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Toxic, Repellent and Attractant Properties of Some Insecticides Towards the Housefly (*Musca domestica* L.)

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Abstract: The LD_{50} of malathion, diazinon, dichlorvos, phosphamidon, lambda-cyhalothrin, cypermethrin, carbosulfan and propoxur on adult housefly were 2.9852, 0.1696, 0.2769, 0.3346, 0.0155, 0.0060, 0.0329 and 0.0137 μg/fly respectively. Dichlorvos, diazinon and cypermethrin showed the highest repellency and phosphamidon, malathion and propoxur showed the highest attractancy to both male and female houseflies. Propoxur and carbosulfan showed lower repellency. Lower doses always showed lower repellency and higher attractancy in all tested insecticides.

Key words: Insecticide, attractancy, repellency, housefly, *Musca domestica*

Introduction

Use of pesticide increased phenomenally since the development of modern synthetic poisons in the 1940s have brought inestimable benefits to humanity in terms of human lives saved, diminished suffering and economic gain (Metcalf, 1968; Smith and Van den Bosch, 1967). Annual losses resulting from insect damage, microbial deterioration and other factors are estimated to be 10-25% of production world wide (Matthews, 1993). However, their widespread use has led to the development of pest strains resistant to insecticides (Subramanyam and Hagstrum, 1995). The effectiveness of an insecticidal treatment is influenced not only by the toxicity of the insecticide but also by the primary response of the insect to its mode of application. Repellent or attractant effects are the principal factors affecting insecticidal efficiency and many common insecticides exhibit one or both of these properties depending on concentration. An insecticide which is highly toxic under laboratory conditions may at times prove ineffective in the field it has repellent properties since the pest will move away before acquiring a lethal dose. On the other hand, an insecticidal formulation that is only moderately toxic may prove highly effective under field conditions due to its lacks of repellent effect. Odours of most insecticides are repellent to certain insects at higher concentrations but act as attractants at lower concentrations (Dethier, 1954). The repellent effects of insecticidal sprays and spray additive have been reviewed by Dethier (1956), Shambaugh *et al.* (1957), Sterling and Howell (1972) and others. Synthetic pyrethroids, the class of insecticides which shows the greatest potential for insect control has been found to possess considerable repellent activity which leads to a reduction in their effectiveness. Residues of pyrethrins are sometimes repellent to various species of insect, for example the cockroaches, *Blatta orientalis, B. germanica* and *Periplaneta americana* (Ebeling et *al.*, 1966). Repellency tests of frequently used insecticides against *B. germanica* have been also reported by Smittle *et al.* (1968).

Many insecticides repel insects and this can be highly disadvantageous to pest control operations. The objective when attempting to eradicate pest infestation is to obtain maximum pick up of toxicant by the pest and the chances of this are reduced when the dust or spray repels the pests. Some insecticides attract insects at very low dosages and this can also be a problem as an area sprayed with such a compound will be more susceptible to re-infestation when only sub-lethal residues remain. There may also be advantages in using attractant or repellent compounds in insect pest management. This present investigation malathion, diazinon, dichlorvos, phosphamidon, lambda-cyhalothrin, cypermethrin, carbosulfan and propoxur were evaluated for their toxicant, repellent and attractant properties towards the housefly, (*Musca domestica* L.).

Materials and Methods

The housefly (local strain) stock cultures maintained in the Crop Protection Laboratory, Department of Zoology, Rajshahi University. The adults were provided with a medium to lay eggs. The medium was presented in plastic cups approximately 10 cm deep. The medium consisted of 9 gm milk powder and 5 gm fresh yeast dissolved in 100 ml of water and added to 100 gm bran following method of Wilkins and Khalequzzaman (1993). The mixture was then thoroughly stirred and put into the pots leaving 3 cm from the top. The pots were placed in the fly rearing cage for 24 h. After that time the females had laid batches of eggs. Batches of approximately 100 eggs were separated out and transferred to similar pots containing the same mixture and fitted with plastic lids with gauze centers. They were then placed in an incubator kept for 28 ± 0.5°C.

The experiments were carried out during July to December 1999. Eight insecticides, viz., malathion (Limithon 57 EC of ACI Ltd.), diazinon (60 EC of Novartis Ltd.), dichlorvos (Nogos 100 EC of Novartis Ltd.), phosphamidon (Dimecron 200 SCW of Novartis Ltd.), cypermethrin (Cymbush 10 EC of ACI Ltd.), lambda-cyhalothrin (Karate 25 EC of ACI Ltd.) carbosulfan (Marshal 20EC of FMC Corp. USA) and propoxur (Acekro 20 EC of McDonald Ltd.) were used in this study.

Bioassay: The flies were anaesthetized with diethylether before the bioassay. Anaesthetized flies were held with padded entomological forceps for treatment. The insecticide and mixtures were applied with the help of a micro syringe (Hamilton gas tight micro syringe No. 630). Five concentrations were used in which seven replications of flies (each having 10 flies) were treated. One batch of control flies was maintained in which only acetone was applied topically. Acetone is an inert solvent, which dried up quickly after application leaving active ingredients only.

Treated flies were kept in the food cup with cotton soaked in glucose solution. Mortality of the flies was recorded after 24 h of treatment. Corrected mortality percentage was calculated using Abbott's formula (Abbott 1925); probit analysis was done according to Finney (1947) and Busvine (1971) using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle upon Tyne, UK.

Repellent effects of insecticides solution towards adult male and female houseflies: Insecticidal repellence was tested by the sandwich bait method of Kilgore and Crowell (1939) as modified by Osmani *et al.* (1972). Thick sugar syrup was spread thinly on pieces of filter paper (11cm) and was dried by heating for 1-2 h. Tissue papers were treated with 1 ml of insecticide solution or water (controls) and air dried for 30 min to evaporate the solvent.

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Each treated tissue was wrapped around a syrup coated filter paper and fixed to a piece of thick card, which was then placed inside a cage (50 x 30 x 20 cm^3).

A replicate of 100 male or 100 female flies, starved for 24 h was placed in each cage. The number of flies alighting on the treated paper strip was recorded at 10 min intervals for 6 h of the experiment. Number of flies on the treated paper strip at any moment in time was averaged over the experimental period for each insecticide and concentration used. An index of repellence (Osmani *et al.*, 1972) was calculated by using the following expression:

$$\text{Index of repellency} = \frac{C-T}{C+T} \times 100$$

Where, T = mean number of insects on the treated paper strip; C = mean number of insects on the control strip was calculated.

Attractant effects of insecticides toward adult male and female houseflies: Insecticide solution (5 ml) was placed in a 11cm petridish which was then covered with mosquito netting to prevent flies form coming into contact with the insecticide film. The dish was placed at the bottom of a beaker (1L). A paper funnel with the stem broken off was inserted into the top of the beaker in such a way that the rim of the funnel rested on the edge of beaker; flies which were attracted by the insecticide could thus enter the beaker but were prevented from leaving it.

The complete assembly was placed inside the cage (50 x 30 x 20 m^3) containing 200 each for male and female starved flies. The number of flies that had entered the beaker was counted every 15 min over a five hours period. These counts were averaged over the duration of experiment. Index of attractancy (Osmani *et al.*, 1972) was calculated as follow:

$$\text{Index of attractancy} = \frac{C-T}{C+T} \times 100$$

Where, T = mean number of insects inside beaker with insecticide solution, C = Mean number of insects inside beaker with water only was calculated.

Results

Lethal effect of insecticides on adult housefly: Different doses of the malathion, diazinon, dichlorvos, phosphamidon, lambda-cyhalothrin, cypermethrin, carbosulfan and propoxur were applied topically on adult houseflies (3 days old) (*M. domestica* L.). The mortality was recorded primarily after 30 min to see if there is any rapid activity exhibited by the treated flies and final mortality was recorded after 24 h of exposure. Calculated LD_{50} values was 2.9852, 0.1696, 0.2769, 0.3346, 0.0155, 0.0060, 0.0329 and 0.0137 µg/fly for malathion, diazinon, dichlorvos, phosphamidon, lambda-cyhalothrin, cypermethrin, carbosulfan and propoxur respectively (Table 1). Results indicate that cypermethrin was the most toxic and malathion was the least toxic to adult houseflies when applied singly. Order of toxicity of the insecticides was cypermethrin > propoxur > lambda-cyhalothrin > carbosulfan > diazinon > dichlorvos > phosphamidon > malathion. All values were insignificant indication good fit of the regression equations. All chi-square values were insignificant indicating good fit of the regression lines (Fig. 1).

Table 1: LD_{50}, 95% confidence limits and regression equations of some insecticide to adult housefly (*M. domestica* L.) after 24 h of treatment

No. of insecticide	LD_{50} (µg/fly)	95% confidence limits (µg/fly) Lower	Upper	Regression equation	X^2
Malathion	2.9852	2.1814	4.0851	Y = 4.428582+1.203048X	0.970
Diazinon	0.1696	0.1088	0.2644	Y = 4.515186+2.111419X	0.315
Dichlorvos	0.2769	0.1729	0.4436	Y = 4.106172+2.020519X	0.121
Phosphamidon	0.3346	0.2280	0.4915	Y = 3.888703+2.118672X	0.013
Lamda-cyhalothrin	0.0155	0.0084	0.0288	Y = 4.812490+0.9809704X	0.466
Cypermethrin	0.0060	0.0048	0.0078	Y = 3.926641+1.375388X	0.538
Carbosulfan	0.0329	0.0258	0.0487	Y = 3.140040+1.225309X	1.150
Propoxur	0.0137	0.0091	0.0141	Y = 3.379209+1.535114X	1.194

Table 2: Repellent effects of insecticide solutions towards adult houseflies

Insecticides	Concentration (mg cm^{-2})	No. of flies visiting insecticide treated paper strip/h		No. of flies visiting control paper strip/h		Index of repellency	
		♂	♀	♂	♀	♂	♀
Malathion	0.149889	11.66	17.83	23.66	28.00	33.95	22.09
	0.074944	18.00	25.50	33.83	38.83	30.54	20.72
	0.037472	31.50	12.16	47.17	18.00	19.92	19.36
Diazinon	0.59998	6.00	5.66	22.76	21.23	58.28	57.90
	0.29999	7.33	7.00	25.13	24.24	54.84	55.19
	0.14999	9.33	10.00	31.60	30.83	54.41	51.02
Dichlorvos	0.99999	5.66	4.83	24.37	23.66	62.25	66.09
	0.49999	8.33	7.50	28.19	27.30	54.37	56.90
	0.24999	10.50	10.00	32.50	31.13	51.16	51.37
Phosphamidon	0.99999	10.00	9.66	21.80	22.51	37.11	39.94
	0.49999	12.00	11.50	27.11	26.12	38.63	38.86
	0.24999	15.00	14.33	30.99	29.76	34.77	35.00
Lambda-cyhalothrin	0.02026	9.66	9.50	21.17	21.16	37.31	38.03
	0.01013	13.33	5.16	18.50	8.50	16.24	24.45
	0.00506	15.83	9.66	20.50	15.16	12.85	22.17
Cypermethrin	0.000210	10.16	10.00	33.83	35.22	53.81	55.75
	0.000105	13.33	15.66	35.50	37.33	45.40	40.89
	0.000052	17.83	19.66	36.33	36.00	34.16	29.36
Carbosulfan	0.000420	20.17	23.50	33.83	36.17	25.30	21.23
	0.000210	22.33	20.00	32.50	30.17	18.55	20.26
	0.000105	24.83	30.66	35.16	44.00	17.22	17.86
Propoxur	0.000420	32.66	17.66	57.16	34.16	27.27	31.84
	0.000210	64.22	42.00	90.83	75.83	17.16	28.71
	0.000105	93.00	36.66	98.16	40.16	2.70	4.56

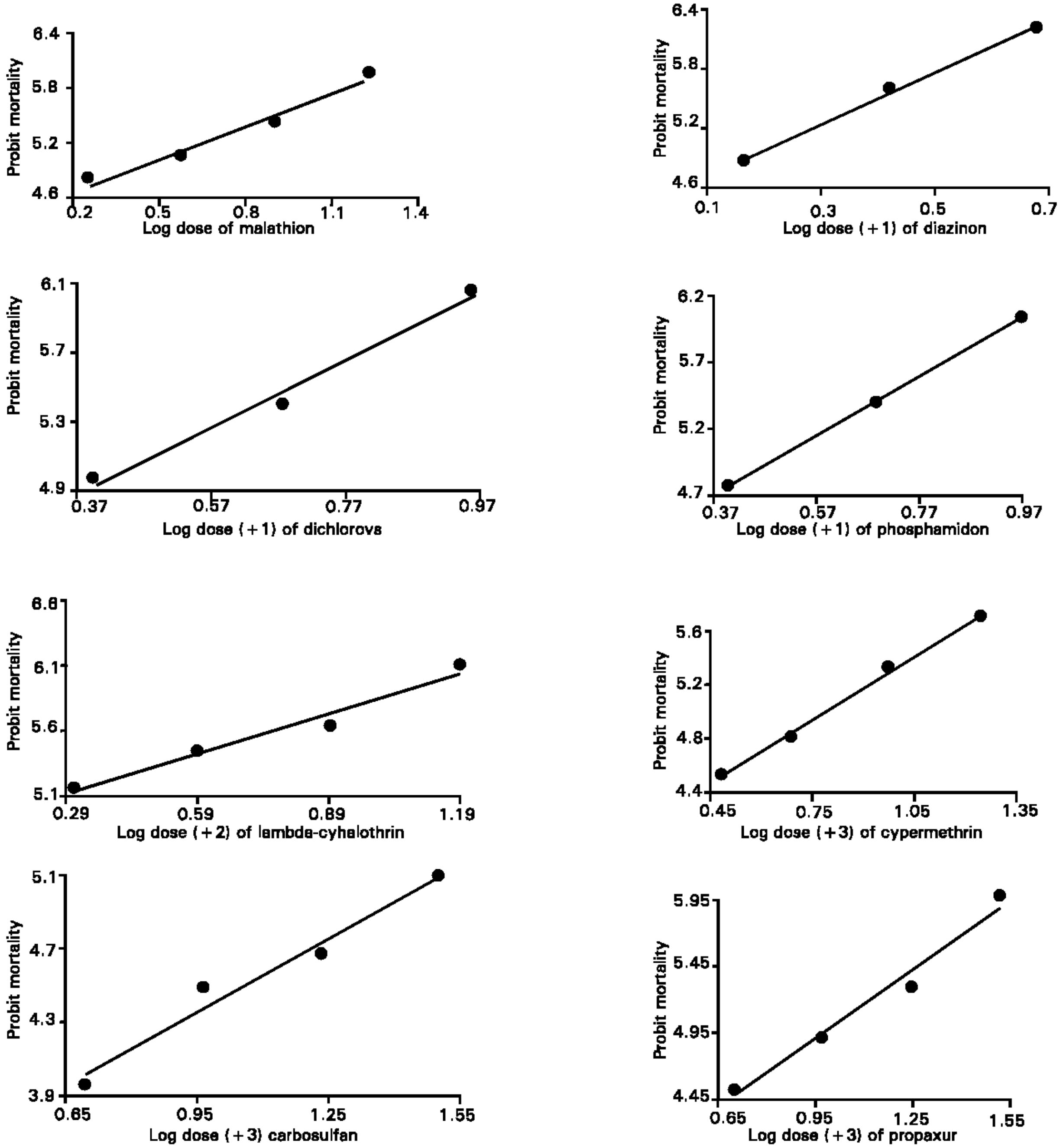

Fig. 1: Regression lines of probit mortality on log dose (µg/fly) of different insecticides used against *M. domestica*

Repellent effect of various insecticides towards adult male and female house files: Dichlorvos, diazinon and cypermethrin exhibited a high degree of repellence to houseflies at the highest dose. The index of repellency for dichlorvos was 62.25, 54.37 and 54.16 for male and 66.09, 56.90 and 51.37 for female files in the doses of 0.999, 0.499 and 0.249 mg cm^{-2} respectively. Diazinon showed the second highest repellency as 58.28, 54.84 and 54.41 for male and 57.90, 55.19 and 51.02 for female flies in 0.599, 0.299 and 0.149 mg cm^{-2} respectively. The third highest repellency showed by cypermethrin, index of repellency for cypermethrin was 53.81, 45.40 and 34.16 for male and 55.75, 40.89 and 29.36 for female flies in the doses of 0.00021, 0.000105 and 0.000052 mg cm^{-2} respectively. Repellent effect was always higher in the concentrated form than that of dilute form. Results showed that all insecticides repelled both sexes of houseflies (Table 2). Comparatively low repellence was shown by carbamates, i.e., carbosulfan and propoxur.

Phosphamidon in the same doses as dichlorvos the repellency was 37.11, 38.63 and 34.77 for male and 39.94, 38.86 and 35.00 respectively. Index of repellency was 33.95, 30.54, 19.92 in male flies and 22.09, 20.72 and 19.36 in female flies in malathion doses of 0.1498, 0.0749 and 0.0374 mg cm^{-2}. In lamda-cyhalothrin it was 37.31, 16.24 and 12.85 and 38.03, 24.45 and 22.17 for male and female flies in doses of 0.02026, 0.01013 and

Table 3: Attractant effects of insecticide solutions towards adult male houseflies

Insecticides	Concentration (mg cm^2)	No. of flies visiting insecticide beaker/h		No. of flies visiting control beaker/h		Index of attractancy	
		♂	♀	♂	♀	♂	♀
Malathion	0.149889	4.20	4.60	3.40	4.00	10.53	6.98
	0.074944	4.80	4.80	3.20	4.00	20.00	9.10
	0.037472	5.40	5.60	3.40	3.80	22.73	19.15
Diazinon	0.59998	3.80	3.10	3.50	2.80	4.12	5.05
	0.29999	4.20	5.20	3.50	4.60	9.09	11.53
	0.14999	6.40	6.10	4.20	5.0	6.08	9.90
Dichlorvos	0.99999	3.00	4.60	2.60	3.90	7.14	8.24
	0.49999	5.10	5.30	3.30	4.20	21.43	11.58
	0.24999	8.20	5.90	4.80	4.20	26.15	16.83
Phosphamidon	0.99999	4.30	3.00	3.20	2.60	14.67	7.14
	0.49999	5.90	4.20	3.60	3.40	24.21	10.53
	0.24999	7.20	5.50	3.60	3.80	33.33	18.28
Lambda-cyhalothrin	0.02026	3.80	4.60	3.40	4.00	5.55	6.97
	0.01013	3.60	4.40	3.40	4.00	2.86	4.76
	0.00506	4.00	5.00	3.20	3.80	11.11	13.64
Cypermethrin	0.000210	3.60	4.40	3.40	3.80	2.86	7.32
	0.000105	4.20	5.00	3.60	4.00	7.69	11.11
	0.000052	4.60	5.40	3.40	3.80	15.00	17.39
Carbosulfan	0.000420	3.20	3.60	3.00	3.20	3.23	5.88
	0.000210	3.00	4.60	2.60	4.00	7.14	6.98
	0.000105	3.60	4.60	3.00	4.00	9.10	6.98
Propoxur	0.000420	2.00	3.60	1.60	3.00	11.11	9.09
	0.000210	5.00	5.00	3.40	4.00	19.04	11.11
	0.000105	7.00	4.40	3.80	3.40	29.63	12.82

0.00506 mg cm^{-2} respectively. In carbosulfan it was 25.30, 18.55 and 17.22 for male and 21.23, 20.26 and 17.86 for female flies in the doses of 0.00042, 0.00021, 0.00010 mg cm^{-2} respectively. In the same doses propoxur showed index of repellency as 27.27, 17.16 and 2.70 for male and 31.84, 28.71 and 4.56 for female flies respectively (Table 2).

Attractant effects of various insecticides towards adult male and female houseflies: It was observed that high attractive effect was shown by phosphamidon, malathion and propoxur to houseflies. In phosphamidon the index of attractancy was 14.67, 24.21 and 33.33 and 7.14 for male 10.53 and 18.28 for female flies in the doses of 0.999, 0.499 and 0.249 mg cm^{-2}respectively. The index of attractancy for malathion has been calculated as 10.53, 20.00 and 22.73 for male and 6.98, 9.10 and 19.15 for female flies in the doses of 0.1498, 0.0749 and 0.0374 mg cm^{-2}respectively. Index of attractancy for propoxur in the doses of 0.00042, 0.00021 and 0.00010 mg cm^{-2}was 11.11, 19.04 and 29.63 for male and 9.09, 11.11 and 12.82 for female flies respectively (Table 3).

Dichlorvos, diazinon and lamda-cyhalothrin showed moderate attractancy to both sexes of the houseflies. In dichlorvos the index of attractancy was 7.14, 21.43 and 26.15 for male and 8.24, 11.58 and 16.83 for female flies in the doses of 0.999, 0.499 and 0.249 mg cm^{-2} respectively. The index of attractancy for diazinon has been calculated as 4.12, 9.09 and 6.08 for male and 5.05, 11.53 and 9.90 for female flies in the doses of 0.599, 0.299 and 0.149 mg cm^{-2} respectively. In case of lamda-cyhalothrin the index of attractancy was 5.55, 2.86 and 11.11 for male and 6.97, 4.76 and 13.6 for female flies in the doses of 0.0202, 0.0101 and 0.0050 mg cm^{-2} respectively. In cypermethrin it was 2.86, 7.69 and 15.00 for male and 7.32, 11.11 and 17.39 for female flies in the doses of 0.00021, 0.00010 and 0.00005 mg cm^{-2} respectively. In carbosulfan the index of attractancy has been calculated as 3.23, 7.14 and 9.10 for male and 5.88, 6.98 and 6.98 for female flies in the doses of 0.00042, 0.00021 and 0.00010 mg cm^{-2} respectively.

Discussion

The common housefly has been extensively utilized as a test organism to screen candidate insecticides, chemosterilants and insect growth regulators by scientists in public or private research institutions. Since the immature stadia can survive in various substrates, entomologists have normally used materials that are available locally (Spiller, 1964, 1966; Louw, 1964; Sawicki, 1964; Keiding and Arevad, 1964; Schoof, 1964). In this investigation three groups of insecticides (organophosphorous, pyrethroid and carbamate) were against adult housefly (*M. domestica* L.); malathion, diazinon and dichlorvos from organophosphorous, cypermethrin and lambda-cyhalothrin from pyrethrords and carbosulfan and propoxur from carbamates. The present investigation revealed that, pyrethroid insecticide was more active than carbamate and organophosphorous insecticides to flies. According to the calculated LD$_{50}$ values the pyrethroid insecticide, cypermethrin was the most toxic (LD$_{50}$ 0.006 μg/fly), while the organophosphorous insecticide malathion was least toxic (LD$_{50}$ 2.98 μg/fly) to adult housefly.

With the similar experiments with cooper and NH-10 strains of housefly Saleem *et al.* (1994) recorded of LD$_{50}$ lambda-cyhalothrin as 0.235 and 0.292 μg/fly respectively. Liu and Plapp (1990) observed the toxicity of cypermethrin to different strains of *M. domestica* in surface film method and recorded the value as 104.0 and 11.5 μg/petridish (9 cm) for susceptible and kdr strains respectively. The LD$_{50}$ of different insecticides varied to some extent with other workers due to the differences in strain of housefly used. Most of the workers used recognized laboratory strains like cooper, boxted, NH-10 etc.

A range of mechanisms have been implicated in OP resistance in houseflies (Devonshire, 1975; Oppenoorth, 1982; Scott *et al.*, 2000). Organophosphorus compounds undergo various metabolic reactions in living organisms. Major biotransformation reactions are common to compounds possessing similar structures and are mediated mainly by mixed-function oxidases, glutathione S-transferases and arylesterases. Studies revealed that these enzymes show clear stereoselectivity in the metabolism of optically active organophosphorus compounds (Ohkawa, 1982). Pyrethroids have disruptive effects on a variety of arthropod sensory preparations. Gammon (1978) reported that allethrin-treated cockroaches developed bursts of afferent discharges in the cerci, which arose as the treated insects became restless and ataxic. Electrical or mechanical stimuli applied to the cerci produced greatly prolonged sensory responded. Repetitive discharges in the crural nerves of the housefly and cockroach resulted from topical treatment of tarsi on isolated legs (Miller and Adams, 1977).

Dichlorvos, diazinon and cypermethrin showed the highest repellency and phosphamidon, malathion and propoxur showed the highest attractancy to both male and female houseflies. However, it was observed that repellent activity was greater in the higher doses and attractant activity was more in the lower doses. These results correspond with those of Dicke *et al.* (1952), whose olfactometric tests on methoxychlor and DDT showed that flies were attracted to the volatilized insecticides.

These findings tend to support Dethier's (1947) threshold concentration theory that a compound which is attractive at one concentration may be repellent at another. An initially acceptable compound may become more attractive as its concentration is increased until an optimum level is attained; beyond this point the attraction decreases with increased concentration until the nature of response is completely reversed. Thus the behaviour of insect towards a compound depends not only on the nature of compound but also on its concentration.

There appears to be no information in the literature on the relative susceptibilities of male and female insects towards repellents. Waiting and Hoskins (1939) using an olfactometer, observed that houseflies reacted more uniformly to odours when the sexed were exposed separately when mixed batches were used; sex differentiation between susceptibilities to repellent insecticides was observed in this experiment, however, female flies showed slightly higher sensitivity towards certain repellent solutions, as is the case with insecticides treatments. In most cases, however, females were less sensitive than males.

The results of this study indicate that many substances which have been assumed to kill insect pests may infact just have driven them away causing an apparent disappearance or reduction in numbers. It is also evident that some compounds at low concentrations actually attract pest species to them so that pesticide residues left in the field after degradation or evaporation may eventually result in resurgence of pest species.

These are not the only reasons why studies on attraction and repellence are of value, for there is considerable scope for the use of attractant and repellent compounds in pest management. Each successful use of such substances will decrease the need for toxic pesticides and will ultimately be of benefit to the environment, ecology and mankind.

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