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TOXICITIES OF SEVERAL INSECTICIDES TO THE HOUSE FLY *MUSCA DOMESTICA* L. FROM DIFFERENT REGIONS IN JORDAN

Azzam Saleh[*] and Husein Elmosa [**]

ABTRACT

The LD_{50} of eight insecticides to adult female house flies *Musca domestica* L. were determined by topical application. A syringe-microburet was used to apply the insecticide solution to the mesonota of individual insects. Female flies 3-5 days old were used for testing. Age, sex, loci of application and post treatment temperature were standardized to obtain accurate and repeatable results. Toxicity data are recorded in micrograms toxicant per gram fly.

F1 adult flies tested in this work were the progeny of flies obtained from poultry farms in five different locations in Jordan, namely Amman, Central Jordan Valley (50 km west of Amman), Irbid (90 km north of Amman), Karak (120 km south of Amman) and Al-Dafyaneh (130 Km north east of Amman). For comparison, toxicities of the same insecticides were determined on a laboratory susceptible strain.

Results from Amman area indicated that the pyrethroid insecticides tested, i.e., lambda-cyhalothrin, deltamethrin, cypermethrin and cyfluthrin were the mosteffective insecticides with LD_{50} 1.27, 4.22, 7.08 and 7.37 µgm/gm fly respectively. These relatively small LD_{50}s for the respective insecticides indicate that the house fly is susceptible to these insecticides. Propoxur and malathion were the least effective with LD_{50} 4230.47 and 3493.30 µgm/gm fly respectively, which shows that the house fly in Amman area has developed resistance to these insecticides. The relatively small slope of log. dose probit lines and the large LD_{50} values for both malathion and propoxur indicate that the house fly in Amman area may develop still greater resistance for both materials.

Cyfluthrin, malathion and propoxur which represent the pyrethroid, organophosphorus and carbamate insecticides respectively, and which proved to be the least effective in Amman area, were tested on flies from four other locations in Jordan. Results revealed that cyfluthrin was much more effective than malathion and propoxur in all locations. Results indicated that flies from the four locations were susceptible to cyfluthrin and were resistant to malathion and propoxur. The relatively small slopes and large LD_{50} show that greater resistance may develop

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I. INTRODUCTION

The common house fly *Musca domestica* L. causes considerable nuisance to people all over the world. Its habit of walking and feeding on garbage and excrement and also on human body and food makes it an ideal agent for the transmission of several infectious diseases such as bacillary dysentery, amoebic dysentery, diarrhea, typhoid, paratyphoid, food poisoning, cholera, helminthes, poliomyelitis, trachoma, cutaneous diphtheria, yaws and leprosy (Service 1980; Keiding 1986). The house fly is an important public health pest in Jordan, especially in places where poor sanitary conditions prevail. In Jordan Valley, where intensive farming is widespread, natural fertilizers including sheep cattle and poultry manure were used extensively. This in addition to the favorable weather conditions enhances the development and increases the house fly population to very high levels causing nuisance and diseases to inhabitants and visitors.

Because of its importance as a public health pest, several methods have been employed to control it in different parts of the world including Jordan. Prior to the advent of DDT, and in addition to sanitation, borax, sodium fluoride, sodium chloride and pyrethrum extract with kerosene have been used to control the fly. After 1944, with the development of the chlorinated hydrocarbon insecticides, the house fly was satisfactorily controlled with these materials. In mid 1950s the organophosphorus and carbamate insecticides were used for the fly control, followed in 1973 by the synthetic pyrethroid insecticides.

As early as 1946, there were reports by several investigators from different parts of the world that the house fly had developed resistance to the chlorinated hydrocarbon insecticides. This was followed by similar but less widespread development of resistance to other groups of insecticides namely organophosphorus, canbamates and synthetic pyrethroids (Chapman and Morgan 1992). In Jordan, several workers reported varying degrees of resistance to various insecticides belonging to the chlorinated hydrocarbon, organophosphorus and carbamate compounds.

As the house fly is the insect species that has shown the greatest ability to develop resistance, it is necessary from time to time, to monitor the resistance of the local flies to the insecticides in use. Taking the preceding points into consideration, the present work started to investigate the toxicities of different insecticides to house flies collected from five different locations in Jordan. The insecticides tested represented the main insecticide groups used to control the insect in Jordan, i.e., ogranophosphorus, carbamates and pyrethroids. It is envisaged that this study will determine the effectiveness of various insecticides used and whether or not resistance developed or apt to develop to these materials.

2. MATERIALS AND METHODS

2.1. *Method of obtaining house flies*

2.1.1. *Field-collected house fly adults*

The flies used in this research were obtained from poultry farms in five different locations in the country. These locations are : Irbid (90 km north of Amman), Karak (120 km south of Amman), Al-Dafyaneh (130 km north east of Amman), The university experiment station in Central Jordan Valley (50 km west of Amman) and the university of Jordan in Amman. Flies collected from different locations will be referred to hereafter as Irbid strain, Karak strain, Al-Defyaneh strain, Jordan Valley strain and Amman strain. Flies collected were taken to the laboratory for rearing to obtain F1 generation which was used for testing.

House flies were reared at room temperature in a room at the college of agriculture, university of Jordan. Cages 40 cm long, 40 cm wide and 40 cm high were used for rearing the house fly. The cages were covered with 16 mesh screen with cloth sleeve opening at the front.

Adult house flies were fed on a diet composed of two parts of defatted powdered milk and one part of sugar. Water was supplied by a cotton pad placed on the surface of 100 ml glass beaker filled with water. The cotton pad was held on the surface by the use of a piece of polystyrene. A Petri dish containing a piece of cotton immersed in 5% solution of the adult flies diet described above was placed inside each cage. After approximately twenty hours of placing the Petri dishes containing the oviposition medium, eggs were collected simultaneously and placed on the surface of the larval diet which consisted of 100 gm of wheat bran, 50 gm of chicken broiler diet, and 150 ml water placed in two liter glass beaker. About 500 eggs were cultured in each beaker. The beakers were then covered with muslin cloth. Two days after egg hatching, a 5 cm thick layer of sand was added on top of the medium to form a cooler and drier place for the larvae to pupate (Sumitomo 1977). Pupae were collected by using a 25 mesh-sieve, and hundred pupae were transferred to each two liter-glass beaker for fly emergence.

Adult flies were supplied with food consisting of honey and water placed on a cotton pad held on top of the muslin cloth covering the beaker. Rearing took place in a temperature controlled cabinet at 22-27°C and a constant illumination of 12:12 LD (Kence and Kence 1993; Saito *et al.* 1991).

2.1.2. *Laboratory strain of house fly adults*

These house flies were reared from a culture of flies obtained courtesy of Mr. Jorgen Jespersen, Danish Pest Infestation Laboratory, Lyngby, Denmark. As far as known this culture of flies had had no previous contact with insecticides. The flies have been reared in the laboratory at the university of Jordan by the previously described method.

2.2. *Insecticides*

Eight insecticides representing organophosphorus, carbamate and pyrethroid groups were tested. The insecticides used, their purity, and the sources from which they were obtained are listed in table 1.

TABLE I. INSECTICIDES USED, THEIR PURITY AND THE SOURCES FROM WHICH THEY WERE OBTAINED.

Insecticide (common name)	Percent Purity	Source
Malathion	96	Cheminova Agro. A/S, Denmark.
Fenitrothion	95.5	Cheminova Agro. A/S, Denmark.
Chlorpyrifos	99.8	Riedel-de Haen AG., Germany.
Propoxur	98	Bayer, Germany.
Cypermethrin	90	Riedel-de Haen AG., Germany.
Cyfluthrin	94.5	Bayer, Germany.
Deltamethrin	98	Riedel-de Haen AG., Germany.
Lambda-cyhalothrin	98	Zeneca, U.K.

2.3. Topical application of insecticides

The apparatus used for topical application in this research was a modified syringe-microburet, made by the Micro-Metric Instrument Co. of Cleveland, Ohio.

A day before testing, female flies 3-5 days old (WHO 1981) were sorted out after anesthetization with carbon dioxide, and were left overnight in an incubator at 25±1°C. The insects were supplied with food consisting of honey and water as previously described.

Arbitrary preliminary dosages were tested in order to determine the lethal dosages tested in this study. Individual insects were treated with the respective insecticides solutions after they had been anesthetized with carbon dioxide. The carbon dioxide was obtained as a liquefied gas in cylinders under pressure. The gas was allowed to escape through a pressure reducing valve at a slow rate through a 15 cm Buchner funnel in which the test insects were confined. The modified syringe-microburet was used to apply the insecticide solution to the mesonota of individual insects. The toxicant was dissolved in acetone and one microliter of solution was applied to each fly. At least five dose levels of each insecticide were used to determine the LD_{50}. Four replicates of ten flies each were used at each dose level. A control treatment of 10 flies was also run with each replicate. Control flies received one microliter of acetone only. After treatment, flies were confined in a 150ml plastic containers covered with cheese cloth held in place by a rubber band to prevent the escape of the treated flies and transferred to an incubator at 25±1°C. Honey and water were supplied to the treated insects on a piece of cotton placed on top of the cheese cloth. Mortality counts were made 24 hours after treatment. The criterion for mortality was the inability of the insect to show active locomotion. The individual weights of fifty 3-5 day old female flies were weighed to determine the average weights of a female house fly from each location in addition to the susceptible strain.

2.4. *Data analysis*

Data were analyzed by a computer program based on the method of Finney (1952). The calculated results include: LD_{50} and its confidence intervals, the slope and the intercept of the regression line.

Significance between LD_{50} for the different insecticides in different locations was based on non overlap of confidence intervals (Scott and Rutz 1988).

2.5. *Resistance ratio*

The resistance ratio (R/S) is used to illustrate the degree of resistance or susceptibility of a field-collected strain compared to a laboratory susceptible strain.

R/S at LD50 can be calculated by dividing the LD50 for a field strain by the LD50 for the susceptible strain (Keiding 1976,1977).

3. RESULTS

3.1. *The Respective toxicities of eight insecticides to Amman strain of house fly adults.*

The LD_{50s} for flies collected from Amman area, as shown in table 2, indicate high degree of variability between the eight insecticides tested. The table shows that the pyrethroid insecticides tested, i.e. lambda-cyhalothrin, deltamethrin, cypermethrin and cyfluthrin in addition to the organophosphorus compound chlorpyrifos were the most effective insecticides with LD_{50} 1,27, 4.22, 7.08, 7.37 and 10.69 µgm/gm fly respectively. Propoxur and malathion were the least effective with LD_{50} 4230.47 and 3493.30 µgm/gm respectively.

The slope of the log. dose probit line is a measure of the diversity of response or the heterogeneity of the insects toward the toxicant used. In the normal susceptible condition of insects, the slope is great, when resistance begins to develop the slope decreases. This decrease in slope will continue until resistance tends to reach a plateau (Hoskins and Gordon 1956), and subsequently the slope increases. This phenomenon seems to be true with the slopes recorded for the insecticides tested. Table 2 shows very large LD_{50} and relatively small slope for propoxur and malathion which indicates that the flies are resistant to the two insecticides, and resistance did not reach its limit. It is expected that the house fly will develop still greater resistance to propoxur and malathion. Table 2 also shows low LD_{50} and relatively small slopes for lambda-cyhalothrin, deltamethrin, cypermethrin, cyfluthrin and chlorpyrfos. One may speculate that there is certain degree of heterogeneity and resistance is apt to develop.

The resistance ratios for flies collected from Amman were: Cypermethrin (1.96 x), lambda-cyhalothrin 4.76x, deltamethrin 4.82x, cyfluthrin 4.98x, chlorpyrifos 8.48x fenitrothion 28.92 x, propoxure 159.82x and malathion 301.15x. According to Keiding (1976), a ten fold or greater increase in the LD_{50} compared with a susceptible strain means that the tested population have various degrees of resistance, whereas levels less than ten fold were considered increased tolerance. Accordingly, the fly population from Amman area showed various degrees of susceptibility toward lambda-cyhalothrin, deltamethrin, cyfluthrin, cypermethrin, and chlorpyrifos, while it appeared to be resistant to fenitrothion and, to greater extent, to malathion and propoxur.

TABLE II. TOXICITIES OF EIGHT INSECTICIDES AS DETERMINED BY TOPICAL APPLICATION TO HOUSE FLIES COLLECTED FROM THE UNIVERSITY OF JORDAN, AMMAN.

Insecticide	LD_{50}* Micrograms/Gm Fly	95% Confidence Limits	Slope of Log. Dose Probit Line (±SE)	Resistance Ratio at LD_{50} (R/S)
Lambda-cyhalothrin	1.27 c	1.04-1.51	2.67±0.33	4.70
Deltamethrin	4.22 e	3.23-5.21	2.39±0.31	4.82
Cypermethrin	7.08 fg	5.08-8.82	2.36±0.38	1.96
Cyfluthrin	7.37 fg	5.21-9.30	2.16±0.35	4.98
Chlorpyrifos	10.69 g	8.49-12.86	2.52±0.40	8.48
Fenitrothion	52.35 i	42.57-61.92	2.65±0.39	28.92
Malathion	3493.30 mn	2809.97-4412.11	2.07±0.29	301.15
Propoxur	4230.47 n	3163.30-5689.16	1.39±0.25	159.82

* Values in the same column followed by the same letter are not significantly different at 95% confidence level.

TABLE III. TOXICITIES OF EIGHT INSECTICIDES AS DETERMINED BY TOPICAL APPLICATION TO A LABORATORY SUSCEPTIBLE STRAIN OF HOUSE FLY ADULTS.

Insecticide	LD_{50} * Micrograms/Gm. Fly	95% Confidence Limits	Slope of Log. Dose Probit Line (±SE)
Lambda-cyhalothrin	0.27 a	0.22-0.32	2.76±0.33
Deltamethrin	0.88 b	0.73-1.03	3.00±0.36
Chlorpyrifos	1.26 c	1.07-1.47	3.12±0.32
Cyfluthrin	1.48 cd	1.30-1.70	3.45±0.48
Fenitrothion	1.81 d	1.54-2.10	3.67±0.15
Cypermethrin	3.61 e	3.01-4.27	2.90±0.40
Malathion	11.60 g	9.86-13.37	3.20±0.42
Propoxure	26.47 h	23.56-29.38	4.76±0.58

* Values in the same column followed by the same letter are not signficantly different at 95% confidence level.

3.2. ***The respective toxicities of cyfluthrn, malathion and propoxur to Jordan valley, Irbid, Karak, and Al-Dafyaneh strains***

Cyfluthrin, malathion and propoxur representing pyrethroid, organophosphorus and carbamate groups respectively and proved to be the least effective against Amman strain were tested on flies from Central Jordan Valley, Irbid, Karak and Al-Dafyaneh. Insecticides were applied to these flies by the same method described previously. The LD_{50} and slopes of the log. dose probit lines were determined and compared with the laboratory susceptible strain. Results are presented in table 4.

For all field strains tested cyfluthrin was found to be much more effective than both malathion and propoxur. The LD_{50}s in µgm/gm fly for cyfluthrin, malathion and propoxur respectively were: 5.84, 2763.92, 4471.17 for Jordan Vally strain; 6.39, 2574.24, 4104.47 for Irbid strain; 4.20, 1637.06, 3329.47 for Karak strain; and 4.16, 1052.90, 1942.10 for Al-Dafyaneh strain.

Although the LD_{50}s of cyfluthrin for the field strains were higher than that for the susceptible strain (1.48 µgm/gm fly), the flies from the four locations are considered to be susceptible to this insecticide. This will be more evident if the relatively large slopes of the log. dose probit lines for the four strains are taken into consideration. The larg LD_{50}s of both malathion and propoxur for all field strains tested , compared to those for the susceptible strain (11.60, 26.47 µgm/gm fly respectively), indicate strongly that the house flies from all locations are resistant to both insecticides, although significantly smaller LD_{50}s of both chemicals were found with Karak and Al-Dafyaneh strains (table 4). The relatively small slopes of the log. dose probit lines of both insecticides for all field strains indicate that resistance did not reach its plateau and higher levels of resistance to malathion and propoxure may develop.

TABLE IV. TOXICITIES OF CYFLUTHRIN, MALATHION, AND PROPOXUR TESTED ON HOUSE FLIES COLLECTED FROM FOUR LOCATIONS IN JORDAN.

Location	Insecticide											
	Cyfluthrin				Malathion				Propoxur			
	LD_{50}	95% confidence limits	Slope	R/S	LD_{50}	95% confidence limits	Slope	R/S	LD_{50}	95% confidence limits	Slope	R/S
Jordan Valley	5.84 ef	4.23-7.21	2.87±0.45	3.95	2763.92 lm	2182.56-3478.56	1.97±0.26	238.27	4471.17 n	3128.02-6583.51	1.16±0.26	168.91
Irbid	6.39 ef	5.26-7.59	2.78±0.34	4.32	2574.24 klm	1959.47-3470.44	1.25±0.16	221.92	4104.47 mn	3151.56-5568.34	1.33±0.16	155.06
Karak	4.20 e	3.44-4.90	3.39±0.47	2.84	1637.06 k	1280.48-1998.33	2.12±0.26	141.13	3329.47 mn	2626.26-4402.51	1.59±0.20	125.78
Al-Dafyaneh	4.16 e	3.46-4.95	2.97±034	2.81	1052.90 j	846.03-1229.33	2.13±0.23	90.76	1942.10 kl	1554.45-2429.53	1.78±0.19	73.37

* Values followed by the same letter are not significantly different at 95% confidence level

4. DISCUSSION

Increased resistance in an insect population is first suspected if the failure of a standard treatment to give control equal to that obtained previously or large LD_{50} is obtained for the insecticide involved. This however, is not a positive proof that the insect is actually resistant to a certain insecticide. In order to ascertain if a particular insect is resistant to a certain insecticide, laboratory tests are usually carried out to give a direct comparison between a known normal strain and the one under suspicion. To confirm if the house fly was resistant to malathion, propoxur and cyfluthrin, samples of the suspected resistant flies from 5 locations in Jordan and samples of susceptible flies were tested in the laboratory, and the LD_{50} for the six populations was established. Results indicate that the pyrethroid insecticides tested were much more effective against the house fly than the organophosphorus and the carbamate compounds. Results also indicate that the insect from all locations was resistant to malathion and propoxur.

The resistance to malathion and propoxur in the different locations may be attributed to selection pressure and cross resistant extending from other insecticides. Saito *et. al* (1991) found that selection of azamethiphos-resistant flies resulted in high resistance to several insecticides including malathion, fenitrothion and propoxur, while there was no resistance to pyrethroids. Also several workers have reported that selection pressure with pyrethroid insecticides enhance the development of resistance to organophosphorus compounds (Goldena and Forgash, 1985; Scott and Georghiou 1985; Funaki and Motoyama 1986). Moreover, the use of other organophosphorus and carbamate insecticides in agriculture may enhance the development of resistance to public health compounds (WHO 1976, 1992). It is to be mentioned that more than 600 tons of public health related insecticides have been imported to the country during the period 1980-1994 (Ministry of Agriculture 1994). It is also important to note that the use of aerosols to control house flies indoors is widespread in Jordan which may enhance the development of resistance. The significantly smaller LD_{50}s of malathion and propoxur for both Karak and Al-Dafyaneh strains may be due to the relatively less use of insecticides and consequently less selection pressure in both locations vis-à-vis other locations.

Sacca (1973) working on flies from Amman area found various levels of resistance to several insecticides including propoxur to which flies showed susceptibility with R/S 2.8x. Later, Brook and Martin (1975) found that flies from Amman were resistant to propoxur with R/S 25.8x, while the experimental evidence in this research revealed that flies from the same area were resistant to propoxur with R/S 159.8x. Also, Abu-Nada (1990) reported that flies from Central Jordan Valley were moderately resistant to propoxur (15.24x). In the present work, R/S for flies from the same location was 168.9x. This indicate that flies in Jordan Valley developed much greater resistance to porpoxur after 1990.

The house flies tested in all locations showed susceptibility to all pyrethroids tested with R/S ranging from 2.0-5.0X. Keiding (1986) reported that high resistance to pyrethroids was only found locally in Denmark, Sweden, Germany, Switzerland and the United Kingdom in populations exposed to frequent treatments of aerosols or residual sprays with pyrethroid insecticides. In most of these places the knock down resistance (kdr) gene, which is the gene that is involved in the mechanism of insecticide resistance by reducing the sensitivity of the nervous system to that insecticide and its analogues, forms an important component of pyrethroid resistance, and it is strongly recommended to monitor for kdr before considering using residual pyrethroids for fly control. He added that kdr gene seems to be rare in most parts of the world outside northern and central Europe, and pyrethroids may be very effective in such areas. This seems to be true since the present work revealed that all fly strains tested from Jordan are susceptible to these insecticides despite over twenty years of continued use. This coincides with the results obtained by various workers in Jordan who reported that house flies were susceptible to the pyrethroid insecticides tested.

As discussed above, several workers in Jordan have found various levels of resistance to many insecticides tested. In addition, the present work revealed that house flies from the five regions investigated showed various levels of resistance to cyfluthrin, malathion and propoxur. Taking into consideration the fact that a certain resistance factor may work against different insecticides, the possibilities of chemical control of a particular population becomes less with each resistance the population acquires. Therefore, successive application of different insecticides to prevent or delay development of resistance seems to be only of limited value. True changes in the direction of house fly control seems only feasible when non chemical methods are regularly combined with conventional insecticides (Rupes *et al.* 1982; Keiding 1986).

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