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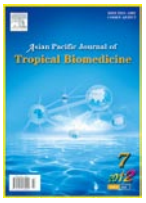

Document heading

Toxicological studies for some agricultural waste extracts on mosquito larvae and experimental animals

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ABSTRACT

Objective: To evaluate some agricultural waste extracts as insecticide and their effects on enzyme activities in liver and kidney of male mice. **Methods:** The insecticidal activity of five tested compounds (one crude extract and 4 waste compounds) was bioassay against the 3rd instars of the *Culex pipiens* (*Cx. pipiens*) larvae in the laboratory. The LC_{50} values of eucalyptol, apricot kernel, Rice bran, corn, black liquor and white liquor are 91.45, 1 166.1, 1 203.3, 21 449.65, 4 025.78 and 6 343.18 ppm, respectively. Selection of the compounds for the subsequent studies was not only dependent on LC_{50} values but also on the persistence of these wastes products on large scale. **Results:** White and black liquor did not produce any gross effect at 200 mg/Kg body weight. No apparent toxic symptoms were observed in tested animals during the whole period of the experiment which run out for 14 days. No statistically significance was observed in the enzyme cholinesterase activity, the activities of liver enzymes and kidney function in treated mice with black and white liquors. While, no and slight inhibition was observed after the 2 weeks of treatment period with deltamethrin and fenitrothion reached to about 24% in plasma cholinesterase enzyme activity. Significantly increase in the activities of liver enzymes and kidney function in treated mice with deltamethrin and fenitrothion. **Conclusions:** Black liquor can be used efficiently to control *Cx. pipiens* larvae under laboratory condition. Environmental problem caused by rice straw can be solved by converting the waste material to beneficial natural selective insecticide.

1. Introduction

Mosquitoes are still the world's number one vectors of human and animal diseases; and are conspicuous nuisance pests as well, even after massive efforts of eradication or control. The extensive use of chemical pesticides or insecticides resulted in inducing resistance by insect pests besides, residue contamination of human food, mammalian toxicity and environmental pollution[1]. These factors have created the need for environmental safe, degradable and target specific agents for pest control purposes. The use of deoiled rice bran and chicken feather waste, as culture medium, is highly economical, for the industrial production of the mosquito pathogenic *bacillus*[2].

Plant extracts have gained importance in insect control, being considered environmentally safe, less hazardous to non target biota, simple inexpensive and can be applied effectively by using techniques more suitable for developing countries[3,4]. Several plant extracts were effective as potential acute or chronic insecticides, insect growth inhibitors or antifeedants against a variety of insect species[5,6]. Only Bakr *et al*., studied the effect of agricultural waste extracts (bran of *Oryza sativa* (*O. sativa*)) on the newly moulted 5th nymphal instars' of *Schistocerca gregaria*[5,6]. Previous studies also discussed changes in protein content and some biochemical effects of some agricultural waste extracts against *Culex pipiens* (*Cx. pipiens*) [7,8].

Several studies have shown that, pyrethroid caused alterations in biochemistry, hematology and reproduction[9]. Repeated daily oral doses of pyrethroid insecticide of α-cypermethrin at 1/10 LD_{50} altered the biochemical parameters, decreased cytochrome P450 content, antioxidant status which correlated with histopathological changes of tissues[10]. Moreover, while there is some evidence

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that chronic exposure to organophosphate pesticides may have adverse effects on health, there is an urgent need for high-quality observational of both occupational and environmental exposure to these compounds[11,13].

Therefore, the present study was undertaken to evaluate some agricultural waste extracts as insecticide and their effects on enzyme activities in liver and kidney of male mice for using these extracts alternate the traditional insecticides in IPM program.

2. Materials and methods

2.1. Tested compounds

2.1.1. Eucalyptol (Eucalyptus oil fraction)

Eucalyptus oil commercial grade from the local market mixed the crude oil with pet. Ether (60-80) and fractionated through silica gel column (70 mesh), the first fraction was eucalyptol.

2.1.2. Apricot kernel extract

Apricot seeds were crushed and the kernels were taken for the extraction process. One hundred grams of kernels were boiled in 1 000 mL of water for 45 min, then the water was evaporated till dryness. The resulted dry crude extracts were weighed and the overall yield from 100 g of each plant by each solvent was calculated before storage at 4 ℃ in screw capped vials, until use.

2.1.3. Rice bran extract

Rice bran was exhaustively extracted with ethanol. The extraction was accomplished by means of a Soxhlet apparatus. The solvent extract was evaporated and dried under vacuum using a rotary evaporator of water bath adjusted at 40-60 ℃. The resulted dry crude extract was weighed and the overall yield from 100 g of plant was calculated before storage at 4 ℃ in screw capped vials, until use.

2.1.4. Corn extract

Produced by patent no. (422/2008).

2.1.5. Black liquor

Black liquor produced from the paper production industry but in new way patent No. (422/2008).

2.1.6. White liquor

White liquor was effluent from bleaching paper that introduced in paper production industry[14] with modification, using sod. hyposulphite at 60 ℃ with stirring for one day.

2.2. Tested mosquitoes

2.2.1. Cx. pipiens (Culicidae: Diptera)

Larvae of *Culex pipiens* provided from Medical Entomology Institute and transferred to the laboratory of Entomology Department-Faculty of Science-Ain Shams University where self-perpetuating colonies were established and maintained during the present study.

Preliminary, toxicological bioassay tests were carried out on tested compounds as a modification for the method described by Wright 1971[15]. Mortality data was analyzed by using log-probity analysis to estimate probity regression line and calculate LC_{50}, LC_{90}, slope function by applying the computer program of Unkelbach[16].

2.3. Toxicological evaluation of the selected compounds on experimental animals

2.3.1. Acute toxicity

Sixty adult Wister rats of both sexes for each compound purchased from animal house colony, in National Research Center, Giza, Egypt. The animals weighing about (200 ± 20) g were divided into six equal groups each containing 10 animals. All rats were kept under controlled conditions of temperature (22 ± 1) ℃ and humidity 60% ± 5%. They were given pellet food and drinking water ad libitum. The experimental protocol met the national guide lines on the proper care and use of animals in the laboratory research.

2.3.2. LD_{50} determination

To determine the lethal dose that kills half (50%) of the animals tested "LD_{50}" for selected compounds, black and white liquor, six doses of each compound ranged from 500 to 5 000 mg/Kg body weight were used. Each dose from both compounds was dissolved in 0.5 mL water and was administered orally to rats using stomach tube with ball tipped oral incubated needle. The LD_{50} value of the two compounds was calculated, for 24 h. mortalities following each treatment.

2.3.3. Sub-acute toxicity

Groups of Swiss albino mice of both sexes (8-10 weeks old) weighing (22 ± 1) g were housed under standard laboratory conditions and had free access to food and water.

In studies of sub-acute toxicity of white and black liquor, a group of 40 mice was used in each treatment for both compounds. In parallel, a group of 30 mice were used as control. Doses equivalent to 4 mg/mice/day of each compound were dissolved in 100 μL of water and administered orally for two weeks.

At the same time another two groups of 40 mice for each group were used to compare the toxic effect of organophosphorus and pyrethroid insecticides Fenitrothion and Deltamethrin against the two previous compounds. Mice treated daily with Fenitrothion and Deltamethrin dissolved in dimethylsulphoxide (DMSO) at dose 0.8 & 12.5 mg/mice/day, respectively corresponding to 1/20 LD_{50}. Animals were observed with regard to behavior, appetite, body weight gain