Effects of Maja Leaf (Aegle marmelos) Extracts on The Morphological of Aedes aegypti Larvae

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The use of synthetic insecticides continuously can cause adverse affects in the form of environmental pollution, such as death of living things, insects become more resistant with pesticide, even gene mutations can occur in insects. Therefore we need insecticide from plants that more safer to use. One of them is a plant from the Rutaceae family known as Maja or Bael (Aegle marmelos) which is used for controlling vector-borne diseases. Maja leaves have the potential as an insecticide because they contain several chemical compounds such as alkaloids, terpenoids, saponin, and tannin. The purpose of the study was to look at the morphological changes of Aedes aegypti larvae due to administration of maja leaves extract with acetone solvent. This experimental study used a completely randomized design with negative control in the form of aquadest, positive control in the form of temephos 1% and maja leaves extract treatment with acetone solvent concentration of 0.1 g/mL, 0.2 g/mL, 0.4 g/mL, 0.6 g/mL, 0.8 g/mL and 1.6 g/mL. Ae. aegypti larvae mortality was assessed for 24 hours and the morphological changes were immediately seen using a light microscope. Within one hour, mortality of *Ae. aegypti* larvae appeared at concentrations of 1.6 g/mL. The negative control group did not show morphological changes. While the positive control group using temephos showed a change in the color of the head to blackish brown, damage to the abdomen and pecten and the rear abdomen changed to a more blackish brown color. Maja leaves extract with acetone concentration of 1.6 g/ mL causes color changes in the head of *Ae. aegypti* larvae and damage to the abdomen. Abdomen larvae of the Ae. aegypti appear to shrank. The effective dose of maja leaves extract needed to kill Ae. aegypti larvae is 1.6 g/mL with damage especially to the abdomen.

Aedes aegypti, Diptera, Culicidae (Linnaeus, 1762); can be found in human dwellings where it obtains food, copulates and spawns. As ubiquitous insect, this species exhibits a high ability to adapt to artificial habitats allowing for their occupation and expansion and, consequently, emergence of epidemias1. The highest epidemiologic importance of this study is that mosquitoes play a role as transmitters of yellow fever and dengue virus2. The control of the dengue vector and important insect species related to public health has been managed in the last decades mainly with synthetic chemical insecticides. The intense use of these synthetic compounds has many exposed mosquito populations to intense selective pressure and hence the prevalence of some populations are resistant to products used to control these vectors. Thus, the use of secondary metabolites of plants that have insecticidal potential is being studied to minimize the impact of synthetic compounds on the environment and human health3. The close relationship between plants and insects and the possible coevolution of plants led to the development of strategies to attack these invertebrates that, are physical or chemical in nature4. Thus, Cnidoscolus phyllacanthus, Ricinus communis (Euphorbiaceae) and Coutarea hexandra (Rubiaceae) are chemical compounds whose biological activities have been described5−8.

In view of the operational and economic difficulties generated by increased mosquito resistance to synthetic insecticides, alternative methods have gained increasing attention9. Several plant species have been investigated and tested with potential larvicides directed against several insect species including Aedes aegypti, Magonia pubescens10, Atlantia monophylla11, Cybistax antisyphilitica12, Anacardium humile13 and Spathelia excelsa14. In this study we report the insecticidal activity (larval and pupal) of C. phyllacanthus, C. hexandra and R. communis on Aedes aegypti.

METHODS

The population of A. aegypti was collected in the District of Monte Santo, Campina Grande, State of Paraíba, Brazil. The collection and establishment of strains in the laboratory occurred between December 2009 and August 2010. Eggs were collected with 50 ovitraps that were installed inside and outside homes distributed in ten blocks where five traps were installed per block.Establishment and maintenance of Aedes aegypti in the laboratory The laboratory bioassays were conducted in a temperature-controlled room (26±2°C and 12h light). Vanes eucatex containing A. aegypti eggs from the field were dried for 48h and then packed in white plastic trays (40x27x7.5cm) with a third of its capacity filled with non chlorinated water. After the outbreak, ornamental fish food (Goldfish growth) was added (100mg/tray). Adults were maintained in cages with a wooden frame coated with organza (40cm x 40cm x 30cm) containing 200 individuals

(100 males and 100 females). Adults were fed with a 20% honey solution and the females were fed blood from the quail

Coturnix japonica three times a week for 30min. After meals were introduced inside each cage, a disposable cup containing 200ml of distilled water with a plastic funnel coated with filter paper served as a substrate for oviposition.

Collection and preparation of solutions

The ethanolic fraction was obtained from cauline tissue from C. phyllacanthus and C. hexandra, which was placed in an incubator at 40°C for 48h. When dry, the shells were crushed and sieved. The powder was moistened with 90% ethanol and subsequently percolated for cold extraction for 48h. After this period, the ethanolic fractions were collected and concentrated by spin evaporation to yield extracts. Seeds from C. phyllacanthus and R communis were used for the extraction of vegetable oil. These were first crushed and macerated and then subjected to a hydraulic press in the cold. The fixed oils were stored in glass bottles covered with aluminum foil and kept in the refrigerator.

Larvicidal and pupicidal bioassaysLarvicidal and pupicidal test were performed according to the methodology of the World Health Organization15 with some adjustments. The biological activity of the extracts of the specimens used in the laboratory was found in late L3 and/or early L4 larval and pupal stages of populations of A. aegypti to obtain of the lethal concentrations (LC50 and LC90) values. We used 30, 60, 125, 250 and 500mg/mL of ethanolic fractions diluted in 1mL of dimethyl sulfoxide (DMSO). For vegetable oils, a 1:1 ratio with tween 20 was made at the following concentrations:

100, 190, 380, 750 and 1.5µl/mL. DMSO and tween and water were used as controls. For each concentration there were four replicates with 25 individuals per replicate, and mortality assessed was after 24 and 48h of exposure. For pupal tests, the occurrence of adults, if they were dead, was counted as pupae. Effect of sublethal (lethal concentrations of cumulative effect), plant extracts were established via the LC50 and LC90 values determined with the test larvicide after 48h of exposure to plant products. There were four replicates with each containing 30 third instar A. aegypti larvae. Daily readings were made with verification of the larval stage, behavior changes, the presence of exuvia, adult emergence, possible mortality of the larvae, pupae and adults, and the water temperature. The experiment was conducted until the last pupa or adult died or until the last adult completely emerged. Mortality data for the larvae and pupae of the studied population were subjected to Probit analysis using the Polo-PC program to determine the lethal concentrations (LC50 and LC90). There was no need to correct the data with the Abbott formula16.

Bioassays of the larvicidal and pupicidal effects of these products at different concentrations and times of exposure were evaluated. The lethal and sublethal effects were determined using different concentrations in larvicidal tests. Mortality data were evaluated by Probit analysis to determine the LC50 and LC90 values.

Biography

Monica Puspa Sari has completed his magister of biomedical at the age of 31 years from Indonesian University. She is the lecturer in parasitology at the Christian University of Krida Krida Wacana, Jakarta Indonesia**.**