THE SUSCEPTIBILITY OF *CULEX PIPIENS MOLESTUS* FORSKAL IN ISRAEL TO SEVERAL INSECTICIDES IN 1958–1960 AND IN 1965

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INTRODUCTION

Culex pipiens molestus is the most widespread mosquito in Israel. It is prevalent during all months of the year and breeds in all kinds of water except brackish water. It is however most commonly found breeding in water highly polluted by sewage. The highest density of breeding of this mosquito has been observed in this country during the summer months —May to September, (Saliternik and Barkai, 1963).

In addition to being a great nuisance to humans it is a suspected vector of the West-Nile fever virus in Israel. Laboratory studies had demonstrated that W.N. virus is transferable by this mosquito in mice (Tahori *et al.*, 1955).

Control of *Culex pipiens molestus* in Israel is carried out mainly by attempts to prevent its breeding. Solar oil and malariol have been applied as common antilarval measures. Only in a few cases were residual formulations of insecticides used as larvicides for field trials.

Since 1949, spraying with DDT has been carried out in several regions of this country against adult anopheline mosquitos. Such spraying could also affect endophilic strains of culicine mosquitos in treated areas.

Various insecticides for household insect control are widespread in Israel. Most of the present aerosol formulations contain 0.2 percent lindane (Gamma BHC) combined with pyrethrins or their analogues. DDT has also been common in aerosol formulations. Since 1960 preparations of malathion and diazinon have been introduced for household insect control.

The spraying of DDT during the antimalarial campaign, and the widespread use of various insecticides for household control as well as for agricultural purposes (including insecticide spraying in cow-sheds and chicken coops), could have induced the development of resistance among local populations of *Culex pipiens molestus*.

The first survey to detect insecticide resistance among local populations of *Culex pipiens molestus* had been carried out by the ministry of health during 1958–1960. A second survey was carried out in 1965. The aim of this paper is to present and to discuss the information obtained in both surveys.

METHODS

COLLECTION OF MOSQUITOS. Larvae were collected in 1958–1960 and in 1965 from various rural and urban localities spread all over the country. Localities chosen for the 1965 survey were not necessarily the same as those chosen previously for the 1958–1960 survey. Most collections had been made from water streams contaminated with sewage and in a few cases from cesspits. Large numbers of fourth stage larvae, from each locality, were transferred on the day of collection to the laboratory. The mortality among the larvae that arrived at the laboratory did not exceed 5 percent.

INSECTICIDAL COMPOUNDS. Susceptibility levels of larvae to DDT, lindane (Gamma BHC), dieldrin, and malathion were tested during the 1958–1960 as well as during the 1965 survey. The 1965 survey included additional tests with diazinon and fenthion. W.H.O. test-kit solutions had been used for assessing susceptibility

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of larvae to the above mentioned compounds. W.H.O. papers impregnated with DDT (4 percent), dieldrin (4 percent) and malathion (3.2 percent), had been used to determine whether resistance to these compounds is detectable among adult mosquito populations.

ASSESSING SUSCEPTIBILITY LEVELS

LARVAE. Tests were performed using the W.H.O. method. Duplicates of 60 fourth stage larvae, from each locality, were tested for each concentration of an insecticide. Mortality rates were recorded after 24 hours of exposure under laboratory conditions at a temperature of 27±1° C. LC50 and LC90 values were determined from percentage mortality curves drawn on a logarithmic probability paper. Microscopic examinations of tested larvae were made at the end of each test and numbers of larvae other than *Culex pipiens molestus* that might have occasionally been involved in the tests were excluded.

ADULTS. Adult mosquitos were allowed to develop from larvae, untreated with insecticides, under laboratory conditions (temp. 27±1° C. and R.H. approximately 75 percent). Sugar-fed (5 percent sugar solution), 3–4-day-old females were submitted to tests. In the 1958–1960 survey, adult females were tested only for their resistance to DDT whereas in the 1965 survey, tests with dieldrin and malathion were also included. Single concentrations of 4 percent DDT, 4 percent dieldrin and 3.2 percent malathion (W.H.O. impregnated papers) were used to determine whether a population from a certain locality was resistant to the insecticide. An assumption was made that a quantal response of less than 50 percent mortality —24 hours after 1 hour exposure to an insecticide at the above mentioned concentration—would indicate resistance to the same insecticide in the tested population. Tests were carried out under the laboratory conditions already mentioned, using the W.H.O. plastic tubes. Each test was performed with 60 females (triplicates of 20) representing a population from a certain locality. Control experiments with sheets of clean paper lining the exposure tubes were made parallel to each test. If mortality was observed in the control experiment of a certain test, this test was not taken into consideration.

RESULTS

LARVAE. Table 1 shows values of the average percentage mortality (±s.e.m.) in each concentration of any tested insecticide. Each arithmetic average was calculated from corresponding values found for separate populations. Such an average represents, therefore, the quantal response of the "average population" of larvae, in this country, to a given concentration of an insecticide. Distribution ranges for LC50 and LC90 values of the various tested populations are presented in a separate column.

Figs. 1 and 2 show dosage mortality curves of the "average population" of larvae for chlorinated hydrocarbon and O-P compounds respectively. These curves were fixed according to the average percentage mortality values that are given in table 1. LC50 and LC90 values of the "average population" which are also presented in table 1 were derived from corresponding curves in figs. 1 and 2.

It can be seen from table 1 and figs. 1 and 2 that for the larval "average population" there is no considerable change of susceptibility levels to the chlorinated hydrocarbon insecticides tested, when the situation in 1965 is compared to that in 1958–1960. Reduced susceptibility to malathion was, however, demonstrable in 1965 in comparison to 1958–1960. The susceptibility to diazinon was found to be similar to that to malathion whereas higher susceptibility to fenthion had been observed.

Fig. 3 shows the resistance spectrum for five insecticides in various larval populations tested during the 1965 survey. Values of relative resistance are given at

TABLE 1.—Results of susceptibility tests with *Culex pipiens molestus* larvae during 1958–1960 and 1965 surveys.

Insecticide	Survey	No. localities tested	Average percentage mortality * (±S.E.M.) at various concentrations (p.p.m.)					LC_{50} (p.p.m.)		LC_{90} (p.p.m.)	
			0.0008	0.004	0.02	0.1	0.5	of "Average population"	Range (tested populations)	of "Average population"	Range (tested populations)
DDT	1958–60	21	..	14.2±3.9	23.2±4.9	53.7±6.2	91.3±2.7	0.09	0.004–0.4	0.39	0.02–>0.5
	1965	12	..	8 ±5.1	22 ±9	55 ±9	93.8±2.8	0.08	0.004–0.19	0.39	0.012–>0.5
Gamma–BHC	1958–60	20	..	14.9±4.6	36.5±5.2	42.2±5.7	74 ±3.9	0.14	0.02 –1.2	>0.5	0.14–>2.5
	1965	12	..	2.3±1.1	8.5±1.2	30.5±7.3	76 ±8.4	0.20	0.02 –0.5	>0.5	0.11–>1
Dieldrin	1958–60	15	..	10.5±2.8	31.4±5.7	61.3±6.5	92.3±3.4	0.055	0.01 –0.22	0.40	0.09–0.65
	1965	9	2.6±1.7	11.2±3.5	33.2±6.5	68.2±10.5	98.8±1.1	0.044	0.01 –0.14	0.22	0.07–0.14
Malathion	1958–60	17	14.9±4.9	36.5±7.6	86.2±5.4	99.2±0.7	100	0.0065	0.001–0.015	0.026	0.004–0.1
	1965	12	1.4±0.9	6.3±4.3	17.3±8.2	92.5±3.3	100	0.04	0.004–0.08	0.1	0.014–0.2
Diazinon	1958–60	..	..	..	..	..	..	..	..	..	..
	1965	12	2.6±0.9	5.6±3.2	20.6±8.3	83.6±8	100	0.043	0.006–0.16	0.12	0.03–0.35
Fenthion	1958–60	..	..	..	..	..	..	..	..	..	..
	1965	12	9.2±6.6	58.5±11	91.5±6.7	100	100	0.0033	0.0008–0.03	0.02	0.0016–0.045

* After 24 hours of exposure to a given concentration (W.H.O. solutions)

the LC_{50} as well as LC_{90} level for each population. The larval population collected in Beit-Haemek (a settlement in the western Galilee) had been found as the most susceptible one to all the tested insecticides and was therefore used as the reference susceptible population. The histograms in fig. 3 represent relative resistance levels of the larval "average population" in 1965. It must be noted that LC_{50} or LC_{90} values of an insecticide in the larval "average population" should not necessarily be equal to the arithmetic average of various LC_{50} or LC_{90} values obtained separately for the same insecticide in the tested populations.

ADULTS. Results of the adult mosquito tests are given in table 2. This table shows that resistance to DDT among adult female mosquitos had already been detected in the 1958–1960 survey. LC_{50} values of DDT and dieldrin would be higher than 4 percent in the 1965 survey, which indicates resistance to these insecticides in the adult "average population." Resistance to malathion had not been determined.

DISCUSSION

Susceptibility levels to chlorinated hydrocarbon insecticides in the larval "average population" had not been significantly

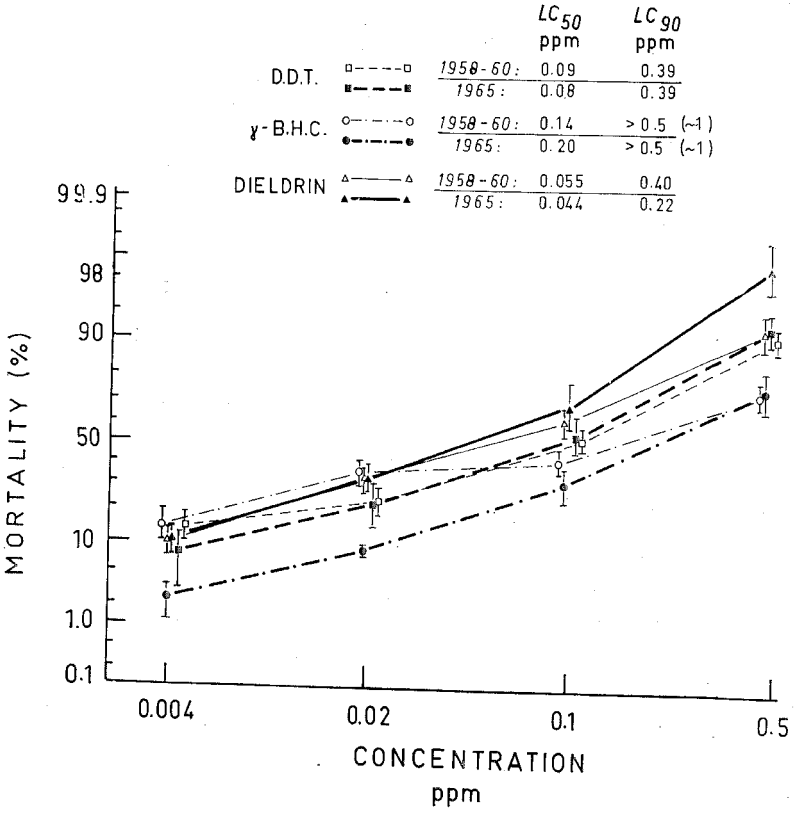


FIG. 1.—Dosage mortality curves for the "average population" of *Culex pipiens molestus* larvae in Israel, to three chlorinated hydrocarbon insecticides in 1958–1960 and in 1965 (according to data given in table 1).

TABLE 2.—Average percentage mortality (±S.E.M.) in *Culex pipiens molestus* adult populations, 24 hours after 1 hour exposure to indicated concentrations * of various insecticides, during 1958–1960 and 1965 surveys.

Year	DDT (4 percent)	Dieldrin (4 percent)	Malathion (3.2 percent)
1958–1960	28 ±6	..	..
1965	30.2±6.6	33.1±7.3	95.8±3.3

* W.H.O. impregnated papers.

changed from 1958–1960 to 1965. These susceptibility levels are not, however, considered as "normal." It has been shown (Davidson 1964) that *Culex pipiens molestus* and *Culex pipiens fatigans* approximate the same "normal" levels of susceptibility. Hamon and Mouchet (1965) indicated that LC100 for larvae of susceptible *Culex pipiens fatigans* populations must be equal or less than 0.07 p.p.m. of dieldrin and 0.36 p.p.m. of DDT when tested by the W.H.O. method. In the present study LC90 values of dieldrin

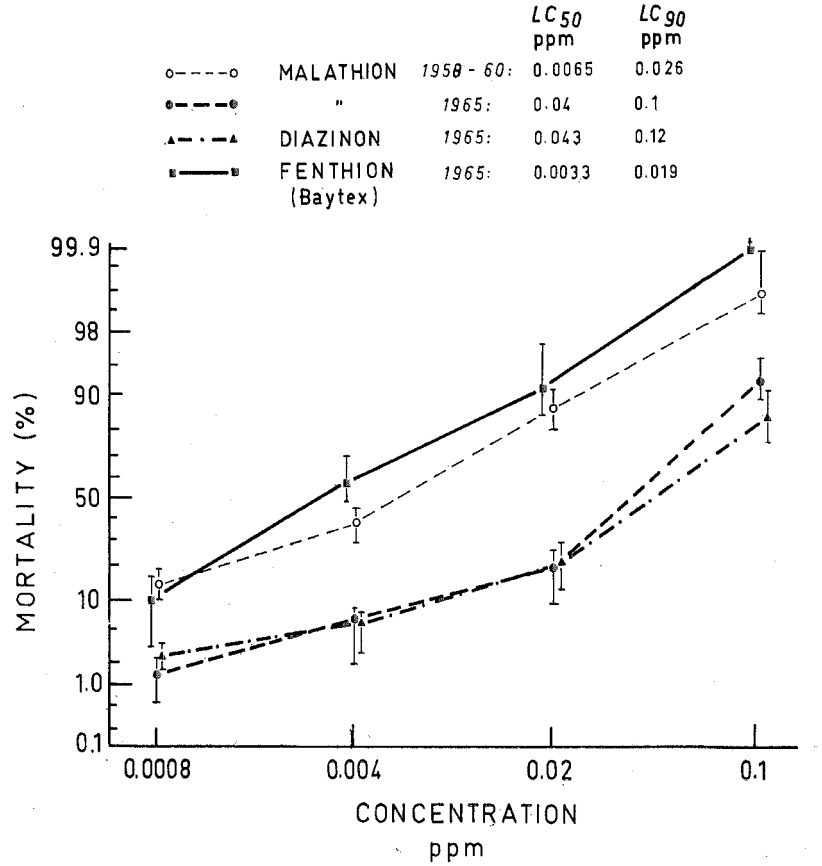
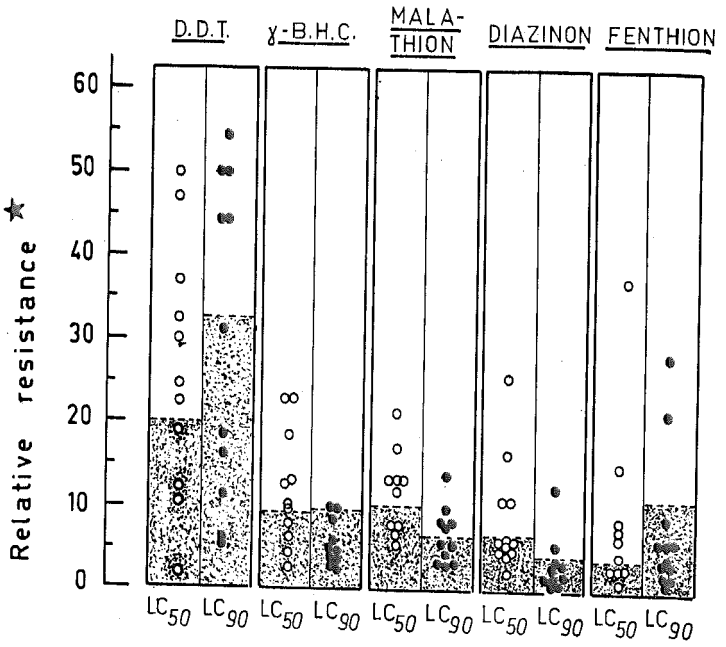


FIG. 2.—Dosage mortality curves for the "average population" of *Culex pipiens molestus* larvae in Israel to three O-P compounds (according to data given in table 1).



★ Relative resistance = Susceptibility level of a given strain / Susceptibility level of the most susceptible strain

FIG. 3.—The distribution of relative resistance to 5 insecticidal compounds among various populations of *Culex pipiens molestus* larvae in 1965, at LC50 and LC90 levels. Histograms represent relative resistance of the larval "average population." Reference strain used was that from Beit-Haemek (further details are given in the text).

and DDT as determined for the larval "average population" were found to be higher than the LC100 indicated by Hamon and Mouchet for susceptible populations of *Culex pipiens fatigans*. It would seem, therefore, that physiological resistance to DDT and dieldrin should be suspected according to these criteria. The W.H.O. criterion for determining resistance (Symes, Thompson and Busvine, 1962) indicates however that when an LC50 above 1 p.p.m. DDT is found for *Culex* spp. larvae, resistance should be suspected. A comparison with available data for susceptible populations of *Culex pipiens fatigans* (Hamon and Mouchet 1965) leads to a conclusion that the "average population" of *Culex pipiens molestus* larvae in Israel has shown reduced susceptibility to the tested chlorinated hydrocarbon compounds, but no resistance to these insecticides has been achieved.

Tahori (1961) using the W.H.O. method, had studied five different populations of *Culex pipiens molestus* in Israel for their susceptibility to DDT. LC50 values of four larval populations in his study were found to be at the same range given for DDT in Table 1 of this paper.

In one case, however, a highly resistant population was detected (LC50 above 10 p.p.m.). This resistant population was collected in a breeding place being regularly DDT-oiled, which is not common in this country as an antilarval measure. Nevertheless this finding proved that DDT resistance could be developed among local larval populations in the field, after treatment with the insecticide. A laboratory investigation (Barkai and Rosen 1964) demonstrated that larvae of *Culex pipiens molestus* from this country acquire genetical mechanisms for the development of resistance to DDT. An LC50 of 4.2 p.p.m. DDT was reached after a selection of fourth stage larvae, during 10 successive generations, through a constant concentration of 1 p.p.m. DDT, the initial LC50 of the population being 0.25 p.p.m.

Resistance of the adult "average population" to DDT and dieldrin, as demonstrated in the present study, is probably a consequence of the widespread use of these insecticides in agriculture as well as for household insect control. Residual spraying with dieldrin in public health operations has not been carried out in this country whereas DDT has been used during the antimalarial campaign in various regions. The possibility of cross resistance between DDT and dieldrin seems unlikely since, as observed for *Culex pipiens fatigans*, the genes that determine resistance to these insecticides are probably independent (Davidson, 1964).

The results obtained in this study with chlorinated hydrocarbon insecticides recall other observations on the phenomenon that resistance in populations of adult mosquitos does not necessarily imply resistance upon larvae of same populations (Hamon and Mouchet 1965. Burnett and Ash 1961).

In spite of the reduced susceptibility to malathion that had been observed in 1965 in comparison to 1958–1960, it may be concluded that the "average population" of *Culex pipiens molestus* larvae in Israel is still susceptible to the three tested O-P insecticides. The adult "average population" seems also to be susceptible to malathion.

An interesting phenomenon of reduced susceptibility to fenthion was observed during the 1965 survey. Three different populations from the region of Wadi-malcha were tested during this survey. In this wadi fenthion has been applied since 1963 as a larvicide in a stream of water contaminated by sewage (Barkai *et al.* 1964). Previous treatments with malathion and parathion as larvicides had also been applied in this stream (Saliternik *et al.* 1963). Samples of larvae had been collected during the 1965 survey from: 1) focus of treatment; 2) downstream, at a distance of 15 km from the focus of treatment; 3) downstream, at a distance of 50 km from the focus of treatment. LC50 values of fenthion in these three populations were the highest among the 12 populations tested for fenthion during 1965—being 0.03, 0.012 and 0.0065 p.p.m. for 1), 2), and 3) respectively. The most susceptible population tested in 1965 had an LC50 of 0.0008 p.p.m. fenthion. It was also interesting to notice that survivors to 3.2 percent malathion among the 12 different populations tested for determining resistance in adult mosquitos, were contributed only by the three populations from the region of Wadi-malcha.

SUMMARY

Surveys to detect insecticide resistance among local poulations of *Culex pipiens molestus* had been carried out in Israel during 1958–1960 and 1965.

During 1958–1960, larval populations were tested for their susceptibility to DDT, dieldrin, lindane and malathion, whereas adult female populations were tested only for DDT resistance. The 1965 survey included additional tests of larval populations with diazinon and fenthion and of adult female populations with dieldrin and malathion. W.H.O. methods were applied for susceptibility tests. Localities chosen for collecting mosquitos dur-

ing the 1965 survey were not necessarily the same as those chosen previously for the 1958–1960 survey. LC50 and LC90 of various tested insecticides were determined for separate larval populations and for the larval "average population" in both surveys. Reduced susceptibility to the tested chlorinated hydrocarbon insecticides had been determined for the larval "average population" during the 1958–1960 survey. No considerable changes were observed for its susceptibility levels to these insecticides in 1965.

This larval "average population" has, however, shown reduced susceptibility to malathion in 1965 compared to the situation in 1958–1960. The susceptibility level of the larval "average population" to fenthion, in 1965, was higher than that to malathion or diazinon. Resistance to DDT had been observed in the adult female "average population" during 1958–1960. A similar level of resistance to DDT and a dieldrin resistance in adult female "average population" had been observed during 1965. No resistance to malathion had been detected in that year among adult female populations.

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References

BARKAI, A., and ROSEN, P. 1964. Development of resistance in *Culex pipiens molestus* by larval exposure to a constant dosage of DDT. Riv. Parass. 25(3):217–224.

BARKAI, A., PICKEL, D., LIDROR, R., and SHUVAL, H. 1964. The treatment of a sewage stream near Jerusalem, with 50 percent fenthion emulsifiable concentrate for the control of *Culex pipiens molestus*. W.H.O./Vector control/74.

BURNETT, G. F., and ASH, L. H. 1961. The susceptibility to insecticides of disease-carrying mosquitos in Fiji. Bull. Wld. Hlth. Org. 24:547–555.

DAVIDSON, G. 1964. DDT-resistance and dieldrin-resistance in *Culex pipiens fatigans* Wiedemann. Ann. Trop. Med. Parasit. 58(2):180–188.

HAMON, J., and MOUCHET, J. 1965. Resistance to insecticides in *Culex pipiens fatigans* Wied. W.H.O./Vector control/151.

SALITERNIK, Z., and BARKAI, A. 1963. Mosquitos in Israel (Hebrew). Israeli Ministry of Health publication.

SALITERNIK, Z., ROSEN, P., and PICKEL, D. (1962). Organic phosphates for control of *Culex pipiens molestus* mosquitos near Jerusalem, Israel. Mosquito News 22(4):373–376.

SYMES, C. B., MUIRHEAD THOMPSON, R. C., and BUSVINE, J. R. Insect control in Public Health. Elsevier 1962.

TAHORI, A. S., STERK, V. V., and GOLDELUM, N. 1955. Studies on the dynamics of experimental transmission of West Nile virus by *Culex molestus*. Amer. J. Trop. Med. Hyg. 4(6):1015–1027.

TAHORI, A. S. 1961. A survey of *Culex molestus* larvae in Israel for DDT resistance. Bull. Wld. Hlth. Org. 24:674–675.