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Research Article

Potency of Papaya Leaf (*Carica papaya* L.) as Toxicant and Repellent against German Cockroach (*Blattella germanica* L.)

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Abstract

Background and Objective: Papaya leaves have the potency to be developed as alternative insecticide to control the German cockroaches that have been resistant to synthetic insecticides. This study aimed to determine the potency of ethanolic extract of papaya leaf as toxicant and repellent against 2 populations of German cockroaches which are known resistant to synthetic insecticide. **Materials and Methods:** The methods that used was contact toxicity test and repellency test. The ethanolic extract of papaya leaf residues were used in contact toxicity test of 4 residues and a sub-lethal residue in repellency test. Three populations of German cockroach were collected in field and kept in laboratory. **Results:** Lethal residue 50% (LR_{50}) of ethanolic extract of papaya leaf between 2.97-4.72 mg cm^{-2} while lethal residue 90% (LR_{90}) between 6.05-8.92 mg cm^{-2} and very effective killed all population of German cockroach. Repellency value of ethanolic extract of papaya leaf with a sub-lethal residue of 0.39 mg cm^{-2} between 88.89 -94.74% and level of repellency was very repellent. **Conclusion:** Ethanolic extract of papaya leaf could be formulated as a potent natural insecticide to control populations of German cockroach that have been resistant to synthetic insecticides.

Key words: Urban pest, tropical plant, insecticide resistance, a natural insecticide, insecticide residue

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

INTRODUCTION

German cockroach (*Blattella germanica* L.) is a pest in urban areas, very harmful to human health and has been resistant to synthetic insecticides in Singapore¹, China², Iran^{3,4}, Malaysia⁵ and USA⁶. The insecticide failed to control German cockroach populations due to resistance of some commercial insecticides⁷, pyrethroid⁸, carbamate and phenylpyrazole⁹ in Indonesia. Therefore, to face the problem of insecticide resistance in Indonesia, exploration of natural insecticides from plants as alternative insecticide to control the German cockroach population that have been resistant to synthetic insecticides is very important to do^{10,11}.

The plant that has potential to be developed as a source of natural insecticides is papaya (*Carica papaya*) because its leaves extract potential as larvicides to chikungunya¹², filaria¹³ and malaria vector mosquitoes^{14,15}. The natural insecticides that have been tested and effective in controlling the population of German cockroach that has been resistant to synthetic insecticides in Indonesia that have been reported are *C. nardus*¹⁶ and *C. flexuosus*¹⁷. However, there has no scientific report on the use of papaya leaves as a toxicant against German cockroaches in Indonesia.

Papaya belongs to Caricaceae family and distributed in the tropical area. Various parts of papaya plant showed some biological activities and also displayed good activities against *Plasmodium falciparum*¹⁴. Papaya extracts can be recommended as a larvicides in mosquito control program¹³. Papaya is a plant with cheap price and easy to find in nature. This study was conducted to test the toxicity and repellency of the ethanolic extract of papaya leaves against German cockroach. The effectiveness of papaya leaves is expected to be a source of information for the community to control German cockroach population that has been resistant to synthetic insecticides using environmentally friendly materials in the tropics area.

MATERIALS AND METHODS

Location and time duration: This study was conducted in Laboratory of Animal Physiology, Department of Biology Universitas Andalas Indonesia upon the 8 months from November, 2016 until July, 2017. The field populations of German cockroach was collected from 2 location in Indonesia that were from Bandung (KRS-BDG population) and Samarinda (SMRD-KALTIM population). The standard population obtained from the Vector Control Research Unit School of Biological Sciences Universiti Sains Malaysia (VCRU-WHO population).

Provision of German cockroaches: The rearing process of German cockroach as described by established method¹⁷. The

German cockroaches used in this study were male individuals from the standard population (VCRU-WHO) and field populations (KRS-BDG and SMRD-KALTIM). German cockroach populations kept at Laboratory of Animal Physiology, Department of Biology Universitas Andalas from 2012.

Provision of ethanolic extract of papaya leaf: This extract was made at the Laboratory of Natural Organic Chemistry, Department of Chemistry Universitas Andalas as described by established method¹⁸. Ethanolic extract of papaya leaf residues were used in contact toxicity test of 2, 5, 7 and 9 mg cm⁻² and sub-lethal residue in repellency test of 0.39 mg cm⁻².

Contact toxicity test: This test as described by established method¹⁶ to determine lethal residue (LR) and lethal time (LT). The LR test used five concentrations of ethanolic extract of papaya leaf with residues of 2, 5, 7 and 9 mg cm⁻² and LT test used the best concentration that obtained from LR₉₀ value after 24 h exposure on previous LR test, the best residue was 8.92 mg cm⁻² because it can kill 90% of German cockroach population before 24 h. The residue value is then rounded off to 9 mg cm⁻² for the easier provision of ethanol extract during contact toxicity test. The knockdown and mortality of cockroaches observed every hour until 6 and 24 h after exposure. The test replied 3 times.

Repellency test: This test as a modification from established method¹⁹ used petri dish (15 cm) and sublethal residue (0.39 mg cm⁻²) of ethanolic extract of papaya leaf that obtained from the preliminary test, it lowest residue does not kill German cockroach during observation. Cockroaches movement were observed at 1, 6 and 24 h after exposure.

Data analysis: Contact toxicity test data analyzed by probit analysis with POLO-PC computer software²⁰ to determine of lethal residue (LR_{50,90}), knockdown time (KT₉₀) and lethal time (LT₉₀) values. The effectiveness of ethanolic extract of papaya leaf as determined by established criteria¹⁷ as follows: Very effective: LT₉₀ ≤ 24 h, Effective: 24 h < LT₉₀ ≤ 48 h, Less effective: 48 h < LT₉₀ ≤ 96 h and ineffective: LT₉₀ > 96 h.

The repellency value of ethanolic extract of papaya leaf used following this equation²¹:

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

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 ethanolic extract of papaya leaf within 24 h used established criteria²² as follows, 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% and Very high repellent: RV 80.1-100%.

RESULTS

Toxicity contact test: The lethal residue of ethanolic extract of papaya leaf that was ranged from 2.97-4.72 and 6.05-8.92 mg cm⁻² could kill 50% (LR₅₀) and 90% (LR₉₀) of German cockroach populations. German cockroaches of KRS-BDG population were more homogeneous than other populations characterized by higher slope value (0.07±0.009). The residue of ethanolic extract of papaya leaf needed to kill 90% of the KRS-BDG was 6.05 mg cm⁻² which was the lowest residue as compared with the residue levels that could kill the other two populations. The highest residue needed to control 90% of German cockroach population was the VCRU-WHO population with 8.92 mg cm⁻² residue (Table 1).

The effectiveness test used 9 mg cm⁻² residue (derived from result of LR₉₀ in Table 1) was carried out to determine the time needed to control 90% of animals test or lethal time 90% (LT₉₀). The test results shown in Table 2, depicting that time needed for killing 90% of each cockroaches population ranged from 3.58-5.83 h. Thus the toxicity of ethanolic extract of papaya leaf was very effective to all population of German cockroach.

The individuals death trend of VCRU-WHO and SMRD-KALTIM populations almost the same with KRS-BDG population. Time of death of VCRU-WHO and SMRD-KALTIM populations was faster in the first 6 h than KRS-BDG population. The 90% of German cockroach populations

death after 6 h and all population death at 24 h of observation. The death of KRS-BDG population are slowly than other 2 populations (Fig. 1).

Repellency test: Ethanolic extract of papaya leaves was not only toxic to German cockroach but also has a repellent activity. The repellency value of ethanolic extract of papaya leaves for VCRU-WHO population was 46.15% at the first hour and increase until 94.74% at 24 h, whereas, in the other populations the repellency of papaya leaves was immediately high was ranged 91.89-97.44% but decreased to 88.89% at 24 h. Repellency criteria of ethanolic extract of papaya leaf were very high to all population after 24 h (Table 3).

Table 1: Lethal residue (LR₅₀, 90) of ethanolic extract of papaya leaf against German cockroach population

Population	LR ₅₀ (mg cm ⁻²)	LR ₉₀ (mg cm ⁻²)	Slope ± SE
VCRU-WHO	4.72	8.92	0.05 ± 0.007
KRS-BDG	2.99	6.05	0.07 ± 0.009
SMRD-KALTIM	2.97	8.40	0.04 ± 0.006

LR₅₀: Residue that could kill 50% of German cockroach population, LR₉₀: Residue that could kill 90% of German cockroach population, SE: Standard error

Table 2: Effectivity of ethanolic extract of papaya leaf residue of 9 mg cm⁻² against German cockroach population used LT₉₀ value

Population	LT ₉₀ (h)	Criteria*	Slope ± SE
VCRU-WHO	3.86	Very effective	0.01 ± 0.001
KRS-BDG	5.83	Very effective	0.01 ± 0.001
SMRD-KALTIM	3.58	Very effective	0.02 ± 0.001

LT₉₀: Time needed to kill 90% of German cockroach population, *Refers to the established criteria as described elsewhere¹⁶

Table 3: Repellency value and level of ethanolic extract of papaya leaf residue 0.39 mg cm⁻² against German cockroach population within 24 h interval of observation

Population	1 h (%) ± SD	6 h (%) ± SD	24 h (%) ± SD	Level*
VCRU-WHO	46.15 ± 50.00	0.00 ± 57.74	94.74 ± 6.42	Very high
KRS-BDG	91.89 ± 11.87	85.71 ± 18.48	88.89 ± 20.27	Very high
SMRD-KALTIM	97.44 ± 5.56	97.44 ± 5.56	88.89 ± 20.27	Very high

*From established criteria²¹

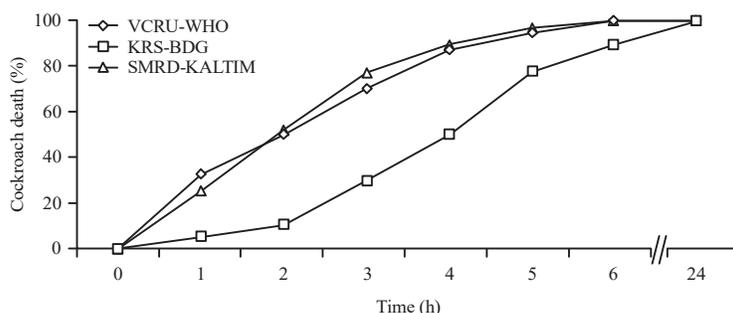


Fig. 1: Mortality rate of German cockroaches against an ethanolic extract of papaya leaf residue of 9 mg cm⁻² used contact toxicity test within 24 h interval of exposure

DISCUSSION

The residue of ethanolic extract of papaya leaves needed to kill German cockroach populations was proportional to the number of deaths cockroach. The residue range from 2.97 until 4.72 mg cm⁻² could kill 50% cockroach population and if that residue increase from 6.05 until 8.92 mg cm⁻² could kill 90% of German cockroach populations. If the residue of ethanolic extract of papaya leaves used is higher, then the toxic substances that enter the body of the cockroach are also getting bigger. The dose needed to kill the KRS-BDG population (field population) was lower than VCRU-WHO population (standard population). That result suggesting the KRS-BDG is more susceptible as compared with VCRU-WHO against ethanolic extract of papaya leaves.

The KRS-BDG population of German cockroach was resistant to synthetic insecticides such as propoxur, permethrin and fipronil⁹ but still susceptible when exposed to the ethanolic extract of papaya leaves, it is indicates the population may have never been exposed to the active compounds of the ethanolic extract of papaya leaves. Papaya leaves contain substances that can disturb the metabolism and probably a novel toxic compound for German cockroaches. Papaya leaves contain the enzyme papain, carpainin alkaloid, carpaine, pseudocarpaine, saponin, tannin, choline, carposid glycosides, vitamins C and E²³, among these substances probably is toxic to cockroaches and has not been received by test cockroaches. Papaya leaf extract is also very effective for controlling caterpillars in mustard greens²⁴ and can reduce attacks from pests such as *Plutella xylostella* larvae²⁵, aphids (*Aphis craccivora*)²⁶, *Nezara viridula*²⁷ and *Anopheles aconitus* larvae²⁸.

The lethal residue (LR₉₀) of ethanolic extract of papaya leaves to kill 90% of German cockroach population ranging from 6.05 until 8.92 mg cm⁻² and higher than others natural extracts such as lemongrass oil with ranging from 2.39 until¹⁷ 3.53 mg cm⁻². The result shows that ethanolic extract of papaya leaves is less toxic than lemongrass oil. The using of crude extract like ethanolic extract of papaya leaves has advantages in the process of making extracts that are easy and fast, but its activity to disturbing the nervous system and the function of octopamine in the insect's body is longer²⁹. Octopamine is a neurotransmitter that is disturbed when insects are exposed to plant extracts or essential oils^{29,30}.

The individuals of German cockroaches try to stay away from the base of Petri dish which is exposed with papaya extract because the cockroaches movement becomes more active. The active movement of cockroaches are decline and

then cockroaches stop moving. The 100% death in VCRU-WHO and SMRD-KALTIM populations of German cockroaches occurred at the 6th h, while the KRS-BDG population occurred at the 24th h. The gradual death respond of German cockroaches showed that each individual had a different response to the ethanolic extract of papaya leaves.

Papaya leaves contain of 5.7% flavonoid, 3% alkaloid, 2% saponin, 0.43 mg/100 g tannin and 0.33 µg g⁻¹ HCN³¹. Secondary metabolites contained in plants could act as a contacted toxin by penetrating the cuticle layer and also as a stomach toxin that could enter the gastrointestinal tract along with the ingested food. However, the use of toxic compounds of these plants must be applied correctly and precisely because those compounds are very easily degraded by sunlight, air and humidity³².

Sub-lethal residues of ethanolic extract of papaya leaves of 0.39 mg cm⁻² did not cause the death of German cockroaches but showed a repellency effect of 88.89-97.74%. This repellent effect is probably due to the odor produced from the ethanolic extract of papaya leaves. The repellency effect of ethanolic extract of papaya leaves on German cockroaches changes at each observation hour. The cause of the repellency effect variation on German cockroach populations at every hour of observation is not known yet.

The repellency criteria of ethanolic extract of papaya leaf at 24 h of observation are very high (88.89-94.74%) suggesting that the compounds in papaya leaves may disturb the cockroach metabolism process. The presence of odor compound causes cockroaches to move away from the part exposed by the ethanolic extract of papaya leaves due to the disturbed chemoreceptors³³. Ethanolic extracts of papaya leaves are very repellent to German cockroaches on a laboratory scale. The other essential oil containing volatile constituents were also reported to be highly repellent against American cockroach on lab scale test³³, hence, in the further study, it is needed to test its potential repellent in the field scale.

CONCLUSION

Ethanolic extract of papaya leaves is very effective in killing the German cockroaches by direct contact and also highly repellent by means indirect contact. Ethanolic extract of papaya leaves could be an alternative insecticide to control German cockroach that resistant to synthetics insecticides. Further research is needed to elucidate the toxic substances contained in papaya leaves and its ethanolic extract as well as its effectivity against German cockroaches in the field scale.

SIGNIFICANCE STATEMENT

This study will help the researchers to uncover the critical areas of the natural products such as papaya leaves could be a solutions to control the German cockroaches population that resistant to the general insecticides used by people that many researchers in Indonesia were not able to explore. Thus a new theory on control management of resistant German cockroaches populations may be arrived at. The natural insecticide from papaya leaves is more recommended to be a repellent than being a contact poison because the requirement for a contact insecticide must be contacted with the target pest, while papaya leaves have a high repellence rate.

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