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SUSCEPTIBILITY OF SEMARANG AND PURWOKERTO STRAINS OF GERMAN COCKROACH, *Blattella germanica* (L.) TO FIPRONIL, INDOXACARB AND IMIDACLOPRID GEL BAITS

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ABSTRACT

The susceptibility status of the German cockroach, *Blattella germanica* (L.), collected from four areas in Semarang and two areas in Purwokerto, Indonesia was investigated to commercial baits containing fipronil, indoxacarb, and imidacloprid, and compared them to the Vector Control Research Unit (VCRU), Universiti Sains Malaysia susceptible strain. The results showed that all German cockroach strains from Semarang had developed resistance to fipronil, indoxacarb, and imidacloprid, while the Purwokerto strains were still susceptible to imidacloprid. The resistance ratio values (RR_{50}) of the Semarang strains to the three insecticides ranged from 1.694 to 8.28 fold (low to moderate resistance). Similarly, the RR_{50} values for the Purwokerto strains to fipronil and indoxacarb ranged from 2.13 to 3.01 fold (low to moderate resistance), but both Purwokerto strains remained susceptible to imidacloprid, with RR_{50} values of 0.88 and 0.77-fold, respectively. Based on these findings, it is recommended that insecticide resistance be monitored and that mechanisms and management of resistance be understood. This is crucial for developing effective cockroach management and control strategies.

Keywords: Fipronil; German cockroaches; indoxacarb; imidacloprid; susceptible

ABSTRAK

Kajian terhadap tahap kerentanan lipas German, *Blattella germanica* (L.), yang dikumpul dari empat kawasan di kota Semarang dan dua kawasan di kota Purwokerto, Indonesia terhadap umpan komersial berbahan aktif fipronil, indoxacarb dan imidacloprid telah dikaji dan dibandingkan dengan strain rentan Unit Penyelidikan Kawalan Vektor (VCRU), Universiti Sains Malaysia. Hasil kajian menunjukkan bahawa kesemua strain lipas dari Semarang telah menunjukkan tahap kerintangan terhadap ketiga-tiga insektisid yang diuji, manakala strain dari Purwokerto masih menunjukkan kerentanan terhadap imidakloprid. Nilai nisbah kerentanan(RR_{50}) bagi strain Semarang terhadap fipronil, indoksakarb dan imidakloprid berada

dalam julat 1.694 hingga 8.280 (tahap resistan rendah hingga sederhana). Sementara itu, nilai RR_{50} bagi strain Purwokerto terhadap fipronil dan indoksakarb masing-masing berada pada julat 2.130 hingga 3.010, (tahap resisten rendah hingga sederhana). Namun begitu, kedua-dua strain Purwokerto masih kekal peka terhadap imidakloprid dengan nilai RR_{50} sebanyak 0.88 dan 0.77 masing-masing. Berdasarkan dapatan ini, adalah disyorkan agar pemantauan berterusan terhadap kerintangan lipas terhadap insektisid dilaksanakan, di samping kajian lanjut untuk memahami mekanisme resisten yang terlibat. Langkah ini penting bagi merangka strategi kawalan dan pengurusan lipas yang lebih berkesan dan mampan.

Kata kunci: Fipronil; indoxacarb; imidacloprid; kerentanan; lipas Jerman

INTRODUCTION

The German cockroach, *Blattella germanica* (L.) is a major insect pest most commonly found in urban areas and almost worldwide (Tang et al. 2018; Wolfe & Scharft 2022). These insects are often found, especially in buildings, kitchens, hotels, and food outlets where sanitation levels are poor. German cockroach infestation in a place is influenced by environmental conditions and the behavior of building occupants (Shahraki et al. 2010; Wang et al. 2019). As residential pests, German cockroaches contaminate food, transmit pathogens, and cause allergic reactions and psychological stress (Brenner 1995).

As mechanical disease vectors, many pathogenic organisms have been reported to contaminate the German cockroach. Davari et al. (2017) reported that German cockroach inhabiting several hospitals, restaurants, and residential areas in the Khorramabad region, Lorestan province in Iran, were most often contaminated with fungi (60.7%), including the genus *Candida, Geotrichum*, and *Penicillium*. Adeleke et al. (2012) reported 23 microorganisms, namely *Klebsiella aerogenes*, *Bacillius cereus*, *Proteus* spp, *Staphyloccocus aureus*, *S. saprophyticus*, *Enteroccocus faecalis*, *Staphylococus epididermis*, *E. coli*, *Listeria monoctogene*, *Proteus mirabilis*, *Citrobacter* sp., *Pseudomonas aeruginosa*, *Psuedomonas* sp., *Seretia mensence*, *Candida albicans*, *Candida* spp., *Aspergilius* spp., *A. flavus*, *A. fumigate*, *Mucor* sp. and *Penicilium* species were isolated from feces and body surfaces of cockroaches from several hospitals and residential areas in Osogbo, South West Nigeria. All isolated pathogenic bacteria had multiple resistance to antibiotics such as Ampicillin, Augumentin, Amoxicillin, and Septrin (30µg). Other organisms that contaminate cockroaches include *Lophomonas blattarum*, which is a protozoan parasite that causes pneumonia (Camargo-Assis et al. 2020) and worms (Dokmaikaw & Suntaravitun 2019). Besides being a vector, cockroaches can also act as a source of disease allergens (Do et al. 2016) Because of its role as a vector and trigger of allergies, causing health problems, the population needs to be controlled.

Until now, insecticide control is the most commonly used method in *B. germanica* (Fazeli-Dinan et al. 2022) and other pest e.g. Kalaavathi et. al (2024). However, continuous use over a long period causes the resistance level of *B. germanica* to several active insecticide ingredients to increase. This is due to its ability to survive, detoxify toxic substances, short life cycles, and high reproductive ability (Buczkowski & Schal 2001; Wu & Appel 2017). *Blattella germanica* has resistance to 42 active ingredients of organochlorine insecticides, organophosphates, carbamates, synthetic pyrethroids, neonicotinoids, oxadiazine, and phenylpyrazole (Tang et al. 2018). Several reports of resistance to insecticides have occurred in Japan (Umeda et al. 1988), Taiwan (Pai et al. 2005), Iran (Ladonni 2000), and Indonesia (Ahmad et al. 2009; Rahayu et al. 2012).

Semarang City is the capital of Central Java province, Indonesia with a fairly high residential density (Rattafary 2016). Various facilities are available in Semarang City, such as hotels, markets, restaurants, and other public facilities. Not all public facilities implement good sanitation. The sanitary condition of an area is correlated with the presence of cockroaches. Areas with poor sanitation support cockroach infestations with abundant food, water, and hiding places (Wang et al. 2019). Until now, there have been no reports regarding the status of the vulnerability of *B. germanica* in Semarang City to insecticides, although there are anecdotal reports from pest control operators regarding the increasing population of *B. germanica* in several areas of Semarang City so that in several areas of Semarang City are thought to be resistant to several classes of insecticides. Meanwhile, based on the report by Ambarningrum et al. (2022), *B. germanica* from three markets in the city of Purwokerto are still susceptible to fipronil. Therefore, it is important to carry out this research to determine the susceptibility status of *B. germanica* in Semarang and Purwokerto strains to fipronil, indoxacarb, and imidacloprid. Pest control operators in the cities of Semarang and Purwokerto often use these three active ingredients to control *B. germanica.*

MATERIALS AND METHODS

Strains Collection

Semarang and Purwokerto strains of German cockroach, *Blattella germanica* (L.) were collected in 2021-2022 from several commercial segments in both regions (Table 1). Maintenance was carried out by placing each strain of cockroach in a plastic jar with a volume of 16 L, diameter of 25 cm, and height of 30 cm. Each jar has a shelter for cockroaches, cat food, and water. The inside of the jar is smeared with petroleum jelly to prevent cockroaches from coming out of the jar, then covered with a cloth tied with rubber. Cat food and drinking water are provided ad libitum. The maintenance room has a temperature of 25–30ºC, humidity of 55–95%, and a photoperiod of 12:12 (12 hours of light and 12 hours of darkness).

Table 1. Source of German cockroach used in this study

No.	Strain	Collection Site	Year Of Collection	City
1.	VCRU	Universiti Sains Malaysia	2007	Penang, Malaysia
2.	SMGa	Commercial segmentation	2021	Semarang
3.	SMGb	Commercial segmentation	2021	Semarang
4.	SMGc	Commercial segmentation	2021	Semarang
5.	SMGd	Commercial segmentation	2022	Semarang
6.	PWTa	Commercial segmentation	2022	Purwokerto
7.	PWTb	Commercial segmentation	2022	Purwokerto,

Noted: VCRU (Vector Control Research Unit); SMGa (Semarang a); SMGb (Semarang b); SMGc (Semarang c); SMGd (Smarang d); PWTa (Purwokerto a); PWTb (Purwokerto b).

Insecticide Gel Baits

The insecticides used were Advion gel bait containing indoxacarb, Maxforce gel bait containing fipronil, and Blattanex gel bait containing imidacloprid.

Bioassay

Bioassay uses the bait method. A total of 10 male cockroaches from each field strain, the Vector Control Research Unit (VCRU), Universiti Sains Malaysia susceptible strain were acclimatized for 24 hours in a test arena (40 x 25 x 15 cm) with the inner wall surface smeared

with petroleum jelly to prevent cockroaches from escaping and inside, there was a shelter as a hiding cockroach place. Gel bait was given as much as 0.1 g and placed at the bait station in the testing arena. Cockroach deaths were observed at 3-hour intervals for 12 hours and then every 24 hours after that until all individuals were dead. The control device only accepts food and water. The experiment for each strain and each feed was repeated 3 times.

Data Analysis

The lethal times (LT_{50} and LT_{90}) were calculated by probit analysis using the POLO-PC software program (LeOra Software 2003)

Resistance Ratio

The Resistance Ratio (RR) was determined by comparing the LT_{50} of each field strain to the LT_{50} of the susceptible strain (Robertson et al. 2007). The resistance levels were grouped into categories: not resistant ($RR_{50}\leq1$), low level of resistance ($1<RR_{50}\leq5$), medium level of resistance ($5<RR_{50}\leq10$), high level of resistance ($10<RR_{50}\leq50$), and very high level of resistance ($50<RR_{50}\leq1000$) (Lee & Lee 2004).

RESULTS AND DISCUSSION

The research showed that all *B. germanica* strains collected from Semarang have become resistant to fipronil, indoxacarb, and imidacloprid. In contrast, the *B. germanica* strains from Purwokerto were still susceptible to imidacloprid but have become resistant to fipronil and indoxacarb. The resistance ratio value (RR_{50}) of the Semarang strain to the three types of insecticides ranges from low to moderate resistance (1.694-8.28 fold), as well as the RR_{50} value of the Purwokerto strain to fipronil and indoxacarb ranges from low to medium (2.13-3.01 fold). However, Purwokerto strain was still susceptible to imidacloprid with RR_{50} values of 0.88 and 0.77 fold, respectively (Tables 2- 4).

Table 2. Toxicity of fipronil to adult male of German cockroaches

Strain	n	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope±SEM	X^2(df)	RR_{50}
		Hours				
VCRU	30	4.9(4.16-5.65)	11.42(9.51-14.93)	3.49±0.52	0.68(3)	1
SMGa	30	8.26 (7.32-9.20)	17.74(15.26-21.94)	3.86±0.51	1.50(5)	1.69
SMGb	30	40.57(37.09-44.05	210.28(181.52-251.05)	1.79±0.12	9.05(44)	8.28
SMGc	30	20.56(18.52-22.56)	69.73(64.52-75.89)	2.42±0.13	41.43(48)	4.2
SMGd	30	8.79(7.66-9.86)	17.59(15.23-21.6)	4.25±0.52	4.93(6)	1.79
PWTa	30	10.44(9.15-11.69)	30.36(26.33-36.37)	2.76±0.27	4.95(11)	2.13
PWTb	30	10.65(9.49-11.76)	24.70(21.81-28.99)	3.51±0.14	4.65(9)	2.17

Noted: VCRU (Vector Control Research Unit); SMGa (Semarang a); SMGb (Semarang b); SMGc (Semarang c); SMGd (Smarang d); PWTa (Purwokerto a); PWTb (Purwokerto b); LT (Lethal Time); RR (Resistance Ratio).

Table 3. Toxicity of indoxacarb to adult male German cockroaches

Strain	n	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope±SEM	X^2(df)	RR_{50}
		Hours				
VCRU	30	8.83(6.69-13.44)	42.05(23.44-135.12)	1.89±0.40	0.05(2)	1
SMGa	30	14.96(12.97-16.84)	39.54(35.83-44.16)	3.04±0.25	4.80(18)	1.69
SMGb	30	9.72(8.06-11.38)	29.09(25.24-34.17)	2.69±0.23	13.27(13)	1.1
SMGc	30	14.46(12.60-16.20)	32.4(29.28-36.39)	3.66±0.34	2.33(12)	1.64
SMGd	30	16.63(14.5-18.62)	42.58(37.76-49.44)	3.14±0.32	1.98(11)	1.88
PWTa	30	26.33(21.26-30.79)	60.21(48.73-88.47)	3.57±0.38	45.79(13)	2.98
PWTb	30	26.61(18.35-36.69)	70.57(47-251.52)	3.03±0.36	67.13(10)	3.01

Noted: VCRU (Vector Control Research Unit); SMGa (Semarang a); SMGb (Semarang b); SMGc (Semarang c); SMGd (Smarang d); PWTa (Purwokerto a); PWTb (Purwokerto b); LT (Lethal Time); RR (Resistance Ratio).

Table 4. Toxicity of imidacloprid to adult male German cockroaches

Strain	n	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope±SEM	X^2(df)	RR_{50}
		Hours				
VCRU	30	16.54(12.98-20.08)	52.45(40.56-78.58)	2.56±0.28	11.43(9)	1
SMGa	30	18.57(14.86-22.64)	236.83(154.98-440.65)	1.16±0.14	13.06(14)	1.12
SMGb	30	46.81(42.81-50.65)	152.35(134.8-177.54)	2.50±0.17	52.55(39)	2.83
SMGc	30	36.57(31.61-41.10)	76.67(65.80-97.60)	3.99±0.38	44.55(18)	2.21
SMGd	30	40.88(26.84-53.46)	144.35(99.81-329.34)	1.92±0.20	47.2.91(34)	2.36
PWTa	30	14.61(8.09-21.48)	303.8(152.33-1194.46)	0.97±0.12	61.28(20)	0.88
PWTb	30	12.68(8.49-17.15)	64.43(43.74-122.63)	1.82±0.18	34.99(11)	0.77

Noted: VCRU (Vector Control Research Unit); SMGa (Semarang a); SMGb (Semarang b); SMGc (Semarang c); SMGd (Smarang d); PWTa (Purwokerto a); PWTb (Purwokerto b); LT (Lethal Time); RR (Resistance Ratio).

Susceptibility of the *Blattella germanica* to Fipronil

The death time that causes 50% of the field cockroach population to die (LT_{50}) due to exposure to fipronil ranges from 8.26–40.57 hours. The death time for field cockroaches is longer than standard cockroaches. (Table 2). The longest LT_{50} for field cockroaches on the SMGb strain was 40.57 hours with an RR_{50} value of 8.28-fold, while the fastest LT_{50} for field cockroaches on the SMGa strain (8.26 hours), with an RR_{50} value of 1.69 fold. The RR_{50} value of this study is higher than the research report by Appel et al. (2022), which is 0.73-4.43 fold, and Hu et al. (2020), which is 1.47-3.76 fold. Ambarningrum et al. (2022) 0.51-0.96-fold, but this result is lower than the results reported by Rahayu et al. (2012) 44.72-fold and Gondhalekar & Scharf 2012) 36.42 fold. The LT_{50} results of this study ranged from 8.26-40.57 hours, longer than the LT_{50} results of the study by El-Monairy et al. (2015) of 0.64-0.96 hours and research by Ang et al. (2013) of 7.50-9.46 hours, but faster than the research results of Tisgratog et al. (2023), namely 1.87-8.27 days.

Fipronil is a type of insecticide that was only used in Indonesia around 2000 and was only bait formulated form (Ahmad & Suliyat 2011), but research results show that the Semarang and Purwokerto strains of cockroaches are resistant to fipronil, although levels were low to moderate, as also reported by Rahayu et al. (2012), with a resistance ratio of 2.11–44.72 fold. Ambarningrum et al. (2019) reported the potential for developing behavioral resistance to bait containing glucose with the active ingredient fipronil in Indonesian cockroach strains PKUb, JKTa, JKTb, and BDGb. This is possible due to the continuous use of fipronil bait over a long period (Fardisi et al. 2019). Another factor may be caused by cross-resistance between fipronil and dieldrin, which may have occurred previously in Indonesia or perhaps from German cockroaches that migrated to Indonesia via transportation routes (Gondhalekar & Scharf 2012; Kristensen et al. 2005). Valles et al. (1997) stated that insects resistant to organophosphates, carbamates, and pyrethroids may resistant to fipronil.

Susceptibility of *Blattella germanica* to Indoxacarb

The RR_{50} and LT_{50} values of the Semarang strain of *B. germanica* against Indoxacarb gel bait were higher than the results of other studies. Davari et al. (2018) reported RR_{50} ranging from 1.2–1.6 fold (low resistance). Liang et al. (2017) showed an RR_{50} of 1.07-fold for the NYA strain, but the RR_{50} for the CA strain was higher than the results of this study, namely 1.62-fold. Both show low levels of resistance. Chai & Lee (2010) reported RR_{50} 1.4–2.9 fold. The LT_{50} of the Semarang and Purwokerto strains of *B. germanica* against indoxacarb was longer (8.83–26.33 hours) compared to the research results of Davari et al. (2018), which is around 7.79–12.84 hours.

The reduced susceptibility of the Semarang and Purwokerto strains of *B. germanica* to indoxacarb may also be caused by long-term exposure to the active ingredient, cross-resistance due to the choice of other insecticides, or a combination of both factors. (Gondhalekar et al. 2013). Resistance to indoxacarb is also possible due to the influence of resistance to fipronil, where this insecticide began to be used before the indoxacarb insecticide was used and is still used interchangeably. This is reinforced by the higher level of resistance shown by fipronil compared to indoxacarb. According to Liang et al. (2017), high levels of resistance to fipronil were observed in all cockroach strains, while levels of resistance to indoxacarb were lower in some strains, so resistance to indoxacarb including those produced by fipronil selection, may depend on some mutations in metabolic detoxification and target proteins. The emergence of insecticide resistance following the administration of toxic baits is not mediated by external entry barriers such as the cuticle. However, the intestinal barrier may contribute if the active ingredient acts outside intestinal cells. Exposure to fipronil bait confers cross-resistance to indoxacarb, although these two insecticides function through different mechanisms. Fipronil is a phenylpyrazole compound that targets the central nervous system by blocking chloride channels, while indoxacarb is an oxydiazine that blocks sodium channels. Fipronil and indoxacarb have molecules with different structures, and both compounds may be metabolized similarly after consumption because both have been proven to be substrates for cytochrome P450 enzymes in cockroaches.

Susceptibility of the *Blattella germanica* to Imidacloprid

The LT_{50} value exposed to imidacloprid gel bait was faster (12.68–46.81 hours) compared to the research results of Hu et al. (2020) 2.77–6.4 days, Tisgratog et al. (2023) 1.77–11.72 days, but longer than the research results of Wan-Norafikah et al. (2017) 1.58 hours. Other researchers reported that a concentration of 10% caused cockroach death by 52.5% within 24 hours, and a concentration of 45% caused death by 95-100% within 24-72 hours (Baniardalani et al. 2019). The RR_{50} value for the Semarang strain ranges from 1.12–2.83 fold. In contrast, for the Purwokerto strain, the RR_{50} value is below 1, which means the Purwokerto strain is more susceptible to imidacloprid than the Semarang strain. According to Hu et al. (2020), the RR_{50} value of *B. germanica* to imidacloprid is low (<4), making it difficult to determine whether this strain has natural tolerance or because cross-resistance has occurred.

Imidacloprid is a systemic insecticide and is classified as a neonicotinoid/chloronicotinyl. Imidacloprid affects the nicotinic acetylcholine receptors in the insect's central nervous system. The similarity of chloronicotinyl to insect nicotinic acetylcholine receptors is an important characteristic of this class of insecticides (Baniardalani et al. 2019). Hu et al. (2020) stated that the performance of gel baits containing the active ingredients fipronil, imidacloprid, and indoxacarb correlated with high levels of resistance to deltamethrin (a group of pyrethroids). The RR_{50} value for deltamethrin ranges from 0–817.5-fold. Resistance of *B. germanica* to deltamethrin was also reported by Hariani (2013) from 32 strains of *B,germanica* in Indonesia that were resistant to deltamethrin (RR_{50} = 11.23–1462.11 fold). Still in the same group as deltamethrin, resistance in several *B. germanica* strains in Indonesia has also been reported to permethrin, cypermethrin, and d-allethrin (Ahmad et al. 2009; Rahayu et al. 2012). Pyrethroid class insecticides have been used in Indonesia continuously since 1980 with increasing doses and frequency, so it is suspected that cross-resistance has occurred between insecticides of the same pyrethroid class (Hariani 2013). The correlation between deltamethrin resistance and the performance of bait containing the active ingredients fipronil, indoxacarb, and imidacloprid appears to be related to metabolic resistance mechanisms that trigger longer lifespan or related to behavioral resistance that triggers aversion to bait. Aversion to bait was also detected in several *B. germanica* strains in Indonesia that were

exposed to bait containing glucose and the active ingredient fipronil (Ambarningrum et al. 2019). Aversion to bait also occurs in flies, as reported by Hubbard and Murillo (2022), who stated that imidacloprid can stimulate bitter taste neuron receptors in flies, causing an aversion to bait.

CONCLUSION

The conclusion of this study shows that Semarang and Purwokerto strains have developed resistance to fipronil and indoxacarb, although still at a low to moderate level. Additionally, the Semarang strain has also become resistant to imidacloprid. Therefore, monitoring resistance to the insecticides commonly used in cockroach control is necessary. Pest control operators need to understand the mechanisms and management of resistance. This understanding is crucial for developing effective cockroach management and control strategies.

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Conflict of Interest
None declared

Ethics Declarations
No ethical issue is required for this research.

Data Availability Statements
This manuscript has no associated data.

Author's Contributions
Trisnowati Budi Ambarningrum (Conceptualization-Supporting, Data curation-Lead, Funding acquisition-Equal, Investigation-Lead, Project administration-Supporting, Resources-Equal, Validation- Equal, Visualization-Equal, Writing – original draft-Supporting), Sorta Basar Ida Simanjuntak (Writing – review& Supporting), Aris Mumpuni (Writing – review & editing-Supporting), Trisno Haryanto (Project administration-Lead & Software-Lead, review & editing), EkoPuji Sri Haryanto (Supporting, review & editing)

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