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Toxicity of Citronella Grass Essential Oil (*Cymbopogon nardus* (L.) Rendle) to Female and Nymph German Cockroaches (*Blattella germanica* (L.))

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ABSTRACT

The synthetic insecticides were reported have been resistant to the German cockroach. Based on this problem, we need to explore the new alternative insecticides of plant essential oils. Citronella grass essential oil has containing toxic substances that are used to kill the German cockroaches. The aim of this study was to determine the toxicity of the citronella grass essential oil against female and nymph of German cockroaches. The method that used in this study was Tarsal Contact Test using 9 cm diameter Petri dish with the essential oil of Citronella grass concentration 80%. The standard strain was used as susceptible strain from Vector Control Research Unit-World Health Organization, and the other strain were collected from four locations in Indonesia (Jakarta, Bandung, Padang, and Payakumbuh). The Lethal Time of 90% (LT₉₀) females and nymphs was ranged from 4.70 to 13.93 hours and from 0.32 to 42.82 hours respectively. The toxicity of citronella grass essential oil was generally effective to nymph of the German cockroaches compared to the female. The citronella grass essential oil was still susceptible to German cockroaches, thus citronella oil have a potential to develop for new insecticide alternative to control German cockroaches populations.

Keywords: Toxicity, Citronella grass, German cockroaches, Female, Nymph

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INTRODUCTION

The German cockroach, *Blattella germanica* (L.) is the household and most common pests in the public facilities in Indonesia ^[1]. The synthetic insecticides are still an option to control the German cockroach populations in Indonesia, despite using of the synthetic insecticides continuously and uncontrolled will make cockroaches become resistance. In Indonesia, many cases were reported that the cockroaches have been resistant to Carbamate, Pyrethroid and Phenylpyrazole ^[2]. This is also occurred in the other household pests insects such as flies that is resistant to Permethrin, Imidacloprid ^[3], Propoxur ^[4] and mosquitoes to Malathion ^[5].

The development of resistance in the cockroaches population makes difficulties to control its population. Therefore, the new alternative insecticide was needed to face this problem, such as insecticide from plant essential oils. The essential oils can be used as a toxic substances, repellent and antifeedant to control insect pest population ^[6]. The essential oils also have a low toxicity for the mammals and easily degraded in the environment ^[7]. One of the plants which are having the essential oils is citronella grass, *Cymbopogon nardus* (L.) Rendle.

The citronella oil was toxic to the pest insects such as *Cryptolestes* sp., *Palorus subdepressus*, *Rhyzopertha dominica*, *Sitophilus zeamais* ^[8], *Frankliniella schultzei*, *Myzus persicae* ^[9], *Aedes aegypti*, *Anopheles dirus* ^[10], *Culex quinquefasciatus* ^[11] and *Helicoverpa armigera* ^[12]. The citronella grass essential oil also toxic and effective to the male German cockroaches ^[13], it also expected to be toxic for female and nymph of German cockroaches.

The female and nymph of cockroaches have more body fat than the male. The body fat is used to inhibit the insecticide compounds reaching the target cells, so the cockroaches with more body fat will have higher tolerance for insecticide ^[14]. The body fat was also playing a role in the metabolism to store of the toxic substances to the body such as insecticides ^[15]. The purpose of the present paper is to study the toxicity and effectiveness of citronella grass essential oil against female and nymph of German cockroaches.

MATERIALS AND METHODS

Cockroaches. The cockroaches that used in this study were female and nymph of German cockroaches. The field strain was collected from four regions in Indonesia (Table 1), and we used VCRU-WHO strain as susceptible standard which is from the Vector Control Research Unit, University Sains Malaysia, Penang, Malaysia. The cockroaches were reared in Animal Physiology Research Laboratory, Biology Department of Andalas University, Indonesia. The rearing process of cockroach conducted at a temperature between 26-28 °C with photoperiod 12:12, and feed with cat food and water were given to cockroaches ad-libitum ^[16].

Table 1. The sources of cockroaches strain and the years of collection

Strains	Collection sites	Collection years
VCRU-WHO	Penang, Malaysia	2007
HHB-JKT	Jakarta, Indonesia	2007
KRS-BDG	Bandung, Indonesia	2007
PLZ-PDG	Padang, Indonesia	2014
RMH-PYK	Payakumbuh, Indonesia	2015

Essential oil. The essential oil that used in this research was extracted from citronella grass plant (*Cymbopogon nardus* (L.) Rendle) provided by the Research Institute for Spices and Medicinal Plants K.P. Laing, Solok, West Sumatra, Indonesia. The preliminary study had been done to determine the concentration of essential oil that used in the experiments. The concentration of essential oil that used was 80% (diluted with ethanol).

Contact Toxicity Test. The test of toxicity contact of citronella grass essential oil toward German cockroaches refers to a method of tarsal contact test ^[17] modified by using a Petri dish (diameter 9 cm). Upon the experiment, Petri dishes were filled with 500 µl of the essential oil to obtain 6.29 mg/cm² solution in the bottom of the Petri dish. Then 10 individuals of the female and nymph from each population were placed into each Petri dish for 96 hours. The mortality of the cockroaches was observed until 96 hours after treatment. All

the experiments were replicated three times. The side of the Petri dish smeared with baby oil and petroleum jelly solution to prevent cockroaches escape during the observation.

Data Analysis. The lethal time (LT) of each cockroaches population were analyzed by Probit Regression Analysis in Polo-PC computer software ^[18]. The susceptibility status of each cockroaches population was determined by calculating the ratio of the resistance (RR_{50}) by comparing the LT_{50} values between the field strain and the susceptible standard strain. The ratio of resistance that obtained were grouped into five categories (RR_{50} of <2 indicates susceptible, RR_{50} ranged from 2–5 indicates the presence of low resistance, RR_{50} ranged from 5–10 indicates moderate level of resistance and RR_{50} of >10 demonstrates high resistance ^[19]). The effectiveness of citronella grass essential oil can be determined if more than 90% of the cockroaches died in less than six hours of observation ^[20].

RESULTS AND DISCUSSIONS

The mortality response of each female population started at 1st hour except the Jakarta strain (HHB-JKT) and Padang strain (PLZ-PDG). The mortality of female cockroach continues to next 6th hour with range from 60% to 93%. The 100% mortality of the female population occurs at 48th hour observation. The mortality rate of the nymphs has also started on the 1st hour with mortality range from 20% to 92%. The mortality was increased range from 63% to 100% at the next 6th hour. The mortality of the entire nymph populations occurs until the next 96th hour. The mortality rate of nymphs occurred more gradually at each time of observation because the responses of individuals in each population toward citronella grass essential oil were diverse (see Fig. 2).



Fig. 1. The sampling sites (red dots) of five strains (VCRU-WHO: Standard strain, HHB-JKT: Jakarta strain, PLZ-PDG: Padang strain, RMH-PYK: Payakumbuh strain and KRS-BDG: Bandung strain) of German cockroaches (Sources by: Google map)

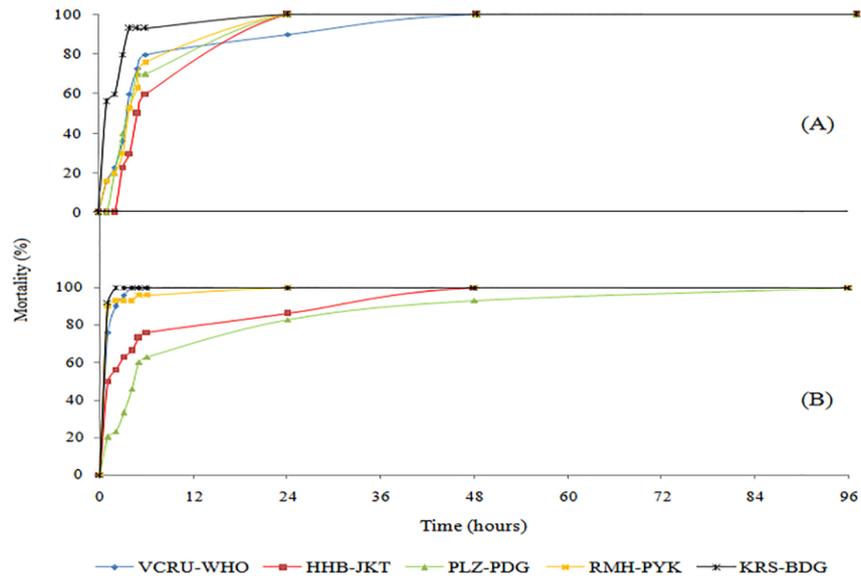


Fig. 2. Mortality rate of five strains (VCRU-WHO: Standard strain, HHB-JKT: Jakarta strain, PLZ-PDG: Padang strain, RMH-PYK: Payakumbuh strain and KRS-BDG: Bandung strain) female (A) and nymph (B) of German cockroaches on citronella grass essential oil concentration 80% for 96 hours of observation

Each strain of cockroaches has different capabilities to respond to the citronella grass essential oil in the tarsal contact test. This also happened in each individual in the same strain. The gradual response in cockroach population toward insecticides can be determined by the variation of individual in the strain. Individual variation of any strain can be seen from the slope value. If the slope value is lower in a strain, the individuals in the same strain were more varied or heterogeneous responses to insecticides, so it takes time gradually to lethal all individuals in the population [21].

The slope values of female cockroaches mortality ranged from 1.79 to 5.64 and were generally bigger than the nymphs only that ranged from 1.35 to 2.63 (Fig. 3). Individuals in the nymph strains of cockroaches have a high variation because the nymph strain slope values are smaller than females. The variation of individuals in the nymph strain affected the stage of mortality, as the Padang strain (PLZ-PDG) 100% mortality occurred in the 96th hours. The heterogeneous individual variation has a low level of sensitivity to insecticides than homogeneous cockroaches [21].

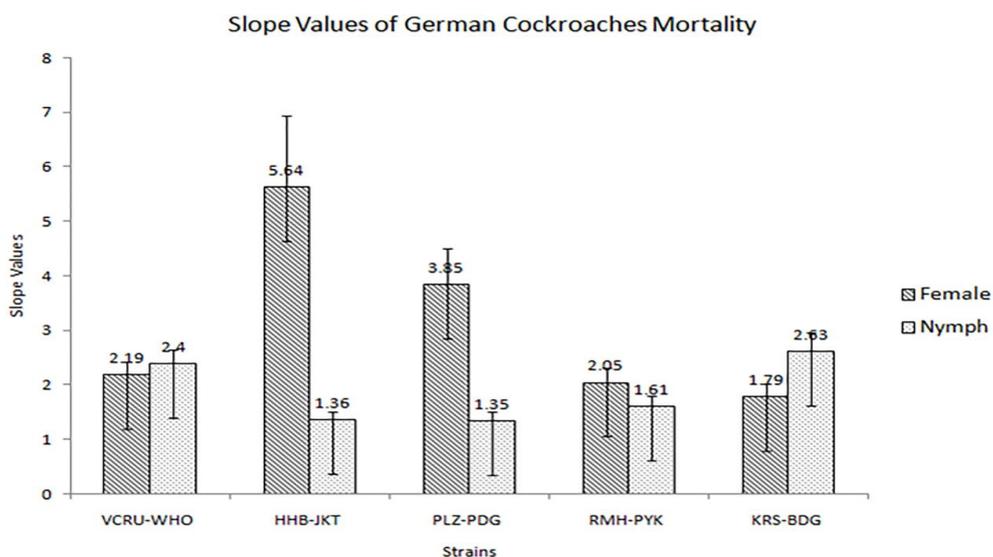


Fig. 3. Mortality slope value of five strains (VCRU-WHO: Standard strain, HHB-JKT: Jakarta strain, PLZ-PDG: Padang strain, RMH-PYK: Payakumbuh strain and KRS-BDG: Bandung strain) female and nymph of German cockroaches against citronella grass essential oils concentration 80%

The effectiveness of citronella grass essential oil to lethal time 90% (LT₉₀) of German cockroaches was shown in Table 2. The citronella grass essential oil was effective only for the female from the Bandung strain (KRS-BDG) and the nymph from standard strain (VCRU-WHO), Payakumbuh strain (RMH-PYK), and Bandung strain (KRS-BDG). The insecticide was considered effective to kill the cockroaches, if it caused the lethal time of cockroaches $\geq 90\%$ within less than 6 hours observation^[20]. In this study, the citronella grass essential oil was generally more effective against nymph compared with female cockroaches.

Table 2. Effectiveness of citronella grass essential oil concentration 80% to the five strains (VCRU-WHO: Standard strain, HHB-JKT: Jakarta strain, PLZ-PDG: Padang strain, RMH-PYK: Payakumbuh strain and KRS-BDG: Bandung strain) of female and nymph of German cockroaches

Cockroaches	Strains	LT ₉₀ (Hours)	Effectiveness criteria*
Females	VCRU-WHO	12.63	Ineffective
	HHB-JKT	8.68	Ineffective
	PLZ-PDG	7.77	Ineffective
	RMH-PYK	13.93	Ineffective
	KRS-BDG	4.70	Effective
Nymphs	VCRU-WHO	1.78	Effective
	HHB-JKT	15.82	Ineffective
	PLZ-PDG	42.82	Ineffective
	RMH-PYK	1.30	Effective
	KRS-BDG	0.32	Effective

- LT₉₀: Lethal Time of 90% cockroaches

*The criteria by the Indonesia Directorate General of Fertilizers and Pesticides (2004) if the lethal of cockroaches $\geq 90\%$ within ≤ 6 hours of observation.

The effectiveness of citronella grass essential oil was different between nymph and female cockroaches. The differences may be influenced by the different amount of the body fat. The body fat was used as a barrier and a natural filter of insecticides in the body of cockroaches. Female cockroaches were generally heavier than the nymph because of the amount of body fat^[22]. Insecticides enter the body through the body fat before it is reached the target cells. A lot of body fat, will blocked the entry of insecticide into the body, and reduced the level of toxicity of insecticides. Therefore, if the cockroaches have more body fat then more insecticides will need to kill them. The larger and heavier individual of cockroaches will be more tolerant toward the amount of insecticide^[14]. The other functions of the body fat was a barrier of insecticide and it plays a role in stored the toxic substances to enter the body^[15].

The slow mortality response of female cockroaches was possibly caused by the low concentration of a citronella grass essential oil concentration. This phenomenon also occurs in the male cockroach, lower concentration of Citronella grass essential oil will cause a slow mortality response^[23]. Therefore, the increasing of the concentration of citronella grass essential oil will increase the mortality response to German cockroaches. The female cockroaches required higher concentrations of insecticides to kill it than nymph cockroaches^[24]. The sensitivity of cockroach to insecticides from the high to low is as follows: a small nymph, large nymph, male, gravid female and female cockroaches^[25].

The German cockroaches susceptibility status by citronella grass essential oil was determined by the lethal time (LT₅₀) from the field strains cockroaches compared to a standard strain (Table 3.). The susceptibility status of the field strain of female cockroaches is still susceptible with RR₅₀ range from 0.24 to 1.36 fold. The Payakumbuh strain (RMH-PYK) and Bandung strain (KRS-BDG) of nymph cockroaches were still susceptible with RR₅₀ <1.00 fold. Generally, the female and nymphs of cockroaches were still susceptible to citronella grass essential oil.

Table 3. Susceptibility status of five strains (VCRU-WHO: Standard strain, HHB-JKT: Jakarta strain, PLZ-PDG: Padang strain, RMH-PYK: Payakumbuh strain and KRS-BDG: Bandung strain) female and nymph of German cockroaches by citronella grass essential oil concentration 80%

Cockroaches	Strains	LT ₅₀ (hours)	RR ₅₀ (Folds)	Susceptibility status
Female	VCRU-WHO	3,56	1,00	Susceptible
	HHB-JKT	4,83	1,36	Susceptible
	PLZ-PDG	3,82	1,07	Susceptible
	RMH-PYK	4,06	1,14	Susceptible
	KRS-BDG	0,85	0,24	Susceptible
Nymph	VCRU-WHO	0,55	1,00	Susceptible
	HHB-JKT	1,45	2,61	low resistance
	PLZ-PDG	4,49	8,09	moderate resistance
	RMH-PYK	0,27	0,48	Susceptible
	KRS-BDG	0,09	0,17	Susceptible

The female and nymph of German cockroaches generally were still susceptible toward citronella grass essential oil. The citronella grass essential oil has the high potential to be developed into new insecticides alternative. This essential oil contains many chemical compounds which are dominated by Citronella (35.97%) and followed by Nerol (17.28%), Citronellol (10:03%), Geranyl acetate (4:44%), Elemol (4:38%), Limonene (3.98%), and Citronellyl acetate (3:51%) [12]. The number of the compounds that contained in citronella grass essential oil makes physiological resistance of the cockroach will develop more slowly. The citronella grass essential oil can be alternative insecticide than the synthetic insecticides which have been widely resistant to pest insects [6]. The essential oils were not toxic to mammals and fish after toxicology tests, economically, it safe for the environment because it contains easily degradable compounds in the environment.

CONCLUSIONS

The Citronella grass essential oil was more effective to the nymph than the female German cockroaches. Female and nymph of German cockroaches generally were still susceptible to citronella grass essential oil. Citronella grass essential oil has a high potential to be developed into new insecticides alternative to control the German cockroaches population.

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