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A SURVEY OF BED-BUG RESISTANCE TO INSECTICIDES IN ISRAEL

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SYNOPSIS

Fourteen strains of bed-bugs collected from various places in Israel and one normally susceptible strain from the laboratories of J. R. Geigy S.A., Basle, Switzerland, were tested for their resistance to DDT and gamma-BHC. A very high degree of resistance to DDT was found in most of the strains, indicating that DDT-resistance is very widespread in Israel. A moderate to low resistance to gamma-BHC was observed in several strains.

Although the common bed-bug, *Cimex lectularius*, is not a disease vector, the annoyance caused by its feeding on man has led to the widespread application of various insecticides to control it. This, as well as its exposure to insecticides applied for other purposes, such as mosquito control, has gradually resulted in the development of bed-bug resistance to insecticides in a number of places. The extent of this resistance has been well summarized by Brown (1958).

Bed-bug resistance to insecticides was first noticed in Israel in 1951 (Levinson, 1953), when attempts to control an infestation in Tel Aviv failed even though concentrations of DDT as high as 2 to 3 g/m² were used. Control, however, was obtained at that time with a spray of gamma-BHC at 0.45 g/m². Later Cwilich, Mer & Meron (1957) reported on two strains of BHC-resistant bed-bugs, one in the Bet-Shaan valley and the other in Tiberias. By 1956, so many reports of the failure of DDT and occasionally BHC to control bed-bugs had been received that it was decided to carry out a broad survey of the extent of the resistance in order better to guide control operations.

The preliminary results of this survey were published recently (Gratz, 1958) and the following report presents the complete data. Fourteen local strains of bed-bugs were tested for resistance and compared with a " normal " or susceptible strain from the laboratories of J. R. Geigy S.A., Basle, Switzerland. Several other strains were tested, but not enough data were obtained for presentation in this paper. All strains were tested against

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varying concentrations of DDT and gamma-BHC. These two insecticides were chosen since their household use has been very widespread in Israel. DDT especially has been widely sprayed as part of the antimalaria campaign and both are common in the household insecticides which are found in almost every home. Dieldrin has only rarely been used in household control operations as its use in household insecticide formulations in Israel has not been permitted.

Methods and Materials

Each group of field-collected bed-bugs was brought into the laboratory and placed in small beakers with several folds of filter-paper within. The mouths of the beakers were covered with fine bolting-silk. The bugs were then offered a blood meal by placing the beakers mouth down on the shaven stomach skin of a rabbit. The first generation (F_1) bugs which developed from the eggs laid by the field-collected parent strains were isolated and held until mature, being fed before each moult on a rabbit; when mature, these bugs were tested for resistance. It was decided to use the F_1 rather than the field-collected bugs, in order to ensure uniformity of age and nutritive status in the test insects. Occasionally, for replicates, F_2 bugs were also used, and no loss of resistance was noted. Several preliminary tests showed no significant difference in the resistance or susceptibility of male and female bugs of uniform age and nutritive status, and thus no distinction was made between the sexes of the insects used in subsequent tests. All bugs were offered a rabbit-blood meal 24 hours before testing and only those that fed were used.

Acetone solutions of DDT, ranging from 0.025% to 5%, were prepared and pipetted into 20 mm × 100 mm Petri dishes in quantities of 0.65 ml per dish; this amount, in relation to the area of the bottom of the Petri dish, would give a deposit equivalent to 1 g/m² with the 1% solution, the deposits at other concentrations being correspondingly higher or lower. Gamma-BHC solutions were prepared at concentrations of from 0.005% to 1% and, again, 0.65 ml of each concentration was added to the Petri dishes to give a deposit equivalent to 1 g/m² with the 1% solution. Four Petri dishes were prepared for each concentration, the acetone solutions being swirled about in the dish while drying in order to obtain an even coverage of the bottom of the dish. In the case of the DDT, as soon as the acetone had evaporated from the Petri dishes, five highly resistant *Musca domestica vicina* were placed in each covered dish and left there for 24 hours. This was done in order to ensure crystallization of the minute drops of DDT by contact with the tarsi of the flies moving about in the dish. Beakers containing the bugs to be tested were placed in a refrigerator at 4°C for a few minutes and then emptied into large Petri dishes floating in bowls of crushed ice. The bugs

which had fed were selected for testing and transferred to the Petri dishes containing the insecticides. This method of handling reduced the mortality due to physical damage and made possible a more speedy selection of the bugs to be tested.

The bed-bugs were held for 24 hours in the Petri dishes containing the insecticides. Ten bugs were tested in each dish and four dishes were used for each concentration. Forty bugs in four Petri dishes of ten bugs each were used as controls for each series of tests. All tests were carried out at 28°C.

Observations of morbidity and mortality were made after 30 minutes, 1 hour, 2 hours, 3 hours and 24 hours, and the number of dead and affected (i.e., lying on back and/or moribund) bugs noted. The data presented in the tables are for the 24-hour check only. Varying numbers of replicates were performed for different concentrations of insecticides and different strains of bugs; the number of replicates is given in the tables above the number of dead and affected bugs for that concentration. The mortality among the controls was generally low or zero and when, for some reason, a number of the controls died, the series of tests in question was rejected and repeated.

Results and Discussion

DDT (see Table 1)

The bugs from the strain collected in Nazareth proved to be more susceptible to DDT than the Geigy " normal " strain. These bugs came from an area which has not been sprayed in the antimalaria campaign and in which few household insecticides are used. At the other extreme were the highly resistant strains from Ein Hashlosha (a collective agricultural settlement in the southern part of the country) and Mandelbaum Gate (Jerusalem), where large quantities of DDT have been used and, in the case of Ein Hashlosha, gamma-BHC as well. The 14 strains were taken from all parts of the country, from Galilee in the north to Beersheba in the south, and the results thus indicate a very widespread resistance to DDT. The ED_{50} (50% effective dose—i.e., the concentration at which 50% of the bugs were either dead or moribund) for the Nazareth strain was only 0.025%, whereas for 6 of the 14 strains it could not be reached even at a concentration of 5%. These 6 strains, then, were at least 200 times more resistant to DDT than the Nazareth strain, and the next most resistant strain, that from Ramleh, was at least 160 times as resistant.

Investigating a reported case of bed-bug resistance in Teheran, C. Mofidi & B. Samimi exposed *C. lectularius* for one hour to filter-papers impregnated with 5% DDT in oil and observed a mortality of 38%; and after one hour's exposure to 5% DDT dust at a concentration of 10 mg/cm², the mortality was 50% (unpublished report—Institute of Parasitology and Malariology, Teheran, 1956). These results may be compared with the zero or very low

TABLE 1. AVERAGE NUMBER OF DEAD AND AFFECTED BED-BUGS AT DIFFERENT CONCENTRATIONS OF DDT*

Strain	Concentration of DDT											
	0.025%	0.05%	0.075%	0.1 %	0.25%	0.5%	0.75%	1%	2%	3%	4%	5%
1. Nazareth	*2* 17(2)	*2* 23(11)		*2* 20(18)	*2* 17(23)	*2* 15(22)		*3* 23(15)	*1* 30(4)	*2* 33(14)	*1* 18(16)	*1* 24(13)
2. Geigy					*2* 28(4)	*3* 29(4)	*1* 24(9)	*5* 24(10)	*3* 25(13)	*5* 22(10)	*1* 27(12)	*1* 27(12)
3. Migdal			*1* 11(10)	*1* 16(13)	*2* 25(10)	*1* 27(5)	*1* 21(12)	*2* 17(17)	*1* 12(21)	*1* 12(25)	*1* 6(28)	*1* 12(21)
4. Rami								*1* 32(2)	*1* 27(7)	*1* 22(14)	*1* 21(14)	*1* 22(13)
5. Beersheba								*2* 14(13)	*2* 7(19)	*2* 11(18)	*2* 10(15)	*2* 16(17)
6. Brosh						*1* 14(12)		*2* 17(6)	*2* 11(8)	*2* 10(7)	*1* 15(12)	*1* 10(15)
7. Sakia								*3* 17(7)	*3* 18(6)	*3* 21(7)	*3* 23(6)	*3* 20(6)
8. Ramleh								*1* 15(2)	*1* 13(4)	*1* 15(4)	*1* 12(8)	*1* 10(6)
9. Bet Shemesh								*2* 11(3)		*2* 9(2)	*1* 6(1)	*1* 12(3)
10. Tel Hashomer								*3* 7(2)	*2* 6(1)	*3* 11(1)	*2* 11(1)	*3* 10(4)
11. Kiryat Schmoneh								*1* 12(1)		*1* 10(5)		*1* 9(1)
12. Shavuot-Am		*1* 3(0)	*1* 5(0)	*1* 4(0)	*1* 6(0)			*1* 2(0)	*2* 1(0)	*1* 0(0)	*2* 2(1)	
13. Natanya						*1* 2(1)		*1* 3(2)		*1* 2(8)		
14. Mandelbaum Gate, Jerusalem								*2* 1(2)	*2* 0(1)	*2* 1(1)	*2* 0(0)	*2* 2(2)
15. Ein Hashlosha								*1* 0(2)	*1* 0(0)	*1* 1(1)	*2* 0(1)	*2* 0(2)

* The figures in italics denote the number of replicates performed.

mortalities observed among some strains after a 24-hour exposure to 5% DDT in the present series of tests. Busvine (1958) tested a number of colonies of bed-bugs from different places throughout the world, including a colony collected in Israel in 1954. When exposed to insecticide-impregnated papers, the bugs from Israel showed no mortality with 4% DDT. This indicates a level of resistance comparable to that of the strains showing high resistance in the tests discussed above; the different methods of testing must, however, be taken into account: Busvine's tests were done on impregnated papers and ours by direct exposure to insecticides on a glass surface.

TABLE 2. AVERAGE NUMBER OF DEAD AND AFFECTED BED-BUGS AT DIFFERENT CONCENTRATIONS OF GAMMA-BHC

Strain	Concentration of gamma-BHC								
	0.005 %	0.0075 %	0.01 %	0.025 %	0.035 %	0.05 %	0.065 %	0.075 %	0.1 %
1. Rami			*1* 7(33)	*1* 4(36)	*1* 4(36)	*1* 6(34)			
2. Kiryat Schmoneh			*1* 14(26)	*1* 4(36)	*1* 1(39)	*1* 1(39)			
3. Mandelbaum Gate, Jerusalem		*1* 14(23)	*2* 16(20)	*2* 0(40)	*2* 0(40)	*1* 0(40)	*1* 0(40)	*1* 0(40)	*1* 0(40)
4. Migdal	*1* 14(13)		*2* 15(18)	*2* 2(38)	*1* 2(38)				
5. Nazareth	*1* 9(2)	*1* 18(22)	*3* 17(16)	*2* 6(34)	*2* 1(39)	*1* 2(38)			
6. Ramleh			*2* 16(10)	*3* 10(30)	*2* 1(39)	*3* 3(37)	*3* 2(38)	*3* 1(39)	*3* 1(39)
7. Shavuot-Am			*2* 5(6)	*2* 6(34)	*2* 3(37)				
8. Brosh			*1* 16(8)	*1* 8(32)	*1* 6(34)	*1* 1(39)			
9. Sakia		*1* 18(0)	*3* 19(4)	*3* 24(14)	*3* 21(19)	*3* 12(28)	*3* 7(33)	*3* 5(35)	*2* 2(38)
10. Natanya			*2* 10(8)	*1* 19(9)	*1* 14(25)	*2* 7(33)		*1* 2(38)	
11. Geigy			*2* 10(2)	*2* 11(15)	*2* 11(25)	*2* 6(34)		*2* 4(36)	
12. Bet-Shemesh			*2* 7(2)	*2* 12(14)	*2* 10(29)	*2* 11(27)		*2* 7(33)	
13. Beersheba			*1* 2(3)	*1* 12(6)	*1* 13(26)	*1* 11(29)	*1* 12(28)	*1* 9(31)	*1* 4(36)
14. Tel Hashomer			*3* 11(0)	*3* 35(1)	*3* 19(12)	*3* 17(23)	*3* 19(21)	*3* 6(34)	
15. Ein Hashlosha			*2* 2(1)	*1* 1(8)	*2* 10(4)	*2* 11(22)		*1* 12(28)	

* The figures in italics denote the number of replicates performed.

Gamma-BHC (see Table 2)

Although resistance to gamma-BHC was not marked at the higher concentrations, it was observed in several of the strains at lower concentrations. Most of the strains, however, proved to be more susceptible to gamma-BHC than the Geigy " normal " strain. The most susceptible strain came from the large Arab village of Rami in western Galilee, where apart from its occasional use by some householder, neither BHC nor any related compound has ever been sprayed. The ED_{50} for the strain collected from the town of Migdal in the southern coastal plain, and which was tested at a lower con-

centration than that of the Rami strain, could not be reached even at 0.005%. The ED_{50} of the Ein Hashlosha strain was between 0.035% and 0.05%; this strain is therefore some ten times more resistant than the Migdal strain. The Ein Hashlosha strain was collected as a result of a complaint about the failure of a commercial pest-control operator to control the infestation at that settlement even by several sprayings with a BHC-based insecticide formulation. The Mandelbaum Gate strain, which was one of the most resistant to DDT, was one of the least resistant to gamma-BHC. In cases of infestations resistant to both DDT and gamma-BHC, control was obtained by the use of malathion.

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RÉSUMÉ

Cimex lectularius, la punaise des lits, n'est pas un vecteur de maladies, mais ses inconvénients sont tels qu'il est intéressant de connaître sa résistance aux insecticides, résistance qui s'est surtout développée depuis leur emploi dans d'autres buts, en particulier la lutte contre les moustiques. Les auteurs ont procédé, sur 14 souches de punaises provenant de différentes régions d'Israël et sur une souche témoin dite normale (sensible aux insecticides) à des essais de résistance à deux insecticides, le DDT et le HCH-gamma.

Les souches recueillies à Nazareth se sont montrées plus sensibles au DDT que la souche normale. Elles provenaient d'une région non touchée par la lutte antipaludique et dans laquelle peu d'insecticides domestiques étaient utilisés. Par contre, les souches provenant d'Ein Hashlosha et de Mandelbaum Gate, secteurs où de grandes quantités de DDT avaient été utilisées se sont montrées vraiment résistantes. La DE_{50} (50% de morts ou de moribonds) de DDT pour la souche de Nazareth était de 0,05% et pour six des 14 souches elle ne pouvait pas même être atteinte avec une concentration de 5%. La résistance au HCH-gamma était faible aux fortes concentrations de ce produit. La plupart des souches étaient plus sensibles que la souche dite normale. A Ein Hashlosha où le HCH-gamma avait déjà été utilisé, les punaises étaient dix fois plus résistantes que celles du secteur où aucun insecticide n'avait jamais été utilisé. On obtient de bons résultats avec le malathion quand il existe une résistance aux deux insecticides à la fois.

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