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RESISTANCE TO 10 COMMON INSECTICIDES OF HOUSEFLIES (MUSCA DOMESTICA) FROM GARBAGE DUMPS IN TAIWAN

Entomology

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ABSTRACT

To understand the resistance tendencies of *M. domestica* populations from garbage dumps, this study conducted a survey to assist with their future management and monitoring. Samples were collected from garbage dumps in northern, central, and southern Taiwan in 2015 and 2018. Bioassay tests were conducted to study the resistance of the houseflies to 10 common insecticides: pyrethrin insecticides (cypermethrin, tetramethrin, permethrin, and deltamethrin), organophosphate insecticides (chlorpyrifos, fenitrothion and pirimiphos-methyl), propoxur, fipronil, and imidacloprid. The results revealed that houseflies' resistance to deltamethrin has become increasingly problematic and that they have developed cross-resistance. Although fenitrothion and pirimiphos-methyl resistance had decreased, chlorpyrifos resistance had increased. In addition, although the houseflies exhibited a general trend of decreasing resistance to fipronil, their resistance to propoxur and imidacloprid increased for some strains. Overall, houseflies from garbage dumps in Taiwan have developed multiple resistance.

KEYWORDS

Musca Domestica, Resistance, Pest Control, Bioassay

INTRODUCTION

The housefly, *Musca domestica* (L.), lives alongside humans worldwide and is a pest that critically affects humans and their domesticated animals. These flies typically feed on waste and food, from which they may acquire and disseminate various diseases. Houseflies act as mechanical carriers for over 100 intestinal diseases specific to humans and animals as well as other bacterial, viral, protozoan, and helminthic infections (Förster et al., 2007; Greenberg, 1971; Malik, Singh, & Satya, 2007).

Numerous reports from various locations worldwide have revealed the housefly's resistance to chemical insecticides (Huang, Kristensen, Qiao, & Jespersen, 2004; Liu & Yue, 2000; Marcon, Thomas, Siegfried, Campbell, & Skoda, 2003; Pospischil et al., 1996; Sharififard & Safdari, 2013; Singh, 1973; Akiner & Çağlar, 2006). Both cross-resistance and multiple resistance of houseflies to insecticides have been discovered (Liu & Yue, 2000; Pospischil et al., 1996).

The climate of Taiwan is warm and humid and thus suitable for the growth of flies. Since 2000, because of improvements in home environments and sanitation, houseflies are less commonly seen in households in metropolitan areas. However, they still often breed in garbage dumps. Chemical control of this insect pest often utilizes insecticides such as pyrethrin, organophosphates, propoxur, fipronil, and imidacloprid. Because garbage dumps are periodically treated with insecticides, a large housefly population within a dump can indicate insecticide resistance. Houseflies are now resistant to nearly all insecticides employed for their control, and this resistance represents a global challenge (Georghiou & Mellon, 1983; Scott, Roush, & Rutz, 1989).

Early detection of insecticide resistance and an effective strategy may efficiently reduce the operational, financial, and social costs associated with housefly control. To update current knowledge on housefly insecticide resistance, this study investigated its resistance to 10 insecticides using samples collected from garbage dumps in Taiwan in 2015 and 2018. The resistance tendency of *M. domestica* populations from garbage dumps was surveyed to assist with the future management and monitoring of this pest.

MATERIALS AND METHODS

INSECTS

Four field strains of *M. domestica* were used in this study, namely northern Taiwan (NT), central Taiwan (CT), southern Taiwan A (STA), and southern Taiwan B (STB) strains. They were collected using a sweep net at various garbage dumps, which were the same for collections in 2015 and 2018. They were maintained at a temperature of 27°C ± 2°C, relative humidity of 60% ± 10%, and photoperiod of 12 h:12 h (light:dark). Water (10% syrup) and food in the form of sugar and powdered milk were provided to adults. Collected female houseflies were then left to lay eggs. Eggs were inoculated in a larval medium comprising rat feed suspended in hot water at a ratio of 1:1 (e.g., 160 g of mouse feed to 160 mL of water). When the larvae were mature, wood chips (approximately 1-cm thick) were laid on the upper layer of the medium, and the pupae were collected from the wood chip layer. The pupae were sifted into a petri dish, placed in a new fly cage, and emerged as adult flies after approximately 5–7 days. The susceptible strain was provided by the Department of Entomology of National Taiwan University (Taipei, Taiwan, ROC) in 2004 and maintained in a laboratory without any chemical exposure for more than 150 generations.

INSECTICIDES

Ten insecticides of technical-grade were diluted to one-fifth concentration with analytical-grade acetone for testing. The following insecticides were purchased from a representative of a chemical service company in Taiwan: pyrethrin insecticides from Aerolead International Ltd. (cypermethrin [92%], tetramethrin [92%], permethrin [92%], and deltamethrin [98%]), organophosphate insecticides (chlorpyrifos [98%, Aerolead International Ltd.], fenitrothion [95%, Tyeng Long Incorporation] and pirimiphos-methyl [90%, Nan Sing Chemical Meg. Co., Ltd.]), propoxur (97%, Tyeng Long Incorporation), fipronil (95%, Aerolead International Ltd.), and imidacloprid (95%, Aerolead International Ltd.).

BIOASSAY TESTS

Bioassay tests were conducted using the topical method (Sharififard & Safdari, 2013; Akiner & Çağlar, 2006). First, the pesticide was diluted to 1% with acetone and then prepared as a series of concentrations corresponding to various mortality rates from 10% to 90%. Second, five concentrations were prepared for each insecticide to determine the 50% mortality rate; 5- to 7-day-old adult female houseflies were anesthetized with carbon dioxide and then temporarily placed in an insect anesthesia device. Each insect was treated topically using a microapplicator (Type MSN-100 microsyringe; Terumo Corporation in Taiwan, New Taipei City). For each insecticide, 1 L was dissolved in acetone and dropped onto the mesonotum of the flies. The control flies were treated with acetone only, and each test used 20 flies for each treatment. After topical application, houseflies were kept in plastic cylindrical tubes (7 cm in diameter; 12-cm long) that were covered with plastic gauze (80 mesh) and secured on both sides with rubber

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bands. Cotton with 10% syrup was placed on the tubes for feeding. Testing was performed in triplicate for each concentration. Final mortality was assessed after 24 h of exposure to insecticides, and the flies were assumed to be dead if they were ataxic.

DATA ANALYSIS

Mortality data were evaluated through probit and logit analysis using Polo Plus v 1.0 (LeOra software, 2002–2019) to determine the lethal dose (LD_{50}), which was defined as the dose causing death of 50% of the adults in the test. The resistance ratios (RRs) were calculated using the following formula: RR = LD_{50} (field population)/LD_{50} (susceptible population). The resistance level was evaluated on the basis of the LD_{50} in four categories (Sharififard & Safdari, 2013): Low RR < 10, moderate RR = 11–40, high RR = 41–160, and very high RR > 160.

RESULTS

In 2015, the LD_{50} values of the strains susceptible to cypermethrin, tetramethrin, permethrin, and deltamethrin were 1.59, 0.27, 2.50, and 0.05 ng/female, respectively (Table 1). In 2018, LD_{50} values for the strains susceptible to cypermethrin, tetramethrin, permethrin, and deltamethrin were 0.90, 1.10, 1.70, and 0.02 ng/female, respectively (Table 1). In 2015, the four field strains of housefly exhibited moderate-to-very-high resistance; the only exception was the STA strain, which exhibited low resistance to deltamethrin (RR = 6.35) (Table 2). The highest RRs were observed in the NT strain, which was resistant to cypermethrin (155.22); the STA strain, which was resistant to tetramethrin (271.67); the CT strain, which was resistant to permethrin (358.45); and the NT strain, which was resistant to deltamethrin (523.20; Table 2). In 2018, the four strains of housefly had moderate-to-very-high-resistance to four common pyrethroid insecticides. Moreover, all four strains exhibited very high resistance to deltamethrin (RR: 541.67–708.33; Table 2). These results revealed that the resistance of houseflies to deltamethrin has become increasingly problematic and that houseflies have developed cross-resistance in Taiwan (Figure 1).

In 2015, the LD_{50} values of the strains susceptible to chlorpyrifos, fenitrothion, and pirimiphos-methyl were 63.81, 9.24, and 2.21 ng/female, respectively (Table 1). In 2018, the LD_{50} values of the strains susceptible to chlorpyrifos, fenitrothion, and pirimiphos-methyl were 17.00, 18.00, and 1.70 ng/female, respectively (Table 1). In 2015, all strains exhibited low resistance to chlorpyrifos (RR < 10). Furthermore, both the NT and STA strains had high resistance to fenitrothion (40 > RR > 160) (Table 2).

Table 1: Median Lethal Dose (LD_{50}) to Houseflies of 10 Common Insecticides with the Topical Bioassay Method

Insect Strains / Insecticides	The year	Susceptible strains LD_{50} (95% CI)	NT strains LD_{50} (95% CI)	CT strains LD_{50} (95% CI)	STA strains LD_{50} (95% CI)	STB strains LD_{50} (95% CI)
Cypermethrin	2015	1.59 (1.20-1.96)	246.80 (171.55-503.47)	16.23 (4.46-26.86)	27.55 (18.86-34.27)	22.58 (9.38-33.96)
	2018	0.90 (0.05-0.12)	47.00 (31.00-62.00)	13.00 (7.00-18.00)	34.00 (22.00-45.00)	40.00 (25.00-52.00)
Tetramethrin	2015	0.27 (0.18-0.34)	36.61 (28.99-44.86)	48.35 (36.31-64.84)	73.35 (54.61-117.55)	25.89 (15.66-35.44)
	2018	1.10 (0.80-1.40)	118.00 (95.00-146.00)	133.00 (109.00-162.00)	28.00 (21.00-35.00)	17.00 (14.00-21.00)
Permethrin	2015	2.50 (1.39-3.67)	88.80 (60.67-125.43)	895.78 (422.65-8788.91)	96.18 (71.32-137.47)	44.78 (29.28-120.74)
	2018	1.70 (1.50-2.10)	56.00 (45.00-67.00)	59.00 (48.00-72.00)	71.00 (59.00-85.00)	56.00 (45.00-67.00)
Deltamethrin	2015	0.05 (0.05-0.06)	28.25 (22.24-34.16)	2.74 (0.10-7.38)	0.34 (0.25-0.49)	1.90 (0.22-4.08)
	2018	0.02 (0.02-0.03)	17.00 (14.00-20.00)	17.00 (14.00-21.00)	13.00 (11.00-16.00)	15.00 (12.00-18.00)
Chlorpyrifos	2015	63.81 (53.40-79.03)	342.18 (268.31-454.90)	193.40 (153.72-274.71)	459.12 (392.49-533.71)	35.49 (10.48-61.96)
	2018	17.00 (14.00-21.00)	222.00 (180.00-272.00)	222.00 (179.00-274.00)	152.00 (118.00-188.00)	165.00 (136.00-198.00)
Fenitrothion	2015	9.24 (3.80-14.44)	791.87 (647.97-1049.74)	115.24 (58.31-167.81)	1245.46 (949.4-2048.0)	77.39 (33.77-119.70)
	2018	18.00 (14.00-21.00)	246.00 (188.00-304.00)	257.00 (204.00-311.00)	322.00 (261.00-388.00)	330.00 (277.00-387.00)
Pirimiphos-methyl	2015	2.21 (1.52-2.85)	428.66 (298.95-813.01)	242.39 (168.07-500.38)	132.00 (101.59-195.38)	718.60 (431.60-2178.72)
	2018	1.70 (1.30-2.00)	176.00 (140.00-216.00)	188.00 (153.00-227.00)	198.00 (164.00-236.00)	145.00 (114.00-177.00)
Propoxur	2015	7.57 (5.39-9.61)	273.39 (154.75-1466.44)	157.48 (105.25-384.67)	103.32 (76.80-175.33)	206.83 (99.82-6201.32)
	2018	6.60 (5.60-7.80)	125.00 (102.00-154.00)	150.00 (123.00-186.00)	259.00 (209.00-324.00)	290.00 (209.00-422.00)
Fipronil	2015	0.81 (0.37-1.23)	11.89 (7.87-15.57)	4.63 (1.83-7.26)	9.03 (3.50-14.37)	11.51 (6.38-16.24)
	2018	1.60 (1.20-2.00)	13.00 (9.00-17.00)	14.00 (11.00-16.00)	15.00 (12.00-17.00)	14.00 (11.00-17.00)
Imidacloprid	2015	79.42 (69.66-94.05)	192.31 (122.66-563.65)	1362.51 (976.7-2610.8)	822.18 (604.27-1371.12)	337.24 (314.02-443.48)
	2018	17.00 (13.00-22.00)	341.00 (284.00-404.00)	385.00 (310.00-473.00)	213.00 (174.00-260.00)	281.00 (227.00-336.00)

Unit of median lethal dose (LD_{50}): ng/female

Table 2: Resistance Ratios (RRs) of Houseflies (*Musca domestica*) to 10 Common Insecticides in Taiwan

Insect Strains Insecticides	The year	NT strains	CT strains	STA strains	STB strains
Cypermethrin	2015	155.22**	10.21*	17.33*	14.20*
	2018	52.22**	14.44*	37.78*	44.44**
Tetramethrin	2015	135.59**	179.07***	271.67***	95.89**
	2018	107.27**	120.91**	25.45*	15.45*
Permethrin	2015	35.54*	358.45***	38.49*	17.92*
	2018	32.94*	34.71*	41.76*	32.94*
Deltamethrin	2015	523.20***	50.81**	6.35	35.24*
	2018	708.33***	708.33***	541.67***	625.00***
Chlorpyrifos	2015	5.36	3.03	7.20	0.56
	2018	13.06*	13.06*	8.94	9.71
Fenitrothion	2015	85.70**	12.47*	134.79**	8.38
	2018	13.67*	14.28*	17.89*	18.33*
Pirimiphos-methyl	2015	193.96***	109.68**	59.73**	325.16***
	2018	103.53**	110.59**	116.47**	85.29**
Propoxur	2015	36.11*	20.80*	13.65*	27.32*
	2018	18.94*	22.73*	39.24*	43.93**
Fipronil	2015	14.68*	5.72	11.15*	14.21*
	2018	8.13	8.75	9.38	8.75
Imidacloprid	2015	2.42	17.16*	10.35*	4.25
	2018	20.06*	22.65*	12.53*	16.53*

*: moderate RR; **: high RR; ***: very high RR

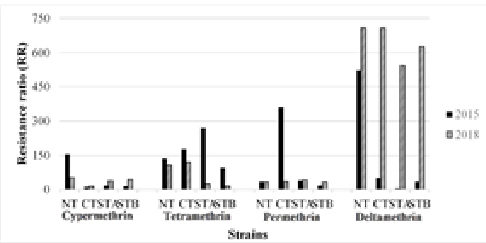

Figure 1: Resistance of Houseflies (*M. domestica*) to common pyrethrin insecticides in Taiwan.

NT: northern Taiwan strain; CT: central Taiwan strain; STA: southern Taiwan A strain; STB: southern Taiwan B strain. black bar: resistance ratios (RRs) of houseflies to insecticides in 2015; Striped bar: RRs of houseflies to insecticides in 2018. RRs = LD_{50} (field population)/LD_{50} (susceptible population).

The CT and STA strains demonstrated high resistance to pirimiphos-methyl (40 > RR > 160) (Table 2), and both the NT and STB strains exhibited very high resistance (RR > 160) to pirimiphos-methyl (Table 2). In 2018, the NT and CT strains were moderately resistant to chlorpyrifos (11 > RR > 40) (Table 2). All four field strains were moderately resistant to fenitrothion (11 > RR > 40) (Table 2) and exhibited high resistance to pirimiphos-methyl (40 > RR > 160) (Table 2). Houseflies in Taiwan were generally resistant to organophosphate insecticides (chlorpyrifos, fenitrothion, and pirimiphos-methyl) and exhibited cross-resistance. Although the houseflies exhibited a general trend of decreasing resistance to insecticides, resistance to chlorpyrifos increased for all strains (Table 2 and Figure 2).

In 2015, the LD_{50} values of the strains susceptible to propoxur, fipronil, and imidacloprid were 7.57, 0.81, and 79.42 ng/female, respectively (Table 1). In 2018, the LD_{50} values of the strains susceptible to propoxur, fipronil, and imidacloprid were 6.60, 1.60, and 17.00 ng/female, respectively (Table 1).

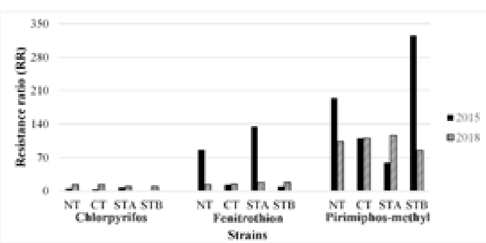

Figure 2: Resistance of houseflies (*M. domestica*) to common organophosphate insecticides in Taiwan.

NT: northern Taiwan strain; CT: central Taiwan strain; STA: southern Taiwan A strain; STB: southern Taiwan B strain. black bar: resistance ratios (RRs) of houseflies to insecticides in 2015; Striped bar: RRs of houseflies to insecticides in 2018. RRs = LD_{50} (field population)/LD_{50} (susceptible population).

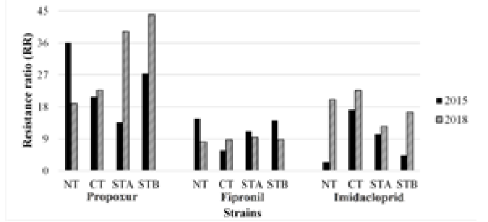

Figure 3: Resistance of houseflies (*M. domestica*) to propoxur, fipronil, and imidacloprid insecticides in Taiwan.

NT: northern Taiwan strain; CT: central Taiwan strain; STA: southern Taiwan A strain; STB: southern Taiwan B strain. black bar: resistance ratios (RRs) of houseflies to insecticides in 2015; Striped bar: RRs of houseflies to insecticides in 2018. RRs = LD_{50} (field population)/LD_{50} (susceptible population).

In 2015, the four field strains of housefly exhibited moderate resistance to propoxur (11 > RR > 40) (Table 2). Three field strains of housefly exhibited moderate resistance, whereas the CT strain exhibited low resistance to fipronil (RR = 5.72) (Table 2). The CT and STA strains of housefly exhibited moderate resistance to imidacloprid (Table 2). In 2018, the STB strains were highly resistant to propoxur (RR = 43.93) (Table 2). All strains exhibited low resistance to fipronil (RR < 10), and all four field strains were moderately resistant to imidacloprid (11 > RR > 40) (Table 2). Although the houseflies exhibited a general trend of decreasing resistance to fipronil, resistance to propoxur and imidacloprid increased for some strains (Table 2 and Figure 3). Thus, houseflies from garbage dumps in Taiwan were determined to have developed multiple resistance.

DISCUSSION

Resistance is caused by innate traits that allow insects to survive exposure to typically lethal dosages of certain insecticides (Hemingway, 1992). This resistance, present in both larvae and adults, results from stable specific gene expression (Whalon, Mota-Sanchez, & Hollingworth, 2008). Because a few resistance-related genetic factors are not bound to specific insecticide molecules, partial cross-resistance to new classes of insecticides can develop (Plapp & Frederick, 1986). Metabolic resistance, target-site resistance, and reduced sequestration and penetration have been identified as being among the various types of resistance (Karunaratne, Jayawardena, Hemingway, & Ketterman, 1993; Oppenoorth, 1982). Houseflies' resistance to pyrethroid, carbamate, organophosphate, and organochlorine insecticides is a challenge faced worldwide, with examples of resistance to insecticides in the mentioned classes of insecticides in England (Chapman & Morgan, 1992), Slovakia (Kočišová, Novák, Toporčák, & Petrovský, 2002), Germany (Pospischil et al., 1996), Iran (Sharififard & Safdari, 2013), Turkey (Akiner & Çağlar, 2005), Pakistan (Khan, Akram, Shad, & Lee, 2013), Africa (Egypt, Saudi Arabia, Sultanate of Oman, Kuwait, Turkey, Iraq, and United Arab Emirates, Bahrain) (Taylor, 1982), the United States (Scott, Alefantis, Kaufman, & Rutz, 2000), Argentina (Acevedo, Zapater, & Toloza, 2009), Japan (Ugaki, Shono, & Fukami, 1985), and Malaysia (Singh, 1973).

In Taiwan, houseflies from garbage dumps are controlled mainly through chemical control and environmental clean-ups. Pyrethrin insecticides have been used for housefly control since the late 1980s in Taiwan, and pyrethrin is currently more commonly used than other insecticide groups. As of February 2019, 454 pesticides were available for housefly control, 352 (77.53%) of which were pyrethrin insecticides (https://mdc.epa.gov.tw/PublicInfo/). Cypermethrin has been widely used in space spraying for fly control in Taiwan, and the highest RR in 2015 was recorded in the NT strain (155.22). All the studied populations had high or very high resistance to tetramethrin; the CT strain had very high resistance to permethrin (RR = 358.84); and the NT strain had very high resistance to deltamethrin (RR = 523.20) in 2015. Deltamethrin has been used since 1997 in these areas. Moreover, resistance levels for deltamethrin were very high in 2018, with the highest (RR = 708.33) being observed in the NT and CT strains (Table 2). The high level of resistance in these strains was considered to be attributable to the extensive usage of deltamethrin. Four field strains of housefly have developed resistance to four pyrethrin insecticides in Taiwan and exhibited very high resistance to deltamethrin. However, all strains of housefly exhibited a decreasing trend of resistance for both tetramethrin and permethrin and an increasing trend for cypermethrin and deltamethrin. Reduced resistance to tetramethrin and permethrin can be explained by the low usage of these insecticides compared with preceding years.

Scott, Alefantis, Kaufman, and Rutz (2000) noted the highest levels of resistance to permethrin among houseflies in the United States. Levels of resistance to the pyrethrin group of insecticides (cypermethrin, permethrin, and deltamethrin) ranged from 23.27 (permethrin-Istanbul fall strain) to 633.09 (cypermethrin-Izmir spring strain) in Turkish housefly populations, indicating the presence of strong selective pressure on the populations (Akiner & Çağlar, 2006). Acevedo, Zapater, and Toloza, (2009) indicated that the RRs for permethrin to various strains of housefly in Argentina ranged from 65.52 to 117.34, indicating high resistance.

The correlation of data concerning the topical application of permethrin and control failures in the field identified by Farnham, O'dell, Denholm, and Sawicki (1984) revealed that control failures usually occur when RRs exceed 15-fold. Another permethrin experiment demonstrated that the level of resistance in houseflies increased 1,800-fold (Liu & Yue, 2000) after five generations. Similarly, Zhang, Shi, and Gao (2008) reported that after 25 generations, selective-resistant strains increased 1,700-fold. These observations indicate that resistance to pyrethrin insecticide in houseflies can develop rapidly.

The organophosphate insecticides fenitrothion and pirimiphos-methyl are substantially less toxic to mammals than methyl parathion. In the early 1960s, these two insecticides were the most commonly used for pest control worldwide for both public health and agricultural purposes. Fenitrothion and pirimiphos-methyl are particularly crucial for dengue vector control in the tropical regions of Taiwan. In 2015, northern and southern Taiwan A strains of houseflies were highly resistant to fenitrothion (RR: 40 > RR > 160). In addition, both the NT and STB strains exhibited very high resistance (RR > 160) to pirimiphos-methyl. The Japanese strain Akita-f exhibited a resistance to fenitrothion that was 3,500 times higher than that of a laboratory-raised susceptible strain (Ugaki, Shono, & Fukami, 1985). Moreover, the Hans strain from Germany was extremely resistant to fenitrothion (RF > 6,700) (Pospischil et al., 1996). When comparing strains from Turkey, Akiner and Çaglar (2012) discovered that the 2004 Şanlıurfa strain was the most resistant to fenitrothion (RR = 50.37). Kočišová et al. (2002) observed that houseflies were highly resistant to pirimiphos-methyl (RF: 79.4 ± 15.9), concluding that traditional insecticide spray routines caused high resistance to develop over the course of one to two seasons.

We discovered that all housefly strains from garbage dumps in Taiwan were susceptible to chlorpyrifos in 2015 (RR = 0.56–7.20). A field population from Pakistan also exhibited low resistance to chlorpyrifos compared with a laboratory-reared susceptible strain (RR = 8.82) (Khan et al., 2013). We have made our observational results available online to enable relevant prevention and control workers to adjust their usage of chemical pesticides as soon as possible. Although the NT and CT strains of houseflies were moderately resistant to chlorpyrifos in 2018, fenitrothion and pirimiphos-methyl resistance had decreased since 2015. Akiner and Çaglar (2012) indicated that this may be attributable to the usage of other organophosphate insecticides.

No research has been conducted on the resistance levels of *M. domestica* L. from Taiwan. In this study, we observed high levels of resistance to a group of pyrethrin insecticides in field populations of *M. domestica* L. Furthermore, all strains of *M. domestica* were resistant to organophosphate and propoxur insecticides and exhibited not only cross-resistance but also multiple resistance.

Although control failure is affecting all insecticides used for housefly control in Taiwan, many compounds are still being used by local authorities. If the usage of these compounds is not restricted, then more serious problems may arise. Therefore, monitoring and establishing more effective programs that cover all aspects of the resistance problem are urgently required. This study suggests that the implementation of regular surveys of garbage dumps could potentially inform effective strategies against houseflies. In addition, the implementation of successful guidelines in Taiwan could prevent the detrimental effects of multiple-resistant insects and future field control failures. Finally, integrated control strategies are required to delay the development of insecticide resistance.

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AUTHOR CONTRIBUTIONS

Hsiu-Hua Pai: (1) Conceived and designed the experiment, (2) drafted the manuscript for content, (3) revised the manuscript critically for important intellectual content, and (4) approved the final version to be submitted and published.

Kai-Chen Lin: (1) Acquired, analyzed, and interpreted data for the study and (2) drafted the manuscript for content.

Syuan Wang: (1) Acquired, analyzed, and interpreted data for the study and (2) drafted the manuscript for content.

Err-Lieh Hsu: (1) Conceived and designed the experiment, (2) revised the manuscript critically for important intellectual content, and (3) approved the final version to be submitted and published.

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