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Full Length Article

High Resistance of Pyrethroid-Resistant *Blattella germanica* (Dictyoptera: Blattellidae) Population of Palembang Disassociated with Cuticle Thickening Mechanism

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Abstract

Blattella germanica L. is highly adaptive to the human environment and reportedly resistant to extensive insecticides. Pyrethroid aerosol is predominantly used to control this pest in households causing more resistance. Reduced insecticide penetration has been reported in *B. germanica* since the 1960s, allowing for cuticle modification in resistant individuals. This study aimed to determine the resistance ratio of cockroaches to six pyrethroid aerosol insecticides in Indonesia (By, Ht, Vp, Fm, Mt, Nm) and detect the presence of cuticle thickening mechanism in pyrethroid-resistant *B. germanica*. This study used the PLZ-PLM strain, originating from Palembang as a field strain, and VCRU-WHO (Vector Control Research Unit-WHO) as a standard strain. The result showed that the resistance ratio of the PLZ-PLM strain to the six aerosol insecticides was 18–45 fold, which confirmed that the PLZ-PLM was highly resistant to all the aerosol insecticides used. There was no significant difference in the average cuticle thickness between resistant PLZ-PLM cockroaches and strains susceptible to pyrethroids (Mann Whitney U-Test, Z= -0.731, $P < 0.05$). The absence of a cuticle thickening in pyrethroid-resistant PLZ-PLM strain indicates that other mechanisms contribute to the resistance. This study illustrates the need to evaluate the use of aerosol insecticides in controlling *B. germanica*. Also, the result confirms that cuticle thickening plays a minor role in the insecticide resistance mechanism. © 2024 Friends Science Publishers

Keywords: Aerosol; *Blattella germanica* L.; Cuticle thickness; Insecticide resistance; Pyrethroid

Introduction

Blattella germanica L., commonly known as the German cockroach, is the most common pest cockroach found in residential areas (Bell *et al.* 2007) and is included in the top 6 urban insect pests (Zhu *et al.* 2016). Population control is of concern because of its negative impact on human health (Pérez 1989) and its very high adaptive capacity to various environmental conditions (Bell *et al.* 2007). Until now, insecticides have been the key to controlling *B. germanica*, but their widespread and excessive use has increased resistance, failing to control this species (Wu and Appel 2017). Since the 1950s, *B. germanica* has been reported to be resistant to dichlorodiphenyltrichloroethane (DDT) (Cochran *et al.* 1953), which then has extended to 45 types of insecticides (Arthropod Pesticide Resistance Database, 2023). In Indonesia, cases of German cockroach resistance to several insecticides have also been reported (Ahmad *et al.* 2009; Rahayu *et al.* 2012; Rahayu *et al.* 2016; Nurseha *et al.* 2019).

Pyrethroids have been commonly used for German cockroach control in recent years due to their low toxicity to mammals (Shiravand *et al.* 2018). In common, this insecticide is commercially formulated as an aerosol passing around the community. Unfortunately, studies have found that some aerosol insecticides are no longer effective in killing German cockroaches in Indonesia (Rahayu *et al.* 2016; Rahayu *et al.* 2021a).

Insects can develop resistance against insecticides through penetration resistance. This process involves thickening or altering the composition of the cuticle, which slows down the entry of insecticides into the insect's body. This, in turn, provides the insect with more time to detoxify the poison. Studies have shown that this resistance mechanism is caused by changes in the cuticle's structure and composition (Balabanidou *et al.* 2018; Chen *et al.* 2019). Since 1967, there have been reports of penetration resistance in *B. germanica* (Ku and Bishop 1967), which were followed by other studies (as shown in Table 1). Some insect pest species that are resistant to insecticides have been found to have thicker cuticles than susceptible ones (Pedrini *et al.* 2009; Wood *et al.* 2010; Lin *et al.* 2012; Balabanidou *et al.* 2016; Lilly *et al.* 2016; Yahuédo *et al.* 2017; Balabanidou *et al.* 2019; Verma *et al.* 2019; Samal and Kumar 2021; Jacobs *et al.* 2023). However, there have been no reports of cuticle thickening in *B. germanica*.

In Indonesia, resistant German cockroaches were reported to possess metabolic resistance mechanisms (Ahmad *et al.* 2009) and KDR mutations (Rahayu and Saputra 2022). However, to our knowledge, there have been no reports regarding penetration resistance. This study investigated a strain of German cockroach, PLZ-PLM, origin from Palembang City, the capital of South Sumatra province and one of the largest metropolitan cities in Indonesia. PLZ-PLM strain has been reported as propoxur-resistant (Nurseha *et al.* 2019), and has an LT90 of more than 192 h on six pyrethroid aerosol insecticides tested (Rahayu *et al.* 2021a). We conducted further data analysis based on Rahayu *et al*. (2021a) to determine the resistance ratio of PLZ-PLM strain to the aerosol insecticides and to detect penetration resistance through the cuticle thickness of *B. germanica*, which is resistant to the pyrethroid-based aerosol insecticides. This monitoring is intended to provide evaluation data for further insecticide resistance management, particularly concerning the Palembang population.

Materials and Methods

Sampling and rearing

Two strains were used in this study, *i.e*., the PLZ-PLM strain obtained from Palembang City, Indonesia, and the VCRU-WHO strain (Vector Control Research Unit-WHO) as the standard strain. Those strains were maintained in the Animal Physiology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Andalas, since 2017 and 2007, respectively. The German cockroaches used were adult males aged about three months. The rearing was carried out in plastic containers (30 cm diameter × 27 cm height). The top edge of the container was smeared with a mixture of petroleum jelly and baby oil and was covered with a thin cloth on the top. Cockroaches were kept at room temperature between 26–28ºC and a photoperiod of 12:12 (12 h of dark and 12 h of light). They were fed cat food (Pedigree®) and water *ad libitum*.

Selection by insecticide efficacy test

The selection was done by applying six commercial aerosol insecticides with different pyrethroid formulations (By, Ht, Vp, Fm, Mt, Nm), separately. Ten samples of cockroaches were placed into a cardboard box (30 cm × 20 cm × 20 cm), where the top inner edge of the box was layered with paper tape and smeared with a mixture of petroleum jelly and baby oil to prevent the cockroaches from escaping. Insecticide was then sprayed from the top of the box for one second at 1 m from the box base (Fig. 1). Observations were made 24 h later. The resistant cockroaches were taken from the PLZ-PLM strain that remained alive, and the susceptible ones were taken from the VCRU-WHO strain. Five samples were taken for each group.

The left middle leg was gently detached from the cockroach's body and put on a glass object. The sample was then dripped with 70% ethanol and cut transversely in the middle of the tibia using a platinum-coated razor blade. Each leg piece was then stored in a labeled separate microtube (1.5 mL) containing 70% ethanol until imaging using SEM.

Preparation and SEM

Samples were immersed in cacodylate buffer solution for about 2 h, agitated in an ultrasonic cleaner for 5 min, and then soaked in a 2.5% glutaraldehyde solution for 24 h. Next, the samples were immersed in 2% tannic acid for 6 h, followed by washing with cacodylate buffer for 5 min, repeated four times. Subsequently, specimens were dehydrated with graded alcohol starting from 50% alcohol for 5 min, repeated four times, continuing with 70, 85 and 95% alcohol, each for 20 min at room temperature. The samples were then immersed in absolute ethanol for 10 min, repeated twice, and then frozen in tert butanol for 10 min, repeated twice. Subsequently, samples were frozen in a freezer and dried with a vacuum drier until dry. After mounting, the specimen was coated with gold metal (Au) and vacuumed for 15 min. Images were taken using a JSM-5000 LV scanning electron microscope at the Biological Research Center-Indonesian Institute of Sciences.

Data analysis

The resistance ratio 90 (RR90) was calculated by referring to data obtained by Rahayu *et al*. (2021b) with the formula as follows:

$$RR90 = \frac{\text{LT90 of the field strain}}{\text{LT90 of the standard strain}}$$

Where RR = resistance ratio, LT90 = time taken for 90% of a test population to die after insecticide exposure.

The resistance ratio values were then grouped into six categories based on Rahayu *et al*. (2012), namely:

RR90 ≤ 1: *absence resistance*
1 < RR90 ≤ 5: *low resistance*
5 < RR90 ≤ 10: *moderate resistance*
10 < RR90 ≤ 50: *high resistance*
50 < RR90 ≤ 1000: *very high resistance*
RR90 > 1000: *extremely high resistance*

Table 1: Penetration resistance of *B. germanica* reported to different insecticides

Study site	Insecticide	Mechanisms	References
Virginia, US	Carbaryl (carbamate)	Slower penetration	Ku and Bishop (1967)
New York, US	Propoxur (carbamate)	Reduced cuticular penetration	Siegfried and Scott (1991)
Florida, US	Permethrin (pyrethroid)	Reduced cuticular penetration	Bull and Patterson (1993)
Florida, US	Permethrin (pyrethroid)	Reduced cuticular penetration	Anspaugh *et al*. (1994)
Indiana, US	Fenvalerate (pyrethroid)	Reduced cuticular penetration	Wu *et al*. (1998)
Florida, US	Cypermethrin (pyrethroid)	Reduced cuticular penetration	Valles *et al*. (2000)
Alabama, US	Permethrin, deltamethrin (pyrethroid)	Reduced cuticular penetration	Wei *et al*. (2001)
Alabama, US	Permethrin, deltamethrin (pyrethroid)	Reduced cuticular penetration	Pridgeon *et al*. (2002)
China	Beta-cypermethrin (pyrethroid)	Elevated expression of putative cuticular protein, and ATP-binding cassette (ABC) transporter	Zhang *et al*. (2014)
China	Beta-cypermethrin (pyrethroid)	Overexpression of *CYP4G19* (related to biosynthesis of hydrocarbon)	Chen *et al*. (2019)

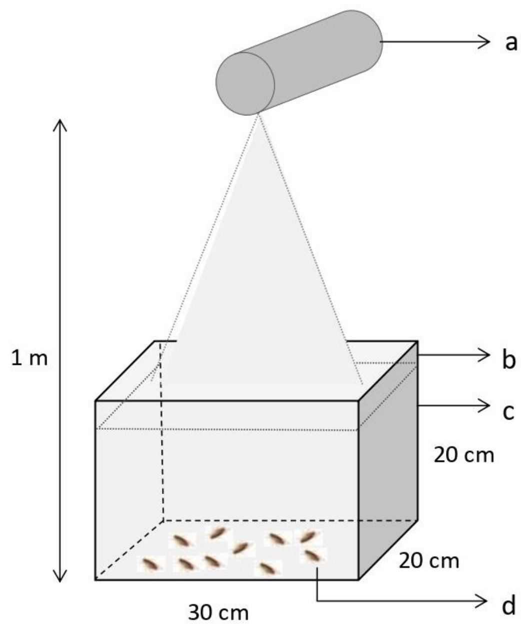

Fig. 1: Diagram of the selection of *B. germanica* by efficacy test, (a) aerosol insecticide, (b) test box area covered with paper tape and smeared by vaseline + baby oil, (c) test box (d) cockroach samples

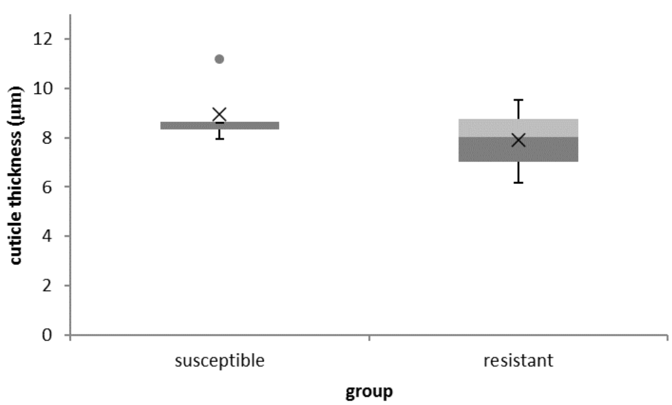

Fig. 2: Boxplot of cuticle thickness in *B. germanica* susceptible and resistant to pyrethroid insecticides. Dots (°) represent outlier data

Micrographs were processed using ImageJ v.1.52p. Cuticle thickness was measured by initially tracing the inner and outer circumference line, whose unclear boundaries due to debris or damage were not measured (Fig. 2, 3). A total of 25 measurement points were taken for each sample. The mean cuticle thickness was obtained from the total cuticle thickness divided by total measurement points. The data collected were compared by the Mann-Whitney test using SPSS 25.

Table 2: Resistance ratio (RR90) of *B. germanica* VCRU-WHO and PLZ-PLM strain to six pyrethroid aerosol insecticides

No	Insecticide	Active ingredient	Strain	RR90 (-fold)	Level of resistance
1.	By	Cypermethrin (0.10%)	VCRU-WHO	1	Absense resistance
		Pralethrin (0.10%)	PLZ-PLM	18	High resistance
		Transfluthrin (0.10%)			
2.	Ht	Transfluthrin (0.17%)	VCRU-WHO	1	Absense resistance
		Pralethrin (0.05%)	PLZ-PLM	42	High resistance
		Cypermethrin (0.10%)			
3.	Vp	Dimefluthrin (0.04%)	VCRU-WHO	1	Absense resistance
		Pralethrin (0.12%)	PLZ-PLM	34	High resistance
		Cyfluthrin (0.03%)			
4.	Fm	Transfluthrin (0.15%)	VCRU-WHO	1	Absense resistance
		Permethrin (0.15%)	PLZ-PLM	21	High resistance
5.	Mt	Permethrin (0.06%)	VCRU-WHO	1	Absense resistance
		Imiprothrin (0.03%)	PLZ-PLM	25	High resistance
		Esbiothrin (0.11%)			
6.	Nm	Transfluthrin (0.06%)	VCRU-WHO	1	Absense resistance
		Cyfluthrin (0.03%)	PLZ-PLM	45	High resistance

Table 3: Average cuticle thickness in susceptible and resistant *B. germanica* to pyrethroid insecticides

Group	n	Mean Cuticle thickness (μm)	SD
Susceptible	5	8,938	1,295
Resistant	5	7,887	1,337

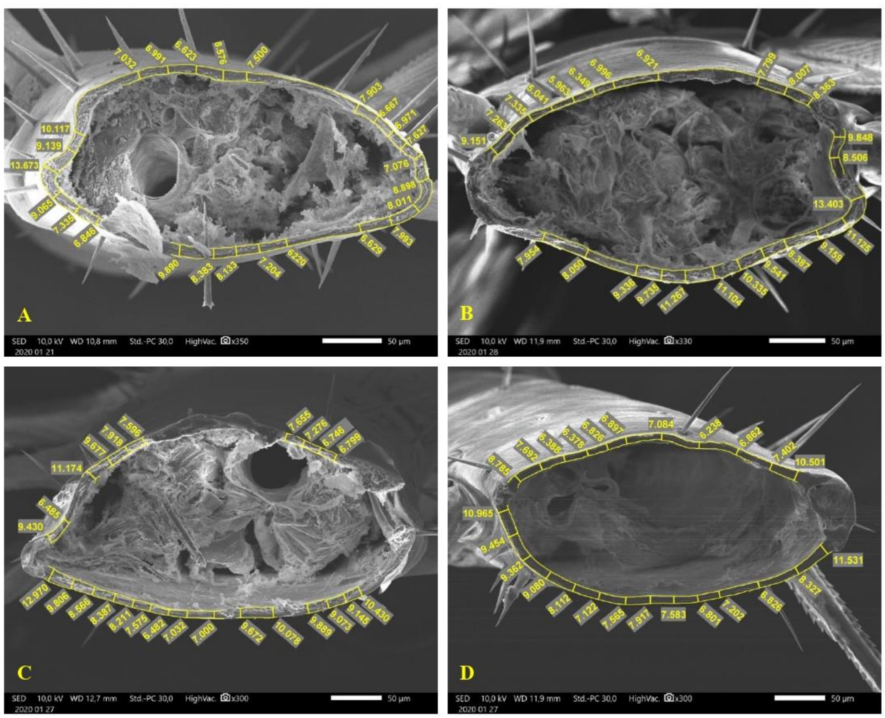

Fig. 3: Micrographs of cross sections of the left middle midleg of the tibia of *Blattella germanica* L. in susceptible (A, B) and resistant (C, D) individuals with 25 points measuring cuticle thickness (μm)

Results

The RR90 of German cockroach strains of VCRU-WHO and PLZ-PLM to six aerosol insecticides can be seen in Table 2. The VCRU-WHO strain was susceptible to all tested insecticides. Meanwhile, the PLZ-PLM strain has an RR90 in the range of 18–45 fold to the aerosol insecticide used which is classified as highly resistant. The highest and the lowest RR90 were found in the Nm and By insecticides, respectively.

The Mann-Whitney test showed an insignificant difference between the cuticle thickness of the susceptible and the resistant group in the *B. germanica* (Z = -0.731, $P < 0.05$; Table 3).

Discussion

Our findings showed the absence of the cuticle thickening mechanism in the pyrethroid-resistant PLZ-PLM strain. On the contrary, the susceptible cockroaches (VCRU-WHO) slightly have thicker cuticles than resistant ones. It seems just the occurrence of individual variation and is not linked to resistance mechanisms. The absence of cuticle thickening suggests the presence of other resistance mechanisms in PLZ-PLM cockroaches, which causes resistant cockroaches to tolerate the insecticide toxins although not having thicker cuticles, either by increased detoxification enzymes or mutations in target proteins. However, despite having a thicker cuticle, the VCRU-WHO strain could not survive insecticide due to the absence or low level of other mechanisms.

Even though we cannot accurately determine the resistance level of each mechanism involved, this study confirmed that the resistance offered by the penetration mechanism is comparatively lower than other mechanisms. Penetration resistance also concurrently occurs with other mechanisms that have been proven to contribute to resistance, as previously reported in *B. germanica* (Wu *et al.* 1998) and other species, such as *Drosophilla melanogaster* (Strycharz *et al.* 2013), *Aedes aegypti* (Kasai *et al.* 2014) and *An. Gambiae* (Yahouédo *et al.* 2017). Nevertheless, the impact of penetration resistance could strengthen the phenotypic and other potential resistance mechanisms, allowing insect resistance to expand to different types of insecticides. Furthermore, this mechanism also allows insects to tolerate higher insecticide concentrations (Balabanidou *et al.* 2016), which results in a higher likelihood of failure in controlling cockroach population. Besides, the absence of cuticle thickening does not eliminate the possibility of other cuticle-related resistance mechanisms in PLZ-PLM cockroaches. There may be a change in the cuticle composition in the resistant cockroaches, which affects insecticide penetration, such as the finding of Bai *et al*. (2022), where cuticle melanization is related to the permeability of the *B. germanica* cuticle. Yet, this possibility requires further investigation.

According to Table 2, the RR90 value showed that PLZ-PLM has high resistance to all tested pyrethroid aerosol insecticides. Even though the insecticide is a mixture of several pyrethroid active ingredients, we found differences in the level of resistance of PLZ-PLM cockroaches to the aerosol insecticides used, where different compositions produced different insecticidal effects. We also found that insecticides containing the same active ingredient but in different concentrations generate distinct insecticidal levels, as observed in the By and Ht (Table 2). It demonstrates that the efficacy of the insecticide is also affected by the active ingredient's concentration and composition.

Additional research is required to investigate other possible factors associated with the penetration resistance of German cockroaches. Handling the resistance of German PLZ-PLM cockroaches to commercial aerosol insecticides demands significant efforts. Developing organic-based bioinsecticides and repellents could be a potential solution to address the challenge of German cockroach insecticide resistance. Several previous studies have revealed several bioinsecticides and repellents that have the potential to control German cockroaches, such as *Schinus molle* (Ferrero *et al.* 2007), *Cymbopogon flexuosus* (Rahayu *et al.* 2018), *Carica papaya* (Rahayu *et al.* 2020), *Morinda citrifolia* L. (Rahayu *et al.* 2021b), *C. nardus* (Jannatan and Rahayu 2021), and organic waste (Jannatan and Rahayu 2023a; Jannatan and Rahayu 2023b).

Conclusion

The current study confirmed that the *B. germanica* PLZ-PLM strain is highly resistant to six pyrethroid aerosol insecticides (By, Ht, Vp, Fm, Mt, Nm), but no evidence of cuticle thickening as a resistance mechanism. Further investigation is needed to determine other resistance mechanisms and the role of the cuticle in the PLZ-PLM strain to develop effective measures to manage the resistance of *B. germanica*.

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Author Contributions

RR designed the study, RR, VHP, and KWPS performed the experiments, RR and VHP analyzed the data, RR wrote the paper.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable.

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