THE EFFECT OF ROTENONE CRUDE EXTRACT FROM *Derris elliptica* ON THE LARVICIDAL ACTIVITY (MORTALITY) OF MOSQUITO

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ABSTRACT

Rotenone is a bio-active compound extracted from *Derris elliptica* (Tuba). It has long been used as bio-pesticide, which is more environmental friendly. Recently, new processing methods have been published on the extraction and usage of rotenone. The objective of this paper is to obtain the effectiveness of *Derris* plant crude extract against mosquito larvae. Rotenone crude extract was extracted using Normal Soaking Extraction (NSE) method. Two different solvent ratios were used to extract rotenone which is: (A) Methyl chloride: methanol (1:1) and (B) Methyl chloride: methanol (1:9). One part of both crude extracts using solvent ratios A and B were concentrated further using the rotary evaporator at 40 ^{0}C and under 800 mbar vacuum pressures. All the samples (normal and concentrated) were subjected to larvicidal activity testing using mosquito larvae. The samples were diluted to 5 different concentrations (0.01 mg/ml, 0.02 mg/ml, 0.03 mg/ml, 0.04 mg/ml and 0.05 mg/ml) for the biological activity treatment (LC_{50}). Six larvae of *Aedes aegypti* were used to test each samples. The study found that concentrated crude extract (B) using solvent ratio (1:1) gave optimum mortality of mosquito larvae of 83.33 % at 0.05 mg/ml after 5 hours post treatment.

Keywords: *Derris elliptica*; rotenone; mosquito larvae; biological activity; mortality

INTRODUCTION

History has recorded the use of rotenone as bio-pesticide by plant and vegetables growers worldwide. The reintroduction of this bio-active compound as an environmental friendly bio-pesticide in organic fruits and vegetables plantations is now an emergent trend. Tuba plant is kind of woody plant, grow along the ground and climbing to other plant and it is leafy. It needs at least 75 % moisture and temperature 25 °C to live. Tuba plant is known by its botanical name as *Derris elliptica*. Rotenone is the bio-active compound extracted from *Derris elliptica* and other important constituents of *Derris* root (deguelin and tephrosin) have been shown to be toxic to insects, however they are less active than rotenone (Waterman, 1980). Commercially important plants like *Derris elliptica* and *D. malaccensis* contains 4.0 % (w/w) to 5.0 % (w/w) rotenone (Parmar, 2001).

Despite significant advances in the techniques used for its control during recent decades, the mosquito continues to pose serious public problems. Mosquitoes are the principal vector of a variety of serious diseases, including malaria, yellow fever, dengue and encephalitis. Alone, malaria is estimated to kill between 1.5 and 2.7 million people every year (Beier, 1998). Insecticides have been used for vector control, mainly organic compounds in origin such as organochlorides, organophosphates, carbamates and pyrethroids. This method of control has proved to be ineffective and undesirable because of development of insect resistance and environmental pollution due to continued accumulation of slowly degradable toxic compounds (Palchick 1996).

METHODOLOGY

Plant collection - Derris elliptica was collected in the state of Johor (Kota Johor Lama), Malaysia.

Mosquitoes larvae - The larvae was collected around residential area in the state of Johor (Taman Universiti, Skudai), Malaysia. The larvae was randomly selected from early to fourth instar larvae and all larvae were

kept into humidified cages and dark in a room temperature at 27 ^{0}C for overnight before the experiments can be carry out.

Extract preparation - The roots of the plants *Derris elliptica* were dried at room temperature, ground in a knife mill. The extraction was carried out in 4 sets of solution by soaking 12 g of the dried powder in 250 ml of two different solvent ratios which is (A) Methyl chloride: methanol (1:1) - Unconcentrated solution, (B) Methyl chloride: methanol (1:1) - Concentrated solution, (C) Methyl chloride: methanol (1:9) - Unconcentrated solution and (D) Methyl chloride: methanol (1:9) - Concentrated solution for 24 hours at room temperature (≅ 28 ^{0}C). The extracts solution of set (B) and (D) were concentrated further using rotary evaporator at 40 ^{0}C to 50 ^{0}C and under 800 mbar vacuum pressures in order to remove approximately 70 % to 80 % volume of solvent.

Analysis of crude extract - The extracts solution were subjected to quantitative analysis using High performance Liquid Chromatography (HPLC) to determine the initial concentration for each sets of solution prior to bioassay treatment.

Bioassay - Each root extract (unconcentrated and concentrated solution) were diluted to the appropriate concentrations (0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml). Six larvae were pipetted into each 20 ml volume (*Petri* dish) and observed for a maximum of 5 hours, when mortality was recorded. Larvae were considered dead or moribund if they stopped moving for a prolonged period even after gentle probing with a small spatula, as described in the World Health Organization's technical report series. Larvae maintained in distilled water were used as a control (Armitage & Berry, 1987).

RESULT & DISCUSSIONS

The analysis of extract solutions were carried out by using an external standard method (Rotenone PESTANAL®, analytical grade, 96.2 % - SIGMA-Aldrich™). The concentrations for each set of solutions were obtained (Table 1.0). Each set of solutions with known concentration will be diluted accordingly to the appropriated concentration in the bioassay.

Table: 1.0: Concentration of each samples solution obtained from the HPLC analysis

Solvent Ratios	Samples	[a]Rotenone concentration (mg/ml)
1:1	(A)	0.21
	(B)	2.77
1:9	(C)	0.13
	(D)	0.59

[a]Rotenone concentration (mg/ml) is obtained from the external standard method [$C_{sample} = A_{sample}/SF$]

Different concentrations of *D. elliptica* root extract were tested against the randomly selected level of larvae instar. Dose-response curves obtained after 5 hours of treatment were exponential (Figure 1.0), showing that both solvent ratios with further concentration process for sets (B) & (D) were very effective against this mosquitoes larvae. The (B) solution was the most active solution compared with others presenting LC_{50} (Lethal Concentration of 50 % mortality) value of 0.03 mg/ml (30 ppm) and the LT_{50} (Lethal Time of 50 % mortality) was approximately 3 hours. Solvent ratios and the concentration process that involved in this study have a significant effect on the larvicidal activity by given the highest mortality of mosquitoes larvae 83.33 % for the solvent ratios of Methyl chloride: methanol (1:1) - Concentrated solution and the concentration applied during the treatment was 0.04 mg/ml to 0.05 mg/ml for 4 to 5 hours. Mortality among the controls was zero for more than 95 % of the assays. Figure 1.2 shows the profile of % mortality against the rotenone extract concentrations (mg/ml).

The mosquito larvae secrete a layer of non-cellular material which is separates the food from the epithelial cells of the gut. This layer is called peritrophic matrix (PM). The PM acts as a protective barrier against various chemical, physical and microbial food components (Peters, 1992). In this study, the larval mortality caused by the methyl chloride: methanol (1:1 and 1:9) of *D. elliptica* appears to be related to disruption of PM structure and rupture the midgut cells.

The extract of *D. elliptica* has already been the subject of phytochemical studies and has been shown to contain insecticidal isoflavonoid-type compounds known as rotenone (Davidson, 1930). In addition to these studies, a bioassay-guided chemical fractionation protocol will be conducted in order to identify further larvicidal components in this extract, mainly those responsible for PM disruption. These metabolites could be used as part of a novel strategy for insect control. Disruption of PM structure could facilitate the transport and enhance the insecticidal activity of different agents such as virus, bacteria, protozoa, toxic proteins and plant secondary metabolites.

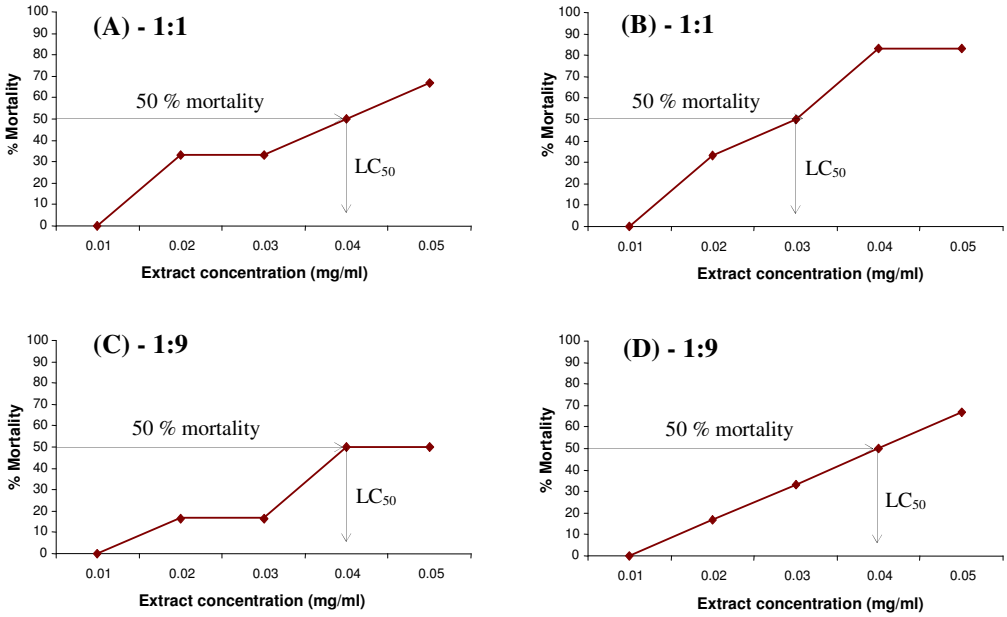

Figure 1.0: Mortality (%) dose-response curves of different rotenone extract ratio

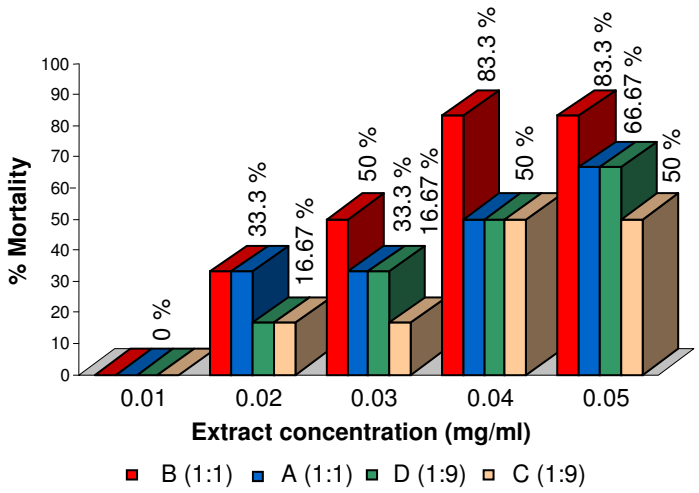

Figure 1.2: Biological activity profiling of different rotenone extract ratio

ACKNOWLEDGEMENT

This research was supported by an IRPA grant 09-02-06-0083 EA261 under the Ministry of Science, Technology and Environment, Malaysia.

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