Jurnal Entomologi Indonesia
Indonesian Journal of Entomology
ISSN: 1829-7722

November 2021, Vol. 18 No. 3, 159–169
Online version: http://jurnal.pei-pusat.org
DOI: 10.5994/jei.18.3.159

Resistance status and the rate of resistance development in *Musca domestica* Linnaeus (Diptera: Muscidae) to permethrin and imidacloprid in Indonesia

Status resistensi dan laju perkembangan resistensi *Musca domestica* Linnaeus (Diptera: Muscidae) terhadap permetrin dan imidakloprid di Indonesia

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(diterima Februari 2021, disetujui Agustus 2021)

ABSTRACT

A previous report has indicated that in many regions of Indonesia, populations of *Musca domestica* Linnaeus have shown very high resistance to permethrin and low resistance to imidacloprid. In this study, the resistance status to permethrin and imidacloprid was updated using a topical application and feeding bioassay. Six housefly strains originated from six highly populated cities in Indonesia, namely Serang (SRG), Jakarta (JKT), Bandung (BDG), Semarang (SMG), Yogyakarta (JOG), and Surabaya (SBY). A seventh strain (Danish Pest Infestation Laboratory (DPIL)) served as the control. Each strain was tested for resistance to the two insecticides. In addition, the rate of development of resistance to the two insecticides was measured over ten generations. The results indicated that all field strains showed very high resistance to permethrin. The highest resistance level recorded was in the SRG strain (RR_{50} = 2880), and the lowest was in the JKT strain (RR_{50} = 520). Repeated application of permethrin over ten generations increased the resistance level by about 2.7–32.73-fold as compared to the level of their respective parental populations. On the other hand, most strains showed low to moderate resistance to imidacloprid, in which the SRG strain had the highest resistance level (RR_{50} = 15.5) and the SBY strain had the lowest (RR_{50} = 2.0). Repeated application of imidacloprid over ten generations increased the resistance level by about 3.25–17.41-fold. The findings, which is the second report of housefly resistance in Indonesia since 2016, provide a crucial foundation for developing appropriate housefly integrated pest management strategies in highly populated areas in Indonesia.

Key words: Indonesia, imidacloprid, *Musca domestica*, permethrin, resistance

ABSTRAK

Di banyak wilayah di Indonesia, populasi *Musca domestica* Linnaeus telah menunjukkan resistensi yang sangat tinggi terhadap permetrin dan resistensi yang rendah terhadap imidakloprid. Dalam penelitian ini, dilakukan pembaruan status resistensi permetrin dan imidakloprid terhadap lalat rumah melalui pengujian status resistensi dengan aplikasi topikal dan uji pemberian pakan. Tingkat resistensi enam strain lalat rumah yang berasal dari enam kota dengan populasi tinggi di Indonesia, yaitu Serang (SRG), Jakarta (JKT), Bandung (BDG), Semarang (SMG), Yogyakarta (JOG), dan Surabaya (SBY), terhadap kedua insektisida uji dibandingkan dengan strain Danish Pest Infestation Laboratory (DPIL) sebagai kontrol. Selain itu, laju perkembangan resistensi selama 10 generasi juga

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dievaluasi. Hasil penelitian menunjukkan bahwa semua strain menunjukkan resistensi yang sangat tinggi terhadap permetrin. Tingkat resistensi tertinggi yang tercatat adalah pada strain SRG (RR_{50} = 2880) dan yang terendah adalah strain JKT (RR_{50} = 520). Perlakuan permetrin secara berulang-ulang selama 10 generasi meningkatkan tingkat resistensi sekitar 2,7–32,73 kali lipat dibandingkan dengan populasi tetua. Di sisi lain, sebagian besar strain menunjukkan resistensi rendah hingga sedang terhadap imidacloprid, yaitu strain SRG memiliki tingkat resistensi tertinggi (RR_{50} = 15,5) dan strain SBY memiliki tingkat resistensi terendah (RR_{50} = 2,0). Perlakuan imidakloprid secara berulang selama 10 generasi meningkatkan tingkat resistensi sekitar 3,25–17,41 kali lipat. Hasil penelitian ini merupakan laporan kedua tentang resistensi lalat rumah di Indonesia sejak 2016, yang dapat memberikan landasan penting bagi pengembangan strategi pengendalian lalat rumah secara terpadu yang tepat di daerah padat penduduk di Indonesia.

Kata kunci: Indonesia, imidakloprid, *Musca domestica*, permetrin, resistensi

INTRODUCTION

The housefly, *Musca domestica* Linnaeus, is the most common insect pest in urban and rural areas in the tropics, especially where people gather for economic activities. Most importantly, this insect can transmit diseases caused by protozoan, bacterial, helminthic, and viral agents including enteric infections (Förster et al. 2007). Applications of synthetic insecticides by professional pest control operators and homeowners have been considered the most common, effective, and cheap method to control the housefly population, especially in urban areas (Zhu et al. 2016). However, repetitive and inappropriate insecticide applications have led to resistance of the housefly to various classes of insecticides worldwide (Khan et al. 2015; Kustiati et al. 2016; Wang et al. 2019) and might lead to a possible outbreak of resistant populations.

Compared to other insecticide groups, pyrethroid insecticides are the most common insecticide applied to control urban pests worldwide due to their extended residual activity, safety for vertebrates, and low cost (Coats 1982). Permethrin, one of the members of pyrethroids, has been widely used for housefly population control in Indonesia since the 1980s (Rahayu et al. 2012). Nonetheless, a recent report showed that housefly populations from many areas in Indonesia have already developed very high resistance to permethrin, with the resistance ratios ranging from 190 to 25,190 fold (Kustiati et al. 2016).

A more recent alternative to control houseflies, imidacloprid, acts on the nicotinic acetylcholine receptor of the insect nervous system; it has been registered in several countries including Indonesia (Kustiati et al. 2016). For example, in China, it was registered for housefly control in the early 1990s (Ai et al. 2009), whereas in the USA, it was registered for housefly prevention in 2004 (Kaufman et al. 2006), while in Indonesia, it was registered in late 2000s (Kustiati et al. 2016). Although it is a relatively new insecticide, various studies showed that houseflies from several countries had developed resistance to imidacloprid, with the level of resistance varying from low to moderate (Li et al. 2012; Kavi et al. 2014; Abbas et al. 2015; Kustiati et al. 2016).

Therefore, to design an effective control program for houseflies, it is necessary to understand the resistance status and the rate of resistance development. However, in Indonesia, the most recent report on the resistance of housefly populations to permethrin and imidacloprid was published in 2016. Data about selection for insecticide resistance to permethrin and imidacloprid in houseflies are lacking. Moreover, in Indonesia, housefly resistance to insecticides has not been monitored regularly by any governmental institution. Rather research has been conducted by researchers at various universities. Further the research has not employed appropriate control strains to help standardize the results. This study aims to update housefly resistance data as a basis of future control programs.

MATERIALS AND METHODS

Housefly strains

The susceptible laboratory strain used in this study was obtained from the Danish Pest Infestation Laboratory (strain DPIL), Denmark, in 2011. The field strains of adult housefly were collected in 2018 from six cities in Indonesia, namely Serang (SRG strain), Jakarta (JKT strain), Bandung (BDG strain), Semarang (SMG strain), Yogyakarta (JOG strain), and Surabaya (SBY strain). All strains were maintained in the Entomology Laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia. In general, the colonies were reared following the methods described by Keiding & Arevad (1964). Briefly, the colonies were maintained inside screen cages (30 cm x 30 cm x 30 cm) at 25 ± 5 °C, 60−70% RH, and a 12:12 light:dark photoperiod. Dry milk, sugar, and water were provided *ad libitum* for feeding adult flies. Larvae were fed with a combination of dried yeast and whole dry milk diluted in water in a 1:1 ratio.

Chemicals

The permethrin insecticide (technical grade, 92% purity) was provided by PT. Inti Everspring, Indonesia. The insecticide imidacloprid (technical grade, 95% purity) was provided by PT. Bayer Indonesia. Both insecticides were diluted with acetone (97% purity) before the application.

Insecticide bioassay

The bioassay used to determine resistance in houseflies was developed by Kristensen & Jespersen (2008). Essentially, the susceptibility to permethrin was determined using a topical bioassay, while the susceptibility to imidacloprid was determined using a feeding bioassay. Serial dilutions of permethrin and imidacloprid were prepared using acetone 24 h before the application. Furthermore, each successive generation was evaluated with a similar method to obtain new concentration values before each subsequent selection to maintain a consistent selection of 50% mortality.

The housefly mortality was assessed at 24, 48, 72, and 96 h. For each preliminary evaluation, 25 houseflies were used for each concentration, and the entire experiment was replicated four times. The 96 h mortality data were subjected to probit analysis using Polo-PC software (LeOra Software 2004), and the results from this test were used to determine the median lethal dose (LD_{50}) and concentration (LC_{50}). The LC_{50} and LD_{50} values were used for further tests on the resistance development after the selection of adult flies.

Resistance development to permethrin

Bioassay for permethrin was conducted by the standard topical method from WHO (Hemingway & Brogdon 1998). Five to 7-day old adult houseflies were anesthetized by cold shock. About 0.2 µl of permethrin solution was applied to the ventral side of the thorax using a 25-µl Hamilton syringe. Control groups received only acetone. The concentrations of permethrin applied in this study were based on the LD_{50} of the previous generation. The houseflies were kept inside a plastic jar (500 ml) with a 15% sugar solution-saturated piece of cotton placed on the bottom of each jar. The individuals were considered dead if they showed no movement 24 h after the treatment. Each experiment was replicated four times. Surviving houseflies were kept inside the rearing cages to reproduce. The experiment was repeated for ten generations.

Resistance development to imidacloprid

The response to imidacloprid was evaluated using a feeding bioassay based on Kristensen & Jespersen (2008). Five serial dilutions of imidacloprid were prepared in 10 ml acetone mixed with 20 g sugar for the treatment group. A mixture of 10 ml acetone and 20 g sugar was made for the control groups. Twenty-five houseflies were kept in a plastic jar (500 ml), covered with a piece of cloth, and secured with a rubber band. A 0.5 g sugar solution in a water-saturated piece of cotton was placed on the bottom of each jar. Mortality was defined by counting the houseflies that showed no movement 72 h after the treatment. The bioassay was replicated four times. Surviving houseflies were kept inside the rearing cages to reproduce. The experiment was repeated for ten generations.

Statistical analysis

Mortality data were subjected to probit analysis to estimate the median lethal concentration (LC_{50}) or the lethal dose (LD_{50}). Probit analysis was carried out with the Polo-PC (LeOra Software 2004). Resistance ratios (RR_{50}) were estimated by comparing the LC_{50} or LD_{50} of each field strain to the susceptible strain (Lee et al. 2000) as shown below

$$RR_{50} = \frac{LD_{50} \text{ or } LC_{50} \text{ of field collected strain}}{LD_{50} \text{ or } LC_{50} \text{ of susceptible strain}}$$

The resistance level of each strain was determined based on Lee & Lee (2004) (Table 1).

In this study, we calculated the rate of resistance development by comparing LC_{50} values of the selected generation with their respective parental generations.

RESULTS

The resistance level of houseflies to permethrin and imidacloprid

Following the classification of insecticide resistance level (Table 1), the results of the topical bioassays showed that, as compared to the control (DPIL) strain, all field strains had a very high resistance level to permethrin (Table 2). The SRG strain showed the highest resistance to permethrin (RR_{50} = 2880), followed by SMG (2624), SBY (1824), BDG (1344), JOG (640), and JKT (520).

On the other hand, using the feeding bioassays, field strains demonstrated low to moderate resistance to imidacloprid, when compared to the control (DPIL) strain (Table 2). The highest level of imidacloprid resistance recorded was on SRG strain (RR_{50} = 15.5), followed by JKT (7.5), BDG (7.1), SMG (5.5), JOG (3.0), and SBY (2.0).

The slope of the dose-response curve to permethrin and imidacloprid of the field strains ranged from 1.785 ± 0.345 to 2.653 ± 0.350 and 1.989 ± 0.169 to 2.735 ± 0.235, respectively (Table 2). The SRG strain had a slope of less than 2 for permethrin, as did the SMG strain for both permethrin and imidacloprid.

Table 1. Classification of insecticide resistance level (Lee & Lee 2004)

Resistance ratio (RR)	Resistance level
$RR_{50} \leq 1$	Absence of resistance
$1 < RR_{50} \leq 5$	Low resistance
$5 < RR_{50} \leq 10$	Moderate resistance
$10 < RR_{50} \leq 50$	High resistance
$RR_{50} > 50$	Very high resistance

Development of resistance to permethrin selected for ten generations

For the DPIL strain, ten generations of selection resulted in very high resistance to permethrin, as shown in Table 3. Similar trends were also observed in the field strains. The RR_{50} values ranged from 6486 to 40,620 (with 2.7 to 32.73-fold resistance level compared to the parental). The RR_{50} values revealed that the SRG strain was the most resistant to permethrin after ten generations of selection (RR_{50} = 40,620), followed by BDG (11,440), JOG (9720), JKT (7960), SMG (7086), and SBY (6486).

Selection with imidacloprid also caused high resistance levels in the DPIL strain (RR_{50} = 12.20). Meanwhile, continuous selection with imidacloprid caused increased RR_{50} values in the field strains that ranged from 24.40 to 123.60 (with 3.25 to 17.41-fold resistance level compared to the parental). The BDG strain had the highest resistance level to imidacloprid (RR_{50} = 123.60), followed by SRG (87.80), SBY (29.80), SMG (28.90), JOG (25.40), and JKT (24.40).

Table 2. Responses of the field strains of houseflies to permethrin and imidacloprid

Population	n	Permethrin		Imidacloprid		RR_{50}	
		LD_{50} (µg/g housefly)	Slope ± SE	LC_{50} (ppm)	Slope ± SE	Permethrin	Imidacloprid
DPIL/S*	300	0.0005	2.006 ± 0.475	0.20	2.448 ± 0.566	-	-
BDG/R*	300	0.6720	2.350 ± 0.878	1.42	2.549 ± 0.667	1344	7.1
SRG/R*	300	1.4400	1.785 ± 0.345	3.10	2.002 ± 0.242	2880	15.5
SMG/R*	300	1.3120	1.878 ± 0.435	1.10	1.989 ± 0.169	2624	5.5
JKT/R*	300	0.2600	2.653 ± 0.350	1.50	2.735 ± 0.235	520	7.5
JOG/R*	300	0.3200	2.455 ± 0.866	0.60	2.478 ± 0.567	640	3.0
SBY/R*	300	0.9120	2.352 ± 0.778	0.40	2.645 ± 0.274	1824	2.0

RR: resistance ratio; S*: susceptible strain; R*: field collected population/strain; DPILS: Danish Pest Infestation Laboratory; SRG: Serang; JKT: Jakarta; BDG: Bandung; SMG: Semarang; JOG: Yogyakarta; and SBY: Surabaya.

Table 3. Changes in the resistance level (RR) and rate of resistance (N) of the houseflies strains to permethrin and imidacloprid selected for ten generations

Strain	Selected generation	n	Permethrin				Imidacloprid			
			LD_{50} (μg/g housefly)	Slope ± SE	RR_{50}	N	LC_{50} (ppm)	Slope ± SE	RR_{50}	N
DPIL/S		300	0.00050	4.456 ± 0.875	1.00		0.20	2.675 ± 0.875	1.00	
DPIL/R	F0	300	0.00078	2.006 ± 0.475	1.56	0.00	0.29	2.896 ± 0.135	1.45	0.00
	F1	300	0.00086	3.052 ± 0.352	1.72	1.10	0.35	3.657 ± 0.754	1.75	1.21
	F2	300	0.00112	3.562 ± 0.567	2.24	1.44	0.47	3.134 ± 0.987	2.35	1.62
	F3	300	0.00135	1.021 ± 0.656	2.70	1.73	0.55	1.543 ± 0.656	2.75	1.90
	F4	300	0.00143	2.752 ± 0.875	2.86	1.83	0.63	2.654 ± 0.685	3.15	2.17
	F5	300	0.00170	6.254 ± 0.856	3.36	2.15	0.75	3.456 ± 0.965	3.75	2.59
	F6	300	0.00230	4.253 ± 0.874	4.68	3.00	0.88	4.678 ± 0.915	4.40	3.03
	F7	300	0.00270	3.175 ± 0.655	5.34	3.42	0.95	3.786 ± 0.543	4.75	3.27
	F8	300	0.01710	2.155 ± 0.756	34.26	21.96	1.51	2.765 ± 0.453	7.55	5.21
	F9	300	0.01980	4.823 ± 0.265	39.50	25.32	1.98	4.645 ± 0.645	9.90	6.83
	F10	300	0.02550	2.564 ± 0.569	51.06	32.73	2.43	2.533 ± 0.134	12.20	8.37
SRG	F0	300	1.44000	2.350 ± 0.878	2880	0.00	3.10	1.875 ± 0.675	15.50	0.00
	F1	300	1.85000	3.875 ± 0.435	3700	2.31	4.30	2.875 ± 0.674	21.50	1.39
	F2	300	2.31000	3.435 ± 0.135	4620	3.44	5.10	2.765 ± 0.786	25.50	1.65
	F3	300	2.78000	2.035 ± 0.676	5560	4.14	6.40	1.875 ± 0.732	32.00	2.06
	F4	300	3.35000	3.756 ± 0.725	6700	4.99	7.50	4.823 ± 0.231	37.50	2.42
	F5	300	5.33000	7.425 ± 0.696	10660	7.93	8.30	2.564 ± 0.785	41.50	2.68
	F6	300	7.07000	5.231 ± 0.453	14140	10.52	8.90	3.367 ± 0.786	44.50	2.87
	F7	300	9.02000	4.213 ± 0.755	18040	13.42	9.75	3.345 ± 0.233	48.80	3.15
	F8	300	14.92000	3.221 ± 0.452	29840	22.20	11.75	2.877 ± 0.345	58.80	3.79
	F9	300	18.72000	2.823 ± 0.452	37440	27.86	13.86	3.765 ± 0.886	69.30	4.47
	F10	300	20.31000	2.125 ± 0.235	40620	30.22	17.56	2.786 ± 0.766	87.80	5.66
BDG	F0	300	0.67000	1.785 ± 0.345	1344	0.00	1.42	2.145 ± 0.897	7.10	0.00
	F1	300	1.55000	2.563 ± 0.467	3100	1.28	1.84	3.657 ± 0.344	9.20	1.30
	F2	300	2.05000	2.978 ± 0.678	4100	1.42	2.67	3.654 ± 0.365	13.40	1.89
	F3	300	2.68000	1.897 ± 0.876	5360	1.86	2.98	2.654 ± 0.564	14.90	2.10

DPILS: Danish Pest Infestation Laboratory; S: susceptible strain; R: field collected population/strain; SRG: Serang; JKT: Jakarta; BDG: Bandung; SMG: Semarang; JOG: Yogyakarta; and SBY: Surabaya.

Table 3. (*Countinue...*) Changes in the resistance level (RR) and rate of resistance (N) of the houseflies strains to permethrin and imidacloprid selected for ten generations

Strain	Selected generation	n	Permethrin				Imidacloprid			
			LD_{50} (µg/g housefly)	Slope ± SE	RR_{50}	N	LC_{50} (ppm)	Slope ± SE	RR_{50}	N
	F4	300	3.01000	4.657 ± 0.567	6020	2.09	3.54	3.645 ± 0.786	17.70	2.49
	F5	300	3.54000	2.675 ± 0.452	7080	2.46	5.78	4.654 ± 0.803	28.90	4.07
	F6	300	3.98000	3.456 ± 0.675	7960	2.76	8.78	5.564 ± 0.985	43.90	6.18
	F7	300	4.17000	3.654 ± 0.654	8340	2.90	10.76	4.564 ± 0.876	53.80	7.58
	F8	300	4.85000	2.876 ± 0.562	9700	3.37	16.78	3.675 ± 0.654	83.90	11.82
	F9	300	5.02000	3.865 ± 0,657	10040	3.48	18.67	2.745 ± 0.345	93.40	13.15
	F10	300	5.75000	2.876 ± 0.675	11440	3.97	24.76	2.654 ± 0.765	123.60	17.41
SMG	F0	300	1.31200	1.878 ± 0.435	2624	0.00	1.10	1.653 ± 0.345	5.50	0.00
	F1	300	1.41700	2.456 ± 0.365	2834	1.08	1.23	2.457 ± 0.895	6.20	1.13
	F2	300	1.52400	3.002 ± 0.675	3048	1.16	1.86	3.432 ± 0.874	9.30	1.69
	F3	300	1.67500	3.820 ± 0.786	3350	1.28	2.54	3.873 ± 0.653	12.70	2.31
	F4	300	1.87900	2.879± 0.678	3578	1.43	2.88	2.965 ± 0.889	14.40	2.62
	F5	300	2.00300	1.789 ± 0.865	4006	1.53	3.23	1.996 ± 0.665	16.15	2.94
	F6	300	2.13500	2.465 ± 0.234	4027	1.53	3.45	2.786 ± 0.654	17.25	3.14
	F7	300	2.56300	2.875 ± 0.712	5126	1.95	3.78	2.886 ± 0.886	18.90	3.44
	F8	300	2.86500	5.345 ± 0.453	5730	2.18	3.99	6.667 ± 0.778	19.95	3.63
	F9	300	3.13200	2.875 ± 0.275	6264	2.39	4.56	2.654 ± 0.288	22.80	4.15
	F10	300	3.54300	3.753± 0.786	7086	2.70	5.78	3.653± 0.796	28.90	5.25
JKT	F0	300	0.26000	2.653 ± 0.350	520	0.00	1.50	2.564 ± 0.405	7.50	0.00
	F1	300	0.56000	2.856 ± 0.768	1120	2.15	1.80	2.903 ± 0.067	9.00	1.20
	F2	300	0.76000	2.765 ± 0.564	1520	2.92	2.30	2.522 ± 0.865	11.50	1.53
	F3	300	0.92000	3.675 ± 0.453	1840	3.54	2.50	3.666 ± 0.876	12.50	1.67
	F4	300	1.67000	2.786 ± 0.453	3340	6.42	2.80	2.886 ± 0.677	14.00	2.27
	F5	300	1.95000	2.876 ± 0.876	3900	7.50	3.00	2.977 ± 0.875	15.00	2.00
	F6	300	2.23000	2.675 ± 0.450	4460	8.58	3.50	2.876 ± 0.765	17.50	2.33

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Strain	Selected generation	n	Permethrin				Imidacloprid			
			LD_{50} (μg/g housefly)	Slope ± SE	RR_{50}	N	LC_{50} (ppm)	Slope ± SE	RR_{50}	N
	F7	300	2.86000	2.875 ± 0.134	5720	11.00	3.70	2.899 ± 0.253	18.50	2.47
	F8	300	3.06000	5.675 ± 0.344	6120	11.76	4.02	5.654 ± 0.677	20.10	2.68
	F9	300	3.65000	2.675 ± 0.564	7300	14.04	4.35	2.668 ± 0.987	21.75	2.90
	F10	300	3.98000	2.877 ± 0.652	7960	15.30	4.88	2.876 ± 0.886	24.40	3.25
JOG	F0	300	0.32000	2.455 ± 0.866	640	0.00	0.60	2.668 ± 0.985	3.00	0.00
	F1	300	0.54000	1.544 ± 0.962	1080	1.69	0.92	1.765 ± 0.764	4.60	1.53
	F2	300	1.32000	2.675 ± 0.876	2640	4.13	1.54	2.665 ± 0.667	7.70	2.57
	F3	300	1.89000	2.767 ± 0.977	3780	5.91	1.88	2.876 ± 0.876	9.40	3.13
	F4	300	2.17000	2.675 ± 0.897	4340	6.78	2.35	2.655 ± 0.986	11.75	3.79
	F5	300	2.56000	3.875 ± 0.657	5120	8.00	2.89	3.887 ± 0.976	14.45	4.82
	F6	300	2.97000	2.675 ± 0.765	5940	9.28	3.24	2.876 ± 0.987	16.20	5.40
	F7	300	3.43000	2.675 ± 0.678	6860	10.72	3.59	2.899 ± 0.779	17.95	5.98
	F8	300	3.86000	2.786 ± 0.912	7720	12.06	4.04	2.998 ± 0.978	20.20	6.73
	F9	300	4.32000	2.876 ± 0.234	8640	13.50	4.68	2.898 ± 0.765	23.40	7.80
	F10	300	4.86000	2.345 ± 0.987	9720	15.19	5.08	2.987 ± 0.886	25.40	8.47
SBY	F0	300	0.91200	2.352 ± 0.778	1824	0.00	0.40	2.876 ± 0.776	2.00	0.00
	F1	300	1.05600	1.234 ± 0.235	2112	1.16	1.52	1.876 ± 0.765	7.60	3.80
	F2	300	1.35400	2.563 ± 0.435	2708	1.48	1.86	2.786 ± 0.653	9.30	4.80
	F3	300	1.65300	3.243 ± 0.433	3306	1.81	1.99	3.876 ± 0.776	9.95	4.98
	F4	300	1.85400	3.654 ± 0.765	3708	2.03	2.56	3.876 ± 0.654	12.80	6.40
	F5	300	2.06500	3.675 ± 0.455	4130	2.26	2.88	3.754 ± 0.676	14.40	7.20
	F6	300	2.34500	2.154 ± 0.875	4690	2.57	3.98	2.765 ± 0.655	19.90	9.95
	F7	300	2.67800	2.453 ± 0.892	5356	2.94	4.43	2.443 ± 0.556	22.15	11.08
	F8	300	2.76500	3.431 ± 0.234	5530	3.03	4.87	3.332 ± 0.675	24.35	12.18
	F9	300	2.98700	2.287 ± 0.453	5974	3.28	5.49	2.675 ± 0.876	27.45	13.73
	F10	300	3.24300	1.786 ± 0.453	6486	3.56	5.96	1.766 ± 0.345	29.80	14.90

DPILS: Danish Pest Infestation Laboratory; S: susceptible strain; R: field collected population/strain; SRG: Serang; JKT: Jakarta; BDG: Bandung; SMG: Semarang; JOG: Yogyakarta; and SBY: Surabaya.

DISCUSSION

Resistance status of houseflies to permethrin and imidacloprid

Following the criteria of Lee & Lee (2004), the current study indicates that housefly populations from Indonesia are highly resistant to permethrin (RR_{50} values ranged from 520 to 2880), and slightly to moderately resistant to imidacloprid (RR_{50} ranged from 2.0 to 15.5). SRG strain showed the highest resistance level to permethrin (RR_{50} = 2880), followed by SMG (2624), SBY (1824), BDG (1344), JOG (640), and JKT (520). In addition, as shown by the value of the slope (which was lower than 2), the SRG and SMG strains gave heterogeneous responses to permethrin treatment.

The finding that current field strains had very high resistance to permethrin is not surprising as equally high levels had been reported earlier by Kustiati et al. (2016). As the first such report, they found resistance ratios of 190−25,190 -fold, obtained from 26 of 32 field strains collected throughout Indonesia. By contrast, studies conducted in three provinces in China from 2011 to 2017 (Wang et al. 2019), where permethrin had been widely used, found that resistance of houseflies to permethrin was relatively low (RR < 50). Wang et al. (2019) also reported higher resistance to two other insecticides than permethrin (deltamethrin, > 100 and beta-cypermethrin, 364.64). Interestingly, they also reported a reversion of the resistance to dichlorvos in 2017. Most of the strains were found to be sensitive to dichlorvos which has been banned in China since 2008. This phenomenon suggests that under natural conditions, without insecticide selection pressure, resistance alleles are unfavorable (Abbas et al. 2016). We suspect that the heavy application of various pyrethroids (including permethrin) since 1980s in urban and veterinary environments, and especially by the farmers in agricultural settings, may explain the occurrence of highly resistant houseflies found in this study. Supporting data from the Ministry of Agriculture Indonesia in 2021 recorded that 47 out of 59 insecticides registered to control *M. domestica* are pyrethroid-based products (Sistem Informasi Pestisida 2021).

Another problem that probably contributes to the high levels of resistance found in this study is that most pest control operators do not understand integrated pest management, including the evolution resistance to insecticide and its management (Rahayu et al. 2012). We noticed that houseflies collected from SRG (from the city of Serang with a population of about 670,000) had the highest RR_{50} (2880), while flies from Jakarta, the capital of Indonesia, with a population of about 10.5 million, had the lowest RR_{50}. The situation with SRG strain could be partly explained by the fact that, the place where we collected the flies, was about 1−2 km from the nearest poultry farms -which regularly spray pyrethroids. In addition, as explained by Kustiati et al. (2016), the exceptionally high resistance levels to permethrin might be related to cross-resistance to DDT which was commonly used before it was banned in 2007.

The mechanism of high resistance to permethrin is primarily due to metabolic resistance and possibly other mechanisms such as reduced cuticular penetration and decreased sensitivity of neuronal sodium channel target (Shono et al. 2002; Chang et al. 2012).

On the other hand, the housefly populations in this study were more susceptible to imidacloprid, similar to previous reports in Indonesia (Kustiati et al. 2016) and other nearby regions, i.e., Malaysia (Jin et al. 2008; Ong et al. 2016). Here the relatively new use of imidacloprid as an insecticide (registered in the late 2000s) and the use of low doses are probably the critical factors responsible for the lower overall level of resistance. Nonetheless, Kaufman et al. (2006), reported that six strains of houseflies in the USA were found resistant to imidacloprid (RR 3.1−8.0-fold), even though imidacloprid had not been previously used. In another study, Markussen and Kristensen (2010) collected housefly strains in Denmark where imidacloprid had not been used and found that two field populations demonstrated high resistance to imidacloprid. They suggested that altered cytochrome P450 activity might play a role.

Resistance development of houseflies to permethrin and imidacloprid

Resistance to permethrin, after ten generations of selection, was higher in this study than reported by others (Georghiou & Taylor 1977; Liu &

Prodgeon 2002; Chang et al. 2012). The situation in Indonesia might be due to an evolved cross-resistance from prior extensive use of insecticides, especially DDT. All these insecticides have a similar mode of action (targeting the voltage-sensitive sodium channel).

Interestingly the susceptible strain also developed high resistance to permethrin after ten generations (albeit lower than field strains); as shown by the slope, which varied from 1.021 ± 0.656 to 6.254 ± 0.856. Nonetheless, it is difficult to confirm that changes in slope correlate with underlying genetic variability; in fact, the slope of the ninth generation was higher than the tenth generation. Therefore, changes in slope might not be good indicators for predicting changes in genetic variation as suggested by Hoskins & Gordon (1956).

There is also a possibility that there were differences in resistance mechanisms to permethrin that evolved between the strains (Shono et al. 2002). Alternatively, the slow development of resistance in the laboratory strain may be due to the lack of random mating within the population (Abbas et al. 2012).

After continuous selection for ten consecutive generations in the laboratory, all the field strains and the control strain showed increased resistance to imidacloprid, with the resistance level ranging from 3.25 to 17.41-fold in all field strains and increasing 12.20-fold in the control. Conversely, Khan et al. (2015) found that when a selected strain was reared without exposure to insecticide for five generations, RR values declined from 33.59 to 21.85, which indicates that the allele (s) that was responsible for resistance might be not stable. Other workers suggested that slower development of imidacloprid resistance could be caused by (1) variation of the resistance mechanisms for imidacloprid (Ma et al. 2017); (2) different genetic background as imidacloprid resistance was incompletely recessive on high exposure and incompletely dominant on low exposure (Khan et al. 2014); (3) possible autosomal trait responsible for imidacloprid resistance (Kavi et al. 2014); and (4) environmental conditions (Bourguet et al. 2000).

From the study, it appears that subjecting each strain to additional selection reveals that they retain enough genetic variation in the wild to evolve additional resistance despite the frequent use of both insecticides. The findings of this study are essential for the management of houseflies not only for the cities in question but also nationwide. Because very high resistance levels to permethrin had occurred, not to mention that it has been used since the 1980s, it is suggested to take preventive actions by stopping the use of permethrin and replacing permethrin with imidacloprid or other newer and safer insecticides. Therefore, using insecticides with different mode of actions for a certain period will avoid the development of higher resistance and control failure, thus maintaining insecticide application effectiveness. In addition, education about insecticide resistance to pest control operators and regular monitoring surveys are needed to fully understand the problem of insecticide resistance and the strategy to manage the resistance in the pest population.

CONCLUSION

In conclusion, we demonstrated the occurrence of insecticide resistance in houseflies collected from six urban areas in Indonesia, with the resistance ratios for permethrin ranging from 520 to 2880-fold, and those for imidacloprid ranging from 2.0 to 15.5-fold.

ACKNOWLEDGEMENTS

We would like to thank PT. Bayer Indonesia and PT. Inti Everspring Indonesia for providing us with the insecticides used in this experiment. This study is part of the Ph.D. thesis of Sri Yusmalinar. This work was partly supported by a competitive grant from the Ministry of Research, Technology and Higher Education (Penelitian Strategis Nasional) given to Intan Ahmad.

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