Original Article

Evaluation of Bifenthrin and *Acorus calamus* Linn. Extract against *Aedes aegypti* L. and *Aedes albopictus* (Skuse)

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Abstract

Background: Bifenthrin and *Acorus calamus* Linn extract were evaluated against dengue vectors in the laboratory.
Methods: Both Bifenthrin and *Acorus calamus* Linn crude hexane extract were bioassayed against the adults and larval stages of dengue vectors *Aedes aegypti* L. and *Aedes albopictus*(Skuse) in the laboratory.
Results: The *A. calamus* crude hexane extract exhibited a larvicidal activity against 4th-instar *Ae. aegypti* larvae with LC_{50} and LC_{90} values of 0.4418 and 11.3935 ppm respectively. The plant crude extract exhibited against *Ae. albopictus* larvae with a higher LC_{50} and LC_{90} values of 21.2555 ppm and 36.1061 ppm, respectively. There was a significant difference on the effect of *A. calamus* extract on both *Aedes* spp. Larvae ($P< 0.05$). However, bifenthrin showed a significant difference on larvicidal effect to that of *A. calamus* hexane extract on both *Aedes* spp ($P< 0.05$). In testing the adulticidal activity, this plant extract exhibited the LC_{50} and LC_{90} values of 17.4075 and 252.9458 ppm against *Ae .aegypti* and a higher LC_{50} and LC_{90} values of 43.9952 and 446.1365 ppm respectively on *Ae. albopictus*. There was no significant difference on the effect of *A. calamus* extract on both *Aedes* spp adults ($P> 0.05$).
Conclusion: Bifenthrin however showed a significant difference on both *Aedes* spp adults ($P< 0.05$). With the wide availability of *A. calamus* in Malaysia, it could be utilized for controlling dengue vectors.

Keywords: *Acorus calamus*, Hexane extract, *Aedes aegypti*, *Aedes albopictus*, Bifenthrin

Introduction

In Southeast Asia, *Aedes albopictus* (Skuse) has been incriminated as a secondary vector of dengue fever and *Ae. aegypti* (Linn.) as the principal vector of dengue viruses(Russell et al. 1969, Chan et al. 1971, Jumali et al.1979, Harinasuta 1984). The dengue viruses may produce occasional fatal diseases, usually among children (Rudnick and Chan 1965, Harinasuta 1984).

Chemicals derived from plants offer promise in future mosquito control programs (Sukumar et al.1991).The search for new environmentally safe, target specific insecticides is being conducted all over the world. To find new modes of action and to develop active agents based on natural products, efforts are being made to isolate, screen and develop phytochemicals possessing pesticidal activity (Mulla and Su, 1999).

There is an urgent need for economically feasible natural and biodegradable compound for the control of mosquito vector. Plants may be a source of alternative agent to replace the more expensive synthetic insecticides for mosquito control.

Thus, the objective of this study was to compare the efficacy of the plant extract *Acorus calamus* Linn (Fam. Araceae) compared to the synthetic pyretroid bifenthrin 80 SC against *Ae. aegypti* and *Ae. albopictus* in the laboratory.

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Material and Methods

Extraction

The rhizome of *Acorus calamus* was grinded to smaller size to enhance extraction yield and was extracted using the Soxhlet apparatus for at least 20 h with hexane. The filtrate was then evaporated to dryness under vacuum pressure. The crude hexane extract of *A. calamus* was bioassayed against the 4th instar larvae and adults of *Ae. aegypti* and *Ae. albopictus.*

Insecticide

The insecticide bifenthrin 80SC was supplied by FMC PT Bina Guna Kimia, Semarang, Indonesia.

Bioassay against adults *Aedes aegypti* and *Aedes albopictus*

The bioassay was conducted according to WHO standard procedures (WHO 1981a) with some modifications. Twenty adult mosquitoes of *Ae. aegypti* and *Ae. albopictus* laboratory colonies, 2-5 d old were exposed for 1 h to filter paper (15x16cm) impregnated with varying concentrations of *A. calamus* extract and bifenthrin 80SC in the range of 1.0 ppm to 8.0 ppm for testing against *Ae. albopictus* and 0.3125 ppm to 5.0 ppm for *Ae. aegypti*. Each concentration was diluted in 0. 1% acetone for bifenthrin and Control impregnated papers; the stock solution for *A. calamus* extract was prepared by diluting the crude extract with Tween 20 and 0.1% hexane. The knockdown was recorded for each 1, 3, 5, 10, 20, 30 and 60 min. All mosquitoes were then transferred to holding tubes with clean filter papers. The mosquitoes were fed with cotton pads soaked in 10% sucrose solution. Each experiment conducted was in duplicate. After 24 h the mortality rate of the adult mosquitoes were recorded. The experiment was repeated three times and analyzed by using Probit Analysis Program (Raymond, 1985) and SPSS Software.

Bioassay against 4th instar larvae of *Ae. aegypti* and *Ae. albopictus*

The bioassay was conducted according to WHO (1981b) with some modification. Twenty-five *Ae. aegypti* and *Ae. albopictus* larvae were exposed in each of 600 ml glass beakers containing 250 ml of prepared *A. calamus* extract and bifenthrin 80 SC in varying concentrations. The *A. calamus* and control were diluted in 0.1% hexane; bifenthrin 80SC and control were diluted in 0.1% acetone. After 24 h the mortality of the larvae were recorded. The experiment was conducted in duplicate and repeated three times. If the control mortality was between 5% and 20%, the percentage mortalities were corrected by Abbott's formula:

$$\frac{\%\ \text{test mortality} - \%\text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

LC_{50} and LC_{90} values were determined by Probit Analysis (Raymond 1985) and SPSS Software.

Results

Table 1 indicated the LC_{50} and LC_{90} values of bifenthrin on 4th-instar *Ae. aegypti* larvae of 0.0034 and 0.0101 ppm; *Ae. albopictus* with LC_{50} and LC_{90} values of 0.1360 and 0.5110 ppm, respectively. There was a significant difference on the effect of bifenthrin to both *Ae.* spp. ($P< 0.05$).The values of LC_{50} and LC_{90} of *A. calamus* extract on 4th instar *Ae. aegypti* larvae were 4.4418 and 11.3935 ppm and *Ae. albopictus* with LC_{50} and LC_{90} values of 3.1330 and 21.2555 ppm respectively. There was also a significant difference on the effect of *A. calamus* extract on both *Ae. aegypti* and *Ae. albopictus* larvae ($P< 0.05$). Bifenthrin showed a significant difference on larvicidal effect to that of *A. calamus* extract to both *Ae.* spp. ($P< 0.05$).

Table 2 indicated the LC_{50} and LC_{90} values of bifenthrin on *Ae. aegypti* adults of

0.7020 and 2.3287 ppm; *Ae. albopictus* with LC_{50} and LC_{90} values of 2.4267 and 4.2532 ppm respectively. There is a significant difference on the effect of bifenthrin to both *Ae.* spp adults ($P< 0.05$). The crude extract of *A. calamus* indicated the LC_{50} and LC_{90} values of 17.4075 and 252.9458 ppm on *Ae. aegypti*; and 43.9952 and 446.1365 ppm on *Ae. albopictus*, respectively. However, there was no significant difference on the effect of *A. calamus* extract on both *Ae.* spp. ($P> 0.05$). Thus, a higher dosage is needed for *A. calamus* extract to be effective as an adulticide compared to bifenthrin.

Table 1. The LC_{50} and LC_{90} of bifenthrin and *Acorus calamus* extract on *Aedes aegypti* and *Ae. albopictus* 4th-instar larvae in the laboratory

Treatment	LC_{50} (ppm)	LC_{90} (ppm)	Slope±SE	LC_{50} (ppm)	LC_{90} (ppm)	Slope±SE
	Aedes aegypti			*Aedes albopictus*		
Bifenthrin	0.0034	0.0101	2.6937±0.2575	0.1360	0.5110	2.2290±0.2642
Acorus calamus extract	4.4418	11.3935	3.1330±0.3571	21.2555	36.1061	5.5701±0.6731

Table 2. The LC_{50} and LC_{90} of bifenthrin and *Acorus calamus* extract on *Aedes aegypti* and *Ae. albopictus* adults in the laboratory

Treatment	LC_{50} (ppm)	LC_{90} (ppm)	Slope±SE	LC_{50} (ppm)	LC_{90} (ppm)	Slope±SE
	Aedes aegypti			*Aedes albopictus*		
Bifenthrin	0.7020	2.3287	2.4610±0.2994	2.4267	4.2532	5.2596±0.7134
Acorus calamus extract	14.4075	252.9458	1.1028±0.1212	43.9952	446.1365	1.2742±0.1303

Discussion

Hidayatulfathi et al. 2004 evaluated methanol extracts of some Malaysian plants for larvacidal activities against mosquitoes. The methanol extract of *A. calamus* Linn. showed a high degree of toxicity to all mosquito species of *Anopheles maculatus* Theobald, *Culex quinquefasciatus* Say, *Ae. aegypti* (L.) and *Ae. albopictus* (Skuse) with LC_{50} of 39.15-58.29 µg/ml. Hidayatulfathi et al. (2005) using the hexane fraction showed the highest larvicidal effect on *Ae. aegypti* 4th instar larvae with LC_{50} value of 1.88 ppm and the LC_{90} value of 10.76 ppm respectively. The present study using hexane fraction indicated LC_{50} of 0.4418 ppm and LC_{90} value of 11.3935, respectively against *Ae. aegypti* 4th instar larvae, while the LC_{50} and LC_{90} values on *Ae. albopictus* 4th instar larvae were 21.2555 and 36.1061 ppm, respectively. Choochote et al. (2005) found that the volatile oil of *Curcuma aromatica* (Fam: Zingiberaceae) possessed a significantly higher larvicidal activity against 4th instar larvae of *Ae. aegypti* than that of hexane extracts with LC_{50} values of 36.30 and 57.15 ppm, respectively. Choochote et al. (2004) also found that *Apium graveolans* (Fam. Apiaceae) seed extract possessed larvicidal activity against 4th instar *Ae. aegypti* larvae with LD_{50} and LD_{95} values of 81.0 and 176.8 mg/L (ppm), respectively. Thomas et al. (2004) also conducted the laboratory bioassay of *Ipomoea*

cairica (Fam. Convolvulaceae) essential oil against *Ae. aegypti* larvae and found that the LC_{50} and LC_{90} values were 22.3 and 92.7 ppm, respectively. Thus, the present study indicated that *A. calamus* extract is more effective than extracts of *Curcuma aromatica, Apium graveolans* and *Ipomoea cairiaca* as a larvicide against *Ae. aegypti* 4th instar larvae evaluated by the above authors.

Hidayatulfathi et al. (2004) found that using the hexane fraction from methanol extract of *A. calamus* rhizome against *Ae. aegypti* adults was the most effective, exhibiting LC_{50} and LC_{90} values of 0.04 mg/cm^2 and 0.09 mg/cm^2, respectively. For *Litsea elliptica* the methanol fraction also displayed good adulticidal property with LC_{50} and LC_{90} values of 0.11 mg/cm^2 and 6.08 mg/cm^2, respectively. Sulaiman et al. (2005) evaluated *A. calamus* extract and bifenthrin in the field at high rise flats in Kuala Lumpur. The impact of both plant extract and insecticide on field populations of *Ae. aegypti* and *Ae. albopictus* was monitored weekly. *A. calamus* extract showed adulticidal effect causing 93.9% (inside flats) to 94.9% (outside flats) adult *Ae. aegypti* mortalities compared to bifenthrin with 98.3% (inside flats) and 99.1% (outside flats) adult mortalities. In the control group, the adults of *Ae. aegypti* mortalities were 19.2% (inside flats) and 18.2% (outside flats), respectively 24 h after ULV spraying. Choochote et al. (2004) evaluated the adulticidal efficacy of the crude seed extract of *Apium graveolans* against *Ae. aegypti* and found a slightly adulticidal potency of this extract with LD_{50} and LD_{95} values of 6.6 and 66.4 mg/cm², respectively. Choochote et al. (2005) also tested for adulticidal activity of hexane-extracted *Curcuma aromatica* against *Ae. aegypti* females, it was found to be slightly more effective with the LC_{50} value of 1.60µg/mg compared to volatile oil with LC_{50} value of 2.86 µg/mg, respectively.

In conclusion, although bifenthrin has more toxic effect on *Ae.* spp larvae and adults than *Acorus calamus* extract, but in search for botanical insecticide, *A. calamus* extract could be utilized for dengue vector control. The wide availability of this plant in tropical area could be exploited for vector control usage.

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