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INSECTICIDE RESISTANCE IN *Blattella germanica* L. (DICTYOPTERA: BLATTELLIDAE) FROM BUKITTINGGI AND PALEMBANG AGAINST PROPOXUR

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ABSTRACT

German cockroaches (*Blattella germanica* L.) are household pest known to be resistant to chemical insecticides. Resistance to propoxur insecticides causes the controlling to be more difficult. The aim of this study was to determine the resistance status of German cockroaches against propoxur. The method that used in this study was Topical Application Test. The German cockroaches used were collected from the cities of Bukittinggi and Palembang, German cockroaches laboratories populations were WHO standards. The results showed that *B. germanica* RMKN-BKT and PLZ-PLM population had been resistant to propoxur. The resistance ratio (RR_{50}) for each population was 1.42 fold for RMKN-BKT and 1.59 fold for PLZ-PLM. The conclusion of this study is the resistance status of *B. germanica* RMKN-BKT and PLZ-PLM population classified as low resistance. Monitoring the repeated use of insecticides in pest management is necessary so that the status of resistance does not increase.

KEYWORDS: German Cockroach, Resistance, Propoxur, Indonesia.

INTRODUCTION

German cockroaches (*Blattella germanica* L.) are one of the household pest species in Indonesia that are often found in several public areas such as restaurants, hotels and other food outlets (Ahmad *et al.*, 2009; Rahayu *et al.*, 2012). Its existence is very disturbing because these insects are able to cause various kinds of health disease for humans (Wu and Apple, 2017; Rahayu *et al.*, 2018). The control of *B. germanica* has been carried out using chemical insecticides such as organophosphate, pyrethroid and carbamate to suppress pest populations, but some types of insecticides are no longer able to effectively kill cockroaches which ultimately causes cockroaches to become resistant (Rahayu *et al.*, 2016 ; Jannatan *et al.*, 2017).

Propoxur is a non-systemic insecticide which belongs to the carbamate group which is a contact and stomach poison. The mode of action of this insecticide by attacking the acetylcholinesterase enzyme in the central nervous system of insects (Shi *et al.*, 2002; Qian *et al.*, 2010; Colovic *et al.*, 2013). Cases of resistance that occur in *B. germanica* to propoxur have been reported in several countries (Lee *et al.*, 1996; Chai and Lee, 2010; Moemenbellah-Fard *et al.*, 2013; Ahmad *et al.*, 2009; Rahayu *et al.* , 2012), but there have not been many reports regarding the level of resistance to propoxur in Indonesia especially the cities of Bukittinggi and Palembang. Collection *B. germanica* in both locations is because it is a tourist city with a lot of public areas and high population mobility and control of cockroaches in that location using insecticides. Therefore, this study was carried out with the aim of determining the resistance status of *B. germanica* from Bukittinggi (RMKN-BKT) and Palembang (PLZ-PLM) populations to propoxur. Thus, the resistance information obtained is able to provide benefits in the process of controlling *B. germanica* and reduce cases of resistance that occur.

MATERIALS AND METHODS

German Cockroaches

The cockroaches used in this study were male German cockroaches aged ± 2 months (Rahayu *et al.*, 2012). Field cockroach populations were collected from several regions in Indonesia (Table 1), and used laboratory / susceptible populations from the Vector Control Research Unit (VCRU), University of Science Malaysia, Penang, Malaysia. Cockroaches are maintained at the Animal Physiology Research Laboratory, Biology Department, FMIPA, Andalas University, Indonesia. The process of maintaining German cockroaches is carried out in a room with a temperature of 26-28^{o}C and photoperiod 12:12. Cockroaches are given feed

(pedigree) and water in ad-libitum (Ahmad and Suliat, 2011).

Table 1: The sources of cockroaches population and the years of collection.

Populasi	Tempat Koleksi	Kota Koleksi	Tahun Koleksi
VCRU - WHO	Laboratory	Penang, Malaysia	2007*
RMKN - BKT	Restaurant	Bukittinggi	2016
PLZ - PLM	Food Outlet	Palembang	2017

*Rahayu, 2011

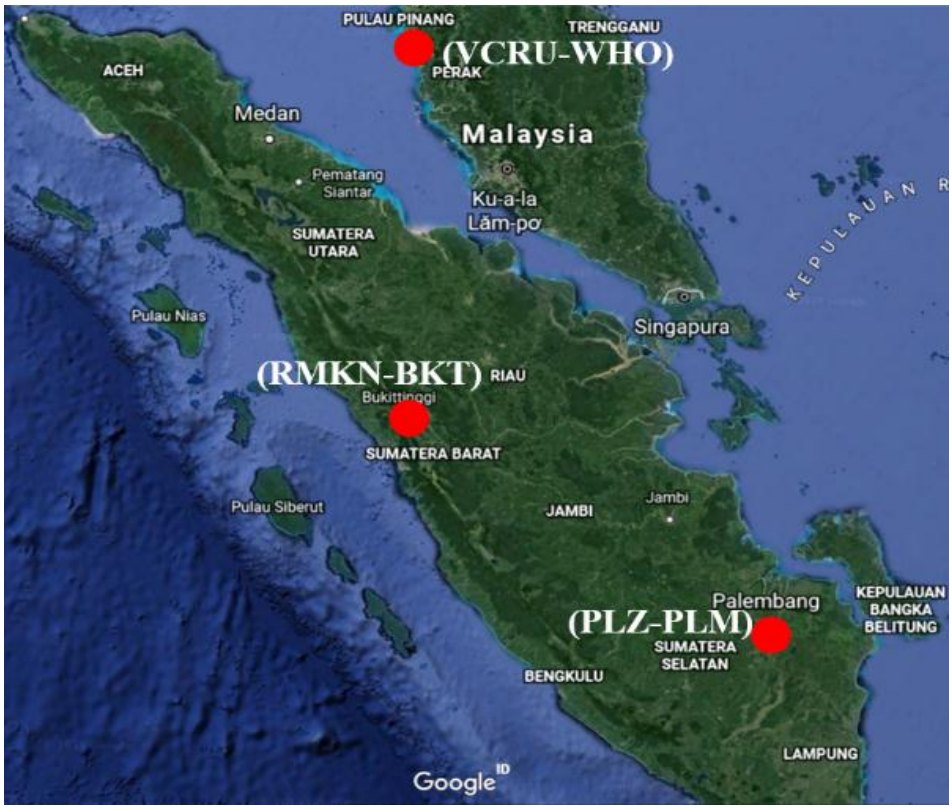

Fig 1. The sampling site (red dot) of *B. germanica* Bukittinggi (RMKN-BKT) dan Palembang (PLZ-PLM) population (Sources by: Google Earth, 2019).

Resistance Test for *Blattella germanica* L.

A resistance test was conducted to determine the time of mortality and the resistance level of *B. germanica*. Adult male *B. germanica* used was treated with insecticides with a concentration of 0.25% which was dissolved in acetone. Adult male *B. germanica* is anesthetized with CO_2 for ± 30 seconds. Then 1 µL of insecticidal solution is given topically in the ventral mesothoracic section using a micropipette. After treatment, cockroaches are kept in a 1 L jar, with wet cotton and feed (pedigree). The mortality of cockroach due to insecticide was observed at 72 hours after treatment. For each population the treatment was carried out in five replications (Wu and Apple, 2017).

Resistance Test Data Analysis

Mortality rates were obtained from probit analysis using Minitab 18 statistical software. Resistance status in cockroaches was determined by the resistance ratio (RR_{50}), which is comparing the value of the LT_{50} field population with the LT_{50} value laboratory population. The resistance ratio category (RR_{50}) refers to Lee and Lee (2004), ≤1 = not resistance, > 1 - ≤5 = low resistance, > 5 - ≤10 = moderate resistance, > 10 - ≤50 = high resistance, > 50 = very high resistance and modified by Rahayu *et al*. (2012) > 50 - ≤ 1000 = very high resistance and > 1000 = very extreme resistance.

RESULTS

Status Resistance of *Blattella germanica* L. RMKN-BKT and PLZ-PLM Population against Propoxur

Resistance levels of *B. germanica* L. population of RMKN-BKT and PLZ-PLM against propoxur insecticides were carried out by comparing *B. germanica* field populations with laboratory populations. The results showed that *B. germanica* population of RMKN-BKT and PLZ-PLM were resistant to propoxur insecticides (Table 2).

Table 2: Level resistance in *B. germanica* L. against propoxur concentration 0, 25%.

Population	n	LT_{50} (hour)	RR_{50}	Slope ± SE	Resistance Status
VCRU-WHO$^{S)}$	50	35,76	1	0,05 ± 0,005	Not Resistance
RMKN-BKT$^{L)}$	50	50,95	1,42	0,05 ± 0,006	Low Resistance
PLZ-PLM$^{L)}$	50	56,88	1,59	0,04 ± 0,005	Low Resistance

S = Laboratory population
L = Field population
n = The amount of *B. germanica* used
LT_{50} = *Lethal Time* to control 50% of the population after being treated until 72 hours
RR_{50} = Resistance Ratio (LT_{50}L / LT_{50}S)

The mortality response of each population starting at 6th hour continues until the 24th hour with a percentage of mortality 6% for the RMKN-BKT, 8% for the PLZ-PLM and 34% for the VCRU-WHO population. Increased of mortality by around 6 fold for each population at 48 hours with a percentage 48% for the population of RMKN-BKT and PLZ-PLM and 2,2 fold for the VCRU-WHO population with a percentage 78%. At the 72nd hour observation the percentage of mortality for each population was 86% for RMKN-BKT, 66% for the PLZ-PLM and 96% for the WHO-VCRU population (see Fig 2). An increase in the percentage of mortality of each population due to the individual response in the population varies with propoxur insecticides.

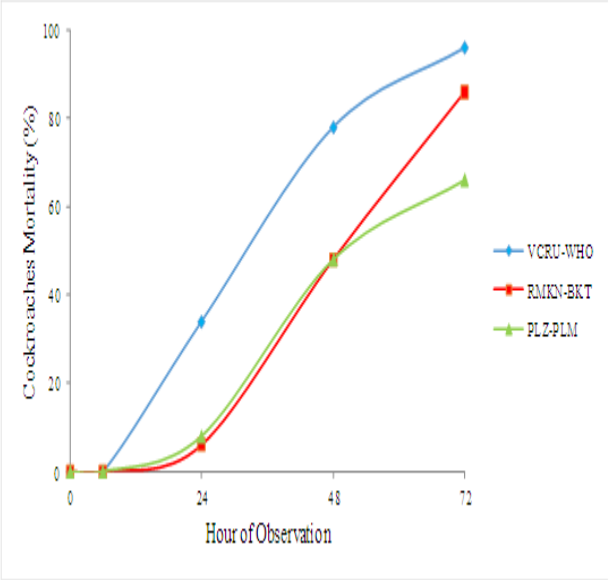

Fig. 2. Mortality rate of *B. germanica* against propoxur for 72 hour of observation.

DISCUSSION

The results showed that the mortality of the VCRU-WHO population was higher than that of the RMKN-BKT and PLZ-PLM populations (Fig 2). That occurs because the VCRU-WHO population is a laboratory population that has never been exposed to propoxur. Meanwhile, for the field population of RMKN-BKT and PLZ-PLM it is thought that it has often been exposed and has been resistant to propoxur. Several studies have also reported that German cockroaches collected from several places in Indonesia have been resistant to chemical insecticides such as propoxur (Ahmad *et al.*, 2009; Rahayu *et al.*, 2012; Bestari *et al.*, 2014; Madona *et al.*, 2015).

The resistance status of *B. germanica* to propoxur was determined by comparing the lethal time (LT_{50}) *B. germanica* field population compared with the laboratory population. Based on the resistance criteria that refer to Lee and Lee (2004) that *B. germanica* population of RMKN-BKT and PLZ-PLM was resistant to propoxur insecticide. *B. germanica* population of RMKN-BKT was 1.42 fold more resistant (low resistance) and *B. germanica* PLZ-PLM population was more resistant 1.59 fold (low resistance) compared to the laboratory population (VCRU-WHO) (Table 2). Cases of resistance in *B. germanica* against propoxur have also been reported in several countries such as Malaysia (Lee *et al.*, 1999; Lee and Lee, 2004), Taiwan (Pai *et al.*, 2005), Singapore (Chai and Lee, 2010), Iran (Limoee *et al.*, 2011; Moemenbellah-Fard *et al.*, 2013), United State (Wu and Apple, 2017) and Indonesia (Rahayu *et al.*, 2012). Resistance that occurred in *B. germanica* population of RMKN-BKT and PLZ-PLM was caused by control measures in the field that routinely sprayed insecticides using the same insecticide. Based on interviews with pest control teams at the field population was collected stated that the pest control process is carried out routinely every month using carbamate insecticides, such as propoxur. Periodic use of insecticides for long periods of time causes increased resistance in pest and requires a greater concentration for controlling and is even able to cause cross-resistance to similar insecticides or different types with the same target-site (Mantolu *et al.*, 2016; Wu and Apple, 2017).

The resistance that occurred in *B. germanica* population of RMKN-BKT and PLZ-PLM was suspected because *B. germanica* had developed a mechanism of resistance both physiologically and behavior. The mechanism of resistance in *B. germanica* also can be done by increasing detoxification metabolism due to increased enzyme activity against insecticidal molecules such as esterase, oxidase and gluthation s-transferase (GST) (Siegfried and Scott, 1992). The involvement of detoxification enzymes as a resistance mechanism in *B. germanica* was also reported by Lee *et al*. (1999), Chai and Lee (2010), Rahayu (2011), Wu and Apple (2017) and Nurseha (2019) who stated that monooxygenase, esterase and acetylcholinesterase enzymes act as one of the mechanisms of resistance that occurs in some *B. germanica* populations who were given propoxur insecticide.

Other factors that cause resistance to *B. germanica* include high selection pressure resulting in the rapid selection of susceptible individuals and resistant individuals developing. Increased lethal concentration of insecticides can also cause an increase in the proportion of resistant insects and loss of the proportion of susceptible insects. In the end, the proportion of resistant individuals will dominate the population because of the increase that occurs from generation to generation. Mechanisms of resistance that develop in the field of various types of insecticides due to an increase in detoxification enzymes, decreasing the penetration rate of insecticides through the skin (cuticles) and the possibility of gene mutations that result in decreased sensitivity of target sites of insecticides (Ahmad *et al.*, 2009; Rahayu, 2011; Saputra, 2019).

CONCLUSION

German cockroaches (*Blattella germanica* L.) RMKN-BKT and PLZ-PLM population were resistant to propoxur with RR_{50} each population was 1.42 and 1.59 fold. The value of the resistance ratio causes *B. germanica* field population to be classified as low resistance.

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