SCREENING OF NATURAL LARVICIDES FROM *SPILANTHES ACMELLA* L. (PÈ-LAYNYIN) AND *MELIA AZEDARACH* L. (PAN-TAMAR)

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Abstract

Two plant materials, aerial part of *Spilanthes acmella* L. (Pè-Laynyin) and bark of *Melia azedarach* L. (Pan-Tamar) were selected for the screening of mosquito larvicidal activity against third and fourth instar larvae (*Aedes aegypti* mosquito). Petroleum ether extract from aerial part of Pè-laynyin (LC_{50} = 0.0065 %, LC_{90} = 0.0146 %) and ethanol extract from Pan-tamar bark (LC_{50} = 1.008 %, LC_{90} = 3.693 %) showed potent larvicidal activities. The larvicidal activity of commercially available synthetic larvicide, Temephos, (Abate) was LC_{50} = 0.0034 ppm and LC_{90} = 0.0075 ppm. Ethanolic extract of *S. acmella* was not observed sign to be toxic in mice at 10 g/kg that is maximum permissible dose. Therefore, *S. acmella* extract did not show harmful effect to mammalian. The maximum giving dose for ethanolic extract of *M. azedarach* in mice was 8 g/kg within survival period for 7 days to be looked forward. At a dose of 10 g/kg, one out of three mice was found to be dead. Therefore, care must be taken if bark of *M. azedarach* is used as larvicide. Petroleum ether extract of *S. acmella* was not observed lethality on fish *Clarias batrachus* (Nga-khu) at 0.025 % concentration level. Therefore, petroleum ether extract was not found harmful effect on aquatic vertebrates and can safely be used as natural larvicide in fresh water.

Keywords: *Spilanthes acmella* L., *Melia azedarach* L., *Aedes aegypti* mosquito, larvicidal activities, synthetic larvicide (Abate), *Clarias batrachus*

Introduction

Among insects, mosquitoes are the most important group in the transmissions of several human diseases, including malaria, yellow fever, dengue, encephalitis and filariasis. Mosquitoes are widely distributed throughout the world. The number of mosquito species exceeds 2500. They are separated into two medically important sub-families – the Anophelinae and Culicinae – the former is smaller but includes the vectors of human malaria and filariasis (Genus Anopheles); the Culicinae includes vectors of viral and filarial disease of man including species which are vicious biters (Genera *Aedes, Culex, Mansonia)* (WHO, 1997).

In tropical countries, *Aedes aegypti* is an important vector of dengue, dengue haemorrhagic fever, yellow fever and other viral diseases. A closely related species, *A. albopichus*, can also transmit dengue. In some areas, *Aedes* species transmit filariasis.

Aedes mosquitos bite mainly in the morning or evening. Most species bite and rest outdoors but in tropical towns, *Aedes aegypti* breeds, feeds and rests in and around houses (Rozendaal, 1997).

Dengue haemorrhagic fever (DHF) is the most common in children less than 15 years of age, but it also occurs in adult. DHF commonly begins with a sudden rise in temperature which is accompanied by facial flush and other non-specific constitutional symptoms, resembling dengue fever such as anorexia, vomiting, headache and muscle or joint pains.

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Insecticides may cause discomfort, sickness and occasionally death to human beings and other living organisms when they are carelessly or incorrectly used. To avoid this effect, research on plant extracts should be carried out to replace synthetic insecticides. The present work investigates the larvicidal ativity of two plant species of *S. acmella* and *M. azedarach* against on *A. aegypti* mosquito larvae.

Selected Myanmar Indigenous Insecticidal Plants

***Spilanthes acemella* L.**

Family	:	Compositae (Asteraceae)
Botanical name	:	*Spilanthes aecmella* L.
Myanmar name	:	Pè-laynyin
English name	:	pelitory (toothache plant)
Part used	:	Aerial part

The genus *Spilanthe*s (Asteraceae) is herb to 50 cm tall, leaves opposite, broadly ovate, 3-6 cm long, flower head terminal, solitary, avoid, about 1 cm cross, long-stalked (12 to 15 cm long) marginal ray-flowers yellow, achenes black. It is native to the tropics of Brazil, through it is grown as an ornamental (and occasionally as a medical) in various parts of the world. The flowering and fruits is November to December (Mohammad *et al*., 2017).

S. acmella (Figure 1) is also known as "toothache plant" or "paracress". *S.acmella* (Asteraceae) has 476 genera and three species in *Spilanthes* : *S. acmella*, *S. oleracea* Jacq. and *S. radicans* Schard.

Figure 1 Photograph of *Spilanthes acmella* L.

Biological Activities of *Spilanthes acmella* L.

S. acmella is sometimes called the toothache plant because chewing on one of the flower buds will numb the mouth and make salivate. The leaves and flower-heads contain

analgesic, antifungal, anthelmintic and antibacterial agents. The leaves may be used topically to treat bacterial and fungal skin diseases such as ringworm (Verma *et al*., 1993).

***Melia azedarach* L.**

Family	:	Meliaceae
Botanical name	:	*Melia azedarach* L.
Myanmar name	:	Pan-Tamar, Thinbaw-Tamar
English name	:	Indian Bead tree, Perisian Lilae
Part used	:	Bark

The genus *Melia* (Meliaceae) is commonly called Perisian Lilae, white cedar, chinaberry or bead. Tree, *M. azedarach* is a deciduous tree, native to India, Southern China and Australia. They are all deciduous or semi-evergreen small trees. The leaves are up to 50 cm long, alternate, long-petiole, 2 or 3 times compound (old-pinnate); the leaflets are dark green above and lighter green below, with serrate margins.

Figure 2 Photograph of *Melia azedarach* L.

Biological Activities of *Melia azedarach* L.

M. azedarach has insecticidal, anti-viral and possible anticancer properties. The flowers and leaves are applied as a poultice to relieve nervous headaches. The oil possess similar properties to that neem oil. The leaves and bark are used internally and externally in leprosy and scrofula. The leaves, bark and fruits are accredited with insect-repellent properties. Leaves are placed inside books and between folds of woolen garments to protect them against insect attack (Adnan, 2009).

Materials and Methods

Mosquito larvicidal activity tests were carried out by the methods described by Swaroop, 1963.

Preparation of Pet Ether Extract from Aerial Part of *S. acmella*

Dried powdered from aerial parts of *S. acmella* (200 g) was macerated in petroleum ether (1000 mL) at room temperature. Petroleum ether was removed by using rotatory evaporator at 50 ºC to obtain petroleum ether crude extract (3.0 g, 1.5 % yield).

Preparation of EtOH Extract from *M. azedarach*

Dried powdered from bark of *M. azedarach* (100 g) was extracted with EtOH (500 mL). This ethanol extract was filtered and then concentrated under reduced pressure at 45 ºC using rotary evaporator to obtain EtOH extract (3.0 g, 3.0 % yield).

Larvae and Bioassays

Laboratory reared 8-10 days old, 3rd and 4th instar *A. aegypti* mosquito larvae were used for larvicidal tests. The mosquito larvae were obtained from Medical Entomology Research Division, Department of Medical Research, Lower Myanmar. For each test 200 larvae were used. Larvae were starved for 24 h before testing. Five replications were carried out for each run.

Test Samples and Doses

(i) 0.025, 0.0125, 0.00625, and 0.003125 % (w/v) solution of petroleum ether extract from aerial part of *S. acmella* in acetone-water

(ii) 3.0, 1.5, 0.75, and 0.375 % (w/v) solution of ethanol extract from *M. azedarach* bark in distilled water

(iii) 0.0125, 0.00625, 0.003125, and 0.0015625 ppm (w/v) solution of commercially available larvicides, Abate in distilled water

Procedure

Late 3rd and 4th instar larvae were exposed to a series of four concentrations of test samples. Larvae were also exposed to either water or acetone-water (solvent used to dissolve the test sample) for control purpose. Mortalities were recorded after 24 h period. In the larvicidal test, the relative humidity (RH) was 75-80 % and temperature was 27 ± 1 ºC. The lethal concentrations (LC_{50} and LC_{90}) were investigated by using dose-effect probit analysis, and commercially available larvicide, Abate, was used for a positive control.

Acute Toxicity Study of *S. acmella* and *M. azedarach*

The purpose of acute toxicity is to identify and categorize those chemical substance that pose a potential hazard to human and other species. The acute toxicity was investigated by the up-and-down method in mice (Bruce, 1985). This method permits a major reduction in the number of animal used. In the up-and-down procedure, the animals were dosed one at a time. If an animal survives, the dose for the next animal is increased; if it dies, the dose is decreased.

Doses are usually adjusted by a constant multiplicative factor, i.e., by dose amounts of 10, 8, 6, 4, 2 and 1 g/kg. The dose for each successive animal is adjusted up or down depending upon the outcome for previous animal. For a limit test, the dosing of three mice of each sex at 5 g/kg was used for substance with low toxicity.

Animals and apparatus

Albino mice of both-sexes (weighing 30-35 g), mouse cages, animal balance, intragastric needles and 2 mL syringe

Procedure

Albino mice (12) were used in this study. The dose was calculated according to the body weight. In the acute toxicity test, EtOH extracts of *S. acmella* and *M. azedarach* were dissolved in distilled water by using intragastric needles, the EtOH extract solutions were offered to a group of those three mice by 10 g/kg per mice and another group of three mice by 8 g/kg per mice. Each group of those mice was housed separately in mice cage. Food and water were allowed to freely assess. They were observed carefully for 24 h. Survivability was observed within a period of 7 days and the lethal dose of the extract was estimated.

Toxicity of Insecticide to Aquatic Vertebrate *Clarias batrachus* (Nga-khu)

Test fish (50) of fresh water organism, *C.batrachus* (Nga-khu) with a length of 5-6 cm, up to 20 days maturity were obtained from Fresh Water Fish Hatchery, Department of Fisheries, Lay-Dauk-Kan, East Dagon Township, Yangon Region.

Apparatus

Container (capacity 1 L), beaker (500 mL), glass rods, micropipette

Procedure

Petroleum ether extract of *S. acmella* was dissolved in a mixture of acetone and distilled water. The required dose (0.025 %) was added into the test fish container filling with the fresh water (pH 7-7.5) at 27-30 ºC. In toxicity testing, 10 fish were used for each test. Five replications were made and observed carefully within 24 h period. Any abnormal behaviour symptoms of tested samples were regularly observed. Acute effects were measured after exposure to insecticide and dead or mortality was measured as acute after 24 h.

Results and Discussion

Mosquito Larvicidal Effect of Crude Extracts and Synthetic Larvicide (Abate)

Mosquito larvicidal activity of crude extracts was tested by Force feeding method. Percent mortality (LC_{50} and LC_{90} values) of test samples were evaluated by statistical analysis of the data of susceptibility of larvae, because this theory can be estimated with greater precision than lethal concentration at either end of the range. Mortality rate in distilled water (or distilled water-acetone) was investigated for untreated control. The number of dead mosquito larvae in each concentration was corrected by using Abott's formula.

$$\text{Corrected Mortality (\%)} = \frac{\text{Observed mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality (\%)}} \times 100$$

The median lethal concentration LC_{50} (the concentration needed to kill 50 % of larvae) and LC_{90} (the concentration needed to kill 90 % of larvae) were investigated from log concentration, probit mortality and linear regression.

A high mortality rate in untreated control (water or acetone-water in this experiment) may indicate that the larvae have been handled carelessly. During 24 h holding period subsequent to the exposure to insecticide, control mortality rates of less than 5 % may be disregarded but rates of 20 % or higher mean that the tests should be repeated. Between these two limit controls mortality rate is used to correct mortality rates in the exposed batches by the application of the formula.

Effect of Crude Extracts

LC_{50} and LC_{90} of *S. acmella* extracts on *A. aegypti* larvae were respectively found to be 0.0065 %, 0.0146 %. In addition, LC_{50} and LC_{90} of *M. azedarach* extract were 1.008 % and 3.693 % respectively. PE extract of *S. acemella* has more potent larvicidal activity than ethanol extract of *M. azedarach*.

The larvicidal activities of different concentrations of two plant extracts were presented in Table 1, 2 & Figure 3, 4. Table 3 and Figure 5 showed the larvicidal activity of synthetic larvicide (Abate). Table 4 represents the comparison of larvicidal activity of crude extracts and Abate (synthetic larvicide) after 24 h exposing period. The activities of two extracts were much more lower than that of Abate. However, natural insecticides are more safe and eco-friendly when compared to synthetic insecticides. All regression lines were found to be good fit that the data were not significantly heterogeneous. These test samples were fitted in regression line and showing the testing goodness of fit.

Table 1 Larvicidal Effect of PE Extract of *S. acmella* Aerial Parts on Immature Stages of *A. aegypti* Larvae

Concentration (%)	Dead/ Tested	Observed mortality (%)	Expected mortality (%)	(Observed - Expected) mortality	Contribution to χ^2 *
0.025	198/200	99	98.6	0.4	0.0010
0.0125	169/200	85	84	1	0.0007
0.00625	87/200	44	48	- 4	0.0060
0.003125	22/200	11	9	2	0.0040
				Total	**0.0117**

(* χ^2 = Chi square)

LC_{50} = 0.0065 % = 95 % Upper confidence limit of LC_{50} = 0.007
95 % Lower confidence limit of LC_{50} = 0.006

LC_{90} = 0.0146 % = 95 % Upper confidence limit of

LC_{90} = 0.0164 95 % Lower confidence limit of LC_{90} = 0.0128

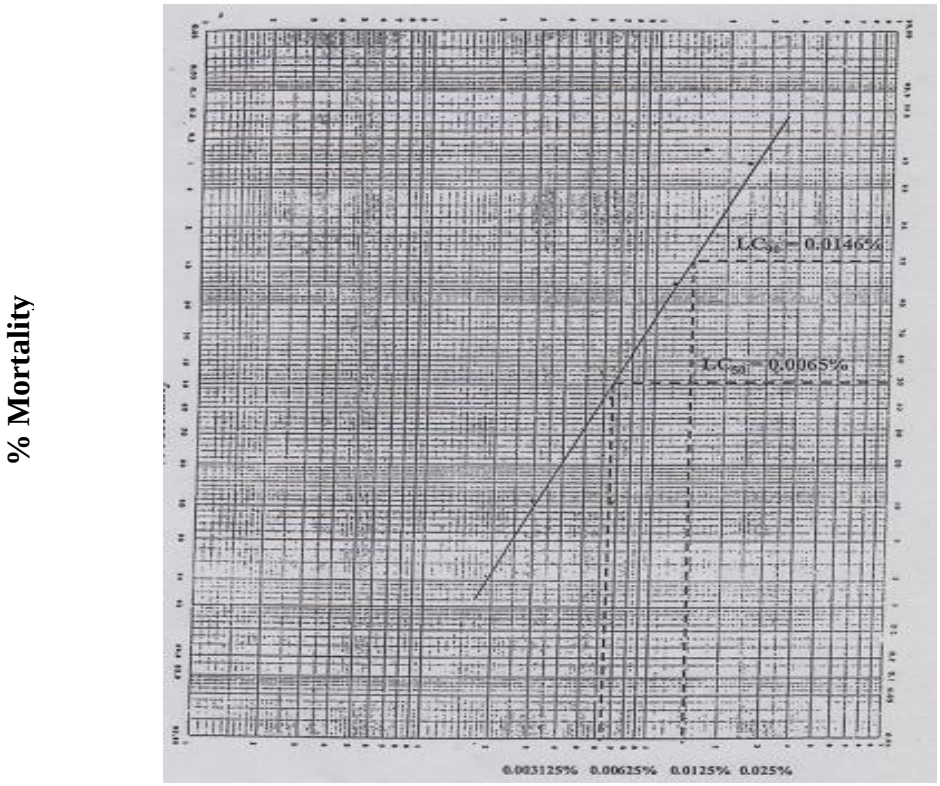

Figure 3 Testing the goodness of fit for dose effect analysis of PE Extract from *S.acmella* aerial parts (% concentration and *A.aegypti* larval mortality)

Table 2 Larvicidal Effect of EtOH Extract of *M. Azedarach* Bark on Immature Stages of *A. aegypti* Larvae

Concentration (%)	Dead/ Tested	Observed mortality (%)	Expected mortality (%)	(Observed - Expected) mortality	Contribution to χ^2
3.0	177/200	88.5	86.5	2	0.0034
1.5	128/200	64	66	-2	0.0017
0.75	68/200	34	38	-4	0.0067
0.375	29/200	14.5	16	-1.5	0.0016
				Total	0.0134

(* χ^2 = Chi square)

LC_{50} =1.008 % = 95 % Upper confidence limit of LC_{50} = 1.146

95 % Lower confidence limit of LC_{50} = 0.87

LC_{90} = 3.693 % = 95 % Upper confidence limit of LC_{90} = 4.518

95 % Lower confidence limit of LC_{90} = 2.868

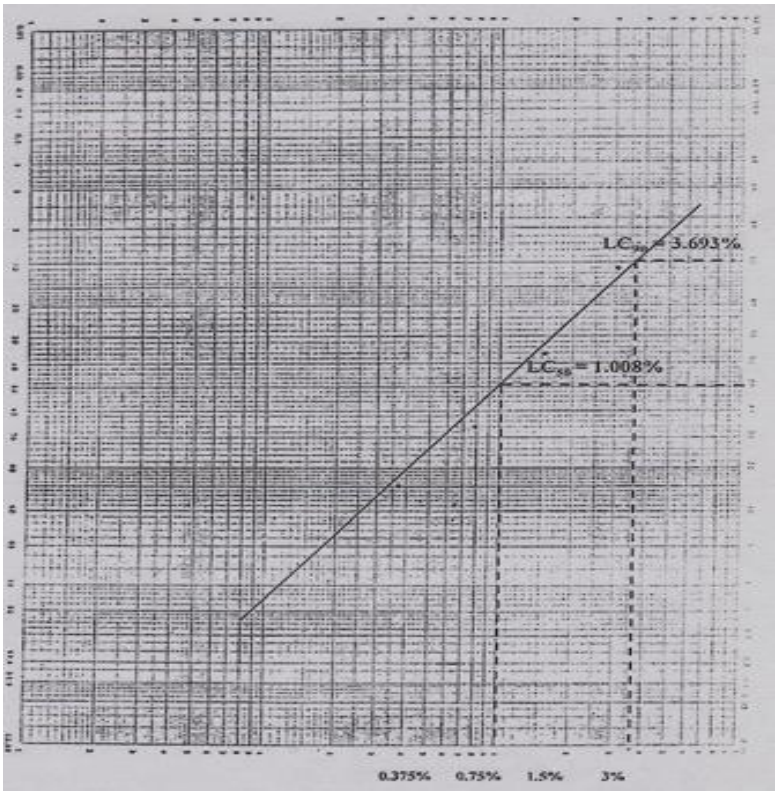

Figure 4 Testing the goodness of fit for dose effect analysis of EtOH extract of *M. azedarach* bark (%concentration and *A. aegypti* larval motality)

Table 3 Larvicidal Effect of Temephos (Abate) on Immature Stages of *A. aegypti* Larvae

Concentration (ppm)	Dead/ Tested	Observed mortality (%)	Expected mortality (%)	(Observed - Expected) mortality	Contribution to χ^2
0.0125	197/200	98.5	99	- 0.5	0.0025
0.00625	174/200	87	86	1	0.0008
0.003125	108/200	54	52	2	0.00163
0.0015625	15/200	7.5	9	-1.5	0.0027
				Total	0.0219

(* χ^2 = Chi square)

LC_{50} = 0.0034 ppm = 95 % Upper confidence limit of LC_{50} = 0.0034

95 % Lower confidence limit of LC_{50} = 0.0033

LC_{90} = 0.0075 ppm = 95 % Upper confidence limit of LC_{90} = 0.0082

95 % Lower confidence limit of LC_{90} = 0.0067

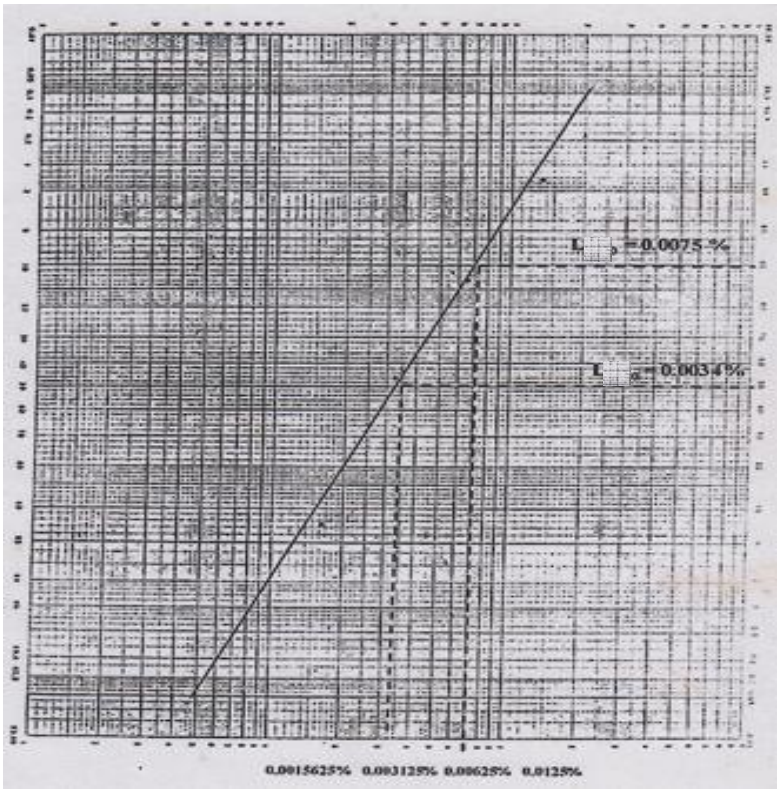

Figure 5 Testing the goodness of fit for dose effect analysis of temephos (Abate) on *A.aegypti* larvae; (% concentration and *A.aegypti* larval mortality)

Table 4 Comparison on LC_{50} and LC_{90} for tested samples

Tested samples	LC_{50} (%)	LC_{90} (%)
PE extract from aerial parts of *S.aemella*	0.0065	0.0146
EtOH extract from bark of *M. azedarach*	1.008	3.693
Temephos (Abate)	0.34×10^{-8}	0.75×10^{-8}

The Effective Persistency of Petroleum Ether Extract from *S. acmella* on *A. aegypti* Mosquito Larvae

Petroleum ether extract from aerial parts of *S. acmella* provided as a promise natural larvicides, the persistent effect of it was investigated on the immature 3rd and 4th instar stage, *A. aegypti* larvae and the results are shown in Table 5. The extract at 0.025 % concentration was found to be persisting up to 4 days (60 % in mortality rate).

Table 5 The Effective persistency of PE extract of *S. acmella* on immature stages of *A. aegypti* mosquito larvae

Concentration (%)	Effective persistency								Remark
	Day-1		Day-2		Day-4		Day-6		
	T	C	T	C	T	C	T	C	
0.025	87	2.5	76.5	1	60.5	0.5	12.5	0	*S. acmella* extract at 0.025 % persists up to 4 days and percent mortality was still 60 % on Day 4.
0.0125	38	2.5	34	1	41.5	0.5	12.5	0	
0.00625	22	2.5	12	1	9.0	0.5	1.0	0	
0.003125	1.5	2.5	4.5	1	8.5	0.5	0.5	0	

T = Test C = Control

Acute Toxicity Study of Ethanol Extracts from *S. acmella* Aerial Parts and *M. azedarach* Bark

The acute toxicity test was done according to the up-and-down procedure and the lethal dose of the ethanolic extracts of *S. acmella* and *M. azedarach* were estimated in mice. The animals are observed periodically during the first 24 h with special attention was given during the first four hours, then at least once a day after 14 days or until they recover. Clinical signs, including time of onset, duration, severity, and reversibility of toxic manifestations, were recorded at each observation period. Body weights were determined pre-treatment, weekly thereafter, and at the death of the animals or termination of the study.

In the acute toxicity test, signs of toxicity include muscle weakness, lethargy, loss of righting reflex and death.

It was observed that ethanolic extract of *S. acmella* in mice was no sign to toxic at 10 g/kg that is maximum permissible dose. Therefore, *S. acmella* extract can be safely used as larvicide and has no harmful effect to mammalian.

On the other hand, it was found that the maximum giving dose for ethanolic extract of *M. azedarach* in mice was 8 g/kg within survival period for 7 days to be looked forward. At dose 10 g/kg, one out of three mice was found to dead. *M. azedarach* showed slight toxicity. Therefore, care must be taken if bark of *M. azedarach* is used as larvicide.

Toxicity of Insecticide to Aquatic Vertebrate *Clarias batrachus* (Nga-khu)

The toxic effect of insecticide (Petroleum ether extract from aerial part of *S. acemella*) on *C. batrachus* (Nga-khu) was investigated for this study. The maximum giving dose was 0.025 % concentration level. The mortality of dead fishes was investigated after 24 h period. PE extract of *S. acemella* showed no toxic effect and no lethality was observed for 0.025 % dose. Therefore, *S. acmella* extract was not observed harmful effect on fishes *C. batrachus*.

Conclusion

PE extract from aerial part of *S. acemella* (LC_{50} = 0.0065 %, LC_{90} = 0.0146 %) and EtOH extract from bark of *M. azedarach* (LC_{50} = 1.008 %, LC_{90} = 3.693 %) showed potent larvicidal activities against the late third and fourth instar larvae (*A. aegypti* mosquito). The larvicidal

activity of synthetic larvicide, Temephos (Abate) was LC_{50}= 0.0034 ppm and LC_{90} = 0.0075 ppm. Ethanolic extract of *S. acmella* in mice was not shown sign to toxic at 10 g/kg that is maximum permissible dose. Therefore, *S. acmella* extract did not show harmful effect to mammalian.

In acute toxicity test, the maximum giving dose for ethanolic extract of *M. azedarach* in mice was 8 g/kg within survival period for 7 days to be looked forward. At dose 10 g/kg, bark of *M. azedarach* showed lethality (1 per 3 mice). Therefore, care must be taken if bark of *M. azedarach* is used as larvicide.

Petroleum ether extract of *S. acmella* was not observed lethality on fishes *C. batrachus* (Nga-khu) at 0.025 % concentration level. Therefore, PE extract of *S. acmella* was not found toxic effect to aquatic vertebrates and can be used safely as natural larvicide in fresh water.

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