

## Effects of sub-lethal concentrations of synthetic insecticides and *Callitris glaucophylla* extracts on the development of *Aedes aegypti*

Essam Abdel-Salam Shaalan<sup>1</sup>, Deon Vahid Canyon<sup>2✉</sup>, Mohamed Wagdy Faried Younes<sup>3</sup>,  
Hoda Abdel-Wahab<sup>1</sup>, and Abdel-Hamid Mansour<sup>1</sup>

<sup>1</sup>Zoology Department, Aswan Faculty of Science, South Valley University, Aswan, Egypt

<sup>2</sup>Anton Breinl Centre, School of Public Health, Tropical Medicine and Rehabilitative Sciences,  
James Cook University, Townsville, Australia

<sup>3</sup>Zoology Department, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt

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**ABSTRACT:** Synthetic and botanical insecticides can have a profound effect on the developmental period, growth, adult emergence, fecundity, fertility, and egg hatch, resulting in effective control at sub-lethal concentrations. This paper investigated sub-lethal concentrations of fenitrothion, lambda-cyhalothrin, and *Callitris glaucophylla* Joy Thomps. & L.P. Johnson (Cupressaceae) extract to characterize their effects on the development of *Aedes aegypti* L. (Diptera: Culicidae) mosquito larvae. The LC<sub>25</sub>, LC<sub>50</sub>, and LC<sub>75</sub> (four replicates) were used for each synthetic insecticide and the LC<sub>25</sub> and LC<sub>75</sub> (four replicates) were used for *C. glaucophylla*. Observations of larval mortality, duration of larval stage, pupal mortality, duration of pupal stage, adult emergence, sex ratio, and malformations were recorded over 14 days. A dose-response effect was observed for all insecticides. Although *C. glaucophylla* extract doses were higher than synthetic insecticide doses, the LC<sub>75</sub> treatment outperformed synthetics by completely prohibiting adult emergence. Consequently, this botanical is recommended for field application either in combination with synthetic or natural insecticides or alone. **Journal of Vector Ecology 30 (2): 295-298. 2005.**

**Keyword Index:** Botanical, phytochemical, insecticide, mosquito, sub-lethal.

### INTRODUCTION

In toxicity studies, the gentle dose-response slope observed for botanicals over 24 h renders many of them unusable by economic standards despite them causing significant mortality at sub-lethal concentrations. Besides toxic larvicidal activity, botanical extracts have been shown to induce pupicidal activity, effects on larval and pupal duration, and often reduce adult emergence. For instance, an extract of *Callistemon lanceolatus* induced concentration-dependent mortalities in juvenile *Culex quinquefasciatus* (Mohsen et al. 1990), while an extract of *Ipomoea carnea* caused mortality and disrupted the development and growth of *Anopheles stephensi* (Saxena and Sumithra 1985). Neem oil and neem seed kernel extract markedly reduced the percentage of pupation and adult emergence of *An. stephensi* (Murugan et al. 1996). Botanical extract-induced malformations, particularly larval-pupal intermediates and half-ecdysed adults, were common (Al-Sharook et al. 1991, Jayaprakasha et al. 1997 and Karmegam et al. 1997).

Synthetic insecticides also induce sub-lethal effects, however, these can be unpredictable. For instance, three pyrethroids (d-phenothrin, d-allethrin, and tetramethrin) reduced *Aedes aegypti* egg production, while only d-phenothrin and d-allethrin reduced blood engorgement (Liu et al. 1986). Topically applied dieldrin caused dose-dependant effects on feeding and affected egg-laying capacity in *Ae. aegypti*, but progeny were unaffected (Duncan 1963). Sub-lethal concentrations of the botanical, *Callitris glaucophylla*,

induced significant larvicidal activity against *Ae. aegypti* compared with fenitrothion and lambda-cyhalothrin (Shaalan et al., unpublished data).

Thus, in the absence of highly toxic natural botanical compounds, the growth or emergence inhibiting activity of a botanical phytochemical may be essential to its uptake by the insecticide industry. Indeed, the rational application of exceptional phytochemicals may not only lead to new IPM strategies but may inhibit the development of insect resistance to existing synthetic insecticides.

This study investigated the effects of sub-lethal concentrations of a liquefied refrigerant gas extract of *C. glaucophylla*, fenitrothion, and lambda-cyhalothrin on the development of *Ae. aegypti* mosquitoes and determined a concentration that led to satisfactory control.

### MATERIALS AND METHODS

#### Test mosquitoes

*Aedes aegypti* were obtained from a colony initiated from mosquitoes collected in 2002 from Townsville, Australia. The colony of mosquitoes were maintained at conditions of 27 ± 2 C° and 70 % ± 5 R.H. under 14L - 10D cycles. *Ae. aegypti* larvae were kept in plastic buckets half filled with tap water and fed on goldfish flakes. Water in rearing containers was refreshed every two days. Male and female adult mosquitoes were maintained on a 10 % sugar solution while female adults were also provided the opportunity to feed on rat blood.

### Test insecticides

Technical grades of the organophosphorous insecticide fenitrothion (96.8 %) and the pyrethroid insecticide lambda-cyhalothrin (90.99 %) were provided by Nufarm Ltd (North Victoria, Australia). Liquefied refrigerant gas extract of *C. glaucophylla* was supplied by Michael Kennedy, Department of Primary Industries, Queensland, Australia (details of extraction awaiting IP protection). It is possible, but not considered likely, that interaction with the compressed refrigerant gas solvent caused or catalyzed chemical changes in the extractive compounds, just as this could possibly happen with conventional solvents and is known to happen during steam distillation. The refrigerant was eliminated from the extract by spontaneous distillation as the pressure was reduced. Minute quantities could remain in the crude extract, but given the volatility of the refrigerant, would be readily lost during application of the extract to the test material during the screening process. Any residual effect on extract activity has not been evaluated.

### Bioassays

Insect growth regulator testing instructions (WHO 1996) were followed to investigate and determine the effects of sub-lethal doses of test insecticides on *Ae. aegypti* larvae. Larvae were subjected to two to three sub-lethal concentrations ( $LC_{25}$ ,  $LC_{50}$ , and  $LC_{75}$ ) in glass beakers. The sub-lethal concentrations for fenitrothion were 0.0025 mg/l ( $LC_{25}$ ), 0.0044 mg/l ( $LC_{50}$ ), and 0.0062 mg/l ( $LC_{75}$ ); for lambda-cyhalothrin they were 0.00004 ( $LC_{25}$ ), 0.00015 ( $LC_{50}$ ), and 0.00026 mg/l ( $LC_{75}$ ); and for *C. glaucophylla* they were 2.6 mg/l ( $LC_{25}$ ) and 14.7 mg/l ( $LC_{75}$ ). All test chemicals were diluted in ethanol. One ml of stock solution was added to 99 ml of de-ionized water. Controls received 1 ml of ethanol only. Four replicates of 25 newly molted 4<sup>th</sup> instar larvae for each concentration were conducted. For accurate determination of the sub-lethal

effects, the larval and pupal mortality as well as adult emergence were recorded daily up to emergence of the adults or death of the last larva or pupa. Due to the long duration of the test, larvae were provided with food at 2-day intervals during the test period.

From the overall results of the test, percentages of both emerged and dead pupae and percentage of adult emergence, sex ratio, larval duration, pupal duration, average developmental period, and growth index were determined. Growth index was calculated according to Saxena and Sumithra (1985): Growth Index (GI) = percentage adult emergence / average developmental period (days). Data analyses were performed using a one-way ANOVA in SPSS version 12.0.1 and significant differences were determined at  $P < 0.05$ .

## RESULTS

Effects of sub-lethal concentrations of *C. glaucophylla*, fenitrothion, and lambda-cyhalothrin on juvenile and adult mosquitoes are shown in Tables 1 and 2.

Fenitrothion results indicated little significant difference between controls and the  $LC_{25}$  dose and between the  $LC_{50}$  and  $LC_{75}$  doses. At the two higher doses, virtually all measured variables were significantly different from controls. Larval survival and adult emergence were reduced 10-fold, total mortality ranged from 88-98 % and growth was significantly limited.

Lambda-cyhalothrin results indicated more of a dose-response with  $LC_{25}$ s often being significantly different from controls. Only  $LC_{50}$  and  $LC_{75}$  doses, however, significantly reduced larval survival and adult emergence. Total mortality was high at all doses and the GI was comparable to that observed for fenitrothion.

*C. glaucophylla* results also showed clear dose-response

Table 1. Effects of sub-lethal concentrations of fenitrothion, lambda-cyhalothrin, and *Callitris glaucophylla* extract on juvenile *Aedes aegypti* development and mortality. Early 4<sup>th</sup> instar larvae were exposed continuously until adulthood.

Insecticide	LC	Average larval period (days)	Larval mortality (%)	Average pupal period (days)	Pupal mortality (%)	Total average development (days)
Fenitrothion	0	5.0±0.1 <sup>c</sup>	0.0±0.0 <sup>a</sup>	2.0±0.3 <sup>b</sup>	6.5±3.8*	6.98±0.3 <sup>b</sup>
	25	4.3±0.8 <sup>bc</sup>	13.0±8.1 <sup>a</sup>	1.9±0.2 <sup>b</sup>	10.5±4.4	6.16±0.7 <sup>b</sup>
	50	2.8±1.6 <sup>ab</sup>	74.5±19.2 <sup>b</sup>	0.6±0.5 <sup>a</sup>	10.0±4.3	3.31±2.0 <sup>a</sup>
	75	1.3±0.5 <sup>a</sup>	92.0±5.2 <sup>c</sup>	0.2±0.3 <sup>a</sup>	4.0±2.8	1.55±1.1 <sup>a</sup>
Lambda-cyhalothrin	0	7.1±0.1 <sup>d</sup>	0.5±1.0 <sup>a</sup>	6.1±0.6 <sup>c</sup>	2.0±2.3 <sup>a</sup>	13.20±0.6 <sup>d</sup>
	25	6.6±0.2 <sup>c</sup>	7.0±3.5 <sup>a</sup>	1.8±1.0 <sup>b</sup>	53.5±18 <sup>c</sup>	8.36±1.2 <sup>c</sup>
	50	4.8±0.2 <sup>b</sup>	33.5±3.0 <sup>b</sup>	0.4±0.4 <sup>a</sup>	53.5±10 <sup>c</sup>	5.27±0.4 <sup>b</sup>
	75	1.7±0.5 <sup>a</sup>	69.5±9.3 <sup>c</sup>	0.1±0.1 <sup>a</sup>	23.0±6.2 <sup>b</sup>	1.82±0.5 <sup>a</sup>
Callitris glaucophylla	0	4.2±0.4 <sup>c</sup>	2.0±2.8 <sup>a</sup>	2.7±0.2 <sup>c</sup>	9.0±2.0 <sup>a</sup>	6.9±0.6 <sup>c</sup>
	25	3.4±0.6 <sup>b</sup>	9.5±4.4 <sup>b</sup>	1.1±0.7 <sup>b</sup>	25.0±12.5 <sup>b</sup>	4.5±0.6 <sup>b</sup>
	75	0.6±0.5 <sup>a</sup>	95.0±2.6 <sup>c</sup>	0.0±0.0 <sup>a</sup>	3.0±2.6 <sup>a</sup>	0.38±0.5 <sup>a</sup>

Values for the same insecticide followed by a different letter within the same column are statistically different ( $P < 0.05$ ).

\* Group not significant.

Table 2. Effects of sub-lethal concentrations of fenitrothion, lambda-cyhalothrin, and *Callitris glaucophylla* extract on adult *Aedes aegypti* development and mortality. Early 4<sup>th</sup> instar larvae were exposed continuously until adulthood.

Insecticide	LC	Adult mortality (%)	Adult emergence (%)	Emergent females	Emergent males	Malformations	Growth Index
Fenitrothion	0	0.0±0.0*	95.5±3.8 <sup>c</sup>	54.0±9.4 <sup>c</sup>	39.5±7.7 <sup>b</sup>	0.0±0.0*	6.72±0.5 <sup>b</sup>
	25	1.5±1.9	72.5±13.3 <sup>b</sup>	39.0±8.2 <sup>b</sup>	33.5±6.4 <sup>b</sup>	2.5±1.9	5.86±0.6 <sup>b</sup>
	50	0.5±1.0	11.5±17.8 <sup>a</sup>	4.0±5.4 <sup>a</sup>	7.5±12.4 <sup>a</sup>	3.3±3.6	1.21±1.3 <sup>a</sup>
	75	0.5±1.0	2.5±3.8 <sup>a</sup>	2.0±4.0 <sup>a</sup>	0.5±1.0 <sup>a</sup>	1.0±2.0	0.47±0.7 <sup>a</sup>
Lambda-cyhalothrin	0	4.0±5.7 <sup>b</sup>	94.0±6.9 <sup>c</sup>	55.5±8.9 <sup>c</sup>	43.0±14.1 <sup>b</sup>	0.0±0.0 <sup>a</sup>	7.1±0.3 <sup>c</sup>
	25	13.5±3.0 <sup>ab</sup>	26.5±15.9 <sup>b</sup>	20.0±10.5 <sup>b</sup>	6.5±6.8 <sup>a</sup>	0.0±0.0 <sup>a</sup>	3.0±1.5 <sup>b</sup>
	50	7.25±6.7 <sup>a</sup>	6.0±4.9 <sup>a</sup>	5.0±4.2 <sup>a</sup>	1.0±2.0 <sup>a</sup>	0.5±1.0 <sup>a</sup>	1.1±0.9 <sup>a</sup>
	75	0.5±1.0 <sup>a</sup>	1.5±1.9 <sup>a</sup>	1.0±1.2 <sup>a</sup>	0.5±1.0 <sup>a</sup>	5.6±3.0 <sup>b</sup>	0.7±0.9 <sup>a</sup>
Callitris glaucophylla	0	7.5±5.3 <sup>a</sup>	81.5±5.3 <sup>c</sup>	45.0±2.6 <sup>c</sup>	36.5±3.0 <sup>c</sup>	0.0±0.0*	11.8±1.2 <sup>b</sup>
	25	30.5±19.2 <sup>b</sup>	35.0±26.1 <sup>b</sup>	22.5±16.0 <sup>b</sup>	12.5±10.1 <sup>b</sup>	0.0±0.0	7.7±6.0 <sup>b</sup>
	75	0.0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0.0±0.0 <sup>a</sup>	2.0±2.3	0.0±0.0 <sup>a</sup>

Values for the same insecticide followed by a different letter within the same column are statistically different ( $P < 0.05$ ).

\*Group not significant.

Growth Index = Adult emergence (%) / Average developmental period (days).

in most measured categories. The LC<sub>75</sub> produced exceptional results, killing all larvae and pupae and preventing any emergence.

Overall, there was no significant difference in the sex of emergent mosquitoes and malformations, for the most part, were uncommon and not related to dose. Mortality experienced by emergent adults was not significant except in the *C. glaucophylla* LC<sub>25</sub> dose where a third perished.

## DISCUSSION

The tested synthetic insecticides and botanical extract induced a wide range of sub-lethal effects on larval mortality, larval duration, pupicidal activity, pupal duration, adult emergence, sex ratio, adult mortality, and malformation.

The insecticide concentrations estimated to cause 25, 50, and 75 % larval mortality in 24 h did not produce expected mortalities with LC<sub>25</sub> doses consistently causing lower mortality and LC<sub>75</sub> doses causing higher mortality in fenitrothion and *C. glaucophylla*. The Probit estimation of LC<sub>25</sub> and LC<sub>75</sub> doses are less accurate unless large data sets are used, which is why publications typically report LC<sub>50</sub>s and not LC<sub>90</sub>s. Pupal mortality did not exhibit a linear relationship with the applied sub-lethal concentrations but was more associated with lower doses. Pupal mortality was lower than controls at the LC<sub>75</sub> dose in fenitrothion and *C. glaucophylla*, but lambda-cyhalothrin was more effective against pupae. Adult mortality results did not present anything of note. Dead adults were mostly half-ecdysed adults. Total mortality was consistently positively correlated with insecticide concentrations and the duration of exposure (Marcard et al. 1986).

Beside immediate toxic larvicidal effects, all insecticides significantly reduced the average larval period compared to controls and, to a large extent, with each other. Larvae were

observed to pupate faster as their environment increased in toxicity. This is clearly a self-preservation mechanism since the pupal form is less susceptible to the environment. All concentrations markedly disrupted pupal duration except for the fenitrothion LC<sub>25</sub> dose. Consequently, the average development period (a factor in the Growth Index formula) was consistently negatively correlated with insecticide concentrations and the duration of exposure. This effect can vary, however, with some researchers showing no effect on the larval and pupal developmental periods (Saxena et al. 1993, Sharma and Saxena 1994) and other researchers showing prolongation of the larval and pupal developmental periods (Karmegam et al. 1997, Saxena and Yadav 1983, Zebitz 1984, Saxena and Sumithra 1985, Mwangi and Rembold 1988, Robert and Olson 1989, Mohsen et al. 1990a, b; Pushpalatha and Muthukrishnan 1995, Pushpalatha and Muthukrishnan 1999). In another study, *Melia volkensii* was observed to prolong the lifespan of *An. arabiensis* larvae but not the pupal period (Mwangi and Mukiyama 1988). Conversely, Supavarn et al. (1974) reported on 11 of 36 botanicals that significantly inhibited pupal development while only a few botanicals affected larval development.

Successful adult emergence is conversely proportional with the insecticide concentration and larval mortality. Of note, *C. glaucophylla* completely inhibited adult emergence compared to fenitrothion (2 – 8 %) and lambda-cyhalothrin (2 – 4 %) at the LC<sub>75</sub> dose. This effect is expected since several studies have shown that botanical extracts either reduce or inhibit adult emergence. For instance, *Descurainia sophia* inhibited *Cx. quinquefasciatus* emergence (Mohsen et al. 1990b) and *Tagetes erectus* significantly reduced adult emergence in *An. stephensi* (Sharma and Saxena 1994).

Changes in the sex ratio of emergent adults tended towards favoring females, however, results were not significantly different. This is not always the case, since Robert

and Olson (1989) found a change in the sex ratio towards more males in *Cx. quinquefasciatus* after sub-lethal exposure propoxur and resmethrin.

The percentage of malformations was very low for all insecticides. The only observed abnormalities were larval-pupal intermediates, half-ecdysed adults, and adults with malformed wings. These morphogenetic abnormalities are commonly caused by botanical extracts and are thought to result from a disturbance to growth regulating hormones (Zebitz 1984; Mwangi and Mukiyama 1988; Pereira and Gurudutt 1990; Saxena et al. 1993).

The growth indices of larvae treated at LC<sub>50</sub> and LC<sub>75</sub> doses were markedly shorter than controls and LC<sub>25</sub> doses for all insecticides with negligible difference between controls and LC<sub>25</sub> doses. Similar results were obtained by Saxena and Sumithra (1985) and Saxena et al. (1993), who found that the GI of mosquitoes treated with *Annona squamosa* alkaloids was longer in controls.

In conclusion, for most measured developmental effects, the response was dose and exposure duration dependant. Significant developmental effects were observed for fenitrothion, lambda-cyhalothrin, and the botanical, *C. glaucophylla*. The latter induced responses at a LC<sub>75</sub> dose that were exceptional and worthy of consideration for field trials pending non-target assessment.

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