

Original Article**Evaluation of *Piper aduncum* Linn. Essential Oil (Fam:Piperaceae) against *Periplaneta americana* (L.)**

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Abstract**Background:** The efficacy of *Piper aduncum* essential oil was evaluated against *Periplaneta americana* adults and nymphs in the laboratory.**Methods:** The plant essential oil at varying concentrations ranging between 10,000 to 80,000 ppm were placed inside glass beakers, rolled horizontally to ensure the essential oil covers all sides of the beakers and exposed to adults and nymphs of *P. americana*. Resigen (R) 1ppm was used as positive control and distilled water as negative control. The LT50 and LT90 was obtained using Log Probit programme.**Results:** Exposure of essential oil to females *P. americana* at concentrations between 10,000 to 80,000 ppm indicated the LT50 and LT90 values between 5.31 h-189.19 h and 14.90 h-2105.31 h, respectively. Treatment with the same concentrations against males *P. americana*, the LT50 and LT90 were 2.08 h-181.73 h and 5.4 h-8460.51 h, respectively. Treatment against the nymphal stage with the same range of concentrations indicated the LT50 and LT 90 of 4.68 h-381.02 h and 28.71 h-5313.36 h, respectively. The nymphs and males were more susceptible than the females cockroaches. Treatment with Resigen (R) at 1ppm indicated much lower LT 50 and LT 90 values of 2.54 h-9.47 h for the females, 1.47 h-4.22 h for the males and 4.69 h-8.92 h for the nymphs. The negative control indicated no mortality for all stages of the cockroach.**Conclusion:** *Piper aduncum* essential oil can be used as an alternative natural product for controlling the cockroach *Periplaneta americana*.**Keywords:** *Piper aduncum essential oil*, *Periplaneta americana*, *Adults*, *Nymphs***Introduction**

Periplaneta americana or also known as the American cockroach is an urban cockroach found in places such as homes and shops (Lee and Lee 2000). It is the mechanical vector to a few pathogens that can cause disease such as food poisoning, typhoid, pneumonia and asthma (Brown 1975). Cockroach also can cause destruction of belongings such as biting clothes and books. Besides that, cockroaches also secrete a mixture of xanturenic acid, kiturenic acid and 8-hydroxycuinalic acid, which are tryptophan derivatives that have mutagenic and carcinogenic properties (Mullins &

Cochan 1973). Research using plant extracts for controlling cockroaches is quite limited. Recently, the essential oil of catnip (*Ne-peta cataria* L.) was reported to have repellency against adult male *B. germanica* (L.). Thava et al. (2007) studied seven commercial essential oils for repellency against cockroaches and found *Citrus hystix* exhibited complete repellency against *P. americana* and *B. germanica*.

The objective of this study was to evaluate the residual effect of *Piper aduncum* essential oil (extract) against adults and nymphal stages of *Periplaneta americana* (L.) in the laboratory.

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Materials and Methods

The cockroaches were bred in the insectarium of the Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia. The *P. aduncum* extract was obtained through the hydro-distillation method using the Clavenger apparatus. The method of testing use was the touch insecticide method recommended by the WHO (1975) to test susceptibility or resistance of the cockroach towards a certain insecticide. 70 cockroaches were fasted for 24 h. A serial dilution of *P. aduncum* extract with concentrations of 10000 ppm, 20000 ppm, 40000 ppm, 60000 ppm and 80000 ppm were prepared and 7 ml of each concentration was placed into separate 2000 ml beakers. The beakers were then rolled horizontally to ensure that the extract covered all the sides. The beakers were then left overnight to allow the extract to dry.

The positive control used in this study was Resigen[®] and the negative control used was distilled water. The inner area of the mouth of the beaker was covered with talcum powder mixed with 70% alcohol to prevent the cockroaches from escaping. Ten cockroaches were placed inside each beaker and observed at time intervals of 1 h, 4 h, 8 h, 24 h, 72 h, and 96 h for knockdown or mortality. This test was repeated six times. The LT_{50} and LT_{90} were obtained using the Log-probit programme (Raymond 1985). This method was conducted using adult male and female cockroaches as well as nymphs.

The percentage of average mortality was counted and compared between *Piper aduncum* essential oil, Resigen[®] and the distilled water as control using SPSS statistical analysis to determine the significant differences between the effectiveness of the plant extract and Resigen[®].

Results

Female cockroaches indicated LT_{50} and LT_{90} values of 5.31-189.19h and 14.90-2105.31 h, male cockroaches showed LT_{50} and LT_{90} of 2.08-181.73 h and 28.71-5313.36 h and the nymphal stage with LT_{50} and LT_{90} of 4.68-381.02 h and 28.71-5313.36 h respectively (Tables 1-3). Resigen (R) indicated lower LT_{50} and LT_{90} values of 2.54 h and 9.47 h for the females, 1.47 h and 4.22 h for the males and 4.69 h and 8.92 h for the nymphs respectively. No mortality occurred for the negative control (Tables 1-3). Comparing the effectiveness of *P. aduncum* essential oil with Resigen (R), there was a significant difference ($P < 0.05$) for the females *P. americana* between Resigen (R) at 1 ppm and *P. aduncum* essential oil of 10000 ppm at all time intervals; ppm at the interval of 1 h, 4 h, 8 h and 24 h and non-significant ($P > 0.05$) after 48 h; 40000 ppm at the interval of 4 h and non-significant ($P > 0.05$) after 8 h exposure respectively. Male cockroaches had the lowest LT_{50} and LT_{90} followed by the female cockroaches and then the nymphs.

For the comparison of the effectiveness of the *P. aduncum* essential oil with Resigen[®] 1 ppm for females *P. americana* there was a significant difference ($P < 0.05$) at the concentration of 10 000 ppm at all time intervals, 20 000 ppm at the interval of 1 h, 4 h, 8 h and 24 h and non significant ($P > 0.05$) after 48 h exposure, 40 000 ppm at interval of 1 h, 4 h and 8 h and non significant ($P > 0.05$) after 24 h exposure, 60000 ppm at 1 h and 4 h and non significant ($P > 0.05$) after 8 h exposure, 80000 ppm at 1 h and 4 h and non significant ($P > 0.05$) after 8 h exposure respectively.

For the males *P. americana* there was a significant difference ($P < 0.05$) between Resigen (R) 1 ppm and *P. aduncum* essential oil at 10000 ppm at all time intervals, 20000 ppm at time intervals between 1 h to 48 h and non significant ($P > 0.05$) after 72 h, 40000 ppm to 80000 ppm at 1 h and non significant ($P > 0.05$)

at 4 h exposure. The nymphs indicated a significant difference between Resigen (R) 1 ppm and *P. aduncum* essential oil at 10000-20000 ppm at all time intervals, 40000 ppm at 1-8 h and non significant ($P > 0.05$) at 24 h exposure,

60000 ppm at 1-8 h and non significant ($P > 0.05$) at 24 h exposure. At 80000 ppm showed no significant difference ($P > 0.05$) between *P. aduncum* essential oil and Resigen (R) 1 PPM at all time intervals.

Table 1. LT_{50} and LT_{90} of *P. americana* females treated with *Piper aduncum* essential oil at various concentrations

<i>Piper aduncum</i> concentrations (ppm)	Female <i>P. americana</i> L		
	LT_{50} (H) (CI 95%)	LT_{90} (H) (CI 95%)	Slope± Standard Error
10 000	189.19 (118.92–444.60)	2105.31 (755.60–16239.51)	1.22 ± 0.21
20 000	24.50 (20.06–29.82)	136.26 (100.68–203.71)	1.72±0.15
40 000	8.01 (4.73–13.37)	25.93 (12.02–61.04)	2.51±0.47
60 000	6.83 (5.80– 8.00)	17.65 (14.26–23.79)	3.11±0.34
80 000	5.31 (4.44–6.28)	14.90 (11.94–20.20)	2.86±0.30
Resigen® 1Ppm	2.54 (1.99–3.13)	9.47 (7.27–13.69)	2.24±0.25
Negative control	0	0	0

Table 2. LT_{50} and LT_{90} of *P. americana* males treated with *Piper aduncum* essential oil at various concentrations

<i>Piper aduncum</i> concentrations (ppm)	Male <i>P. americana</i> L		
	LT_{50} (H) (CI 95%)	LT_{90} (H) (CI 95%)	Slope±Standard Error
10 000	181.73 (100.21–506.34)	8460.51 (2018.82–126526.80)	0.77±0.12
20 000	8.91 (4.86–16.13)	69.39 (29.55–170.17)	1.44± 0.23
40 000	3.17 (0.80–12.58)	10.83 (1.70–69.16)	2.40±1.27
60 000	2.64 (2.22–3.07)	5.02 (4.27–6.18)	4.60±0.55
80 000	2.08 (1.72–2.48)	5.14 (4.18–6.80)	3.27±0.36
Resigen® 1 ppm	1.47 (1.15– 1.80)	4.22 (3.33–5.91)	2.80±0.36
Negative control	0	0	0

Table 3. LT₅₀ and LT₉₀ of *P. americana* nymphs treated with *Piper aduncum* essential oil at various concentrations

<i>Piper aduncum</i> concentrations (ppm)	<i>P. americana</i> L Nymphs		
	LT ₅₀ (h) (CI 95%)	LT ₉₀ (h) (CI 95%)	Slope±Standard Error
10 000	381.02 (188.21–1925.04)	5313.36 (1252.66–172882.10)	1.12±0.24
20 000	52.56 (42.40–68.98)	360.66 (225.29–742.01)	1.54±0.18
40 000	15.36 (9.56–68.97)	59.50 (31.35–115.47)	2.18±0.35
60 000	10.08 (8.25–12.13)	42.54 (33.75–56.68)	2.05±0.16
80 000	4.68 (3.58–5.91)	28.71 (21.76–40.66)	1.63±0.14
Resigen® 1 Ppm	4.69 (4.05–5.31)	8.92 (7.54–11.81)	4.60±0.73
Negative control	0	0	0

Discussion

Plant extracts have been used worldwide as an alternative method to control pests. Thavara et al. (2007) using *Citrus hystrix* DC exhibited complete repellency (100%) against *Periplaneta americana* (L.) and *B. germanica* (L.) and 87.5% repellency on *Neostylopyga rhombifolia* (Stoll) in the laboratory. For field trial in Thailand, *C.hystrix* essential oil formulated as 20% active ingredient in ethanol and some additives provided satisfactory repellency of up to 80% reduction in cockroaches, mostly *P.americana* and *N. rhombifolia* with a residual effect lasting a week after treatment. Peterson et al. (2002) using essential oil of catnip (*Nepeta cataria* L.) isomers of nepetalactone tested the repellent activity to *B.germanica*. The isomer *E, Z*-Nepetalactone was the most effective and being significantly more active than DEET. Sanchez-Chopa et al. (2006) tested the *Schinus molle* var.*areira* (L.) essential oil against *B. germanica* in Argentina. The essential oil extracted from leaves and fruits tested using filter papers at 176. 70 and 35.35 mg/cm² and

compared with DEET. The leaf extract of *S. molle* var. *areira* essential oil did not show repellency to *B. germanica*. However, the fruits extracted showed repellency to *B. germanica* and did not differ from DEET at higher doses.

Jung et al. (2007) evaluated in Korea the hexane fraction of extracts from seeds of *Myristica fragrans* against *B. germanica* (L.) and found that the (1S)-(-)-β-pinene (0.06mg/ cm²) was the most toxic insecticide and comparable to permethin (0.05 mg/cm²). Ngoh et al. (1999) studied the insecticidal activity and repellent properties of nine volatile constituents of essential oils against *P.americana*. Contact and fumigant toxicities to adult females and repellency to nymphs were determined. The decreasing order of knockdown activity via contact was methyl-eugenol> isosafrole= eugenol> safrole. The killing effect via contact was in the order eugenol= methyl-euge-nol= isosafrole> safrole. Fumigant toxicity was only observed for safrole and isosafrole, with safrole being more potent. The decreasing order of repellency to nymphs was safrole> isosafrole> methyl-

eugenol= α -pinene > eugenol > isoeugenol. According to Othman (2006), *Piper aduncum* major constituents include (E)- β -osimena, trans-kariofilena, (z)- β -osimena, β -pinena, α -pinena, germakrena-D, piperitona, γ -terpinena and limonena. However each individual constituent had not been tested to *P. americana*.

The LT50 and LT90 for males were the lowest followed by nymphs and lastly females. Similar finding by Koehler et. al (1993) proves that male cockroaches have the highest susceptibility. This could be due to the differences in metabolic rate found in the adult male and female and the nymphs. The higher metabolism would lead to faster excretion of the toxic substance from the body (Koehler et. al 1993). It can be said that the effectiveness of the *P. aduncum* essential oil is equal with that of Resigen® only at very high concentrations.

The present study indicated that *P. aduncum* essential oil had toxic effect on both adult females and males and nymphs of *P. americana* but at high concentrations compared to Resigen®. Due to large availability of the plant in tropical countries in Southeast Asia, this could be an alternative for controlling cockroaches.

In conclusion, *P. aduncum* essential oil can be use as a pesticide against *P. americana*. The essential oil at higher concentrations had nearly the same effectiveness as Resigen® in killing the cockroach.

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