Evaluation of resistance or susceptibility of the house fly, *Musca domestica* L., of semi-industrial livestock farms to some pyrethroid insecticides in Ahvaz, southwestern Iran

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Abstract

Introduction: The house fly, *Musca domestica* L., is known as one of the most important hygiene problems worldwide. It has shown a high potential to develop resistance to chemical insecticides. This study was undertaken to determine the susceptibility or resistance of the house fly, which were collected from 3 livestock farms near the city of Ahvaz, to prevalent pyrethroid insecticides (delthamethrin, lambda-cyhalothrin, cypermethrin).

Methods and Materials: Females of 3 field strains, Molasani, Saiahi and Shoeibi, and one susceptible population, were exposed to each insecticide with two bioassay methods: topical and residual methods.

Results: All three populations developed resistance to the prevalent pyrethroid insecticides**.** This phenomenon was determined by both topical and residual methods. The highest resistance level to delthamethrin insecticide was observed in the Molasani population with a resistance factor of RF>500 determined by both bioassay methods. The Siahi population showed the highest level of resistance to both the lambda-cyhalothrin and cypermethrin insecticides. Resistance factor values of this population to the above-named insecticides recorded by both bioassay methods were RF>1000 and RF>450, respectively. The highest resistance level of all was observed in the Siahi strain to cypermethrin.

Conclusions: Continuous use of insecticides with the same modes of action results in the development of class- or cross-resistance in target pests. Rotational use of insecticides with different modes of action could lead to the destruction of the resistant population and reduce the development of resistance in the house fly population of these places.

Keywords: Resistance, Susceptibility, *Musca domestica*, Livestock Farm, Ahvaz.

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Introduction

The house fly, *Musca domestica* L., is one of the most important pests from both a medical and a social standpoint. It can affect performance of livestock and poultry, cause irritation to humans, and possess considerable potential for mechanical transmission of various pathogens to man and livestock (1). Chemical control of this insect pest often focuses on the use of insecticides such as pyrethroids and organophosphorates. However, this insect has successfully adapted to most insecticides [1, 2, 3, 4, 5, 6, 7, 8, 9]. From the estimated 10000 arthropod pests, 553 species are reported to have insecticide resistance. This phenomenon was first reported in 1947 against D.D.T. (9, 10). The house fly is one of 20 species that have shown the highest resistance to insecticides, and it is placed in the fifth row. This insect has become resistant to 44 different chemical insecticides, and its resistance is found to be due to a specific gene expression in the adult and larvae (10). The insecticide resistance of insect pests has many socioeconomic impacts, including administrative, operational, financial, social, and agricultural implications (9). Chemical control plays a fundamental role in integrated fly management programs. Monitoring of the house fly population`s susceptibility to insecticides is required for the effective use of insecticides. Early detection of an insect pest's resistance to chemical insecticides and selecting a more effective strategy to control them can efficiently reduce operational, financial, and social losses. Therefore, the objectives of this study were to determine the susceptibility or resistance of the house fly to prevalent insecticides using semi-industrial livestock farms near the city of Ahvaz and to present strategies to prevent or reduce the development of this phenomenon in the house fly populations.

Methods and Materials

Insect: Adults of 3 field house fly populations were collected from different livestock farms (Molasani, Shoaibei, and Siahi livestock farms). They were reared at 26°C, 50±5% RH, and a photoperiod of 14:10 (L: D). Water and food in the form of sugar and powdered milk were provided to adults. Larval media comprised wheat bran, date extract, and dried alfalfa suspended in water. The susceptible strain was provided by the Department of Medical Entomology of Tehran University. For the tests, only two- and three-day-old females were used.

Insecticides: Pyrethroid insecticides of technical grade, including lambda-cyhalothrin (99.5%), cypermethrin (99%), and deltamethrin (99.3%), were purchased from the representative of Chem Service Company in Iran (Danesh Afzare Pishro Jahan).

Bioassay tests: Residual Exposure: In this method, glass Petri dishes were used (interior bottom diameter = 9cm) and the insecticides were dissolved in acetone to made stock solution. Serial dilutions were prepared from the stock solution. 1ml of each concentration was applied to the bottoms of 4 Petri dishes and distributed uniformly by gently rotating the dishes as the concentration dried. Groups of 25 females were anesthetized using CO_2 and placed onto the covers of the treated Petri dishes to allow them to recover from the anesthesia before being exposed to the treated surface. Flies were then exposed to the treated surface for 1 hour. Next, they were transferred to non-treated containers and maintained at a constant temperature of 27ºC. Control groups were exposed to surfaces treated with acetone alone. Mortality was recorded after 24h.

Topical method: Adults of *M. domestica* were narcotized with carbon dioxide, and each insect was treated topically using a micro-applicator. One microliter of each insecticide dissolved in acetone was dropped on the mesonotum of the flies. The control flies were treated with acetone only. Mortality was recorded 24h post-treatment. This test was repeated 4 times.

Data Analysis:

In order to determining lethal doses or concentrations (LD_{50} and LD_{90}), dose-mortality data was submitted to probit analysis using toxicology software (Polo Software, 2007). The resistance factors or resistance ratios calculated using the following formula:

Resistance Factor (RF) = LC_{50} (field population) / LC_{50} (Susceptible population)

Resistance was evaluated on the basis of RF for LC_{50} in four categories: Low RF<10, moderate RF=11-40, high RF= 41-160, and very high RF>160.

Results

Probit analysis of the mortality data of the house fly to deltamethrin, lambda-cyhalothrin, and cypermethrin demonstrated that all three field strains have developed different levels of resistance to the mentioned insecticides .The lethal doses (LD_{50}) of delthamethrin in the susceptible, Molasani, Siahi, and Shoaibei strains using the residual method were 0.022, 13.01, 6.08, and 8.05 µg (ai) per cm^2, and the resistance factors of the field strains were 591.4, 276.4, and 365.9, respectively. According to the resistance factor values, it is clear that the three field populations have developed very high level of resistance to deltamethrin (RF>160). The highest resistance of *M. domestica* to deltamethrin was observed in the Molasani strain (Table 1). Susceptibility of the house fly to lambda-cyhalothrin showed also that all three field-collected strains have become resistant to this insecticide. The calculated lethal dose (LD_{50}) values were 0.027, 15.98, 38.88, and 10.74 µg (ai) per cm^2 for susceptible, Molasani, Saiahi, and Shoaibi strains using the residual method, and resistance factors of the field strains were 591.8, 1440, and 397.8 respectively. The highest resistance of the house fly to lambda-cyhalothrin was recorded in the Saiahi population (Table1).

Lethal doses (LD_{50}) of cypermethrin to the house fly were 0.083, 34.1, 64.3, and 32.22 µg (ai) per cm^2 for susceptible, Molasani, Saiahi, and Shoaibi strains, respectively, and the resistance factors of the field- collected populations were respectively 410.8, 739, and 388/2. Similar to lambda-cyhalothrin, the highest resistance to cypermethrin was observed in the house fly strain collected from the Siahi livestock farm.

The bioassay of the field-collected house fly using topical method confirmed that all the three house fly strains have developed very high resistance to pyrethroid insecticides in comparison to the susceptible strain (RF> 160) (Table2). Although the RF values obtained with the topical method were lower than those obtained with the residual method, but the previous results were confirmed.

Table1: Toxicity of selected insecticides to the house fly, *M. domestica* from Ahvaz city with residual bioassay method (µg (ai) per cm^2)

Insecticides	Insect strains	LD_{50} (FL 95%)	Slope± SE	Heterogeneity Factor	RF (LD_{50})
	Susceptible	0.022(0.019-0.027)	1.84±0.14	0.792	-
Deltamethrin	Molasani	13.01(10.05-17.5)	1.07±0.12	0.716	591.4
	Siahi	6.08(4.99-7.33)	1.54±0.14	0.887	276.4
	Shoaibi	8.05(6.61-9.79)	1.47±0.13	0.839	365.9
	Susceptible	0.027(0.023-0.031)	0.34±0.20	0.96	-
Lambda-Cyhalothrin	Molasani	15.98(13.21-19.13)	1.63±0.14	0.59	591.8
	Siahi	38.88(28.94-55.74)	0.95±0.12	0.68	1440
	Shoaibi	10.74(8.81-13.06)	1.47±0.13	0.84	397.8
	Susceptible	0.083(0.068-0.101)	1.51±0.13	0.41	-
Cypermethrin	Molasani	34.1(27.12-42.9)	1.23±0.13	0.59	410.8
	Siahi	61.34(45.75-88.24)	0.94±0.12	0.74	739
	Shoaibi	32.22(169.09-384.79)	1.47±0.13	0.84	388.2

Resistance Factor (RF)= LD_{50}(Field strain)/ LD_{50}(susceptible strain)

Table2: Toxicity of selected insecticides to the house fly, M. domestica from Ahvaz city with topical bioassay method (µg (ai) per fly)

Insecticides	Insect strains	LD_{50} (FL 95%)	Slope± SE	Heterogeneity Factor	RF (LD_{50})
	Susceptible	0.012(0.011-0.013)	4.30±0.47	0.95	-
Deltamethrin	Molasani	6.69(5.28-8.13)	1.78±0.23	0.78	557.5
	Siahi	3.09(2.89-5.13)	1.79±0.25	0.887	140.45
	Shoaibi	4.4(3.6-5.29)	1.84±0.20	0.839	366.67
	Susceptible	0.017(0.014-0.020)	2.32±0.20	0.99	-
Lambda-Cyhalothrin	Molasani	8.06 (6.75- 9.5)	2.06±0.24	0.52	474.12
	Siahi	17.31(15.41-19.02)	4.02±0.51	0.63	1018.23
	Shoaibi	3.55(2.75-4.63)	1.57±0.19	0.87	208.82
	Susceptible	0.043(0.068-0.101)	1.51±0.17	0.40	-
Cypermethrin	Molasani	11.84(9.92-14.27)	1.77±0.18	0.95	275.35
	Siahi	19.64(16.11- 24.86)	1.78±0.21	0.46	456.74
	Shoaibi	12.3(10.55-14.37)	2.32±0.22	0.62	286.05

Resistance Factor (RF)= LD_{50}(Field strain)/ LD_{50}(susceptible strain)

Discussion

Pyrethroids are the main group of chemical insecticides used to control the house fly and other arthropod pests on the livestock farms surveyed in this study. All three field-collected house fly strains have developed a very high level of resistance to pyrethroid insecticides. This can be confirmed using either the residual method or the topical application method. Many studies have shown the house fly's resistance to chemical insecticides (2, 3, 4, 6, 7, 11, 12, 14, 15, 16, 17, and 19). It was determined using both the topical and residual methods that the Molasani strain of *M. domestica* has developed a very high resistance to deltamethrin (RF>550), while the highest level of resistance to lambda-cyhalothrin and cypermethrin was observed in the Siahi strain with resistance factors of RF >1000 and RF> 450, respectively. The continuous use of insecticides to control pests can cause the development of resistance in target or non-target insects in the same treated area (15). This phenomenon was reported in the house fly population from an agricultural area in Malaysia in which pesticides have been used to control agricultural pests (16). The resistance of *M. domestica* collected from a livestock farm in Albany to permethrin increased from 260-fold in the initial population to 1800-fold within 5 generations after exposure to this insecticide. This house fly population showed a very high potential for developing class- and cross-resistance to pyrethroids or other groups of insecticides with the same mode of action. Its resistance ratios against cyfluthrin, cypermethrin, deltamethrin, and propoxur were respectively 2400, 4200, 10000, and more than 290-fold (17). Conversely, four field strains of *M. domestica* collected from Thailand were susceptible to permethrin with LD_{50} values of 0.0049, 0.0019, 0.0003, and 0.0005 µg (ai) /fly and to deltamethrin with LD_{50} values of 0.1058, 0.0185, 0.1251, and 0.0981µg (ai) /fly (15). Moreover, house flies collected from two beef cattle feedlots in southeastern Nebraska were moderately resistant to permethrin (RF = 4.9-fold and RF = 7.3-fold using topical and residual methods, respectively) and extremely resistant to stirofos and methoxychlor (12). The residual exposure method was more sensitive in resistance detection (i.e., higher resistance ratio) (12), and our results also confirm this conclusion. The status of resistance to cyromyzine, DDVP, and permethrin in house flies from three Argentinean poultry farms was studied. All three studied populations showed resistance ratios (RRs) to cyromyzine of 3.9, 10.98, and 62.5, respectively. The highest levels of resistance were observed toward DDVP and permethrin. The RRs to DDVP ranged from 45.5 to 62.5 and from 65.52 to 117.34 to permethrin (5). Multiple resistances of *M. domestica* to pyrethroids, organophosphates, and carbamates were tested by impregnated filter paper and topical application in Germany. The fly strains tested showed a strong resistance to the insecticide groups, ranging from 37- to > 10000-fold for organophosphates, more than >530 for carbamates, and 150- to >6600-fold for pyrethroids (11). All these studies confirm our results that the house fly has the potential to develop resistance.

It seems that the continuous use of pyrethroid insecticides for controlling the house fly and other ectoparasites, such as ticks, fleas, and lice, on Ahwaz livestock farms (personal discussions with the chief of veterinary office, Ahvaz) led to the development of resistance to other insecticides of the same group by the house fly. Rotational use of insecticides with different modes of action is one of the main strategies used to reduce the development of resistance in insect pests such as house flies in such places. This strategy can also lead to a reduction in use of pesticide volume, and economic costs as well as increased safety to humans and non-target organisms.

The study results of Sharififard *et al.* (2009) showed that all strains of *M. domestica* collected from selected poultry and livestock farms of different cities in Khuzestan Province were susceptible to spinosad insecticide (18). The mode of action of this insecticide varies slightly with that of pyrethroid insecticides (2, 19), so it can be used rotationally with pyrethroids in integrated house fly management programs. Abuse of chemical insecticides in areas where the target pest is resistant to them can lead to a high volume overshoot of insecticides in addition to the failure to control the target pest and much harm to humans and the environment. Therefore, it is necessary to detect the resistance phenomenon in pests and vectors and provide programs to reduce or eliminate it.

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