	22.77	0.055 (0.050–0.060)	0.105 (0.093–0.122)	0.177 (0.147–0.230)
P. langeroni	570	2.63 ± 0.48	37.25	0.125 (0.083–0.157)	0.383 (0.283–0.762)	0.957 (0.551–3.850)
P. papatasi	610	4.50 ± 0.43	20.92	0.053 (0.048–0.058)	0.103 (0.092–0.120)	0.175 (0.146–0.229)
P. sergenti	570	4.82 ± 0.47	16.69	0.051 (0.047–0.056)	0.095 (0.085–0.110)	0.156 (0.131–0.200)

[1] LD_{50}, median lethal dose; LD_{90}, 90% lethal dose; LD_{99}, 99% lethal dose.

tible to DDT and dieldrin (Sayedi-Rashti et al. 1988). Another study in a different locale in Iran found *P. papatasi* susceptible to DDT (Sayedi-Rashti et al. 1992). More recently, Aboul-Ela et al. (1993) reported that *P. papatasi* collected from the greater Cairo area in Egypt were susceptible to DDT, benzene hexachloride, malathion, permethrin, and propoxur. No records are available of insecticide susceptibility or resistance tests being performed on *Phlebotomus bergeroti* (Parrot), *P. langeroni* (Nitzulescu), or *P. sergenti* (Parrot). These latter species are primary or potential vectors of human disease. Likewise, diagnostic dose data for various insecticides against any of the species in this region of the world are absent. As defined by the WHO, the diagnostic dose is a doubling of the lowest dose of that chemical agent being evaluated that consistently gives a complete kill in all tests used to establish the baseline (WHO 1986a, 1986b). This dosage figure is used to monitor resistance, which is defined by WHO as greater than or equal to 20% survival of test insects after exposure to the diagnostic dose of the chemical for 1 h (Davidson and Zahar 1973).

The purpose of this study was to determine the baseline susceptibilities and calculate diagnostic doses for a variety of insecticides against a selection of medically important sand fly species endemic to North Africa and the Middle East. The sand fly species examined were *P. bergeroti, P. papatasi, P. langeroni,* and *P. sergenti.*

MATERIALS AND METHODS

All sand flies used in this study were reared and maintained at U.S. Naval Medical Research Unit Number Three, Cairo, Egypt, following the methods of Modi and Tesh (1983). The *P. bergeroti* colony was established from females collected in the Republic of Djibouti in 1989. The *P. papatasi* and *P. sergenti* were colonized from specimens collected in North and South Sinai, Egypt, in 1989 and 1996, respectively. Ain Shams University, Cairo, Egypt, colonized the *P. langeroni* from specimens collected in Alexandria, Egypt, in 1990. All flies were reared in an insectary maintained at 27°C, 90% relative humidity, and a light:dark cycle of 12:12 h. Susceptibility tests were conducted in a separate room providing similar insectary conditions. Breeding stock for all of these colonies was collected from areas with little to no known insecticide use for sand fly control and was considered to be susceptible.

Susceptibility tests were performed using WHO test kits for measuring insecticide resistance. Bioassays were done according to standard WHO methodology (WHO 1981), utilizing 7- to 10-day-old female sand flies that had been maintained in cages with males and provided with cotton pads soaked in a 30% sucrose and water solution. Insecticide-impregnated papers were used to expose the sand flies to the insecticides. These test papers were procured from the U.S. Army Center for Health Promotion and Preventive Medicine Command at Aberdeen Proving Ground, MD. The papers were treated with insecticides as described by Zeichner (1999). The following insecticides were screened: bendiocarb, cyfluthrin, DDT, malathion, permethrin, resmethrin, and deltamethrin.

Susceptibility tests were conducted by exposing 6 groups of 20–25 female sand flies to 5 different concentrations of each insecticide and 1 control. Before conducting the test, sand flies were kept in a holding chamber for 1 h to verify that the insects

Table 2. Response of *Phlebotomus* spp. sand flies to cyfluthrin.[1]

Species	*n*	Slope ± SE	χ^2	LD_{50} mg AI/ml (95% CI)	LD_{90} mg AI/ml (95% CI)	LD_{99} mg AI/ml (95% CI)
P. bergeroti	899	2.27 ± 0.17	113.9	0.042 (0.028–0.057)	0.155 (0.124–0.202)	0.446 (0.303–0.807)
P. langeroni	614	1.71 ± 0.13	37.84	0.036 (0.028–0.045)	0.202 (0.153–0.286)	0.823 (0.532–1.498)
P. papatasi	595	1.78 ± 0.13	129.5	0.038 (0.023–0.057)	0.200 (0.126–0.408)	0.774 (0.385–2.704)
P. sergenti	601	3.82 ± 0.48	15.10	0.052 (0.039–0.064)	0.113 (0.096–0.137)	0.212 (0.170–0.293)

[1] LD_{50}, median lethal dose; LD_{90}, 90% lethal dose; LD_{99}, 99% lethal dose.

Table 3. Response of *Phlebotomus* spp. sand flies to DDT.[1]

Species	*n*	Slope ± SE	χ^2	LD_{50} mg AI/ml (95% CI)	LD_{90} mg AI/ml (95% CI)	LD_{99} mg AI/ml (95% CI)
P. bergeroti	703	2.90 ± 0.27	28.58	1.104 (0.953–1.250)	3.052 (2.613–3.741)	6.992 (5.387–10.09)
P. langeroni	623	2.26 ± 0.26	34.72	1.884 (1.377–2.348)	6.970 (5.551–9.691)	20.25 (13.52–39.36)
P. papatasi	609	3.62 ± 0.39	24.70	0.943 (0.807–1.070)	2.130 (1.850–2.569)	4.139 (3.288–5.813)
P. sergenti	584	4.58 ± 0.45	13.87	3.288 (2.953–3.613)	6.266 (5.583–7.290)	10.60 (8.835–13.72)

[1] LD_{50}, median lethal dose; LD_{90}, 90% lethal dose; LD_{99}, 99% lethal dose.

were in good condition. Any dead or moribund insects were aspirated out before initiation of the test. Each test group and the control were introduced into the WHO test chamber. Test chambers were lined with either an insecticide-impregnated or control paper. Sand flies were transferred from the holding chamber to the test chamber via a small puff of air. Sand flies were held in vertically positioned treatment tubes for 1 h. They were then transferred to holding tubes containing cotton wads soaked in 30% sugar water. The tubes were kept covered with plastic sheets to stabilize humidity levels. Mortality was assessed at 24 h after exposure by counting the number of dead or moribund insects. Tests were replicated a minimum of 6 times for each insecticide and sand fly combination.

Statistical analyses were performed according to the methods of Robertson and Preisler (1992) using the Polo-PC (Probit or Logit Analysis for Personal Computers) program by LeOra Software, Berkeley, CA. This program analyzed the test data and generated linear regressions. Lethal dose (LD) statistics for LD_{50}, LD_{90}, and LD_{99} values were calculated and utilized to characterize the susceptibility of each sand fly species to the insecticides (Russell et al. 1977). The LC_{99} values were used to calculate the diagnostic doses. The diagnostic dose has frequently been determined by multiplying the dose required to provide 99.9% mortality times 2. However, the POLO-PC software only calculates LDs to whole integers, that is, LD_{99}. We calculated the diagnostic doses by multiplying the LD_{99}s by 2.

RESULTS AND DISCUSSION

The responses of all 4 species to the insecticides (Tables 1–6) were remarkably similar, considering the geographically divergent origins of the colonies. *Phlebotomus langeroni* and *P. papatasi* had the same order of relative susceptibilities to the chemicals. The 3 least toxic insecticides for all species were, in order of toxicity, permethrin, malathion, and DDT, with DDT being the least toxic. The LD_{50}s for all species were very similar in response to each of these chemicals. The responses to the other 3 chemicals (bendiocarb, cyfluthrin, and resmethrin) were not so uniform between species. For *P. langeroni* and *P. papatasi* the most toxic compound was bendiocarb, followed by resmethrin and cyfluthrin. For *P. bergeroti* the most toxic was bendiocarb, followed by cyfluthrin and resmethrin, whereas for *P. sergenti* the most toxic was resmethrin, followed by cyfluthrin and bendiocarb. The low doses needed to produce a 50% kill indicate that all chemicals, with the possible exception of DDT, are very toxic to these sand flies and could have value as control agents. All sand fly species seem to be much more susceptible to pyrethroids than to the other classes of insecticides.

The LD_{50} results obtained with DDT and *P. papatasi* (0.943 mg AI/ml) are very similar to the data reported by Penner and Wilamovsky (1987) (1.21 mg AI/ml). This is of note considering the great distance from the Sinai where our colony originated and the Jordan Valley of Israel where theirs was established. The baseline LD_{50} we report for *P. papatasi* and permethrin (0.363 mg AI/ml) is 3.6 times that reported by Penner and Wilamovsky (1987) (0.10 mg AI/ml). In addition, our LD_{50} data for DDT and *P. papatasi* were almost the same as those reported by Aboul-Ela et al. (1993). They reported an LD_{50} of 1.0 mg AI/ml and we report 0.943 mg AI/ml. Their results with *P. papatasi* and permethrin are widely different from ours. Using the same WHO methodology, they reported an LD_{50} of 0.008 mg AI/ml, whereas the value obtained in this study was 0.363 mg AI/ml. We cannot explain this disparity except to suggest that it is because

Table 4. Response of *Phlebotomus* spp. sand flies to malathion.[1]

Species	*n*	Slope ± SE	χ^2	LD_{50} mg AI/ml (95% CI)	LD_{90} mg AI/ml (95% CI)	LD_{99} mg AI/ml (95% CI)
P. bergeroti	477	4.74 ± 0.51	22.50	0.406 (0.350–0.452)	0.756 (0.683–0.863)	1.256 (1.061–1.622)
P. langeroni	490	3.55 ± 0.34	15.12	0.305 (0.266–0.339)	0.700 (0.625–0.810)	1.380 (1.132–1.829)
P papatasi	754	3.64 ± 0.36	67.50	0.548 (0.467–0.617)	1.231 (1.046–1.603)	2.383 (1.781–3.962)
P. sergenti	736	3.27 ± 0.28	121.1	0.499 (0.406–0.585)	1.231 (0.985–1.828)	2.568 (1.752–5.357)

[1] LD_{50}, median lethal dose; LD_{90}, 90% lethal dose; LD_{99}, 99% lethal dose.

Table 5. Response of *Phlebotomus* spp. sand flies to permethrin.[1]

Species	*n*	Slope ± SE	χ^2	LD_{50} mg AI/ml (95% CI)	LD_{90} mg AI/ml (95% CI)	LD_{99} mg AI/ml (95% CI)
P. bergeroti	614	4.20 ± 0.80	33.78	0.280 (0.217–0.316)	0.565 (0.487–0.789)	1.002 (0.738–2.125)
P. langeroni	626	7.94 ± 1.38	68.10	0.287 (0.205–0.323)	0.416 (0.377–0.513)	0.564 (0.474–0.980)
P. papatasi	910	5.35 ± 0.75	44.34	0.363 (0.335–0.386)	0.630 (0.564–0.762)	0.988 (0.806–1.419)
P. sergenti	616	3.68 ± 0.48	33.61	0.387 (0.346–0.426)	0.864 (0.717–1.194)	1.662 (1.200–2.990)

[1] LD_{50}, median lethal dose; LD_{90}, 90% lethal dose; LD_{99}, 99% lethal dose.

the flies came from widely separated geographic foci. The LD_{50} for malathion on *P. papatasi* was 0.548 mg AI/ml and Aboul-Ela et al. (1993) reported a similar value of 0.36 mg AI/ml.

We submit these data as baseline susceptibilities of these species for 6 of the 7 selected insecticides. These baselines can be used to compare the responses of field populations of sand flies that may have been exposed to insecticides. Using these baseline values and earlier guidance of WHO, the diagnostic doses for these chemicals can be set at 2 times the LD_{99} dosages listed in Tables 1–6 and insecticide resistance in field populations can be evaluated. Calculation of the LC_{99} for *P. langeroni* and resmethrin with a reliable confidence limit was not possible. The authors also attempted to test deltamethrin on all 4 species. Even at the lowest dosage available (0.01 mg AI/ml), the mortality was 55% and mortality was 100% at all higher concentrations starting at 0.04 mg AI/ml. This is a strong indication that deltamethrin is extremely toxic to these sand flies and could also be valuable as a potential control agent.

ACKNOWLEDGMENTS

Our sincere appreciation is extended to Fetouh Ali and Ahmed Fawzy from the Vector Biology Research Program at U.S. Naval Medical Research Unit No. 3, Cairo, Egypt, for their valuable technical assistance in maintaining the sand fly colonies throughout the study. This work was supported by the U.S. Naval Medical Research Center, Bethesda, MD, work unit 102A-M00101-BUX-3408. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government.

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Table 6. Response of *Phlebotomus* spp. sand flies to resmethrin.[1]

Species	*n*	Slope ± SE	χ^2	LD_{50} mg AI/ml (95% CI)	LD_{90} mg AI/ml (95% CI)	LD_{99} mg AI/ml (95% CI)
P. bergeroti	609	2.84 ± 0.25	14.31	0.024 (0.020–0.027)	0.067 (0.058–0.080)	0.156 (0.123–0.217)
P. langeroni	630	0.98 ± 0.17	19.45	0.123 (0.086–0.227)	2.519 (0.897–19.53)	NA
P. papatasi	608	2.48 ± 0.32	25.00	0.039 (0.031–0.046)	0.130 (0.106–0.176)	0.342 (0.236–0.633)
P. sergenti	918	5.91 ± 0.72	75.84	0.065 (0.057–0.072)	0.108 (0.096–0.129)	0.162 (0.133–0.232)

[1] LD_{50}, median lethal dose; LD_{90}, 90% lethal dose; LD_{99}, 99% lethal dose; NA, not available.

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