Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Entomology

ISSN 1812-5670
DOI: 10.3923/je.2018.149.154

Research Article
Efficacy and Residual Activity of Lemongrass Essential Oil (*Cymbopogon flexuosus*) against German Cockroaches (*Blattella germanica*)

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Abstract

Background and Objective: The German cockroach (*Blattella germanica*) is a well-known urban insect pest that has become resistant to synthetic insecticides. Hence, the alternative potent insecticides, synthesized from natural substances such as lemongrass essential oil are urgently needed. Its main objective was to experimentally test the efficacy and residual activity of lemongrass essential oil (*Cymbopogon flexuosus*) against German cockroache's population. **Materials and Methods:** This study was conducted at the Research Laboratory of Animal Physiology, Department of Biology, Andalas University, Indonesia. It used lemongrass essential oil with different concentrations through Contact Toxicity Test (3.15 mg cm^{-2}), Fumigation Toxicity Test (1 g cm^{-3}) and Repellency Test (0.16 mg cm^{-2}). **Results:** Lemongrass essential oil was effective in killing German cockroaches through Contact Toxicity Test (LT_{90}<24 h), lemongrass essential oil at sub-lethal concentration behaved as repellent to German cockroaches with 100% repellency values, observed effectively for 48 h of trial. **Conclusion:** Lemongrass essential oil is potential to be an alternative insecticide in controlling the resistance of German cockroaches through contact and repellency applications.

Key words: Urban insect, synthetic insecticides, repellency, fumigation, lemon grass essential oil, alternative insecticide, German cockroaches

Citation: Resti Rahayu, Mairawita and Robby Jannatan, 2018. Efficacy and residual activity of lemongrass essential oil (*Cymbopogon flexuosus*) against German cockroaches (*Blattella germanica*). J. Entomol., 15: 149-154.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The German cockroach (*Blattella germanica*) has been an urban pest and vector for many pathogens that threaten human livelihood. It has role in trigger vertigo, asthma and nausea reactions in human[1]. Increasing use of synthetic insecticides to control German cockroach population has resulted in the resistance across German cockroach populations. It has been resistant to permethrin, propoxur[2] and several pyrethroid insecticides[3] in Indonesia. Those commercial insecticides, applied through contact or spray methods, may have been no longer effective for controlling German cockroach populations in Indonesia[4]. Essential oils from natural products, in the other hand, raise hope to control German cockroache populations in Indonesia. The essential oil from citronella plant is proven effective against the males[5], females and nymphs[6] German cockroach. Another essential oil from crude extract of papaya leaves, which currently under testing, promises effectiveness for cockroach pest control.

The lemongrass plants contained many secondary metabolites such as alkaloids, terpenoids, flavonoids, carotenoids and tannins[7], indicating its potent as bioinsecticide. The efficacy of some essential oil was tested by previous studies[8-11]. Monoterpenes, derivates of terpenoids extracted from lemongrass *Cymbopogon flexuosus*, can disrupt the works of neurotransmitter in insects[12,13]. Tannin compounds are the inhibitors of enzymes' activities in insect digestion.

Despite the potential substances for controlling cockroach population have been confirmed from lemongrass, there is still lack of information regarding the precise methods for using it. Hence, this study focused on finding the most effective way to apply essential oil from lemongrass to control the resistant German cockroach population. It also assessed the effectivity and efficacy of lemongrass essential oil as the bioinsecticide.

MATERIALS AND METHODS

German cockroach provision and rearing process: This study had been conducted from August 2016 until February 2017 in Research Laboratory of Animal Physiology, Department of Biology Universitas Andalas. The field strains of German cockroaches originated from two cities in Indonesia, Bandung (called as KRS-BDG strain) and Jakarta (HHB-JKT strain) (Fig. 1). A standardized (according to World Health Organization, hereinafter WHO) control population of cockroach was obtained from the Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia (hence called as VCRU-WHO strain). All cockroaches were reared in the Research Laboratory of Animal Physiology, Department of Biology Universitas Andalas according to the established protocol[4]. The cockroaches were feed with cat food (brand Pedigree) and water, with ad libitum ration; the rearing room was maintained at temperature 24-28°C, relative humidity 84-86% and photoperiod of 12:12. Only males German cockroach were used in this study as they physiologically remain stable from hormonal fluctuation, especially when exposed with the bioinsecticide substance[2].

Provision of lemongrass essential oils: Lemongrass essential oil was commercially obtained from Indonesian Research Center for Spices and Medicinal Plants KP. Laing Solok, West Sumatra, Indonesia. The dosage used for contact toxicity test was 3.15 mg cm^{-2} of lethal residue (LR_{90}), which averagely killed 90% cockroaches within 6 h. The repellency test used the sub-lethal residue dosage which was 10% of LR_{90} dosage; while fumigation toxicity test used 100% concentration of lemongrass essential oil, where 1 mL oil was stained on cotton and placed in 1 L plastic container to create fume. The dosage for lemongrass oil used in this study was predetermined by the values of lethal residues (LR) gained from the preliminary test. The values of lethal residue (LR_{50} and LR_{90}) by preliminary test of lemongrass essential oil against German cockroaches on 6 h observation with contact toxicity test are given in Table 1.

Contact toxicity test: Lemongrass essential oil against was applied using Tarsal Contact Test to German cockroaches, following the established procedure[6]. The dosage 3.15 mg cm^{-2} lemongrass essential oil residue was used as the cockroaches will gradually die within 24 h interval after exposure. Knockdown (condition where cockroach was unable to walk, but still move if touched) and mortality of German

Table 1: Values of lethal residue (LR_{50} and LR_{90}) by preliminary test of lemongrass essential oil against German cockroaches on 6 h observation with contact toxicity test

Strains	LR_{50} (mg cm^{-2})	LR_{90} (mg cm^{-2})	RR_{50}	RR_{90}	Slope±SE
VCRU-WHO	1.61	2.39	1.00	1.00	7.45±1.26
KRS-BDG	1.88	3.53	1.17	1.48	4.69±0.68
HHB-JKT	1.75	3.03	1.09	1.28	5.39±0.74

RR: Resistance ratio, RR: LR_{50} fields strain/LR_{50} standard strain, LR_{50} or LR_{90}: Lethal residue to control 50 or 90% of test animals

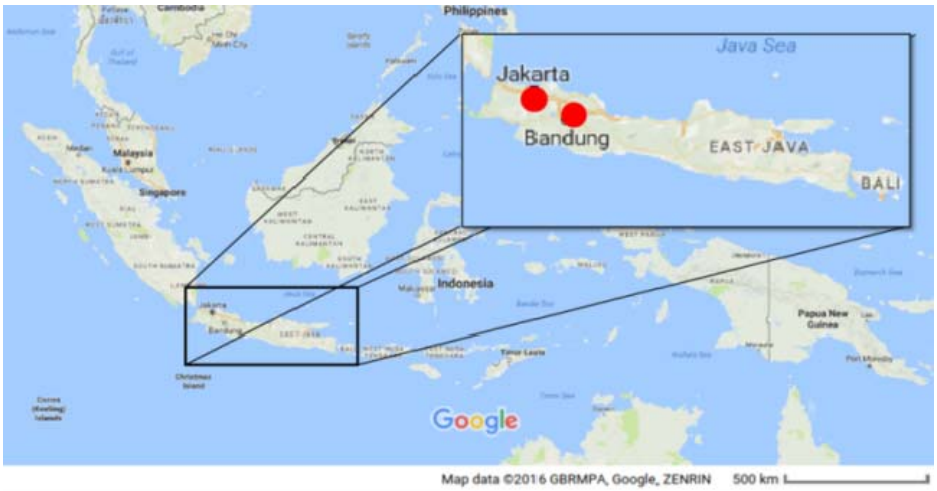

Fig. 1: Sampling sites (red dots) of two fields German cockroaches (HHB-JKT; Jakarta and KRS-BDG; Bandung)
Source: Google Maps, 2016

cockroach were observed at 6 and 24 h after exposure. The treatment was replied three times.

Fumigation toxicity test: This test followed previously established protocol[6], where the mortality of German cockroaches was observed in every 24 h. Upon 6 days of fumigation, unless the mortality of German cockroaches reached 100%, the test should be stopped due to the expiration of essential oil beyond this time-frame. The treatment was repeated three times.

Repellency test: Repellency test used sub-lethal concentration of lemongrass essential oil (residue 0.16 mg cm^{-2}) as referred to the established protocol[6]. The distribution of cockroaches was observed in every hour within the first 6 h, then once at 24 and 48 h after exposure. The treatment was repeated three times.

Data analysis: Probit analysis with POLO-PC computer software[14] used to analyze the data of knockdown and mortality from contact toxicity test and fumigation toxicity test. The probit analysis determined knockdown time (LT_{90}) and lethal time 90% (LT_{90}). The determination of the effectiveness of essential oils following these criteria:

- Very effective : $LT_{90} \leq 24$ h
- Effective : 24 h< $LT_{90} \leq 48$ h
- Less effective : 48 h< $LT_{90} \leq 96$ h
- Ineffective : $LT_{90} > 96$ h

The repellency value of essential oils residue is formulated as following[15]:

$$RV(\%) - 100 - \left(\frac{T \times 100}{N}\right)$$

Where:

RV = Repellency value (%)
T = Number of cockroaches in the treatment area (individuals)
N = Number of cockroaches in control area (individuals)

The repellency level of lemongrass essential oil was determined from modified Dales[16] criteria, observed within 24 h:

- 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%

RESULTS

Contact toxicity test: The knockdown time for VCRU-WHO strain started at minute four with 0.6% of total population, while the paralysis on KRS-BDG and HHB-JKT strains starting observed at minute six, with 12.0 and 0.6% of total population

Table 2: Knockdown time (KT) and lethal time (LT) 90% each strains of German cockroach against lemongrass essential oil with residue 3.15 mg cm^{-2}

Strains	KT_{90} (min)	Slope±SE	LT_{90} (h)	Criteria	Slope±SE
VCRU-WHO	84.88	1.84±0.11	2.06	Very effective	1.93±0.11
KRS-BDG	151.04	1.75±0.10	3.79	Very effective	1.73±0.91
HHB-JKT	107.56	1.85±0.10	2.82	Very effective	1.78±0.96

KT_{90}: Knockdown time (the time required to immobilize 90% of test animals), LT_{90}: Lethal time (the time required to kill 90% of test animals)

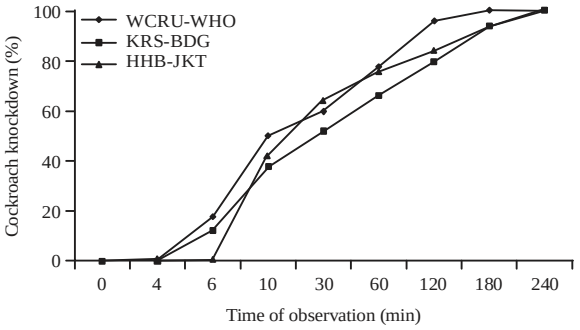

Fig. 2: Average of knockdown time (KT) rate of German cockroach against lemongrass essential oil with residue 3.15 mg cm^{-2} using the contact toxicity test

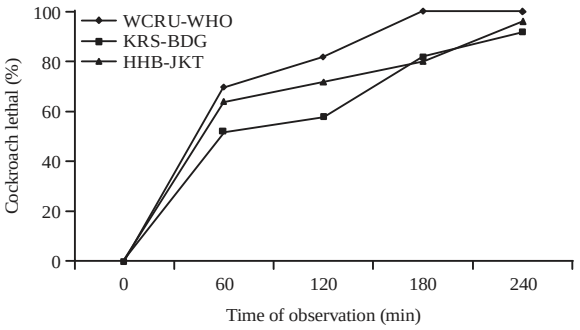

Fig. 3: Average of lethal time (LT) rate of German cockroach against lemongrass essential oil with residue 3.15 mg cm^{-2} using the contact toxicity test with observation time until 6 h

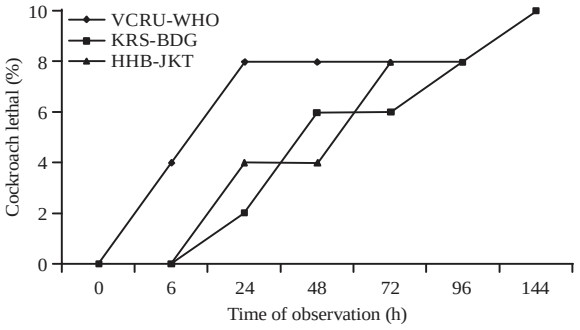

Fig. 4: Average of lethal time (LT) rate of German cockroach against lemongrass essential oil used fumigation toxicity test with observation time until 144 h

Table 3: Repellency value (%) of lemongrass essential oil (*C. flexuosus*) at sub-lethal residue with residue 0.16 mg cm^{-2} against German cockroach (*B. germanica*)

	Repellency value (%) (h)				
Strains	1	6	24	48	Repellency level*
VCRU-WHO	100	100	100	100	Very high repellent
KRS-BDG	100	100	100	100	Very high repellent
HHB-JKT	100	100	100	100	Very high repellent

*Repellency level modified by Dales[17] at 24 h of observation

respectively. The paralysis of VCRU-WHO strain occurred at 120 min as many as 96%, whereas the paralysis of KRS-BDG and HHB-JKT strains at 180 min as many as 94% (Fig. 2). Lemongrass essential oil killed strains of German cockroach between 52-72% at the 1 h. Mortality of all individuals in each strain occurred before 6 h (Fig. 3). The mortality of VCRU-WHO strain occurred at 3 h as many as 100%, whereas the mortality of KRS-BDG and in HHB-JKT strain at 4 h only as many as 92 and 96%. Knockdown Time (KT) and Lethal Time (LT) 90% each strains of German cockroach against lemongrass essential oil with residue 3.15 mg cm^{-2} and the effectiveness criteria used Contact Toxicity Test was given in Table 2. This result indicated the effectiveness of lemongrass essential oil against all experimented strains of German cockroach through the contact toxicity test because 90% of German cockroach population was killed before 6 h.

Fumigation toxicity test: The initial mortality of 4% in VCRU-WHO strain occurred at 6 h, while in KRS-BDG and HHB-JKT strains occurred at 24 h with respectively rate of 2 and 4%. Increased mortality in occurred after 96 h only 8% of all strains and observation was stopped at hour 144 post exposure. The Fig. 4 showed that lemongrass essential oil was ineffective when applied through fumigation.

Repellency test: Lemongrass essential oil showed no lethal effect against German cockroach when applied in this test using sub-lethal concentration (0.16 mg cm^{-2}). The test, however, indicated the repellency of lemongrass essential oil at this dosage was effective in evicting 100% of German cockroach within the 48 h observation (Table 3).

DISCUSSIONS

The test showed that the mortality of VCRU-WHO strain was higher and faster than those of KRS-BDG and HHB-JKT strains. It was caused by the VCRU-WHO strain has never been previously exposed to any synthetics or natural insecticide. The other two strains, which were collected from the field, may have been frequently exposed and become resistant to various insecticides. Previous research reported that some German cockroache populations in Indonesia have become more resistant than the standard cockroaches[4]. Furthermore, the nymphs of German cockroach were proven to be more resistant than the standard cockroaches (VCRU-WHO strain) when experimentally tested with citronella grass essential oil[6].

Some commercial insecticides with active substances such as cypermethrin, imiprothrin, prallethrin, d-allethrin, esbiothrin, permethrin, transfluthrin, cyfluthrin and prallethrin have recently become ineffective to kill German cockroaches within 6 h of application, which indicated their resistance to synthetic insecticides. Lemongrass essential may provide solution to control German cockroach as it showed its eradicating effectivity. It was presumed that the field strains of German cockroaches tested in this study, despite the degree of their resistance to insecticides, was still susceptible to chemical compounds contained in lemongrass essential. The monoterpenes contained in lemongrass essential oil was composed by geranial (46.86%) and neral (33.40%)[8] that served as neurotoxin for insects and caused mortality in cockroaches. In addition, it also contained tannin compounds, which inhibit metabolism in insects by obstructing the activity of digestive enzymes[7].

This study showed that lemongrass essential oil was more effective against German cockroaches on male, previous study also found that citronella grass essential oil also effective on male German cockroach in different strains between current studies[5]. It was also worked more effectively as bio insecticide than the crude extract of papaya leaf against male German cockroaches.

Using fumigation to apply lemongrass essential oil as insecticide show some degree of ineffectivity toward the German cockroach, which presumably caused by the volatile compound it contained. The neurotoxin potent of lemongrass essential oil was drastically reduced when applied through fumigation. Previous fumigation test on German cockroach with an array of essential oils revealed that geranial group, which is the major component of monoterpene in lemongrass essential oil, had the lowest toxicity compared to the other groups. Citronella, another essential oil has been known contained in lemongrass and tested to German cockroach with no effective result in killing it[17].

In the fumigation application, the toxins work by penetrating insect's body through its respiratory tract as soon as the fumigant makes contact with insect body. In this study, the substances contained in lemongrass essential oil are too volatile, dissipating through evaporation. As result, insufficient amount of essential oil became ineffective in killing German cockroaches. Therefore, this study proved that the fumigation method is not suitable to apply the lemongrass essential oil as insecticide for German cockroach.

The compounds of citral, geraniol and linalool in lemongrass essential oils have a distinctive aroma which is unfavorable for cockroaches[7]. Lemongrass essential oil has a high repellency level, even when it was used at low and non-toxic concentrations (Table 3). Phytochemical content in lemongrass essential oil produce distinctive odor which stimulates the chemoreceptor in insects and urge them to evade the source of odor. Chemoreceptors are chemical tasting organs associated with tastes and smells and parts of the behavioral sensory system in insects[18].

All German cockroach strains used in this study initially reacted with rapid and irregular movement when exposed to lemongrass essential oil during the repellency test. The cockroaches tried to find part of testing container that is not applied with repellent where they then settled. Cockroaches seemed to hastily walk through the parts container applied with repellent in searching for secure spot. Similar behavior was also shown by cockroaches during the repellency test with American pepper *Schinus mole*[19]. The rapid and irregular movement made by cockroaches when walking across an area applied with repellent caused by cockroaches' irritability to the repellent[10,20]. Different essential oil may have different length of activity, such as the essential oil from *C. citratus* with 100% repellency to American cockroaches for 24 h exposure[12] and one from Citronella that repelled German cockroaches for 24 h. The result of this study indicated that lemongrass essential oil has stronger and longer repellency than the previous essential oils used in similar testing.

CONCLUSION

Lemongrass essential oil showed some effectiveness, as well as potential to eradicate German cockroaches. The experimentation suggested that it can be contact toxic and repellent against the German cockroaches, but not through the fumigation application. These results will significantly add into the effort of exploration and development of alternative bioinsecticides which needed to control the resistant German cockroaches.

ACKNOWLEDGMENT

This project was partially funded by Program DIPA 2017 with contract number: 28/UN.16.03.D/PP/FMIPA/2017. We would like thank the Indonesian Research Center for Spices and Medicinal Plants KP. Laing Solok, West Sumatra, Indonesia for providing the lemongrass essential oil, as well as to Syafri Yana, for her assistance in the laboratory, to Rijal Satria and M. Nazri Janra for they valuable suggestion.

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