Original Article

Monitoring of Thiamethoxam Resistance in Turkish House Fly Strains, *Musca domestica* (Diptera: Muscidae)

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Abstract

Background: The house fly, *Musca domestica*, is vector for pathogens and parasites and causes economic damage to livestock by reducing forage conversion efficiency, negatively impacting weight gain and milk production. It has shown resistance to multiple insecticide classes. The aim of this research was to determine the susceptibility levels of seventeen field *M. domestica* strains to thiamethoxam, a neonicotinoid insecticide, in Türkiye.

Methods: Insecticide susceptibility of the house flies to thiamethoxam was determined using the WHO glass jar method. A probit analysis program was used to determine LD_{50} values, and then the resistance ratios were compared with insecticide-susceptible strain.

Results: All strains were ≥18.5-fold resistant to thiamethoxam. The data showed that 10 out of 17 strains had either high or very high resistance levels. Our findings revealed that house flies from solid waste landfills in Samsun, Ankara, and Kocaeli exhibited higher resistance ratios compared to those found in animal shelters. Conversely, in Gaziantep, Antalya, İzmir and Erzurum, the exact opposite trend was observed. Regarding the LD_{50} values among solid waste storage areas, the lowest rate was obtained from Gaziantep (0.72 gr ai/m^2), and the highest rate was obtained from Ankara (9.35 gr ai/m^2). Furthermore, regarding the LD_{50} values among animal shelters, the lowest was obtained from Samsun (0.37 gr ai/m^2), and the highest was obtained from Denizli (21800 gr ai/m^2).

Conclusion: The use of integrated control systems is recommended for controlling house fly populations, including insecticide class rotations for preventing, or at least, delaying the onset of resistance.

Keywords: House fly; *Musca domestica*; Resistance; Thiamethoxam; Türkiye

Introduction

The house fly *Musca domestica* Linnaeus (Diptera: Muscidae) is an important vector of many pathogens (such as bacteria, viruses, and fungi) and parasites that can negatively affect the health of humans and animals (1, 2). This fly species can impact animal production systems on dairy farms and is also responsible for decreased meat and milk production (3). Its high reproductive success, short developmental cycle, as well as its adaptive capacity for developing in and feeding upon different kinds of organic substrates makes the control of *M. domestica* very difficult (4). Using only physical or biological methods for managing house flies is found not enough; therefore, chemical control methods are used along with these methods. However, insecticides continue to be relied upon primarily for the management of these pests, in many regions of the world. This situation has resulted in the development of house fly resistance to several of the chemicals used for their control, prompting researchers to

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investigate the presence and extent of resistance in field populations (5–7).

Resistance to insecticides in house flies was first addressed in the 1940s along with DDT resistance. Since then, reports have continued from different regions of the world on difficulties and major problems regarding the failure of control for this pest (5, 7, 8). Therefore, scientists are investigating alternative insecticides substances that prevent the rapid progress of resistance while possessing low toxicity on non-target organisms. The majority of biocides used in Türkiye against adult house flies consist of synthetic pyrethroid and neonicotinoid insecticides. But the researchers indicate that house flies have developed resistance to these insecticides in many regions of the country (9, 10). It is plausible that regions of Türkiye, where stockbreeding and agriculture are the main resources, might harbor house fly strains with higher resistance ratios. The research conducted in the Kumluca region in Antalya, Türkiye, pointed to house flies in that region showing resistance to juvenile hormone analogues (Pyriproxyfen and Methoprene). The higher Deltamethrin resistance ratios were obtained from greenhouse areas than urban areas (11, 12).

More than twenty commercially licensed products are listed as insecticides against house flies including thiamethoxam, one type of a neonicotinoid insecticide. Products containing this active ingredient formulated as sprays or paint-on applications are used in Türkiye. However, anecdotal observations have reported thiamethoxam failing to control house flies in many regions of Türkiye in recent years. In light of this problem, we sampled *M. domestica* field populations from ten Turkish cities to determine their susceptibility to thiamethoxam and report here on those results.

Materials and Methods

House fly strains

Seventeen house fly (*M. domestica*) strains were collected using sweep nets from animal shelters and/or solid waste landfills of ten cities in Türkiye, listed in Table 1 and shown in Fig. 1. About 250–300 adults were transported to the laboratory in fine muslin cages (22x22x22 cm) where they were provided cotton soaked in milk for oviposition. Eggs were collected daily and then transferred into the larval medium that consisted of milk (100 ml) and wheat bran (100 gr) (11). Two-four-day-old F_1 adults were used in all experiments. An insecticide-susceptible house fly strain was supplied from Italy, Pavia University, by Dr Oner KOCAK (Hacettepe University, Pesticide Testing Laboratories) and was similarly reared to provide base-line data for thiamethoxam toxicity studies comparison tests for determination of resistance ratios (13).

Insecticide susceptibility tests

The susceptibility of the house flies to thiamethoxam was determined using the WHO glass jar residual contact method (WHO/VBC/75.593). Thiamethoxam (of 97≥ purity, CAS number 153719-23-4 was obtained from Jiangsu Inter-Chian Group Co.) was prepared as a stock solution with acetone, 1mL of the solution was dispersed along the inner surface and the jars rotated until the acetone was vaporized to produce a film 24 h before testing. Preliminary tests were conducted using a minimum of four doses, above and below the Ministry of Health label recommended dose (0.25 gr active ingredient (ai)/m^2), resulting in 5 to 100% mortality.

Twenty house fly adults (mixed gender) were placed inside each treated jar (250 ml capacity, 260 cm^2 surface area). After 1h exposure, all individuals were transported to clean jars and provided with cotton soaked 10% sucrose solution. At 24 h mortality was recorded. Individuals who did not show any motility behavior were considered dead. All tests were repeated three times. Controls consisted of similarly exposing flies to acetone-treated only glass jars (13).

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Statistics

Lethal dose fifty (LD_{50}) values were calculated using Probit Analysis in the StatPlus software program (AnalystSoft Inc. Walnut, CA). Resistance ratios (RR) were calculated by dividing the LD_{50} values of the field populations into the LD_{50} values of the susceptible population. Six categories were used in categorizing resistance using the criteria of Wang et al. (14). According to these categories; RR< 5 no resistance, RR=5–10 very low resistance, RR=11–20 low resistance, RR=21–50 moderate resistance, RR=51–100 high resistance, and RR>100 very high resistance (14).

Results

Ten out of 17 strains indicated high or very high resistance levels to thiamethoxam (Table 2). Our data indicated that the house flies in the solid waste landfills in Samsun, Ankara, and Kocaeli have higher resistance ratios than those in animal shelters. In Gaziantep, Antalya, İzmir, and Erzurum, the exact opposite situation was observed. Regarding the LD_{50} values among solid waste storage areas, the lowest rate was obtained from Gaziantep (0.72 gr ai/m^2), and the highest rate was obtained from Ankara (9.35 gr ai/m^2); furthermore, regarding the LD_{50} values among animal shelters, the lowest was obtained from Samsun (0.37 gr ai/m^2) and the highest was obtained from Denizli (21800 gr ai/m^2). The LD_{50} value of the susceptible population was calculated as 0.02 ai/m^2.

According to the results obtained, when compared to house flies susceptible to insecticides, 18.5 and 69.5-fold resistance were found in the Black Sea region, 56, 60 and >1000-fold resistance in the Aegean region, 30, 47.5 and 59.5-fold resistance in the Mediterranean region, 36, 47 and 74.5-fold resistance in the Southeastern Anatolia region, 74.5-fold resistance in the Eastern Anatolia region, 32 and 467.5-fold resistance in the Central Anatolia region, 58.5 and 78.5-fold resistance in the Marmara region were detected. There was no correlation between the altitudes of the areas where house flies were collected and resistance ratios.

Table 1. Global Positioning System coordinates, sample dates, locality and habitat type from which adult *Musca domestica* were sampled in the current study, Türkiye

Strain no	Sampling date	Locality (City, district, neighborhood)	Area type*	Coordinates	Altitudes (m)
1	04.06.2018	İzmir, Harmandalı	SWL	N 38°31'59.9" E 27°04'18.7"	310
2	04.06.2018	İzmir, Menemen, Süzbeyli	AS	N 38°32'58.9" E 26°56'13.0"	54
3	27.06.2018	Samsun, İlk adım	SWL	N 41°14'37.7" E 36°12'56.49"	482
4	27.06.2018	Samsun, Sarıışık	AS	N 41°17'16.6.7" E 36°12'05.23"	174
5	28.06.2018	Ankara, Sincan, Yenimahalle	SWL	N 39°56'45.013" E 32°28'13.366"	836
6	28.06.2018	Ankara, Sincan, Saraycık	AS	N 39°55'02.793" E 32°34'10.097"	891
7	03.07.2018	Antalya, Kepez, Kızıllı	SWL	N 37°05'41.183" E 30°45'26.934"	100
8	03.07.2018	Antalya, Kepez, Ayanoğlu	AS	N 36°57'59.257" E 30°43'20.320"	104
9	01.08.2018	Erzurum, Aziziye, Adaçay	SWL	N 39°51'21.8" E 41°08'03.7"	1920

Table 1. Continued ...

10	01.08.2018	Erzurum, Aşkale, Kükürtlü	AS	N39°51'09.925" E40°36'29.395"	2016
11	15.08.2018	Gaziantep, Şahinbey, Mavikent	SWL	N37°02'22.665'' E37°31'27.909"	806
12	15.08.2018	Gaziantep, Çaybaşı, Şehit Kamil, Sinan	AS	N36°59'25.303" E37°24'04.069"	915
13	06.09.2018	Kocaeli, İzmit, İzaydaş, Alikahya, Atatürk	SWL	N40°47'11.517" E30°01'31.730"	137
14	06.09.2018	Kocaeli, İzmit, Kabaoğlu. Üçtepeler	AS	N40°48'00.715'' E29°55'48.578"	316
15	21.05.2019	Denizli, Pamukkale, Korucuk	AS	N 37°49'55.1" E 29°09'05.0"	225
16	28.05.2019	Diyarbakır, Sur, Bağıvar	AS	N 37°86'08.350" E 40°24'36.810"	609
17	29.05.2019	Adana, Ceyhan, Kızıldere	AS	N 36º94'14.870" E 35º66'26.630"	30

*SWSA: Solid Waste landfills * AS: Animal shelter

Table 2. The lethal dose fifty (LD_{50}) values of adult *Musca domestica* populations, as well as their resistance ratios and resistance status to thiamethoxam, Türkiye, 2018

Region	City	Locality type	LD_{50} (gr ai/m^2)	95% CI	Resistance ratio	Resistance Status
The Black Sea	Samsun	AS	0.37	0.09–1.48	18.5	LOW
		SWL	1.39	1.11–1.77	69.5	HIGH
Aegean	İzmir	AS	1.20	0.33–4.36	60.0	HIGH
		SWL	1.12	0.29–7.73	56.0	HIGH
Aegean	Denizli	AS	21800	Undetermined	>1000	VERY HIGH
Mediterranean	Antalya	AS	1.19	0.66–2.43	59.5	HIGH
		SWL	0.95	0.50–2.21	47.5	MODERATE
Mediterranean	Adana	AS	6.6	5.9–7.25	30.0	MODERATE
Southeastern Anatolia	Gaziantep	AS	0.94	0.44–2.21	47.0	MODERATE
		SWL	0.72	0.27–1.64	36.0	MODERATE
Southeastern Anatolia	Diyarbakır	AS	1.49	0.26–8.50	74.5	HIGH
Eastern Anatolia	Erzurum	AS	1.46	1.07–2.09	73.0	HIGH
		SWL	0.93	0.66–1.34	46.5	MODERATE
Central Anatolia	Ankara	AS	0.64	0.13–3.16	32.0	MODERATE
		SWL	9.35	0.05–1479.05	467.5	VERY HIGH
Marmara	Kocaeli	AS	1.17	0.24–5.64	58.5	HIGH
		SWL	1.57	1.23–2.08	78.5	HIGH
Susceptible (WHO)			0.02	0.002–0.14	1	

SWSA: Solid Waste Landfills AS: Animal shelter CI=Confidence Interval

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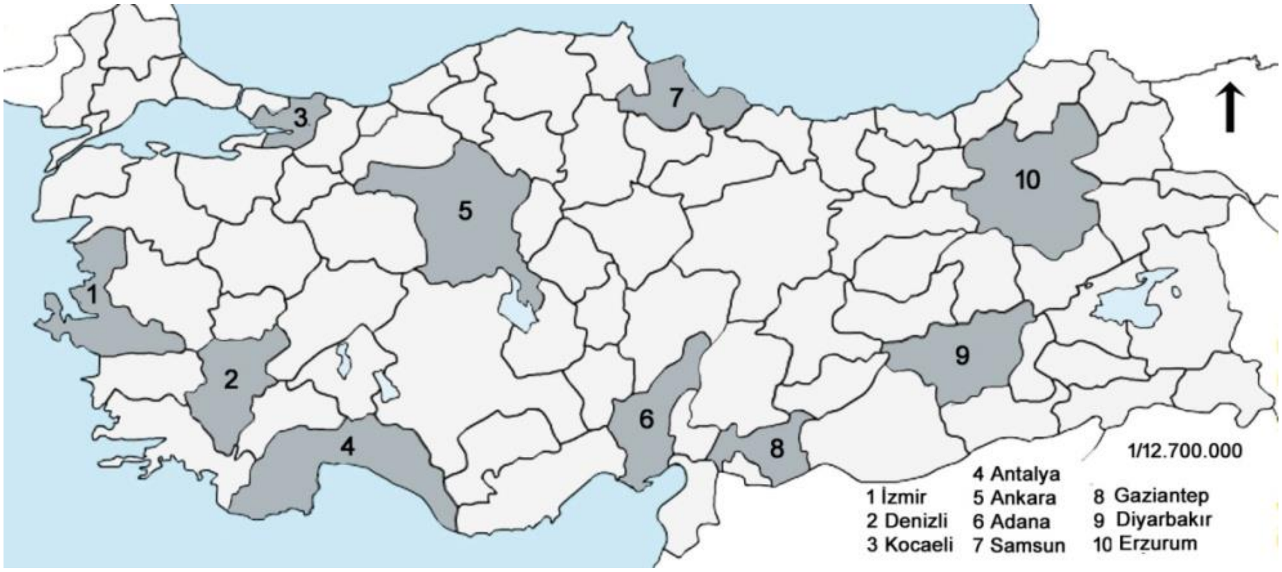

Fig. 1. Turkish city locations where adult *Musca domestica* were sampled for current study

Discussion

In a research on house fly resistance to neonicotinoid insecticides in Türkiye, resistance to neonicotinoid insecticides imidacloprid and methomyl was reported in house flies in Antalya, Ankara and Izmir (9). Indeed, house flies from Antalya had the highest resistance to imidacloprid, followed by the population of Ankara. Methomyl resistance was highest in İzmir followed by Ankara and Antalya. Our results regarding thiamethoxam susceptibility showed that, house flies collected from animal shelters in Izmir possessed the highest resistance to this insecticide (60-fold), followed by Antalya (59.5-fold) and Ankara (32-fold); flies collected from Ankara landfills showed the highest resistance (467.5 fold), followed by İzmir (56 fold) and Antalya (47.5 fold).

The fact that even the oldest solid waste landfills in Ankara are still active, and insecticide applications are carried out by either the metropolitan municipality or the district municipality pest control applicators periodically indicates the possibility that these could be the reasons behind the highest levels of resistance found in our study. Moreover, a master's thesis written by Cakir (15) on the resistance levels of house flies sampled from animal shelters in Antalya showed high levels of resistance to thiamethoxam. There are no other studies conducted on house flies of Türkiye and their resistance to neonicotinoids, other than studies done by Memmi (9) and Cakir (15). It is plausible that regions of Türkiye, where stockbreeding and agriculture are the main production resources, might harbor house fly strains with higher insecticide resistance ratios. Previous research conducted by Cetin et al. (11) in similar areas of the Kumluca region in Antalya, Türkiye, pointed to house flies in that region, showing resistance to juvenile hormone analogues (pyriproxyfen and methoprene). In addition, higher deltamethrin resistance ratios have been reported from greenhouse areas than urban areas (13). Research conducted by Akiner and Çağlar (10) on cities of Antalya, İzmir, Adana, Ankara, Istanbul and Sanlıurfa house fly populations pointed to dairy farm populations being more resistant to synthetic pyrethroids (cypermethrin, cyphenothrin, deltamethrin, permethrin, and resmethrin) and organophosphate (fenitrothion) than landfill populations. The data from this research and from our study, which

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yielded the result whether Antalya, İzmir, and Ankara populations have low, moderate or high resistance levels provided us with the notion that heavy insecticide usage might still be in order after over 17 years (since 2002 when the study was conducted) in these localities.

Resistance to some insecticides has been reported in many house fly populations in other countries (7, 8, 14). Ahmadi and Khajehali (16) studied resistance levels of populations collected from Isfahan, Koohrang and Mobarake regions in Iran. They found that very high level resistance to dichlorvos in the Mobarake population (RR= 33–80.25-fold), and Isfahan population (RR= 43–107.30-fold). Some *M. domestica* strains from dairy farms in Punjab, Pakistan were evaluated for resistance to selected carbamate (methomyl), organochlorine (endosulfan), organophosphate (profenofos, chlorpyrifos) and synthetic pyrethroid (cypermethrin and deltamethrin) insecticides by Khan et al. (17). They reported resistance ratios were for 7.66–23.24-fold for profenofos, 5.60–22.02-fold for endosulfan, 2.47–7.44 fold for chlorpyrifos, 30.22-70.02-fold for cypermethrin, 5.73-18.31-fold for deltamethrin, and 4.39-15.50-fold for methomyl. Resistance to thiamethoxam in house flies from eight localities of Punjab, Pakistan was reported by Khan et al. (7). The results revealed that the field house fly strains showed varying levels of resistance to thiamethoxam (7.66–20.13 folds).

In Brandenburg/Germany, a study was conducted from June to November 2008, around 60 dairy farms. The results show forages that contain thiamethoxam and imidacloprid did not reach their full potential, yet, as the duration of exposure increased, so did the mortality rate (6). In another study, insecticides were given to house flies via the feeding method and a selection was conducted in order to determine the resistance levels. Results showed no increase in resistance to spinosad, however, resistance to thiamethoxam, imidacloprid and fipronil dropped from 23-fold to 6-fold. In the same study, in the populations subjected to selection via thiamethoxam, resistance levels to spinosad were determined to be 8 fold in males and 13 fold in females, compared to the susceptible population (18). In the study conducted by Burgess and King (19) resistance to methomyl, imidacloprid, permethrin, thiamethoxam, spinosad, dinotefuran and nitenpyram was assessed in *Spalangia endius* Walker species via surface contact method, and in *M. domestica* via the feeding method. LC_{50} values were calculated to be 41.49 ng/cm^2 and 3.23 µg/g respectively. Khan et al. (7) researched the toxicity of thiamethoxam via the feeding method on eight house fly populations and found 7.6 to 20.1-fold resistance level. In the population subjected to selection for five generations via thiamethoxam, the initial 7.6-fold resistance coefficient was determined to be 33.5-fold in the 5th generation. In the same population, imidacloprid resistance emerged as a cross-development.

As a result, studies conducted in Türkiye and other countries clearly show that adult house flies have developed resistance to almost all conventional insecticides. Our results showed that the necessity of coming up with better strategic plans in the fight against house flies, especially in solid waste landfills, are now more apparent, due to reduced effectiveness of neonicotinoid applications which are still used against this fly pest. One of these strategies includes evaluating synergists for breaking resistance to neonicotinoids or other groups (20). Indeed, a study conducted with Diyarbakır, Adana, Gaziantep and Kahramanmaras house fly populations, by Polat and Cetin (21) revealed that PBO increased neonicotinoid toxicity in thiamethoxam resistant flies. A rise in production numbers for neonicotinoid products that include PBO is expected.

Conclusion

In this study, susceptibility levels of field-collected house flies to thiamethoxam were assessed in several areas of Türkiye. Results indicated that there were varying levels of resistance to this insecticide designated as moderate (30–47.5-fold), high (56–78.5-fold) and very high (≥467.5 fold). Generally, most house fly strains in Türkiye have high resistance to thiamethoxam as well as other classes of insecticides the latter reported by other authors. As a result, focus should be shifted from adult control to that of the larval stage. Therefore, physical, and cultural changes in manure transportation and storage, frequency of refuse collection and disposal methods need to be conducted in such a way that would not allow for the house fly to develop in organically rich environments. Additional research is required in order to assess larval susceptibility in these environments in order to delay the rapid onset of insecticide resistance in the adult fly populations. Further studies should continue to investigate resistance levels in house flies to other neonicotinoid insecticides, and the effects of using resistance-breaking agents (such as PBO, DEF and DEM) in combination with insecticides should be investigated in different populations.

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Ethical consideration

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Conflict of interest

The authors declare that there is no conflict of interest.

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