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## Research Article Efficacy of Noni (*Morinda citrifolia* L.) Ethanolic Leaf Extract Against German Cockroach (*Blattella germanica* L.)

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### Abstract

**Background and Objective:** The ethanol extract of noni leaves (*Morinda citrifolia* L.) can be used as insecticides to control populations of German cockroaches that have been resistant to synthetic insecticides. This study aimed to determine the potency of the ethanol extract of noni leaves to kill and repel German cockroaches and affect the amount of food consumed. **Materials and Methods:** The methods used in this study were the contact toxicity test, the repellency test and the food consumption test. The noni leaves extract and German cockroach populations were provided in the laboratory. **Results:** The noni leaves extract concentration of 20% (residue of 3.14 mg cm<sup>2-1</sup>) was very effective in killing the standard population and effective in killing the field population of German cockroaches. The sub-lethal concentration noni leaves extract of 0.36% (residue 0.056 mg cm<sup>2-1</sup>) and 1.08% (residue 0.169 mg cm<sup>2-1</sup>) was very high grade as repellent of German cockroaches. The sub-lethal concentration of noni leaves extract did not inhibit the amount of food consumption in German cockroaches. **Conclusion:** Leaves of noni plants can be used as bioinsecticides to control German cockroach populations that have been resistant to commercial insecticides.

Key words: Antifeedant, bioinsecticide, contact toxicity, food consumption, repellency, resistance, noni plant

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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 one species of insect pests and commonly found in urban areas<sup>1,2</sup>. German cockroach becomes a vector for many pathogenic bacteria that cause diseases in humans such as *Salmonella* spp., *Shigella flexneri, E. coil, S. aureus* and *B. cereus*<sup>3</sup>. The control of the German cockroach population uses synthetic insecticides that have been sold commercially to the public. However, its use uncontrolled makes German cockroaches resistant to many types of synthetic insecticides<sup>4,5</sup>. Insecticide resistance makes the population of German cockroaches is difficult to control, therefore alternative insecticides are needed to overcome this problem.

One alternative insecticide to control the population of German cockroaches that resistant to synthetic insecticides is plant essential oils. It is can be used as an insecticide source, antifeedant, repellent, oviposition deterrent, growth regulation and anti-vector activities against insect pests<sup>6</sup>. Some extracts from tropical plants are effective as insecticide and repellent against German cockroaches in Indonesia such as citronella grass (*Cymbopogon nardus*)<sup>7</sup>, lemongrass (*Cymbopogon flexuosus*)<sup>8</sup> and papaya leaves extract<sup>9</sup>. Other essential oils that have been tested against insect pest populations were essential oils from the family Lamiaceae, Verbenaceae and Apiaceae<sup>10</sup>. One extract from a tropical plant that also has potential against German cockroach is ethanol extracts of noni leaves (*Morinda citrifolia* L.).

Noni plant is a tropical plant originating from Southeast Asia and its parts are widely used as a source of traditional medicine by people in South East Asia, the Pacific Islands and Australia. In the last few years, noni plants have economic value that continues to grow significantly because of their benefits<sup>11</sup>. One benefit of the noni plant leaves is to be an insecticide, noni plant leaves extracts are effective as larvicidal and pupicidal of malaria vectors (*Anopheles stephensi*), dengue vectors (*Aedes aegypti*) and filarial vectors mosquitoes (*Culex quinguefasciatus*)<sup>12,13</sup>.

The use of plant essential oils as alternative insecticides is highly recommended because they have low toxicity to humans and are easily degraded in the environment<sup>6</sup>. Noni plants are also widely distributed and are easy to find in tropical areas and there are no scientific reports yet about the noni ethanolic leaf extract against German cockroaches. The present study is investigated the noni ethanolic leaf extract as toxicant, repellent and antifeedant to the German cockroach population.

#### **MATERIALS AND METHODS**

Provision of German cockroach populations: The experiments and provisions of the German cockroach population were conducted at the Research Laboratory of Animal Physiology, Department of Biology, Universitas Andalas, Indonesia. This research project was conducted from March, 2018-2019. German cockroach kept in a plastic container volume of 16 L and fed the cat food (Pedigree) and water ad libitum. Humidity ranges between 84-86%, temperature ranges between 24-28°C and photoperiod 12:12<sup>8</sup>. The population of German cockroach used is a standard population from Vector Control Research Unit (VCRU) School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia which is the WHO (World Health Organization) standard<sup>14</sup> and field populations (KRS-BDG) were collected from Bandung and reared in the laboratory. Cockroaches used are male cockroaches.

Provision of ethanol extract of noni leaves: The making of ethanol extract of noni leaves was conducted at the Natural Organic Chemistry Laboratory, Chemistry Department, Universitas Andalas, Indonesia. Noni plant leaves were collected as much as 6 kg. Samples were sorted and dried at room temperature (25-28°C) for seven days. Noni leaf was crushed with a grinding machine. Noni leaves powder as many as 800 grams was put into a bottle and macerated with 96% ethanol as much as 5 L and stirred until homogeneous. Bottles were closed and stored in a room without sunlight for five days. Ethanol extract from the macerated sample was filtered with filter paper to separate from the filtrate. The noni leaves pulp was re-macerated. The first and second filtrates were combined and thickened using a rotary evaporator at a temperature of 30-40°C until noni leaves extract was obtained.

#### Preliminary studies of noni ethanol extract concentration:

A preliminary test was conducted to determine the concentration that caused each German cockroach population's death gradually. Noni leaves extract tested were concentrations of 10, 20, 40 and 80%. Each concentration of noni leaves extract of 1 mL was put into a petri dish (9 cm) and levelled on the entire inner surface of the petri dish. Noni leaves extract was air-dried for 24 hrs. Vaseline petroleum oil was applied to the inner wall of a petri dish to prevent cockroaches escape during observation. Ten individuals of male German cockroaches in each population were put into a petri dish and observed the mortality until 100%.

**Contact toxicity test of noni ethanol extract against German cockroach:** The test using a petri dish (9 cm) and the concentration tested was obtained from a preliminary test that caused the cockroach death gradually, its concentrations were 2.5, 5, 10 and 20%. The test was to determine the Lethal Concentration (LC) and Lethal Time (LT) and followed a previously established procedure<sup>15</sup>. The mortality of cockroaches was observed every 24 hrs until its death reached 100%. The treatment replied five times.

Repellency test of noni ethanol extract residues against German cockroach: The repellency test method of Ferrero et al.<sup>16</sup> was adopted with some modifications. The repellency test of ethanol extract of noni leaves using a petri dish (15 cm) and filter paper with the same size and arranged into two equal parts. Ethanol extract of noni leaves concentrations tested was sub-lethal obtained from 10 and 30% LC<sub>50</sub> values of contact toxicity test, sublethal concentrations were 0.36 and 1.08%. Noni leaf extract of 1 mL was put into the first half of filter paper as treatment and ethanol in the second half of filter paper as control. The filter paper was dried for 60 min until a residue of  $0.056 \text{ mg cm}^{2-1}$  (0.36% concentration) and 0.169 mg cm $^{2-1}$ (1.08% concentration) was obtained. Both filter papers were put into a Petri dish. The inner walls of the Petri dish are smeared with petroleum oil. Male German cockroach as many as ten individuals were put into the middle of the Petri dish and observed every 24 until 72 hrs. The treatment replied five times.

Antifeedant test of ethanol extract of noni leaves to German cockroach: The concentration of ethanol extract of noni leaves used in this test was sub-lethal so it did not kill cockroaches during the treatment. The sub-lethal concentration used was 0.36% (10% of  $LC_{50}$  in the contact toxicity test). Noni leaves extract of 1 mL was put into a petri dish and air-dried for 24 hrs (residue 0.056 mg cm<sup>2 –1</sup>). Feed cockroaches (pedigree) each weighed 0.5 g and put into a petri dish. Ten individuals of male cockroaches were put into a petri dish. For control, only ethanol was dried in a petri dish. German cockroaches were fasted for three days before being treated and only given water. The observation was 72 hrs after the experiments and replied five times.

**Data analysis:** The mortality of German cockroach in contact toxicity test was analyzed by probit using a computer program<sup>17</sup> to determine lethal times values ( $LT_{50}$  and  $LT_{90}$ ). The criteria for the effectiveness of ethanol extract of noni leaves

against German cockroach as follows, very effective if lethal time 90% ( $LT_{90}$ ) reached  $\leq$ 24 hrs, effective if  $LT_{90}$  achieved >24 and  $\leq$ 48 hrs, less effective if  $LT_{90}$  achieved >48 and  $\leq$ 96 hrs, not effective if  $LT_{90}$  achieved >96 hrs after treatments<sup>8</sup>.

The repellency test values was analyzed used formula by Thavara *et al.*<sup>18</sup> were categorized as follows, class 0 (not repellent) if repellency value <0.1%, class I (very low repellent) if repellency value 0.1-20%, class II (low repellent) if repellency value 20.1-40%, class III (medium repellent) if repellency value 40.1-60%, class IV (high repellent) if repellency value 60.1-80% and class V (very high repellent) if repellency value 80.1-100%<sup>19</sup>.

Food consumption test was analyzed to obtain the antifeedant values used formula by Park *et al.*<sup>20</sup> were categorized as follows, (-) antifeedant activity was very weak or does not inhibit (0-40%), (+) antifeedant activity is weak (41-60%), (++) medium antifeedant activity (61-80%) and (+++) strong antifeedant activity (81-100%).

#### RESULTS

**German cockroach mortality in contact toxicity test:** The increase in Cockroach mortality was proportional to the amount of extract concentration and the duration of exposure. The highest mortality of cockroaches in the VCRU-WHO and KRS-BDG populations occurred at a concentration of 20% at 24 hrs observations. The mortality of cockroaches in 24 hrs observations has reached 96% in the VCRU-WHO population and 86% in the KRS-BDG population. Cockroach mortality increased until 72 hrs observation, the mortality of the VCRU-WHO population reached 100% and the KRS-BDG population only reached 98% (Table 1).

Both cockroach populations died gradually over 120 hrs of exposure. Noni leaves extract was only able to kill the cockroach population  $\leq$ 40% until 5 hrs. The mortality of cockroach increased until 24 hrs and the highest mortality was reached after 48 hrs. This indicates that noni leaves extract was able to reach the lethal target of cockroach populations at 24 until 48 hrs (Fig. 1).

The noni leaves extract concentration of 2.5-10% was not effective in killing cockroach populations. The extract was effective at a concentration of 20% in the KRS-BDG population and very effective in the VCRU-WHO population. The effectiveness of noni leaves extract was reached at 22.56 hrs in the VCRU-WHO population and 34.97 hrs in the KRS-BDG population. The time of noni leaves extract in killing the VCRU-WHO population was faster than the KRS-BDG population (Table 2).



Fig. 1: Mortality rate of German cockroach after ethanol extract of noni leaves exposure with a concentration of 20% (residue  $3.14 \text{ mg cm}^{2-1}$ ) using contact toxicity test during 120 hrs of observation

Table 1. Mortality of definian cockloach ( <i>blattena germanica</i> L.) after ethanor extract of norm leaves ( <i>morinda citinolia</i> L.) exposu
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				Mortality±SD (%)		
Population	Ν	Concentration (%)	Residue (mg cm <sup>2-1</sup> )	 24 hrs	48 hrs	72 hrs
VCRU-WHO	50	0	0.00	0.00±0.00	$0.00 \pm 0.00$	0.00±0.00
	50	2.5	0.39	24.00±11.40	46.00±5.50*	52.00±1.10*
	50	5	0.79	52.00±26.80	68.00±22.80*	78.00±1.80*
	50	10	1.57	72.00±8.40	84.00±11.40	84.00±8.90
	50	20	3.14	96.00±8.90	$100.00 \pm 0.00$	$100.00 \pm 0.00$
KRS-BDG	50	0	0.00	0.00±0.00	$0.00 \pm 0.00$	0.00±0.00
	50	2.5	0.39	14.45±7.88	18.18±6.18*	22.34±11.28*
	50	5	0.79	21.87±13.54	30.41±5.70*	38.99±7.53*
	50	10	1.57	38.28±23.14	$62.00 \pm 22.80$	70.00±18.70
	50	20	3.14	86.00±16.70	98.00±4.50	98.00±4.50

VCRU-WHO: Standard German cockroach population, KRS-BDG: Field German cockroach population, N: Total individual cockroach, SD: Standard deviation, \*Number followed by the sign (\*) is significantly different between standard (VCRU-WHO) and field population (KRS-BDG) at the same concentration and time using the Independent sample t-test 5%

Table 2: Effectiveness of ethanol extract of noni leaves a concentration of 20% (residue 3.14 mg cm<sup>2-1</sup>) using  $LT_{s0}$  and  $LT_{90}$  values

Populations	LT <sub>so</sub> (hrs)	LT <sub>oo</sub> (hrs)	Slope±SE	Criteria*
VCRU-WHO	18.63	22.56	0.23±0.03	Very effective
KRS-BDG	17.62	34.97	0.17±0.02	Effective

VCRU-WHO: Standard German cockroach population, KRS-BDG: Field German cockroach population, LT<sub>50/90</sub>: Time to kill 50 or 90% of the German cockroach population SE: Standard error, \*Effectiveness of bioinsecticide criteria<sup>8</sup>

Table 3: Repellency values of ethanol extract (*Morinda citrifolia*L) concentration of 0.36% (residue 0.056 mg cm<sup>2-1</sup>) and 1.08% (residue 0.169 mg cm<sup>2-1</sup>) against German cockroach (*Blattella germanica*L)

	Population	Repellency values (%)			
Concentration		24 hrs	48 hrs	72 hrs	Repellent criteria*
0.36 (%)	VCRU-WHO	82.00±19.24	84.00±16.73	78.00±19.24	Very high
	KRS-BDG	82.00±17.89	82.00±26.83	82.00±16.43	Very high
1.08 (%)	VCRU-WHO	96.00±5.48	96.00±8.94	88.00±10.95	Very high
	KRS-BDG	96.00±5.48	96.00±5.48	92.00±8.37	Very high

VCRU-WHO: Standard German cockroach population, KRS-BDG: Field German cockroach population, SD: Standard deviation, \*Repellent criteria at 24 hrs<sup>19</sup>

**Repellency of noni ethanolic leaf extract:** Noni leaves extract with sub-lethal concentration of 0.36% (residue  $0.056 \text{ mg cm}^{2-1}$ ) and 1.08% (residue  $0.169 \text{ mg cm}^{2-1}$ ) showed repellent activities of 82% in VCRU-WHO population and 96%

in KRS-BDG population. Noni leaves extract was very high repellent against German cockroaches (Table 3).

The first response of cockroaches with residue extracted in filter paper inside a Petri dish (treatment zone) was

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COCKTOACTI ( <i>Biatteria germanica</i> L.)							
		Amount of food consumpt	Amount of food consumption (mg) $\pm$ SD				
Population	Ν	Without extract	With extract	Δ	Criteria*		
VCRU-WHO	50	167.60±30.44	173.40±20.06	(+) 5.8	Negative		
KRS-BDG	50	$125.60 \pm 23.64$	147.00±30.81	(+) 21.4	Negative		
VCDLL WILLO, Chain al	مسار مستعمية ممارسم ما	a manufation KDC DDC Field Common as	duna a ala manuta si ana CD. Csamala nalada d	يرامحم المريانية والمعمالية والمعمانية	a a ala CD. Caa a al a u al		

Table 4: Effect of ethanol extract of noni leaves (*Morinda citrifolia* L.) sub-lethal concentration of 0.36% (Residue 0.056 mg cm<sup>2-1</sup>) on food consumption of German cockroach (*Blattella germanica* L.)

VCRU-WHO: Standard German cockroach population, KRS-BDG: Field German cockroach population, SD: Standard deviation, N: Total individual cockroach, SD: Standard deviation, Δ: Changes in the amount of food consumed, the sign (+) indicates an increase in the amount of food consumed after extract exposure, \*Antifeedant criteria<sup>20</sup>

erratically movements. The cockroach movement slows down and silences in areas that are not given extract (control zone). Most of the cockroaches in the treatment zone move to the control zone then stayed there until the last observation.

**Antifeedant activity of noni ethanolic leaf extract:** The German cockroach food consumption increased after exposed to sub-lethal concentrations of noni leaves extract of 0.36% (residue 0.056 mg cm<sup>2-1</sup>). The increase in food consumption of the VCRU-WHO population was 5.8 mg while the KRS-BDG population was 21.4 mg. Noni leaves extract has no potential to inhibit the food consumption of German cockroaches (Table 4).

#### DISCUSSIONS

Noni leaves extract concentration of 20% is very effective in killing German cockroaches in standard population and effective in killing field population. Noni leaf extract is also effective as larvicide and pupicide in vector mosquitoes such as *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*<sup>21</sup>.

The mortality of the standard population is higher than the field population because the standard population is susceptible and has never been exposed to insecticides. The field population has been resistant to several synthetic insecticides. Insects resistant can detoxify toxic compounds in insecticides because their detoxification enzyme activity is higher than susceptible insects<sup>4</sup>.

Insects that have been resistant to synthetic insecticides can be controlled by bioinsecticides because the metabolism of both insecticides in the insect body is different. Synthetic insecticides attack the acetylcholine-esterase system which is a specific target site that has mutated in resistant insects<sup>22</sup>. Bioinsecticides have various modes of action depends on the content of secondary metabolites contained therein, most of the bioinsecticide compounds attack the octopamine system in the nervous system of insects<sup>6</sup>. The different modes of action of both insecticides make bioinsecticide an alternative in controlling German cockroaches that are resistant to synthetic insecticides. The toxic compounds enter the body of cockroaches is through cuticles in the contact toxicity test. The contact between the cockroach cuticle and the secondary metabolite of noni leaves extract causes the cockroach hyperactive, paralyzed and death. Kostyukovsky *et al.*<sup>23</sup> also reported insects exposed to toxic compounds of essential oils will become tremors, hyperactivity, paralysis to death.

The repellence level of noni leaves extract with two sub-lethal concentrations tested on German cockroaches was very high. The higher sub-lethal concentration is also directly proportional to the repellence value. After 72 hrs of observation, the repellence value of noni leaves extracts only decreased by 82-78% at a concentration of 0.36 and 96-88% at a concentration of 1.08%.

Noni leaves extract is volatile and has a distinctive odour that is not preferred by German cockroaches. Insects can only detect odours when the protein odour receptor bind to volatile chemicals derived from plant secondary metabolites if an unpleasant odour from a volatile chemical detects by an insect, the insect will stay away from it<sup>24</sup>.

Volatile compounds of plant extract can be a repellent for the presence of German cockroaches. Secondary metabolite compounds of citronella grass extract also have a very high repellence value of 79.63-96.30%<sup>7</sup> and lemongrass extract have a high repellence value of 100% against German cockroach<sup>8</sup>.

Secondary metabolites of essential oils that can be repellent for insects are alkaloids, saponins and triterpenoids. Active compounds such as alkaloids, terpenoids, steroids, glycosides and some sterols have potential as repellent agents<sup>25</sup>. The safrole, isosafrole, methyl eugenol =  $\alpha$  pinene, eugenol, isoeugenol also repellent to *Periplaneta americana*<sup>26</sup>. The responses of German cockroach if the smell volatile compounds of noni leave extract are rapid and erratic movements. The movement then slows down and cockroaches will keep quiet in an area without extract (control). Most of the cockroaches that were initially in an area with extract (treatment zone) will move to the area without extract (control zone) until the end of observation. The response of cockroaches that make contact with insecticides is to explore the areas with insecticide exposure that show

signs of irritability<sup>27</sup>. Noni leaves extract contains large amounts of flavonoids that smell sharp and area repellent for the presence of German cockroaches. The total flavonoid content in Noni leaves is 254 mg/100 g<sup>28</sup>. The volatile component of the noni leaves extract can act as a repellent agent for the presence of German cockroaches.

Noni leaves extract did not inhibit the food consumption of cockroaches but it is increased after exposure with noni leaves extract concentration of 0.36% (residue 0.056 mg cm<sup>2-1</sup>). We presume the increased food consumption of cockroaches is related to the fitness cost of cockroaches if exposed to the toxicant. Jensen et al.29 found lower survival to the German cockroach in the insecticideselected population, which was exacerbated by poor nutrition. The amount of food consumption in the standard population is higher than the field population in treatment with or without noni leaves extract. However, the amount of food consumed by both populations increased after the noni leaves extract was exposed. The increase in the amount of food consumption occurs due to the adaptation of cockroaches to the toxic compounds that enter and accumulate in the body during observation. If the insect is exposed to a toxic compound then the insect will adapt by detoxifying that compound to become inactive<sup>30</sup>.

The increase in the amount of food consumption in the field population is four times higher than the standard population. The field population is resistant to the insecticide which has developed a detoxification mechanism for toxic compounds better than the standard population. Panini *et al.*<sup>31</sup> reported the resistant insects metabolize the insecticide faster because as a result of increased transcription or gene amplification, they have forms of the enzyme with a higher catalytic rate or greater amounts of the enzymes than susceptible insects.

Noni leaves extract has the potential to develop as an insecticide and repellent source against German cockroach that has been resistant to synthetic insecticides. Further research is needed to find out the toxic and volatile compounds contains in noni leaves extract and the physiological mechanism that compounds in the cockroach body.

#### CONCLUSION

The ethanol extract of noni leaves (*Morinda citrifolia* L.) is effective to control German cockroach populations and can be used as a repellent. For further research, the active compound of noni extract could be investigated to develop it as bioinsecticides to control populations of German cockroaches that have been resistant to synthetic insecticides in a tropical area.

#### SIGNIFICANCE STATEMENT

This study will help the researchers to investigate the benefit of tropical plants such as noni as a bioinsecticide. The noni leaves could be solutions to control the German cockroach's population that resistant to the general insecticides (commercial insecticides) that are used by people. Thus, a new strategy on control management of resistant German cockroach populations may be arrived at. The natural insecticide from noni leaves is recommended to be toxicant and repellent.

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