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Fumigant toxicity and repellency of citronella grass essential oil (*Cymbopogon nardus* (L.) Rendle) to German cockroaches (*Blattella germanica* L.)

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ABSTRACT: Citronella grass (*Cymbopogon nardus* (L.) Rendle) is a tropical plant that can develop as an insect pest fumigant and repellent, especially for the control of the populations of German cockroaches (*Blattella germanica* L.). The research aims to investigate the fumigation toxicity and repellency of citronella grass essential oil against German cockroach males and nymphs. Fumigation toxicity and repellency tests are the protocol that uses in the present research. The field populations of cockroaches collected in Indonesia from several locations. The essential oil of citronella grass is not fumigant. In contrast, the citronella grass essential oil effectively repels the cockroach, and the repellency ranges from 65.72–100.00% at 1 hour and still effective after 24 hours. The citronella grass essential oil can develop as a repellent product than as a fumigant to the German cockroach pest.

Keywords: Citronella; German cockroach; Repellent; Fumigant; Indonesia.

1. INTRODUCTION

The German cockroach was the common household pest in Indonesia and has been resistant to synthetic insecticides [1-2]. It will be more challenging to control German cockroach populations if resistance to insecticides grows. Therefore, to control German cockroach populations, we need new insecticide alternatives, such as plant essential oils. It is possible to use essential oils as a toxicant, repellent, and antifeedant [3], with a non-toxic or low toxicity effect to mammalian and easily degrade in the environment [4-5]. One of the tropical plants that produce essential oils is citronella grass (*Cymbopogon nardus* (L.) Rendle).

The citronella grass essential oil is toxic to German cockroach males and nymphs using the contact test [6]. Another strategy uses to control German cockroach populations is using the fumigation method. The public has widely used the fumigation technique to control insect pests. The fumigation method's advantage is that the insecticides quickly penetrate the insect body via the trachea and leave a little residue in the environment [7].

Repellent use to repel and prevent cockroaches comes to human settlements and public buildings. Some essential oil components were repellent to cockroaches at a laboratory scale [8-9]. The citronella grass essential oil has several compounds repellent to agricultural pests [10] and maybe repellent to urban pests. Investigation the Citronella grass essential oil as a fumigant and repellent is a good source of alternative insecticide to control German cockroach populations in the tropical area. Therefore, this study aims to determine the fumigation toxicity and the level of repellency of citronella grass essential oil against German cockroaches males, and nymphs.

2. MATERIALS AND METHODS

2.1. Provision of German cockroach populations

The Vector Control Research Unit (VCRU), Universiti Sains Malaysia, Malaysia, provided a susceptible population of German cockroaches as a World Health Organization (WHO) standard population. The field cockroach populations used in the present research were collected in Indonesia from four locations (Table 1). The cockroach populations were reared in Animal Physiology Laboratory, Biology Department, Universitas Andalas, Indonesia. The rearing process was conducted in photoperiod 12:12 at a temperature of 26–28ºC, the cockroaches feed with cat food and water ad-libitum [11]. The cockroach sex and stage used in this study were male and nymphs.

Table 1. The information of sources and collection years of German cockroach populations.

Population	Collection location	Collection years	Resistant level history		
			Propoxur	Permethrin	Fipronil
VCRU-WHO	Penang	2007	Susceptible	Susceptible	Susceptible
HHB-JKT*	Jakarta	2007	High	Extremely high	High
KRS-BDG*	Bandung	2007	Low	Low	Low
PLZ-PDG	Padang	2014	-	-	-
RMH-PYK	Payakumbuh	2015	-	-	-

*the information of German cockroach resistance [1].

2.2. Provision of citronella grass essential oil

The Research Institute for Spices and Medicinal Plants K.P. Laing, Solok, West Sumatra, Indonesia, provided the citronella grass essential oil. The concentration of essential oil used in the fumigant toxicity test was 100%. The amount of essential oil used in the repellency test was 0.57 mg/cm^2 and was a sub-lethal concentration and not killing the cockroaches during observation. The oil concentrations were determined from the preliminary test. The components of citronella grass oil were not determined in the present research, the main components volatility of the oil that have been reported were geraniol (35.7% of total volatiles), *trans*-citral (22.7%), *cis*-citral (14.2%), geranyl acetate (9.7%), citronellal (5.8%) and citronellol (4.6%) [12].

2.3. Fumigant toxicity test

The test of fumigant toxicity of citronella grass essential oil to German cockroaches was referred from the previous study [9] with some modifications. This test used a plastic container (volume: 1 liter) and a cotton ball (diameter: 1 cm). The cotton ball injected with 100 µl essential oil using a micropipette. It is then suspended with yarn in the middle of the plastic container's top to prevent the cockroaches from being in direct contact with

essential oil. The plastic container's top inside wall was smeared with Vaseline and petroleum oil solution to prevent cockroaches escape during observation. The top of the container was covered with gauze. About ten individuals from each German cockroach's population were placed into the plastic container. The mortality of the cockroaches was observed every 24 hours until 96 hours after treatments. Each treatment was replicated three times.

2.4. Repellency test

The repellency test of citronella grass essential oil to German cockroaches was referred from the previous study [9] with some modifications. The filter paper (diameter: 15 cm) was divided into two parts. Part 1 filled with essential oil as much as 0.57 mg/cm^2, and part 2 was only filled with ethanol as much as 0.57 mg/cm^2. The concentrations used were obtained from the preliminary test and were a sub-lethal concentration (did not paralyze or killed the cockroaches). Therefore, the movement of cockroaches can observe during the treatment. The filter paper dried at room temperature for 24 hours until the ethanol evaporated. Both of the papers were placed into a petri dish (same diameter as filter paper). The inside wall of the petri dish was smeared with Vaseline and petroleum oil solution to prevent the cockroach escape during observation. About ten individuals from each population of German cockroaches were placed in the center of the petri dish. Distribution and movement of the cockroaches were observed every one hour until 24 hours after treatment. Each treatment was replicated three times.

2.5. Data analysis

Each cockroach population's lethal time was analyzed using Probit Regression Analysis in Polo-PC computer software [13] to determine the lethal time 50% (LT50) of cockroaches. The susceptibility status of each cockroach's population was determined by calculating the ratio resistance (RR50) by comparing the LT50 between the field population and the standard strain. The ratio resistance was grouped into four categories (RR50 < 2 indicates susceptible, RR50 ranged from 2–5 indicates the presence of low resistance, RR50 ranged from 5–10 indicates a moderate level of resistance, and RR50 > 10 demonstrates high resistance) [14]. 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 [15]. To determine the repellency value of citronella grass essential oil was used the formula: Repellency (%) = 100 - (T × 100/N) [16], T = number of individuals distributed in the treatment paper filter, N = number of individuals distributed in the ethanol paper. The repellency value (RV) of citronella grass essential oil was determined from criteria: not repellent: RV < 0.1%, very low repellent: RV 0.1–20%, low repellent: RV 20.1–40%, repellent: RV 40.1–60%, high repellent: RV 60.1–80%, very high repellent: RV 80.1–100% [11]. The lethal time 90% (LT90) and repellency effect of citronella grass essential oil between standard population and field populations of German cockroaches were determined using Analysis of Variance (ANOVA) and Duncan Multiple Range Test 5%.

3. RESULTS

3.1. Fumigation toxicity test

The lethal time 90% (LT90) of male German cockroaches in standard population was occurred at 11.43 hours and in field population at 65.00 hours until 107.03 hours. The LT90 of nymph was slower than male in the standard population. However, the mortality of nymph field populations faster than males. In general, the mortality of cockroaches did not occur at 6 hours in each experimental population. The LT90 of male and nymph of German cockroaches were significantly different between standard and field populations (Table 2).

Table 2. The Lethal time 90% (LT90) of male, and nymph of German cockroach, and the effectiveness category of citronella grass essential oil used fumigant toxicity test.

Stages	Population	LT90 (Hours)	Effectiveness category
Male	VCRU-WHO	11.43	Ineffective
	HHB-JKT	72.95*	Ineffective
	PLZ-PDG	74.72*	Ineffective
	RMH-PYK	107.03*	Ineffective
	KRS-BDG	65.00*	Ineffective
Nymph	VCRU-WHO	37.73	Ineffective
	HHB-JKT	22.50^{ns}	Ineffective
	PLZ-PDG	48.70*	Ineffective
	RMH-PYK	53.74*	Ineffective
	KRS-BDG	53.74*	Ineffective

The LT90 values were followed by (*) sign that significantly difference between standard population (VCRU-WHO) and field population used Duncan New Multiple Range Test 5%.

The German cockroach's susceptibility level to citronella grass essential oil was moderate for males, with RR50 varying from 6.81–9.10 folds. In comparison, in field populations with RR50, the nymph's susceptibility level was still susceptible from 1.45–1.46 folds (Table 3).

Table 3. The susceptibility status of citronella grass essential oil against German cockroaches male and nymph in fumigation toxicity test.

Stages	Population	LT50 (Hours)	RR50 (Folds)	Susceptibility status	
Male	VCRU-WHO	6.77	1.00	RR50 $\leq$2	Susceptible
	HHB-JKT	50.14	7.41	5<RR50$\leq$10	Moderate resistant
	PLZ-PDG	55.35	8.18	5<RR50$\leq$10	Moderate resistant
	RMH-PYK	61.58	9.10	5<RR50$\leq$10	Moderate resistant
	KRS-BDG	46.09	6.81	5<RR50$\leq$10	Moderate resistant
Nymph	VCRU-WHO	17.13	1.00	RR50$\leq$2	Susceptible
	HHB-JKT	12.45	0.73	RR50$\leq$2	Susceptible
	PLZ-PDG	24.97	1.46	RR50$\leq$2	Susceptible
	RMH-PYK	24.82	1.45	RR50$\leq$2	Susceptible
	KRS-BDG	24.82	1.45	RR50$\leq$2	Susceptible

Note: LT50 (Lethal Time 50%) = the time of 50% cockroaches mortality; RR50 (Resistance Ratio 50) = LT50 field population/LT50 standard population.

3.2. Repellency test

The repellency of citronella grass essential oil differed between German cockroaches stages and sexes at one-hour observation. The result was high in females (87.96–100.00%) and followed by nymphs (82.01–96.30%) and males (65.72–83.33%). After 24 hours, this repellency test was decreased in males (57.14–65%), in females of the Bandung population, KRS-BDG (82.01%), and nymphs (57.14–83.33%).

Table 4. The repellency (%) of citronella grass essential oil to male, female and nymph of German cockroaches at 24 hours of observation.

Strains	Male (%)	Female (%)	Nymph (%)
VCRU-WHO	69.05±10.31	100.00±0.00	93.00±14.00
HHB-JKT	57.14±0.00 ^{ns}	100.00±0.00 ^{ns}	83.33±14.43 ^{ns}
PLZ-PDG	84.26±8.01 ^{ns}	91.67±14.43 ^{ns}	73.68±15.91 ^{ns}
RMH-PYK	79.63±8.02 ^{ns}	100.00±0.00 ^{ns}	73.68±15.91 ^{ns}
KRS-BDG	59.79±27.87 ^{ns}	82.01±22.24 ^{ns}	57.14±0.00 ^{ns}

Note: The repellency values followed by signs (ns) was not significantly difference between standard and field populations in male, female and nymph of German cockroaches used ANOVA test 5%.

4. DISCUSSION

The citronella grass essential oil is not effective in killing the German cockroach population using the fumigation toxicity test. We assume that German cockroaches field populations have the same physiological responses to citronella grass essential oil. Citronella grass essential oil as a fumigant cannot poisonous to the nervous system of cockroaches. The compound of citronella grass essential oil is dominated by citronella at 35.97% [10]. Some studies also reported the dominated compound of citronella grass oil varied among the cultivated varieties of citronella grass plant. Citronella grass essential oil from Sri Lanka and Indonesia dominated by 40.5–60.7% of citronellal, whereas the oil from India contained the small amounts of citronellal (17.2–33.2%) [17]. Previous research reported that the citronellal is tested to a cockroach in the fumigant method and did not cause mortality [18].

The essential oil from the citronella grass is volatile and quickly evaporated into the air. The higher volatile compound of citronella oil is geraniol (35.7%) [12], geraniol has low toxicity level to German cockroach [7]. Via the tracheal system of cockroaches, the essential oil will penetrate. In a cockroach's body tissue, the chemical compounds of essential oil are inserted into spiracles and delivered through the diffusion process. The tracheal cockroaches system is derived from the integument as the tubular form and served to exchange gases [19].

The octopamine system usually targets the essential oils in insects. The octopamine was a nervous system neurotransmitter [4]. We presume that in German cockroaches, the volume of essential oil that reaches the tracheal system might not be capable of paralyzing the cockroach in the octopamine system.

The evaporation point possibly affected the citronella grass essential oil's ineffectiveness against cockroaches used the fumigation test. This point affects the volatility of the essential oil compounds entered through the trachea during the respiration process. The essential oil compound is more volatile and toxic at the high evaporation point than the low point. Insects bodyweight does not influence the toxicity of essential oil in a fumigation test, so the differences in body weight between males and nymphs will not affect the toxicity of essential oil compounds in the fumigation test [7]. In the present study, we also found that the citronella grass essential oil has a low toxicity level and not fumigant to German cockroaches.

The citronella grass essential oil has a low susceptibility level. It most likely takes a long time for cockroaches to develop resistance to citronella grass essential oil because these essential oil compounds are more complex than synthetic insecticides. Several chemical compounds containing citronella, nerol, citronellol, geranium acetate, elemol, limonene, and citronellil acetate in the composition of citronella grass essential oil [10].

A variety of essential oil chemical compounds can develop physiological resistance more slowly than synthetic insecticides commonly resistant to insect pests [3].

In Indonesia, German cockroaches are resistant to synthetic insecticides, such as carbamate, pyrethroid, and phenylpyrazole. The resistance of German cockroaches to permethrin is extremely high [1], which is very high compared to the susceptibility of citronella grass essential oil using the contact [7] and the fumigation method.

In the repellency test, the fast and erratic movements are German cockroach responses before a one-hour observation in this study. When they find the area without essential oil, cockroach's movements become slower, so most of them will stay there. The interaction of cockroaches with an insecticide is shown to be irritable and marked by rapid motion patterns. It is known as an insect repellent in essential oils when the insects made natural movements to keep away from the surface containing insecticide [8].

In all-male, female, and nymph of German cockroaches, the repellency of citronella grass essential oil generally still effective within 24 hours. Meanwhile, the repellency decreased by the boiling point of the essential oil of citronella grass. The essential oil of citronella grass has dominated by citronella [3, 10]. Citronella has a 121°C boiling point, but apart with a low boiling point can evaporate faster [7].

After 24 hours, the repellency of citronella grass essential oil was still higher than 50 percent. The essential oil of citronella grass also has a high repellency against *Aedes aegypti* [20], and *Helicoverpa armigera* [10]. The essential oil of citronella grass can be developed as a repellent for cockroaches. The essential oil product would prevent cockroaches in human settlements or public places such as hospitals and restaurants. As a cockroach repellent, the citronella grass essential oil was more effective in present research than killing them as a fumigant [21].

5. CONCLUSIONS

Citronella grass essential oil as a fumigant cannot poison the nervous system of cockroaches and ineffective in controlling the German cockroach population. Erratic movements and rapid motion patterns show the interaction of cockroaches with citronella grass essential oil. The German cockroach makes natural movements to keep away from the surface containing citronella grass essential oil. Citronella grass essential oil was more effective to the German cockroach population in present research as a repellent than killing them as a fumigant.

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Conflict of Interest: The author has no conflict of interest to declare.

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