TOXICITY OF DICHLORVOS 50EC AND CHLORPYRIFOS 40EC AGAINST HOUSE FLY, *MUSCA DOMESTICA* L.

Sohail Ahmed and Mohammad Irfanullah
Department of Agricultural Entomology, University of Agriculture, Fasialabad

Base line data of susceptibility of a laboratory reared strain of *Musca domestica* to dichlorvos 50EC and chlorpyrifos 40EC in a range of concentrations from 250 to 5000 ppm of the formulated insecticides in acetone was determined. These concentrations in a volume of 5 ml were added to 25 gm of granulated sugar in a petri dish. Sesame oil was used, as the synergist. Insecticide was mixed with sesame oil in 1:1 and 1:2 ratio to obtain KD_{50}/LC_{50} of the insecticide + synergist combination. House fly was allowed to feed on the insecticide coated sugar for 48 hr. Knockdown and mortality data was observed after 1, 2, 4, 6, 8, 12, 24 and 48 hours. The results showed that dichlorvos was the most effective with LC_{50} value, 122 ppm and with the synergist combination, none of the two insecticides proved effective. Toxicity relationship of these insecticides with sesame oil is discussed.
Key words: dichlorvos; chlorpyrifos; bioassay via feeding; *Musca domestica*; synergist, sesame oil

INTRODUCTION

The house fly, *Musca domestica* L. is a well known cosmopolitan insect pest of both farm and house. This species is always found in association with humans or activities of humans. Not only are they a nuisance, but they can also transmit disease-causing organisms (Scott, 1998). More than 100 pathogens associated with the house fly may cause diseases in humans and animals. These diseases include typhoid, cholera, tuberculosis anthrax, mastitis, bacillary dysentery and infantile diarrhea (Service, 1980).

The control of *M. domestica* is vital to human health. The threshold density for determining when to control flies depends on the area where control measures will be taken. The threshold density of the house fly at waste management sites was estimated to be 150 individuals per fly paper in 30 minutes (Hogsette, 1993).

The most common control measures involved with the management of house fly are sanitation, use of traps and insecticides but in some cases integrated fly control has also been proposed. Fly traps may be useful in some fly control programmes when enough traps are used, provided, they are placed correctly. House fly has many natural enemies and among the most important in poultry facilities are the wasps (Hymenoptera: Pteromalidae) *Muscidiforax raptor* and *Spalangia cameroni*.

Until now control of this important public health pest is mainly relied on the insecticides. These are applied against adults and larvae directly or indirectly to suppress their densities (Imai, 1985). Residual wall sprays can be applied where the flies congregate. Treatment with the insecticides may be in the form of surface sprays, baits, aerosols and wet sprays etc. (Mabbet, 1996).

Organophosphates, carbamates, pyrethroids and Insect Growth Regulators (IGRs) have been used to control house fly while many aerosols containing pyrethroid insecticides such as permethrin, fenvalerate, allethrin etc. are being used (Pan *et al.*, 1997; Kocisova *et al.*, 2000; Lin *et al.*, 2000; Liu *et al.*, 2000; Gu *et al.*, 2001; Jin & Feng 2001; Azzam & Hussein 2002). Resistance of synthetic pyrethroids in the house fly has also been amply documented in these above studies. However, use of deltamethrin for control of flies during 1979-90 has caused low level of resistance to pyrethroids (Keiding, 1995). Therefore, testing of newly developed insecticide is a routine resistance monitoring in house fly. As residual /surface/aerosols application have increased chances of development of insecticidal resistance in the house fly in many parts of the world. These sprays can also contaminate food and water. Hence, baiting is a good choice for the abatement of house fly. Efficacy of the insecticides should be known before adding them into the bait. The insecticides that can give quick and safer control are the best for a baiting system (Saito *et al.*, 1991).

Taking the preceding points into consideration the present work was started to investigate the toxicities of the different insecticides to house fly. For this purpose, a laboratory reared strain of house fly was exposed to dichlorvos 50EC and chlorpyrifos in concentrations ranging from 250-5000 ppm mixed with sugar.

Insecticide synergism is the technique that can be used to control or study insecticide resistance. The synergists are considered straightforward tools for overcoming metabolic resistance, and can also delay manifestation of resistance (BBernard & Philogene, 1993; Yuan & Chambers, 1996).

The insecticides were also co-applied with a synergist (Sesame oil 100% pure) to find out improvement in the efficacy of the insecticides through feeding technique.

MATERIALS AND METHODS

Insecticides

Trade names, common names, formulations and concentrations of insecticides used in the present study are given as under:

Trade name	Common Name	Formulation	Concentration
Thunder	Dichlorvos	50EC	1250-5000 ppm.
Lorsban	Chlorpyrifos	40EC	1000-4000 ppm

The other materials used in this study were beakers, Petri dishes, cotton wool, distilled water, mesh cage, aspirator, granulated sugar, acetone.

Rearing of flies

House flies were reared for bioassay at room temperature in the Crop Pest Management Laboratory, Department of Agri. Entomology, University of Agriculture Faisalabad. Cages, 40 cm long, 40cm wide and 40cm high, were used for the rearing of house fly. The cages were covered with mesh screen with cloth sleeve opening at front. The adult flies were maintained in a mesh cage with granulated sugar and soaked cotton wool in Petri dishes.

The newly emerged flies were also fed with full fat fresh milk soaked in cotton wool for three days after emergence to enhance egg production. Then they were given milk sugar solution in the way mentioned above. After 3 days of fly emergence, the beakers containing larval food were placed for egg lying. The beakers were removed from cages after 2-3 days when eggs were visible and attached to food along the sides of beakers. The food was changed after 2-4 days depending upon the numbers of larvae per beaker. The beakers were kept in separate cage for fly emergence.

Diet

Larval media consisted of yeast, dry milk powder, wheat bran and water. The beaker of 500 ml was filled with this larval media and put in the cage with flies. After 2-3 days these beakers were removed from cages, and a piece of nylon mesh was fastened to the mouth of beakers held in a position by rubber band. The larval media was changed depending upon the number of larvae. When the pupae were formed, these beakers were kept in another cage for adult emergence.

Bioassay Procedures

A: Studies with insecticides

A range of concentrations from 250-5000 ppm of the formulated insecticides was made in acetone to a volume of 10 ml. The 25 gm of granulated sugar was put in a Petri dish, 5 ml of each concentration was added into the sugar on Petri dish and these Petri dishes were left in fume cupboard for overnight to allow the evaporation of acetone. A batch of 50 flies was released in a smaller mesh cage with one concentration of an insecticide and these were repeated thrice.

B: studies with synergist

Sesame oil was used as the synergist. Insecticide was mixed with sesame oil in 1:1 and 1:2 ratio to obtain KD_{50}/LC_{50} of the insecticide + synergist combination. Data on the knockdown effect and mortality were taken after 1, 2, 4, 6, 8, 12, 24 hrs and 48 hrs after treatment.

Statistical Analysis

The dosage-mortality and knockdown data were analyzed using Probit Analysis (Hewlett and Plackett, 1979) to obtain KD_{50}/LC_{50}, x^2 value, slope, and fiducial limits.

RESULTS

Table I shows KD_{50} and LC_{50} values for the effect of various concentration of dichlorvos on the adults of *M. domestica* via feeding method. KD_{50} value was very high during 1 hr, after 2 hr it decreased to 23552 ppm. KD_{50} value decreased during 4, 6, 8 hr from 37080 to 9346 ppm. The slope values during these hours were less than 2 but more than one. At 24 hr, x^2 and H values were 4.53 and 2.26, which indicate random response of flies at this time point. There was no knock down at 48 hr. LC_{50} value from 6^{th} to 48^{th} hr decreased from 168805 to 122 ppm. Slope value during 1^{st} hr value were very high. The slope value during 6, 8, 12, 24 and 48 hr was more than one but less than 2. x^2 and H values during 8^{th} hr were 4.84 and 2.42 and at 12^{th} hr were 3.48 and 1.74, respectively.

Table II shows KD_{50} and LC_{50} values for the effect of various concentrations of chlopyrifos. The KD_{50} value increased during 1 and 2 hr from 1902 to 10771 ppm. This KD_{50} value was dropped down from 9151 to 7651 ppm in the 4th and 6th hr. The slope values during these hrs were > 1 but < 2. KD_{50} value was very high during 12, 24 and 48 hr with x2 value 4.76, 1.87 and

Table I. LC_{50} and KD_{50} of dichlorvos against *Musca domestica* L.

	Fit of Probit Line					Fit of Probit Line				
Time (hr)	KD_{50}	FL 95%	Slope±SE	χ^2	H	LC_{50}	FL 95%	Slope±SE	χ^2	H
1	>124620		1.06±0.42	1.05	0.53	41362	*	2.01±0.42	1.05	0.53
2	23552	11223-240235	1.74±0.47	0.73	0.37	>125260	*	1.23±0.59	0.40	0.20
4	37080	13978-794333	1.20±0.32	0.72	0.36	>365370	*	0.84±0.41	0.06	0.03
6	14059	7414-65936	1.02±0.22	1.01	0.51	16805	8864-75209	1.22±0.26	0.97	0.49
8	9346	6360-18952	1.60±0.27	0.50	0.25	4290	*	1.46±0.21	4.84	2.42
12	9920	6776-20120	1.76±0.29	0.13	0.07	3779	*	1.47±0.21	3.48	1.74
24	18740	*	0.42±0.48	4.53	2.26	1059		0.72±0.19	0.92	0.46
48	No knockdown					122	*	0.27±0.19	0.92	0.46

FL: Fiducial limits ; H, Heterogeneity
* Fiducial limits are not calculated for data has shown large "g" value, g is the index of significance for potency estimation.

Table II. LC_{50} and KD_{50} of chlorpyrifos against *Musca domestica* L.

	Fit of Probit Line					Fit of Probit Line				
Time (hr)	KD_{50}	FL 95%	Slope±SE	χ^2	H	LC_{50}	FL 95%	Slope±SE	χ^2	H
1	1902	9096-144490	1.53±0.37	0.18	0.09	63778	*	1.66±1.09	0.43	0.22
2	10771	6540-30653	1.58±0.30	1.22	0.61	22389	*	2.31±1.16	0.18	0.09
4	9151	5785-22713	1.49±0.27	1.56	0.78	94127	*	1.09±0.44	0.46	0.23
6	7671	5043-16998	1.43±0.25	0.11	0.06	19021	9096-144490	1.53±0.37	0.18	0.09
8	80169	*	0.73±0.25	2.92	1.46	7644	5228-15406	1.69±0.28	0.18	0.41
12	>390960	*	0.60±0.28	4.76	2.38	6678	4660-12669	1.58±0.25	0.80	0.40
24	>172420	*	0.90±0.38	1.87	0.94	5990	3904-13941	1.12±0.21	1.45	0.73
48	>266500	*	1.21±1.05	0.83	0.42	2921	1680-20123	0.52±0.18	0.17	0.09

FL: Fiducial limits ; H, Heterogeneity
- Fiducial limits are not calculated for data has shown large "g" value, g is the index of significance for potency estimation.

Table III. LC_{50} and KD_{50} of dichlorvos+synergist (sesame oil) in 1:1 against *Musca domestica* L.

Fit of Probit Line						Fit of Probit Line				
Time (hr)	KD_{50}	FL 95%	Slope±SE	X^2	H	LC_{50}	FL 95%	Slope±SE	X^2	H
1	103883	21154-769295	0.97±0.35	0.55	0.28	73796	*	1.33±0.59	0.35	0.18
2	>379260	*	0.66±0.30	1.42	0.71	>120330	*	1.11±0.45	0.97	0.49
4	51475	15864-3712197	1.41±0.26	0.39	0.20	37938	14136-1152828	1.35±0.19	014	0.07
6	13629	*	1.41±0.26	3.32	1.66	29052	12393-345625	1.31±0.33	0.32	0.16
8	11409	*	1.32±0.24	2.74	1.37	21614	10622-128159	1.34±0.30	0.03	0.02
12	13040	*	1.46±0.27	2.29	1.14	19980	9750-117735	1.14±0.26	0.41	0.21
24	44072	14505-1887996	0.95±0.27	0.94	0.47	7822	5121-17853	1.18±0.21	0.41	0.21
48	>420500	*	0.38±0.46	0.76	0.38	1500	1178-1933	1.03±0.19	0.00	0.00

FL: Fiducial limits ; H, Heterogeneity
* Fiducial limits are not calculated for data has shown large "g" value, g is the index of significance for potency estimation.

Table IV. LC_{50} and KD_{50} of dichlorvos+synergist (sesame oil) in 1:2 against *Musca domestica* L.

Fit of Probit Line						Fit of Probit Line				
Time (hr)	KD_{50}	FL 95%	Slope±SE	X^2	H	LC_{50}	FL 95%	Slope ± SE	X^2	H
1	>11766	*	0.73±0.45	0.28	0.14	79722	*	1.66±1.09	0.43	0.22
2	>754920	*	0.73±0.40	0.17	0.09	67190	*	1.59±0.82	1.14	0.57
4	>135420	*	0.89±0.34	0.09	0.03	>593030	*	0.73±0.38	0.27	0.14
6	83428	19034-8036600	0.85±0.28	0.26	0.03	>161610	*	0.83±0.32	0.07	0.04
8	24902	1.663-790539	1.00±0.24	0.19	0.10	>129790	*	0.75±0.28	0.03	0.02
12	36517	13485-790539	1.04±0.27	0.85	0.43	60261	15611-2062239	0.77±0.25	0.39	0.20
24	>154870	*	0.81±0.31	0.39	0.20	12756	7265-43804	1.15±0.23	0.17	0.09
48	>138960	*	0.42±0.44	0.01	0.07	1061	*	0.17±0.17	0.25	0.13

FL: Fiducial limits ; H, Heterogeneity

- Fiducial limits are not calculated for data has shown large "g" value, g is the index of significance for potency estimation.

Table V. LC_{50} and KD_{50} of chlorpyifos+synergist (sesame oil) in 1:1 against *Musca domestica* L.

	Fit of Probit Line					Fit of Probit Line				
Time (hr)	KD_{50}	FL 95%	Slope±SE	X^2	H	LC_{50}	FL 95%	Slope±SE	X^2	H
1	13853	7674-55038	1.59±0.34	0.24	0.12	62788	*	1.32±0.55	0.55	0.28
2	10025	6216-26732	1.57±0.29	1.10	0.55	52784	*	1.27±0.46	0.89	0.45
4	8402	5524-18738	1.59±0.28	0.26	0.13	>133320	*	0.92±0.38	0.24	0.12
6	14696	7557-71300	1.24±0.27	0.85	0.43	31327	11349-839903	1.41±0.31	0.05	0.03
8	34259	12919-2901847	0.92±0.26	0.21	0.11	12962	7179-4826	1.37±0.28	0.25	0.13
12	40991	12919-2901847	1.08±0.32	0.09	0.05	10302	5979-32917	1.24±0.24	0.38	0.19
24	>320000	*	0.68±0.31	0.11	0.06	7675	4621-22683	1.07±0.21	0.96	0.48
48	>420500	*	0.38±0.46	0.75	0.38	1500	1119-2046	1.03±0.19	0.00	0.00

FL: Fiducial limits ; H, Heterogeneity
* Fiducial limits are not calculated for data has shown large "g" value, g is the index of significance for potency estimation.

Table VI. LC_{50} and KD_{50} of chlorpyifos+synergist (sesame oil) in 1:2 against *Musca domestica* L.

	Fit of Probit Line					Fit of Probit Line				
Time (hr)	KD_{50}	FL 95%	Slope±SE	X^2	H	LC_{50}	FL 95%	Slope±SE	X^2	H
1	>322390	*	0.65±0.30	0.11	0.06	>313730	*	0.46±0.02	2.95	1.47
2	>139630	6216-26732	0.68±0.27	0.04	0.02	>696120	*	0.43±0.41	0.96	0.48
4	57022	13470-46127853	0.75±0.25	0.32	0.16	>443210	*	0.57±0.41	1.37	0.69
6	59516	13359-10290360	0.71±0.24	0.01	0.01	>252650	*	0.82±0.37	1.35	0.68
8	>454140	*	0.63±0.31	0.14	0.07	66981	14653-115044	0.76±0.26	1.06	0.53
12	>260990	*	0.84±0.40	0.04	0.02	29206	9167-283266	0.71±0.22	1.97	0.99
24	>459420	*	0.58±0.31	0.20	0.10	9785	*	0.96±0.21	2.41	1.20
48	No knockdown					2044	*	0.64±0.19	6.48	3.24

FL: Fiducial limits ; H, Heterogeneity
* Fiducial limits are not calculated for data has has shown large "g" value, g is the index of significance for potency estimation.

0.83 while H value was 2.83, 0.94 and 0.42, respectively. The slope during these hrs was less than one but at 48 hr it was more than one. LC_{50} value was high in 1 hr but decreased in 2nd hr with 22389 ppm values and again increased at 4th hr. During 6, 8, 12, 24 and finally after 48 hr, it decreased gradually with slope more than one except in 48th hr with slope less than one; χ^2 and H value at 48th hr was 0.17 and 0.09, respectively. Slope of LC_{50} at 48th hr was less than one which shows a steep line of the Probit data.

Table III shows KD_{50} and LC_{50} values for the effect of various concentrations of dichlorvos+sesame oil in 1:1. There was no knockdown at 48th hr. KD_{50} value was very high in the 2nd hr. χ^2 value was 3.32 during the 6th hr. LC_{50} value was high at 2 hr and then decreased from 37938 to 7822 ppm, the latter being at 48th hr. The slope limits during these hrs ranged from 0.03 to 0.41.

Table IV shows KD_{50} and LC_{50} values for the effect of various concentrations of dichlorvos+sesame oil in 1:2. KD_{50} value was very high for the 1, 2 and 4th hour with slope less than one and KD_{50} value was also high for 24 and 48 hrs. Similarly the LC_{50} value for the 4, 6, 8hrs was also very high and slope was also less than one. Slope for the 24th hour was more than one but less than 2.

Table V shows KD_{50} and LC_{50} values for the effect of various concentrations of chlorpyrifos+sesame oil in 1:1. KD_{50} decreased from 13853 to 8402 ppm in the 1, 2, 4 hr and then increased at 12th hour. KD_{50} value was very high during 48 hr with χ^2 and H value 0.75 and 0.38, respectively. LC_{50} value was very high at 4th hour and slope value was less than one. From 6 to 24 hr, LC_{50} decreased gradually. The slope during these hrs was less than 2 but more than one.

Table VI shows KD_{50} and LC_{50} values for the effect of various concentrations of chlorpyrifos+sesame oil in 1:2. KD_{50} value was high for the 1st and 2nd hr and high too at 8, 12, 24 hrs. There was no knockdown at 48th hr. LC_{50} value was high for 1, 2, 4, 6 hrs. Slope line for all the hrs was less than one.

DISCUSSION

House fly, *M. domestica* is an important insect pest of household and public health because it transmits a number of deadly diseases like typhoid, cholera, tuberculosis anthrax, mastitis, bacillary dysentery and infantile diarrhea (Service, 1980).

Control of house fly has been mainly dependent upon the use of inorganic and synthetic organic insecticides with the field (Saito *et al.* 1991; Hogsette, 1993; Keiding, 1995; Mabbet, 1996; Yuan and Chambers, 1996; Kocisova *et al.*, 2000).

In the laboratory the exposure of the house fly to the insecticide has been a routine research work in order to extrapolate the insecticide resistance level and its mechanisms. Most of the studies are based on the topical application of insecticides to the adults of house fly, *Musca domestica* (Keiding, 1995).

Very few studies show the insecticide toxicity bioassay via feeding. This type of bioassay can help to develop an effective baiting system. The insecticide having the least KD_{50} value in less time should be the most effective. Chlorpyrifos had a knockdown value 1902 ppm at 1st hour whereas dichlorvos had >124620 ppm KD value in the 1st hour after exposure.

Pyrethroids have been used with the assumption that they were the least hazardous to the environment. Pyrethroids used in aerosols provide an instant kill of the house flies but for the long lasting and persistent control, it is not clear whether pyrethroids can be effective. The insecticide having the least LC_{50} in less time was considered as the effective. The dichlorvos was the most effective with LC_{50} 122 ppm at 48th hr and chlorpyrifos with LC_{50} 183 ppm at the 48th hr.

Insecticide synergism is the technique that can be used to control or study insecticide resistance. It contributed significantly to improve the efficacy of the insecticides, particularly when problems of resistance have arisen (BBernard and Philogene, 1993). Insecticide synergism is the type of interaction of insecticide with another chemical, usually of low toxicity, which results in an unexpectedly high mortality. Sesame oil has been used as synergist to the pyrethroid for the control of house flies. Piperonyl butoxide contain methylenedioxyphenyl moiety, a molecule found in sesame oil and thus termed as sesamin. Thus, sesame oil was used as readily available synergist locally (Yuan and Chambers, 1996). There is also little information regarding synergistic effect of the sesame oil on the house flies via feeding method. If at 48th hr LC_{50} has become less or low then the synergistic effect is there and vice versa. The present date showed antagonistic effect of sesame oil on the two insecticides where the LC_{50} values had become 10 times the values with insecticide alone. No comparable data so far published, parallel to the present studies was available; therefore, further investigation may be carried out to confirm the present data.

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