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Larvicidal Properties of the Essential Oils of Some Malaysian Plants on Three Vector Mosquitoes

Ibrahim Jantan, Zaridah Mohd Zaki and Rohani Ahmad*

*Forest Research Institute Malaysia,
Kepong, 52109 Kuala Lumpur, Malaysia*

**Institute for Medical Research Malaysia,
50586 Kuala Lumpur, Malaysia*

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ABSTRAK

Ciri toksik minyak pati dari lapan belas spesies tumbuhan keatas instar keempat larva tiga nyamuk vektor (*Anopheles maculatus, Aedes aegypti* dan *Culex quinquefasciatus*) telah dikaji. Minyak pati daripada daun *Litsea elliptica* adalah yang paling efektif, menunjukkan LC_{50} 13.61 μg ml^{-1} keatas *An. maculatus*, 16.01 μg ml^{-1} ke atas *Ae. aegypti* dan 14.63 μg ml^{-1} ke atas *C. quinquefasciatus*. Minyak-minyak pati dari species yang lain juga menunjukkan nilai toksik yang tinggi, dengan LC_{50} berkisar dari 16.58 ke 161.12 μg ml^{-1}.

ABSTRACT

The toxicity of the essential oils of eighteen Malaysian plants on the 4th-instar larvae of three vector mosquitoes (*Anopheles maculatus, Aedes aegypti* and *Culex quinquefasciatus*) was studied. The leaf oil of *Litsea elliptica* was the most effective, exhibiting LC_{50} of 13.61 μg ml^{-1} for *An. maculatus*, 16.01 μg ml^{-1} for *Ae. aegypti* and 14.63 μg ml^{-1} for *C. quinquefasciatus*. The essential oils from the other species were also toxic with LC_{50} of 16.58 – 161.12 μg ml^{-1}.

Keywords: essential oils, *Litsea elliptica*, bioassay, larvicidal activities, toxicities, mosquito larvae

INTRODUCTION

In the search for new measures to control vector insects, the common phenomenon of vector resistance to insecticides and the residual effects of these chemicals on the environment are causes for concern. The recent public awareness of the hazardous effects of highly toxic and non-biodegradable synthetic insecticides on human health has prompted scientists to seek safer alternatives in the form of natural products to be used directly or as starting materials to synthesize more potent derivatives or as models for the developement of synthetic chemicals.

Natural products of plant origin such as rotenone, nicotine and pyrethrins have long been used to control destructive insects and vectors of diseases (Matsumura 1975). One of the earliest reports on the toxicity of plant extracts on mosquito larvae was by Campbell and Sullivan (1933) who reported that the plant alkaloids, nicotine, anabasine, methylanabasine and lupinine killed larvae of *Culex pipiens, Cx. territans* and *Cx. quinquefasciatus.* Subsequently, many researchers have reported on the effectiveness of plant extracts against mosquito larvae (Haller 1940; Hartzell and Wilcoxon 1941; Amonkar and Reeves 1970; Supavan *et al.* 1974; Chavan 1983). Recently, Zebitz (1986) reported that neem seed kernel extract (*Azadirachta indica*) is active against 4th-instar larvae of *Aedes togoi* and *Ae. aegypti* with LC_{50} of 1.19 – 18.10 $\mu g\ ml^{-1}$.

This paper reports on the toxic effects of the extracts of eighteen Malaysian plants on 4th-instar larvae of *Anopheles maculatus, Aedes aegypti* and *Culex quinquefasciatus.*

MATERIALS AND METHODS

Plant materials were collected from various locations in Peninsular Malaysia. The plants were identified and voucher specimens were deposited at the herbarium of Forest Research Institute Malaysia. The essential oils were prepared by subjecting the plant materials (200 g, mesh size 40 - 60) to water distillation for 8 h.

Three species of vector mosquitoes, *Anopheles maculatus, Aedes aegypti* and *Culex quinquefasciatus,* served as the test organisms. The larvae colonies of these mosquitoes were established and collected from the Insectary of the Institute for Medical Research, Kuala Lumpur.

Each essential oil in 0.2 ml ethanol was dissolved in distilled water to prepare 1000 $\mu g\ ml^{-}1$ stock solution from which concentrations of 500, 200, 150, 100, 50 and 10 $\mu g\ ml^{-1}$ were prepared by dilution. Twenty ml of each sample were placed in a vial. Ten 4th-instar larvae of each vector species were transferred into each vial using a disposable pipette. The bioassay was carried out in two stages. Initially, all plant extracts were screened using concentrations of 1000 and 500 $\mu g\ ml^{-1}$. Extracts producing high mortality rates were further tested at lower concentrations of 200, 150, 100, 50 and 10 $\mu g\ ml^{-1}$. The treatment on each concentration was replicated 3 times in a completely randomized design. A control sample was prepared by the addition of 0.2 ml ethanol to the water in each vial which contained ten larvae. Solutions of DDT dissolved in water at 1 – 200 $\mu g\ ml^{-1}$ concentrations were used as a standard toxicant.

Mortality was observed after 24 h and corrected mortality obtained by applying Abbott's formulae (1925). LC_{50} and 95% confidence intervals were determined by the probit analysis method as described by Finney (1971).

RESULTS AND DISCUSSION

The bioassay of the essential oils against mosquito larvae demonstrated their effectiveness, with LC_{50} of 13.6 – 161.1 μg ml^{-1} (Table 1). However, the efficacy of each oil towards the various larvae was non-selective as LC_{50} showed little variation. The only exception was the essential oil of *Goniothalamus andersonii* which showed relatively strong activity against *Culex quinquefasciatus* (LC_{50} 60.85 μg ml^{-1}), but weaker activity against the other two vectors with LC_{50} of 113.85 and 116.85 μg ml^{-1} respectively.

TABLE 1
Toxicity of essential oils of some Malaysian plants to mosquito larvae

Sample	LC_{50} (95% CI)		
	A.e.	C.q.	A.m.
Cinnamomum impressicostatum	116.85 (111.09 – 122.40)	121.32 (110.79 – 136.03)	
Cinnamomum mollisimum	119.25 (110.10 – 129.38)	132.85 (125.47 – 140.64)	
Cinnamomum iners	62.91 (59.42 – 66.80)	63.07 (57.19 – 68.50)	
Cinnamomum zeylanicum	87.47 (77.94 – 97.79)	72.71 (67.02 – 79.24)	
Piper aduncum	23.38 (21.07 – 25.76)	16.58 (15.69 – 17.38)	18.97 (16.94 – 20.96)
Piper betle	75.67 (67.36 – 82.88)	59.01 (53.93 – 62.82)	74.00 (69.59 – 79.44)
Piper penangense	161.12 (150.62 – 169.82)	128.83 (122.05 – 135.09)	102.59 (84.82 – 122.49)
Piper lanatum	82.18 (78.91 – 85.30)	92.32 (86.99 – 100.43)	62.14 (57.83 – 66.65)
Piper pedicellosum	84.44 (77.45 – 93.97)	70.25 (67.82 – 72.85)	49.03 (31.63 – 62.06)
Litsea elliptica	16.01 (14.29 – 18.05)	14.63 (13.85 – 15.79)	13.61 (10.79 – 15.59)
Goniothalamus andersonii	116.85 (111.09 – 122.40)	60.93 (50.81 – 69.73)	113.85 (98.12 – 126.16)

Table 2: Cont'd

Leptospermum javanicum	68.51 (63.75 – 73.41)	68.38 (64.43 – 71.63)	91.15 (75.57 – 119.53)
Pogostemon cablin	67.46 (61.85 – 72.72)	56.01 (50.36 – 61.07)	
Cymbopogon nardus	74.51 (67.23 – 83.03)	71.09 (67.41 – 74.64)	
Melaleuca cajupati	82.04 (79.83 – 84.12)	60.29 (57.61 – 62.81)	
Vetiveria zizanioides	90.27 (77.30 – 105.39)	95.85 (88.19 – 103.22)	
Polygonum minus	47.94 (43.23 – 52.79)	38.43 (33.75 – 44.05)	
Dipterocarpus kerrii	146.10 (139.66 – 152.29)	126.83 (110.57 – 136.38)	

**Note:*
A.e. = *Aedes aegypti*
C.q. = *Culex quinquefasciatus*
A.m. = *Anopheles maculatus*

The essential oils of *Litsea elliptica*, *Piper aduncum* and *Polygonum minus* showed $LC_{50} < 50$ $\mu g\ ml^{-1}$, indicating significant levels of larvicidal properties. The leaf oil of *Litsea elliptica* plant extract was the most effective, exhibiting LC_{50} of 13.61 $\mu g\ ml^{-1}$ for *Anopheles maculatus*, 16.01 $\mu g\ ml^{-1}$ for *Aedes aegypti* and 14.63 $\mu g\ ml^{-1}$ for *Culex quinquefasciatus*; this showed that the plant extracts contained active principles responsible for the larvicidal activity.

The active principles of the essential oils, when isolated in pure form, might possess high larvicidal activity. The results should encourage further efforts to purify the active constituents and study their pathological effects on mosquito larvae. The toxic properties of the essential oils could also be due to the combined effect of the compounds in the crude extracts which were of diverse chemical structures and could exhibit a different mode of action towards the test organisms, resulting in high toxicities.

CONCLUSION

The results indicate that some of the plants studied can be effectively used in mosquito control as an alternative to synthetic insecticides. Although the plant extracts were less toxic than the chlorinated insecticide, DDT, they are environment-friendly and less harmful than the latter, which has been reported to cause undesirable side effects to human (Reynolds 1989). However, the residual lifespan as well as their performance under field conditions need to be determined to assess their potential as commercial insecticidal agents. The leaf oil of *Litsea elliptica* with the lowest value of LC_{50} has the greatest potential, followed by the leaf oils of *Polygonum minus* and *Piper aduncum* which showed $LC_{50} < 50\ \mu g\ ml^{-1}$. The active ingredients of each extract and the minimum amount needed in formulations need to be determined.

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