NRESISTANCE LEVEL OF THREE STRAINS OF GERMAN COCKROACHES, *Blattella germanica* FROM SEMARANG CITY TO FIPRONIL

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Abstract. German cockroaches (*Blattella germanica*) are one of the common household pest insects frequently encountered in urban environments, including the city of Semarang. It has been reported that German cockroaches have developed resistance to 42 active insecticide ingredients, including fipronil. The aim of this study is to evaluate the toxicity level of 0.05% fipronil against German cockroaches from Semarang city and determine their susceptibility to fipronil. Three strains of German cockroaches from Semarang, as well as one World Health Organization (WHO) standard strain from Vector Control Research Unit (VCRU) Universiti Sains Malaysia, were utilized for this experiment. The German cockroaches were tested using the baiting method. The research results indicate that 50% mortality of the field cockroach population occurred within the range of 9-27 hours after the application. Resistance ratio calculations showed that the resistance level of the three strains ranged from 2.55 to 7.57 times higher compared to the VCRU strain. This suggests that the three strains of German cockroaches from Semarang exhibit low-level resistance to 0.05% fipronil.

Keywords : baiting method, fipronil, german cockroach, pest insect, resistance

1. Introduction

German cockroaches (*Blattella germanica* L.) are one of the residential pest insects which generally infest kitchen areas in apartments, restaurants, hotels, hospitals and other public places [1]. The negative impacts caused by the presence of German cockroaches are generally related to human health because of their role as mechanical vectors of a number of pathogenic organisms. As disease vectors, German cockroaches can carry and spread pathogenic organisms including *Escherichia coli* which causes diarrhea, Enterobacter cloacae which causes nosocomial infections, Pseudomonas aeruginosa which causes nosocomial infections, Staphylococcus aureus which causes skin diseases, Klebsiella spp. causes pneumoniae, Salmonella typhi causes typhoid fever, and *Shigella dysenteriae* causes dysentery [2]. Apart from that, German cockroaches also produce allergens which can trigger asthma [3]. The main sources of German cockroach allergens are saliva, feces, spermatophore and skin. German cockroach excretion particles can enter the lungs by sticking to the nasal cavity or mouth, which can trigger allergies [4]. Therefore, the German cockroach population needs to be controlled.

Until now, German cockroach population control still uses insecticides, both by pest control operators and on a household scale [5]. Insecticides are chosen because they are easy to use, work quickly and are cheap. However, the dependence and high frequency of application causes the resistance level of German cockroaches to some insecticides to increase. This is due to the ability of German cockroaches to survive and digest chemicals, as well as their short life cycle [6]. Apart from that, German cockroaches have a high reproduction rate, reaching three to four generations per year. This is why German cockroaches are very difficult to control [7].

German cockroaches are reported to have experienced resistance to 42 insecticide active ingredients, many of which occur in the main insecticide groups, including organochlorines, organophosphates, carbamates, synthetic pyrethroids, neonicotinoids, oxadiazines, and phenylpyrazoles [8]. German cockroaches have been proven to develop resistance to insecticides because there have been reports from several countries including Japan, Taiwan, Iran, and Cuba [9-13]. German cockroach resistance reported in Taiwan is resistance to organophosphates, carbamates and pyrethroids which is relatively high [10]. The first case of German cockroach resistance in Indonesia was reported in 2009 with low levels of resistance to pyrethroid insecticides in German cockroaches from Jakarta, Bandung and Surabaya [14].

Several strains of German cockroaches originating from three cities in Indonesia were also reported to have the potential to develop behavioral resistance in response to insecticides formulated in the form of bait [15]. Behavioral resistance in German cockroaches, also called bait aversion, was first discovered in the United States in the 1990s [16]. Ambarningrum [17] stated that there are four strains of German cockroaches from 21 cockroach strains from 12 provinces that are indicated to have the potential to develop behavioral resistance. The four strains are the Jakarta-a (JKT-a), Jakarta-b (JKT-b), Bandung-b (BDG-b), and Pekanbaru-b (PKU-b) strains. Reports regarding the resistance status of German cockroaches to fipronil in Central Java have only been reported from German cockroaches from the Purwokerto market which did not show resistance to fipronil [18].

Semarang City is the second city with poor sanitation. According to the Semarang City Regional Development Planning Agency (Bappeda), approximately 63% of residents do not have good sanitation facilities. This is related to the city of Semarang which is a densely populated city [19]. The city of Semarang is the capital of Central Java Province, so it is not surprising that there are many large hotels as a place to stop for official business or just traveling. Not all hotels in Semarang implement good sanitation, especially in the kitchen area. This is because the quantity of food that will be served to consumers is the main focus for hotel management [20]. The sanitary condition of a place is closely related to the presence of cockroaches in that place. Most research results show that an increase in cockroach populations occurs when sanitation in an area decreases. Environments with poor sanitation favor cockroach infestations due to the abundance of food, water, and easily accessible hiding places than environments with good sanitation [21].

Until now there have been no reports regarding the resistance of the Semarang strain of German cockroaches to insecticides. The research that has been reported regarding cockroaches is regarding the density of cockroaches in places selling foodstuffs and traditional markets in Semarang City. A total of 66 grocery and food store outlets in the traditional markets of Semarang City were still found to have a cockroach density of 20%. According to Minister of Health Regulation Number 50 of 2017, the density of cockroaches in food outlets in traditional markets does not exceed 2 per number of cockroaches caught in traps for 24 hours. Traders have not been able to determine and control cockroaches properly. This was a factor in the discovery of a higher density of cockroaches in groups that carried out cockroach control than those who did not control cockroaches [22]. Therefore, it is important to carry out this research to determine the level of resistance of the Semarang city strain of German cockroaches to fipronil and indoxacarb. These two active ingredients are often used in controlling German cockroaches by pest control operators in the city of Semarang.

2. Methods

2.1 Time and Place

The research was carried out at the Entomology-Parasitology Laboratory, Faculty of Biology, Jenderal Soedirman University, from December 2022 – July 2023. Samples of field strain German cockroaches were obtained from three four-star hotels in Semarang City as shown in Figure 1.

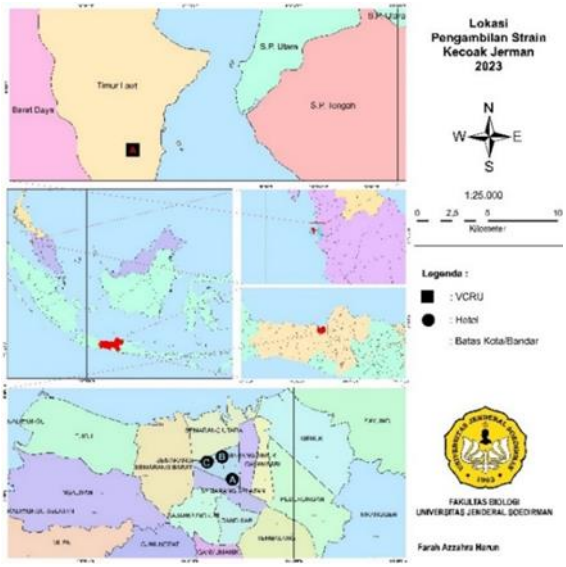

Figure 1. Location

2.2 Tools and Materials

The tools used in this research include a 1 L plastic jar, a 5 L plastic jar, a 16 L plastic jar, a single chamber (tub) measuring l x w x h = 40 x 25 x 15 cm, plastic bucket, cloth, rubber tape, used cardboard, rubber, used bottle caps, used water containers, sponges, teapots, trays, scissors, oil containers, spoons, small plastic tubes, cotton wool, labels, stationery and cameras.

2.3 Collection and Rearing of German Cockroaches

The German cockroaches used in the study were collected from three hotels in the city of Semarang as field strains and from the Vector Control Research Unit (VCRU), School of Biological Sciences, Universiti Sain Malaysia, Penang as World Health Organization (WHO) standard susceptible strains. VCRU strain has been kept for more than 30 years and has not been exposed to insecticides. Field strains of German cockroaches were collected directly from the field, then reared in the Entomology-Parasitology Laboratory, Faculty of Biology, Universitas Jenderal Soedirman from May 2022. Maintenance was carried out by placing approximately 300 strains of each German cockroach in a plastic jar with a volume of 16 L, diameter 25 cm, and height 30 cm. Each jar is provided with harborage for German cockroaches, cat food and water. The inner rim of the jar is smeared with oil that has been combined with vaseline so that German cockroaches don't come out of the jar, then covered with a cloth and secured with rubber tape. Cat food is given around 25 g and 40 mL of water, twice a week ad libitum. The rearing room has a temperature ranging from 25 – 30oC, humidity ranging from 55 – 95%, and a photoperiod of 12:12 (12 hours of light and 12 hours of darkness).

2.4 Testing the time of death of German cockroaches to Fipronil using the bait method

Testing was carried out using the baiting method using a single chamber. Ten male German cockroaches were put into a 5 L jar containing harborage and water, then fasted from food for 24 hours. After 24 hours, the German cockroaches were moved to a single chamber containing a harborage and water, then gel bait with a 0.05% fipronil formulation weighing 0.1 g was placed using a syringe. Observations were made every three hours until all individuals died. The treatment was repeated five times.

2.5 Data analysis

The mortality data obtained were analyzed using probit analysis using POLO-PC software LeOra (2004) to determine the lethal time 50 (LT50) value. Then, the resistance level (Resistance Ratio/RR50) was calculated by comparing the LT50 value of the field strain with the LT50 value of the susceptible strain (VCRU). The following is the resistance level formula (Resistance Ratio/RR50):

RR50= (LT50 field strain)/ (LT 50 VCRU strain)

Resistance level categories are classified as follows: (Lee & Lee, 2004)

RR50≤1 : not resistant
1<RR50≤5 : low resistance
5<RR50≤10 : moderate resistance
10<RR50≤50 : high resistance
50<RR50≤1000 : very high resistance.

3. Results And Discussion

Based on the tests and analysis of the level of resistance of German cockroaches to fipronil 0.05% that were carried out, the results were obtained in the form of LT50 and RR50 values for the four cockroach strains as shown in Table 4.1. The death time for 50% of German cockroaches in the population is shown by the LT50 value ranging from 3,614 – 27,351 hours. If we look at the RR50 value, the three field strains (Semarang 1, Semarang 2, and Semarang 3) show low to moderate resistance criteria, namely between 2.553 – 7.568 times with a slope value of >2.

Table 1. Lethal Time 50 (LT50) Value and Resistance Level of German Cockroaches to Fipronil 0.05%

Strain	Resistance Level			
	LT_{50} (hour)	Slope±SEM	RR_{50}	Categories
VCRU	3,614 (2,956 – 4,229)	4,770±0,540	1	-
Semarang 1	9,225 (7,981 – 10,410)	3,741±0,311	2,553	low resistance
Semarang 2	27,351 (23,860 – 30,756)	1,773±0,085	7,568	moderate resistance
Semarang 3	20,602 (18,306 – 22,834)	2,669±0,108	5,701	moderate resistance

The results of the Semarang strain of German cockroach RR50 research with 0.05% fipronil gel bait were higher than the results of several other studies which also used fipronil gel bait. Some of these studies include research by Davari [23] with an RR50 of 1.3 – 1.5 times (low resistance), research by Ambarningrum [18] with an RR50 of 0.70 – 0.96 times (not resistant), and research by Appel [24] with RR50 0.88 – 4.21 times (not resistant). The LT50 of German cockroaches against the insecticide fipronil 0.05% is longer than several

studies, where research by El-Monairy [25] with LT50 0.64 – 0.96 hours and research by Ang [26] with an LT50 of 7.50 – 9.45 hours, while it is shorter than Davari&Nasirian [23] research, namely between 30.5 – 47.1 hours.

Based on the results of this research, the three field strains originating from three hotels in Semarang City all showed resistance to both the insecticides fipronil 0.05% and indoxacarb 0.6%. These results are in accordance with the statement of Davari [23] which states that field strains of German cockroaches are more tolerant to gel bait than standard strains of German cockroaches. This can also be seen from the LT50 standard strain German cockroaches dying faster than field strain German cockroaches. The reason is because the susceptible strain of German cockroaches has not been exposed to insecticides for more than 30 years.

Repeated exposure to poisoned baits over generations will increase the frequency of the genes coding for these traits. In German cockroach populations that have glucose avoidance behavior, it has been reported that there is an increased frequency of individuals who have better gustatory sensillary function that underlies glucose avoidance behavior. Insects generally have a sense of taste, which specifically functions to detect the sweet taste of sugar. The gustatory sensillum provides information about nutrients and harmful food sources [27]. Cockroaches have Gustatory Receptor Neurons 1 (GRN1) as a sugar detector and Gustatory Receptor Neurons 2 (GRN2) as a receptor for deterrent compounds. Individuals who develop glucose aversion behavior after exposure to glucose will stimulate GRN2 but suppress GRN1, then glucose is detected as a bitter compound which cockroaches do not like and finally German cockroaches avoid the presence of glucose. Glucose avoidance is an adaptive behavior due to the plasticity of the sensory system to adapt to environmental changes [15]. The few field populations of German cockroaches that have been shown to not feed on gel baits are generally found in locations with poor sanitary conditions and frequent use of gel baits. Spinning gel baits containing different active ingredients in the bait are unlikely to avoid behavioral resistance. German cockroaches will likely develop similar behavioral resistance in response to gel bait matrix materials after repeated exposure [28]. A part from the regular use of insecticides to control German cockroaches, resistance can also be caused by cross resistance between insecticides with the same mode of action and double resistance between different insecticides. Remembering that before the use of fipronil and indoxacarb gel baits, German cockroaches in several hotels were controlled by spraying with deltamethrin. This is in accordance with the statement of Fardisi [29] that the use of spray insecticides not only failed to reduce the number of German cockroaches, but also caused the emergence of resistance. Rahayu [30] also stated that one of the factors causing resistance to insecticides is due to cross resistance with insecticides that were previously used and have the same way of working.

Double resistance to fipronil is caused by the use of deltamethrin due to a point mutation in the voltage-gated sodium channel (kdr resistance) [31]. In research, house flies that were highly resistant to deltamethrin showed longer resistance after exposure to fipronil, compared to house flies that were not resistant to deltamethrin [32]. However, several studies state that pyrethroid-resistant German cockroaches do not show cross-resistance to fipronil [33], male German cockroaches that are tolerant to pyrethroids are susceptible to fipronil [34], pyrethroid-resistant German cockroaches with activity Elevated cytochrome P450 monooxygenase (P450s) enzymes are more susceptible to fipronil [7]. Detoxification mechanisms involving P450s and esterases also confer relatively low metabolic resistance [35].

Cross-resistance could also be the reason why the Semarang strain of German cockroaches are resistant to fipronil gel bait because they may have previously been exposed to insecticides from the organochlorine group. Research by Kristensen [36] found that strains Zo9600302 and strain Ga021001 originating from Europe were resistant to fipronil which had previously been controlled using cyclodiene insecticide, but had not been used again for the past 20 years. This cross resistance is because both are class 2 insecticides, where the cyclodiene insecticide also works antagonistically with resistance to the dieldrin (Rdl) subunit of the GABA receptor. Cyclodiene resistance in several insect species is known to be caused by alanine to serine substitution (A302S) which is commonly known as the Rdl mutation in the Rdl gene. Rdl mutations related to cyclodiene resistance are also known to confer cross-resistance to fipronil because the Rdl gene is still strong in German cockroaches [37]. Despite conferring high resistance to dieldrin, Rdl gene mutations appear to confer relatively low cross-resistance to fipronil [35].

So far the bait method is still the most effective method for controlling cockroaches because it works orally and directly hits the target animal, so it is not harmful to the environment and other animals. Gel bait made from the active ingredients fipronil is slow action which allows German cockroaches to die in their hiding places, giving rise to a domino effect in the population [18]. When the active ingredient is included in the bait, German cockroaches will consume a lethal dose from a single meal. Therefore, baits are considered less likely to elicit high levels of cockroach resistance than spray-applied insecticides and other formulations. However, the existence of avoidance behavior towards bait containing glucose and physiological resistance due to previous use of insecticides make it difficult to control German cockroaches [21]. For this reason, it is possible to replace the feed formulation containing glucose with another type of sugar as an attractant.

4. Conclusion

German cockroaches originating from three hotels in Semarang City have developed resistance to the 0.05% fipronil bait insecticide with low to moderate levels of resistance, while the 0.6% indoxacarb bait insecticide is all low. Therefore, an integrated approach is needed in controlling German cockroaches, as well as a wiser use of insecticides. Resistance monitoring needs to be carried out to detect changes in resistance levels.

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