

Original Article

Dichlorvos Resistance in the House Fly Populations, *Musca domestica*, of Iranian Cattle Farms

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Abstract

Background: Insecticide resistance is one of the most important problems associated with the control of *Musca domestica*, due to the potential of the rapid development of resistance to different chemical insecticides. The present study was carried out to evaluate dichlorvos resistance in the house fly populations collected from central regions of Iran, Isfahan Province and Chaharmahal and Bakhtiari Province, during 2017 to 2019.

Methods: Bioassays were carried out using a standard topical application method as well as a fumigation method. The Koohrang population (susceptible) with the lowest LD₅₀ values to dichlorvos was chosen to calculate the resistance ratios (RR). Altered sensitivity of acetylcholinesterase (AChE), a target enzyme for dichlorvos, was investigated.

Results: According to the results, very high levels of dichlorvos resistance were observed in the Mobarake population (RR= 80.25-fold by topical application and 33-fold by fumigation bioassay), and Isfahan population (RR= 107.30-fold by topical application and 43-fold by fumigation bioassay) compared to the Koohrang population. Acetylcholinesterase of the Koohrang population was the most sensitive to inhibition by dichlorvos based on the determination of median inhibitory concentration (IC₅₀), but AChE of Mobarake and Isfahan populations were 741.93- and 343.94- fold less sensitive to inhibition.

Conclusion: The insensitivity of AChE was possibly involved in dichlorvos resistance in the house fly populations.

Keywords: Organophosphorus insecticides; Target site resistance; Acetylcholinesterase; Median inhibitory concentration

Introduction

The house fly, *Musca domestica* Linnaeus, is a crucial pest in medical and veterinary. House fly is a vector of different kinds of pathogens in humans and animals. The flies not only act as a source of annoyance but also transfer pathogens mechanically when moving to residential, commercial, livestock, and poultry places (1). The chemical control, often against the adult stage of house flies, is mainly by synthetic insecticides such as pyrethroids, neonicotinoids, organophosphates, and carbamates (2-5). In the dairy and poultry industry, house flies are considered as a major pest, and for its control, pyrethroids and organophosphates are extensively applied in Iran.

Several organophosphorus (OPs) compounds including of dichlorvos, diazinon, fenchlorphos, malathion, fenthion, dimethoate, and trichlorfon are used for house fly control (4). Organ-

ophosphorus bind to AChE, which leads to an accumulation of acetylcholine (ACh) in cholinergic synapses and subsequently disrupt nervous functions, resulting in paralysis and death (6).

Among OPs, dichlorvos (O, O- dimethyl-O-2,2-dichlorovinylphosphate or DDVP) has been recognized as one of the widely used insecticides for the management of house fly and other arthropod pests (7). However, the World Health Organization has classified dichlorvos as a highly hazardous pesticide (8), and harmful to human and animal health by long-term low-level dietary uptake of food containing dichlorvos residues (9). Furthermore, there are several reports on the development of dichlorvos resistance in this species all over the world (10-16). Development of resistance may cause increasing the dosage and frequency of

insecticide applications in the residential, commercial, livestock, and poultry places, which enhances the cost of control, and it also has negative impacts on the environment (17, 18).

Also, metabolic resistance via the enhanced activity of detoxification enzymes has been associated with resistance to OPs, and modified AChE has been reported as the main mechanism of resistance (19, 20). Biochemical characterization of altered AChEs has shown that there is a wide range of insensitivity between insect species and between OPs compounds (21). Insensitivity of AChE to OPs insecticides in house fly was documented for the first time in 1973 (22). Several mutations in the AChE gene of the house fly have been proved to be involved in OPs resistance (21). Despite numerous cases of insecticide resistance in the house fly, there is a broad spectrum in insecticide sensitivity between populations. Thus, the assessment of resistance to different insecticides in regional populations of house flies can provide useful information for the fly control and insecticide-resistant management programs (17, 23). In the present study, efforts were made to understand dichlorvos resistance status and mechanisms in the different house fly populations.

Materials and Methods

Chemicals

Technical grade of dichlorvos insecticide (98.7%) was provided by Golsam Sepahan Company (Iran), acetylthiocholine iodide (ATChI), Coomassie Brilliant Blue G-250, bovine serum albumin, 3,3',5,5'-, fast blue RR salt were purchased from Sigma-Aldrich (Germany).

Insect rearing

The house fly populations were collected from dairies in Mobarake (Isfahan Province-32.3347°N, 51.5571°E) and Isfahan (Isfahan Province -32.6546° N, 51.6680° E). The population of Koohrang (Chaharmahal and Bakhtiari Province -32.3297° N, 50.1112° E) was col-

lected from a rural area where insecticides had not been used. Adults were reared under laboratory conditions of 25±2 °C, 16:8 (L: D), and 60±5% relative humidity. The adult diet consisted of sesame meal and wheat bran (1: 3) in a plastic container. Also, a mixture of water and sugar (10%) was provided in another plastic container. Larvae were transferred to plastic buckets containing 20g a diet, included sesame meal and wheat bran (1: 3), 1.5g milk powder, 1.5g honey mixed with 8ml of water. Female house flies were used for bioassays.

Topical and fumigation bioassays

Topical bioassays were followed, as described by Kasai et al. (24). Briefly, technical grade dichlorvos solved in acetone, and then 1µL were topically applied by micropipette (Nichiryo Model 8100, Tokyo, Japan) on the notum of CO₂-anesthetized flies (3–5-day-old females, n= 20 flies per concentration/per replication). In control groups, flies only received topical application of acetone. The treated flies were released in plastic jars (250ml) containing cotton moistened with a 20% sugar solution. Mortality data were recorded after 24h exposure to insecticide. For bioassays, 5 to 6 concentrations of insecticide were prepared as serial dilutions in acetone and replicated three times.

The fumigant bioassay was conducted, according to Rossi et al. (25), with slight modifications. Briefly, female house flies (3–5-days adult) were placed in a fitted glass jar (650ml). Serial dilutions prepared dichlorvos concentrations in acetone were then placed on a cotton pad inside a Petri dish; the dish was sealed to prevent fly contact and was placed inside a glass bottle. The bottle was sealed tightly and kept in a home temperature (25±2 °C) for 30 min. Mortality data were recorded after 30min of exposure. Each test was replicated three times.

Acetylcholinesterase activity and inhibition by dichlorvos

Twenty heads of 3–5-day old females from each population were homogenized in 1mL of

sodium phosphate buffer (0.1M, pH 7.5), containing 0.1% (w/v) Triton X-100 in ice-cold conditions. After centrifugation (12,000g at 4 °C for 15min), the supernatant was used for the enzyme assay. The AChE activity was assayed based on the method of Ellman et al. (26) with slight modifications. The ATChI (10 mM) was used as the substrate. To determine median inhibitory concentrations (IC_{50s}) aliquots (10 μ L) series of dichlorvos concentrations mixed with 20 μ L enzyme source and 70 μ L sodium phosphate buffer solution and the mixture was incubated for 5min at room temperature (25 ± 2 °C). The reaction was started by adding 900 μ L of substrate - reagent solution, containing 1mL ATChI, 250 μ L DTNB reagent (10mM) dissolved in 8.750mL sodium phosphate buffer (0.1M, pH 7.5). The control treatments were prepared by adding 10 μ L of acetone without insecticide. The acetone concentration of all reactions was 1%. The change in absorbance was measured using a spectrophotometer (Unico, Model UV-2100, USA) at 412nm for 10min with a read interval of the 30s at room temperature (25 ± 2 °C). The tests were replicated three times. Protein content was determined by the Bradford method (25), and bovine serum albumin was used as the standard. To convert absorbance into molarity an extinction coefficient of $13.6mM^{-1} cm^{-1}$ was used. The specific activity of AChE expressed as nmol of acetylthiocholine iodide hydrolyzed per min per mg protein ($nmol min^{-1} mg$ of protein $^{-1}$). The inhibition rate was calculated as a percentage with respect to the control by the following formula (26):

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{Enzyme Activity of Treatment}}{\text{Enzyme Activity of Control}} \times 100 \right]$$

Statistical analyses

Percentage mortality data of the topical application and fumigant assay were corrected by using Abbott formula (27), and data were inputted to the POLO-Plus software for analysis (28). Median inhibitory concentration (IC_{50}) values were determined by probit analysis between the inhibition percentages against the insecticide concentrations (29). The AChE enzyme activi-

ties were subjected to ANOVA, and differences among means were compared by the LSD test ($P < 0.05$) using SAS 9.4 software (30).

Results

Topical and fumigation bioassays

The LD_{50} values of dichlorvos in the Isfahan, Mobarake, and Koohrang populations by the topical application were estimated 515.29, 385.22, and 4.80 μ g/fly, respectively. The Koohrang population exhibited the lowest LD_{50} value and was used as the reference strain to evaluate the resistance ratios (RR). Therefore, high RR values were observed in Mobarake (80.25-fold) and Isfahan (107.30-fold) populations (Table 1).

The fumigation assay of the dichlorvos insecticide on female house flies on different populations was performed, and the LD_{50} values against dichlorvos in the Isfahan, Mobarake, and Koohrang populations were 1.34, 1.00, and 0.03 (μ L/L), respectively (Table 2). Fumigant bioassay revealed high resistance levels to dichlorvos in Mobarake and Isfahan populations, 33- and 43-fold, respectively.

Acetylcholinesterase activity and inhibition by dichlorvos

The activity of AChE was highest in the Mobarakh population ($155.65\pm 2.83nmol/min/mg$ Protein) and Isfahan population ($234.79\pm 7.07nmol/min/mg$ protein). It was lowest in the Koohrang population ($79.25\pm 2.28nmol/min/mg$ protein) (Table 3). The AChE activities in the Mobarakh and Isfahan populations were significantly higher than that of the Koohrang population ($P < 0.05$). Sensitivities of three AChEs to dichlorvos as an inhibitor were also determined. The estimated IC_{50} values by series of dichlorvos concentrations showed the insensitivity of AChE to the insecticide in the resistant populations. Acetylcholinesterase of the Koohrang population was the most sensitive, and AChEs of Mobarakh and Isfahan populations showed insensitivity to dichlorvos (Table 3).

Table 1. Contact toxicity of dichlorvos insecticide on the field populations of house flies

Insecticide	populations	n	LD ₅₀ (µg/fly) (95% FL)	Slope±SE	Chi- square	Df	RR
dichlorvos	Koohrang	360	4.80 (4–5.8)	1.885±0.173	0.72	4	-
	Mobarake	322	385.22 (296–502)	1.337±0.156	0.8	4	80.25
	Isfahan	300	515.29 (387–710)	1.242±0.161	0.078	4	107.3

n: number of flies used in bioassays

FL: fiducial limits

DF: degrees of freedom

RR: LD₅₀ of Mobarake or Isfahan/ LD₅₀ of Koohrang

Table 2. Fumigant toxicity of dichlorvos on the field populations of house flies

Insecticide	populations	n ¹	LD ₅₀ (µL/L) (95% FL)	Slope±SE	Chi-square	Df	RR
dichlorvos	Koohrang	360	0.03 (0.029–0.044)	1.651±0.167	0.845	4	-
	Mobarake	360	1.00 (0.87–1.3)	1.565±0.158	0.174	4	33
	Isfahan	360	1.3 (1–1.6)	1.479±0.154	0.229	4	43

n: number of flies used in bioassays

FL: fiducial limits

DF: degrees of freedom

RR: LD₅₀ of Mobarake or Isfahan/ LD₅₀ of the Koohrang population

Table 3. Mean AChE activity (nmol/min/mg protein) and its inhibition by dichlorvos

populations	AChE	Ratio ^A	IC ₅₀ (M)	Ratio ^B
Koohrang	79.25±2.28 ^c	-	3.14×10 ⁻⁷ ±1.56	-
Mobarake	155.65±2.83 ^b	1.96	2.32×10 ⁻⁴ ±5.78	741.93
Isfahan	234.79±7.07 ^a	2.96	1.08×10 ⁻⁴ ±2.33	343.94

A: ratio of enzyme activity in the resistant population/ enzyme activity in the susceptible population

B: ratio of IC₅₀ in the resistant population/ IC₅₀ in the susceptible population

^{a, b, c} significantly different by applying LSD (P< 0.05)

Discussion

Dichlorvos is one of the most common insecticides used for house fly control in cattle farms of Isfahan Province. Therefore, monitoring of susceptibility of the fly to dichlorvos is necessary for managing programs of *M. do-*

mestica. To our knowledge, no information is available on the resistance status of house flies of Iranian cattle farms. In the present study, levels of resistance to dichlorvos were determined through both topical and fumigation applica-

tion methods in different field-collected populations.

Because of a known reference strain was not available, in this study the most susceptible strain, i.e. the Koohrang population, was considered as the reference strain. Although this strain possibly was not fully susceptible, field populations showed significantly high resistance ratios, justifying the resistance mechanisms. According to the results, high RR values to dichlorvos were observed in both cattle farm populations of Mobarake and Isfahan, ranging from 33- to 107.30-fold. The RR values in topical bioassay were estimated higher than fumigant bioassay in the resistance populations of the house fly. It could be associated with the cuticular penetration factor involved in the resistance that can decrease insecticide penetration (31, 32). Several studies have already documented house fly resistance to dichlorvos and other OPs. Wang et al. (35) reported a 14- to 28-fold resistance to dichlorvos. Moderate levels of resistance have also been reported from the house fly population in Argentina (33) and Denmark (34). Resistance to dichlorvos has also been found in other medically important Diptera, such as *Aedes aegypti* (35). Also, low RR value to temephos was observed in *Anopheles stephensi* in the Chabahar sea of Iran (36). Furthermore, OPs resistance has been investigated in crop pests. High levels of resistance to OPs insecticides were reported in *Spodoptera litura* (229-fold) (37) and *Tetranychus urticae* (4164 -fold) (38).

The main mechanism of OPs resistance is altered AChE, which led to enzyme insensitivity to inhibition by insecticides. Also, 2- to 5- fold increases in the activity of AChE were associated with OPs resistance in *Drosophila melanogaster* and *M. domestica*, respectively. The higher AChE activity in strains with altered AChE could be directly contributed to OPs resistance or compensate for decrease AChE hydrolysis (11, 39). Soltani et al. (43) have reported the altered AChE in *Anopheles stephensi* in the south of Iran, which causes resistance to

temephos insecticide. In this study also an increase in AChE activity was observed in resistant populations of Mobarake (1.96-fold) and Isfahan (2.96- fold).

Walsh et al. (21) have reported a higher than 500-fold insensitivity of AChE to dichlorvos in house fly resistant populations. Moreover, it has reported that resistance to OPs in *Schizaphis graminum* contributed to increased AChE activity through elevated expression of the AChE gene (40) and AChE insensitivity (41). Based on the biochemical assays on the AChE inhibition by dichlorvos and obtained IC₅₀ values, target-site modification is possibly involved in resistance to dichlorvos in house fly populations. The AChE of the Koohrang population was most sensitive, while AChE of Mobarake and Isfahan populations showed 741.93- and 343.94- fold insensitivity to dichlorvos. Several point mutations in the AChE gene can confer enzyme insensitivity to inhibition by OPs insecticides (38, 42-44).

Metabolic detoxification has been demonstrated to be a key OPs resistance mechanism in the house fly, which is mediated by cytochrome P450 monooxygenases (P450s), the glutathione S-transferases (GSTs) and the carboxylesterases (CarEs) (45, 46). Ahmadi et al. (5) determined detoxification enzyme activities in Koohrang, Mobarake, and Isfahan populations and reported significantly lower activities of P450s, GSTs, and CarEs in the Koohrang population than that of Mobarake and Isfahan populations. P450s have been implicated to play major role in conferring OPs resistance in house fly strains. For example, OPs resistance in the Rutgers strain has been linked to the overexpression of P450s (47). Thus, enhanced activity of P450s in Mobarake and Isfahan populations (more than 2.2 folds) possibly contribute to dichlorvos resistance. In house flies, enhanced production of CarEs has been implicated as contributing to resistance to OPs and other insecticides (48). In the ALHF house fly strain, OPs resistance have been found due to increased ac-

tivities of CarE (49). The CarE activity in the OPs resistant strains of Mobarake and Isfahan was reported significantly higher than that of Koohrang population (3.7- and 2.01-fold, respectively). OPs resistance in house flies has been also linked to increased activities of GSTs (45). Previously, 4.9 and 5.2- fold higher activities of GSTs were found in Mobarake and Isfahan populations compared to the Koohrang population (5). It seems that enhanced detoxification by P450s, GSTs, and CarEs is an important mechanism of dichlorvos resistance in Mobarake and Isfahan populations. The enhanced detoxification could also be responsible for cross-resistance to other insecticides in house fly populations (50).

Insecticide resistance can increase costs and doses of insecticides in the house fly control, as well as decrease inefficiency of control (18). It also has environmental pollution problems and human health risks and negatively effects the non-target organisms (51).

Conclusion

The present study revealed high levels of dichlorvos resistance in house fly populations. AChE insensitivity and enhanced metabolic detoxification identified as the conferring mechanisms. However, the molecular mechanisms involved in AChE insensitivity and enzyme detoxification remain uncharacterized. Further studies in Iranian cattle farms are needed to confirm these findings and to design management strategies to delay the development of insecticide resistance in house fly populations.

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The authors declare that there is no conflict of interests.

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