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Detection of organophosphate resistance in house flies population (*Musca domestica* L.) by laboratory bioassay method

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Abstract
House fly menace is one of the major problems in poultry industry in the recent years. High density of flies not only cause stress to birds and farmworkers, they can also cause great annoyance to nearby human habitations which poses a serious public health problem. Adult house flies were collected from five selected poultry farms using sweep net and reared in laboratory. Three to five days old F_1 generation flies produced by the healthy parental population, maintained in the laboratory were used for topical and contact residual bioassays. In this study, different concentrations of dichlorvos were used for topical bioassay such as, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4 and 0.8 µg (a.i)/µl and 0.14, 0.28, 0.56, 1.12, 2.24 and 4.48 µg (a.i)/cm^2 for residual contact bioassay. Mild to moderate resistance was observed in F_1 generation house flies treated with dichlorvos from all the five farms. The resistance ratio ranged between 1.46 to 15.15 and 6.23 to 22.45 fold by topical and residual contact bioassay respectively. In the present study to identify the level of insecticide resistance in house flies to common insecticides by laboratory bioassay methods.

Keywords: House flies, organophosphate resistance, bioaasay method, Tamil Nadu

Introduction
House fly, *Musca domestica* L. (Diptera: *Muscidae*) is a well-known pest commonly feeds and breeds on decaying matter, human waste and in poultry manure. In poultry farms, manure accumulation under the cages coupled with prevailing temperature and humidity provide an ideal environment for the breeding and development of house flies. Moreover, housefly is capable of transmitting many human and animal pathogens mechanically (Forster et al. 2007) [1]. The poultry farmers generally rely on insecticides as a first choice for the control of house flies. Organophosphates (OP) are being used widely as insecticides for housefly control in many countries (Gao *et al.* 2012) [2] and continued to be the most frequently used insecticides for housefly control in commercial poultry farms in India. Consequently, insecticide resistance has developed among all population of *M. domestica* around the world, causing an important problem for house fly control (Scott *et al.* 1989) [7].

Materials and methods
Maintenance of housefly colonies
Adult flies (>100 individuals designated as parental generations) were collected from five selected poultry farms by sweep net and grown in a fly breeding chamber in the laboratory**.** These flies were reared at 33±1˚C with 60-70 per cent relative humidity and a photoperiod of 12:12 hours light and dark until the F_1 generations were produced (Fig 1). Each 100 gm of larval medium composed of calf feed - 65 gm, yeast-1gm, water - 34 ml (Pinto and Prado, 2001) [5]. Three to five days old F_1 generation flies produced by the healthy parental population, maintained in the laboratory were used for topical and contact residual bioassays.

Bioassays
Dichlorvos topical bioassay
The topical bioassay was conducted as per the guidelines of WHO, 1980 [4] and Srinivasan *et al.* 2008 [9] with minor modifications using F_1 generation of 3-5 day old house flies with ≥ similar size. Prior to topical application, the flies were anaesthetized by keeping in freezer for 2-3 minutes. One microliter of Dichlorvos, containing the required quantity of active ingredient (a.i) in microgram (µg) diluted with acetone was applied on the mesothoracic notum

of the flies using a micropipette. The test was carried out at different doses such as 0.02. 0.04, 0.08, 0.1, 0.2, 0.4 and 0.8 µg (a.i) per fly. The control group houseflies were treated with acetone alone. Twenty adult houseflies in triplicate were used for each concentration. The insecticide treated flies were transferred to plastic container containing honey and sugar coated bread and covered with muslin cloth. Both treated and control groups were maintained under 33±1˚C with 60-70 per cent relative humidity (Fig 2). Data on mortality, number of house flies that remained motionless or paralysed were recorded at periodic intervals until 24 hours post treatment in the observation sheet. Flies that survived above the diagnostic dose were preserved in 70 per cent alcohol for further molecular studies.

Dichlorvos residual contact bioassay

First generation house flies (3-5 days old) were bio-assayed at six different doses of dichlorvos viz, 0.14, 0.28, 0.56, 1.12, 2.24 and 4.48 µg (a.i)/cm^2 coated bottles (250 ml capacity) using technical grade insecticide by residual bioassay method as per WHO (1980) [4] guidelines and Popsilchil *et al.* (1996) [6]. Twenty adult flies each were placed inside insecticide coated bottles for one hour and then transferred to normal container containing honey and sugar coated bread for feeding and covered with muslin cloth. Each concentration was tested in triplicates using twenty adult houseflies along with control group in bottles coated with acetone alone. Both treated and control groups were maintained at 33±1 ˚C with 60-70 per cent relative humidity (Fig 3). Data on mortality, including ataxic flies were considered as dead and recorded at periodic intervals until 24 hours post treatment in the observation sheet. Survived population of houseflies at higher doses were preserved in 70 per cent alcohol for further studies. The observed mortalities were corrected to the control mortalities using the Abbotts formula, if any and then subjected to calculate the lethal doses (LD_{50}/ LD_{99}) by standard probit analysis (Finney, 1971) using SPSS software programme. Resistance ratios (RR) was determined by dividing the LD_{50} or LD_{99} of field collected population by the corresponding value of susceptible strain (Keiding, 1976) [3].

Results

Topical bioassay for organophosphate insecticides

The LD_{50} of dichlorvos tested against F_1 generation flies from selected farms ranged from 0.057 to 0.607 µg (a.i) /fly and the LD_{99} varied from 0.241 to 1.669 µg (a.i) /fly. The results of topical bioassays are shown in Table 1. The LD_{50} and LD_{99} values of dichlorvos were calculated from the regression equation and resistance ratio was calculated by dividing the LD_{50} of the field populations by the LD_{50} of published susceptible population (0.039 µg/ fly) and the level of resistance in each farm was scaled based on WHO recommendation and shown in Table 2. The findings implied a moderate level of resistance in farm- II and V with a RR15.56, 10.33 respectively whereas a mild resistance in farm-I, III (RR-5.28), (RR-3.43) and farm-IV (RR-1.46) respectively.

Residual contact bioassay for organophosphate insecticides

Bioassay of house flies with different concentrations of dichlorvos by residual contact method showed the mortality rate ranged from 40 to 100 per cent. The LD_{99} of dichlorvos tested against F_1 generation flies varied from 0.935 to 3.368 µg (a.i) /cm^2 in different farms and shown in Table 3. The levels of resistance in different farms are shown in Table 4. The moderate level of resistance was noticed in farm-II (RR-22.45) followed by farm-V (RR-14.55) and farm-I (RR-12.10) and mild resistance was observed in farm-III (RR-7.18) and farm-IV (RR-6.23).

Discussion

The results of this study indicate that house fly population from Namakkal region are mild to moderate level of insecticide resistance has been developed. Insecticide resistance develops more quickly when insecticides are applied frequently or in heavy doses to control of house fly in poultry farms.

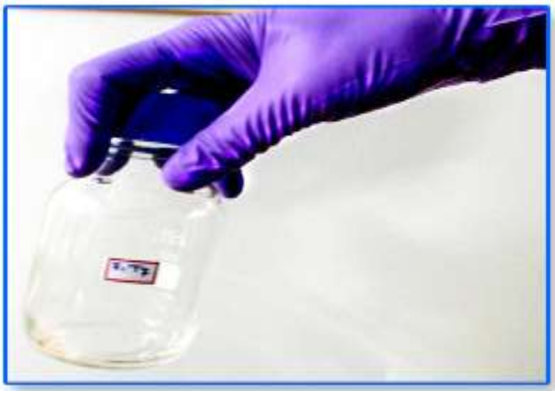
Topical bioassay of houseflies with different concentrations of dichlorvos in the present study showed that, the adult fly mortality had increased as the concentration of the dose increased. The LD_{50} of dichlorvos tested against F_1 generation flies from selected farms ranged from 0.057 to 0.607 µg (a.i)/fly and the LD_{99} varied from 0.241 to 1.669 µg (a.i)/fly. The LD_{50} values of flies in all selected farms were 1.46 to 15.5 times higher than susceptible population, indicating that, all the five field populations are tolerant to this drug. The tolerance could be attributed to the continuous and generous use of organophosphate-dichlorvos for house fly control in the poultry farms ever since it was introduced way back in 1970's. Interestingly, direct application of dichlorvos to knock down heavy population of houseflies during fly breeding season, impelled the farmers for overuse of this insecticide resulting in the development of resistance among field populations of house fly. These findings are in close accordance with Scott *et al.* (2000) [8], stating low to moderate resistant levels to organophosphates from several house fly strains collected from the poultry farms of New York. Likewise, Srinivasan *et al.* (2008) [9] reported the tolerance level of house flies against dichlorvos was 3.5 to 3.9 fold higher than laboratory susceptible population in southern India. Similarly, Wang *et al.* (2012) [10] observed 14 to 28 fold resistance to dichlorvos in Chinese house fly population.

The results of the residual bioassay showed that 6.23 to 22.45 fold resistance in all field populations of housefly. These findings emphasize the unsavoury truth of the prevalence of dichlorvos resistance among house fly populations of Namakkal region. The failure of measures implemented to control house fly populations revealed by the poultry farmers in this belt could be ascribed to the development of tolerance to dichlorvos in house fly due to repeated and over usage of these drugs. These inference were identical to the pattern of resistance was observed by Pospischil *et al.* (1996) [6] and Scott *et al.* (2000) [8] that increase in resistance to organophosphate insecticides in their countries due to repeated usage.

Fig 1: A. Laboratory house fly rearing chamber, B. Larval medium for fly rearing, C. Eggs of house fly in larval medium, D. Laboratory reared larvae of house fly in larval medium, E. Pupae of house fly collected from larval medium, F. F_1 generation of house flies for bioassay.

C

Fig 2: A. Anaesthetized house flies for application of insecticide. B. Topical application of insecticide over the thorax of house flies. C. Maintenance of treated flies in the laboratory.



A

B

C

D

E

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Fig 3: A. Insecticide coating in the bottom, B. Insecticide coating in the top, C. Insecticide coating in the side, D. Drying of insecticide coated bottles, E. Residual contact bioassay-house flies exposed to insecticide coated bottles, F. Maintenance of treated flies in the laboratory.

Table 1: Mean values (Mean ± SE) and percentage of adult house fly mortality in response to dichlorvos-topical bioassay

Dose Conce. µg (a.i) per fly	No. of flies treated/replicate	Farm-I		Farm-II		Farm-III		Farm-IV		Farm-V	
		No. of flies dead	%	No. of flies dead	%	No. of flies dead	%	No. of flies dead	%	No. of flies dead	%
0.02	20	4 ± 0.33	20	0 ± 0.33	0	5 ± 0.33	25	8 ± 0.33	40	0 ± 0.33	0
0.04	20	5 ± 0.33	25	1 ± 0.58	5	6 ± 0.33	30	9 ± 0.58	45	2 ± 0.33	10
0.08	20	8 ± 0.58	40	3 ± 0.88	15	9 ± 0.58	45	14 ± 0.33	70	4 ± 0.58	20
0.1	20	9 ± 0.33	45	4 ± 0.58	20	12 ± 1.20	60	16 ± 0.33	80	5 ± 0.58	25
0.2	20	14 ± 0.33	70	7 ± 0.67	35	15 ± 0.88	75	19 ± 0.58	95	9 ± 0.67	45
0.4	20	17 ± 0.88	85	9 ± 0.67	45	17 ± 0.67	85	20 ± 0.33	100	14 ± 0.58	70
0.8	20	18 ± 0.33	90	11 ± 0.58	55	20 ± 0.33	100	20 ± 0	100	15 ± 0.58	75
Control	20	0	0	0	0	0	0	0	0	0	0

Table 2: LD_{50}, LD_{99} and RR values of dichlorvos in topical bioassay

Insecticide	Populations	n*	LD_{50} (µg (a.i) /fly)	LD_{99} (µg (a.i) /fly)	RR
Dichlorvos	Farm-I	20	0.206	0.883	5.28
	Farm-II	20	0.607	1.669	15.56
	Farm-III	20	0.134	0.542	3.43
	Farm-IV	20	0.057	0.241	1.46
	Farm-V	20	0.403	1.190	10.33
	Reference value (Srinivasan *et al.*, 2008)	20	0.039	-	-

* n= no. of flies treated

Table 3: Mean values (Mean ± SE) and percentage of adult house fly mortality in response to dichlorvos- contact bioassay

Dose Conce. µg (a.i) per cm^2	No. of flies treated/ replicate	Farm-I		Farm-II		Farm-III		Farm-IV		Farm-V	
		No. of flies dead	%	No. of flies dead	%	No. of flies dead	%	No. of flies dead	%	No. of flies dead	%
0.14	20	12 ± 0.58	60	8 ± 0.33	40	10 ± 0.88	50	11 ± 0.33	55	9 ± 0.88	45
0.28	20	14 ± 0.33	70	10 ± 0.67	50	12 ± 0.58	60	13 ± 0.33	65	12 ± 0.33	60
0.56	20	15 ± 0.33	75	12 ± 0.88	60	13 ± 0.58	65	15 ± 0.58	75	13 ± 0.58	65
1.12	20	16 ± 0.58	80	14 ± 0.58	70	20 ± 0.33	100	20 ± 0.33	100	14 ± 0.33	70
2.24	20	20 ± 0.33	100	17 ± 1.15	85	20 ± 0.33	100	20 ± 0.33	100	20 ± 0.33	100
4.48	20	20 ± 0	100	20 ± 0.33	100	20 ± 0	100	20 ± 0	100	20 ± 0	100
control	20	0	0	0	0	0	0	0	0	0	0

Table 4: LD_{99} and RR values of dichlorvos in residual contact bioassay

Insecticide	Populations	n*	LD_{99} (µg (a.i) /cm^2)	RR
Dichlorvos	Farm-I	20	1.815	12.10
	Farm-II	20	3.368	22.45
	Farm-III	20	1.078	7.18
	Farm-IV	20	0.935	6.23
	Farm-V	20	2.183	14.55
	Reference value (Popspichil *et al.*, 1996)	20	0.15	-

* n= no. of flies treated

Conclusion

Based on the results obtained in the present study, topical and residual contact methods were performed in house flies (F_1 generation) from selected farms using commonly used drugs dichlorvos to revealed that all the five studied field populations showed mild to moderate resistance to dichlorvos as evidenced in topical (RR - 1.46 to 15.5 fold) and residual contact (RR - 6.23 to 22.45 fold) methods.

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