ISSN 2089 - 2136

Tropical Medicine Journal

Volume 03, No. 2, 2013

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TMJ	Volume 03	Number 02	Page 95 - 195	ISSN 2089 - 2136

Center for Tropical Medicine, Faculty of Medicine, Universitas Gadjah Mada
in collaboration with Indonesian Society of Tropical Medicine and Infectious Disease (PETRI)

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Universitas Gadjah Mada, Yogyakarta 55281, Phone : +62-274-588483, E-mail: tropmedjournal@gmail.com

TROPICAL MEDICINE JOURNAL

ISSN : 2089-2136
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Volume 03, Number 02

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Effication Test of Srikaya Seeds Extract (*Annona squamosa* L.) to Kill *Aedes aegypti* Larvae in Laboratory

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ABSTRACT

Introduction: Dengue fever is still becoming national health problem. Control of it have been done but the cases still increase significantly. One way to control the disease is vector control of *Aedes* sp. Generally, people prefer to choose synthetics insecticides than botanical insecticides and environmental management. Used of synthetics insecticides causing mosquito resistence.
Objectives: To know the potency of Srikaya seeds as botanical larviciding to *Aedes aegypti* larvae as main vector of dengue fever.
Methods: Experimental study with cross sectional design have done with three times repeat of efication test. Extraction process of Srikaya seeds according to Kardinan (2000). Concentration of Srikaya seeds extracts are 25%, 50% and 75% with control. Population of the study is *Ae.aegypti* larvae (L3) from Entomology Laboratory of Balai P2B2 Banjarnegara. Data was observed are mortality and growth of larvae into pupa and imago stages. Probit analyzed was done and describes by graphic and table.
Results: Srikaya seeds extracts effect to *Ae. aegypti* larvae and causing larvae death for each concentration test (concentration 25%, 50%, and 75%). Growth of *Ae.aegypti* larvae also decreases that shown by pupa failure to reach imago stages after 6th day. This result shows that Srikaya seeds extracts have potency as larvicide might be due to annonain and squamosin content as acetogenine that can cause mortality of some insects Ordo.
Conclusion: Srikaya seeds extracts have potency as larvicide on *Aedes aegypti* larvae. Srikaya seeds extract have a potency as botanical insecticides, not only to pest control on farm and poultry but also in vector diseases control.

Keywords: Srikaya seeds extracts, larvicide, *Aedes aegypti*, mortality

INTISARI

Pendahuluan: Demam berdarah masih menjadi masalah kesehatan nasional. Pengendalian itu telah dilakukan tetapi kasus masih meningkat secara signifikan. Salah satu cara untuk mengendalikan penyakit ini ialah dengan pengendalian vektor *Aedes sp.* Umumnya, orang lebih suka memilih insektisida sintetis daripada insektisida botani dan pengelolaan lingkungan. Penggunaan insektisida sintetis menyebabkan resistensi nyamuk.
Tujuan: Untuk mengetahui potensi biji Srikaya sebagai penggunaan larvasida botani untuk Aedesaegypti larva sebagai vektor utama demam berdarah.
Metode: Penelitian eksperimental dengan rancangan potong lintang ini dilakukan dengan tiga kali pengulangan uji efikasi. Proses ekstraksi biji Srikaya menurut Kardinan (2000). Konsentrasi ekstrak biji Srikaya adalah 25%, 50% dan 75% dengan kontrol. Populasi dari penelitian ini adalah larva Ae.aegypti (L3) dari Laboratorium Entomologi Balai P2B2 Banjarnegara. Data yang diamati adalah kematian dan pertumbuhan larva menjadi pupa dan tahap imago. Probit dianalisis dilakukan dan dijelaskan dengan gambar dan tabel.

Hasil: Ekstrak biji Srikaya berefek pada larva *Ae. aegypti* dan menyebabkan kematian untuk setiap konsentrasi uji. Pertumbuhan larva *Ae.aegypti* juga menurun menunjukkan oleh kegagalan pupa menjadi tahap imago setelah hari ke-6. Hasil ini menunjukkan bahwa ekstrak biji Srikaya berpotensi sebagai penggunaan larvasida kemungkinan karena mengandung annonain dan squamosin sebagai acetogenine yang dapat menyebabkan kematian dari beberapa serangga Ordo.
Simpulan: Ekstrak biji Srikaya berpotensi sebagai penggunaan larvasida pada larva *Ae. Aegypti*. Ekstrak biji Srikaya mempunyai potensi sebagai insektisida botani, tidak hanya untuk mengendalikan hama pertanian dan peternakan tapi juga mengendalikan vektor penyakit.

Kata kunci: ekstrak biji Srikaya, larvasida, *Aedes aegypti*, kematian

INTRODUCTION

Dengue fever is a disease that caused by dengue viruses that transmitted from one person to another by biting of *Aedes* mosquitos as a vector, mainly *Aedes aegypti*[1,2]. Dengue viruses transmitted by biting of imago stages of female *Aedes* as daily feeder with activity time of biting on morning and afternoon. There are two kinds of *Aedes* that have potency as dengue vector, they are *Ae. aegypti* and *Ae. albopictus*. The mosquitos that found almost on people residences is *Ae. aegypti* and mainly in urban area. This mosquitos have ovipotition sites in a containers either artificial or natural[2,3].

Dengue fever is a diseases that still becoming a health problem in the world because the vaccine and antiviruses agent not yet found[4]. The increase in dengue epidemic can be attributed to the rising level of urbanization, which promote contact between humans and *Aedes* sp.,inadequate domestic water supplies, and increasing international or national travel, migration, trade, which help disseminate vectors and virus[2]. Another factors are opening of new residence that makes the density of residences and people increased, low control practises of breeding places on household and community levels, there are mosquito vector in almost of Indonesia area, and the four dengue serotypes, known as dengue 1, 2, 3 and 4 that is always circulating all of the years.

Many control methods have been done. Some of them are by fogging and controling of breeding places, but it is still not effective because of many factors. Actually, there are three methods of mosquitos vector control which are by chemical, biological and environmental management that could be practiced with rational system and attention to endemicity level of the area[5].

One of method to control dengue fever is by vector control. Generally, control of mosquito used synthetics insecticides. But, in 1997, the government regulated the used of some insecticides and put off the subsidy so the cost of insecticides become expensive. Those regulation according to some research papers shows that some synthetics insecticides makes negative effects likes intoxication to human and poultry, environmental pollutions, improvement of new variety of pest resistances, to appear a secondary pests, and also killed the natural predator of pests and non-target animals. This conditions became promoting factors to find out alternative insecticides, such as botanical insecticides [6,7].

Botanical insecticides actually has been long times used on farmer to controlled the pests. There are 650 kinds of plants that have potency of insecticides by traditional applications with many types of material likes shelling, pounding, burning or pressing [8,6,7].

One kind of plants that have potency as botanical insecticides sources is Srikaya (*Annona squamosa* L.). The main of active material in Srikaya seeds are annonain and squamosin that included asetogenine material[9,10]. Asetogenine from Annonaceae groups reported higly enough of toxicity that effect to some insect Ordo likes Lepidoptera, Coleoptera, Homoptera and Diptera[11,12]. Another report also revealed that annonain and reticulin from Annonaceae groups have a potency as Acariciding[13].

The seeds of Srikaya containing higly enough of lipid (42-45%)[7], so can be solved in alcohol because alcohol as non-polar solvent is able to dissolve lipid and another lipofilic materials. The effect of the Srikaya seeds extract to the death and growth of *Ae. aegypti* larvae never been reported. Therefore, the aims of this research is to observe the potency of Srikaya seeds extracts as botanical insecticides to *Ae. aegypti* larvae in laboratory.

MATERIAL AND METHODS

This research was experimental study with cross sectional design. This research was conducted on August-November in 2012. The samples are *Ae. aegypti* larvae (3rd instar/L3) from Entomology laboratory of Balai P2B2 Banjarnegara, Central Java. Srikaya fruits was collected from local traditional market. The seeds that black or dark brown colour was pill out from bear-fruits and then dried on sun light until 7 days. All of seeds were peeled out from the skin-seeds and was blended to get powder of the Srikaya seeds[7].

Extraction process is wet-extraction. A hundred gram of Srikaya seeds powder was prepared in a beaker glass, added 100 mL aquadest, and mixed. Filter it used paper filter and the result is 100% concentration extracts.

- To make 25% concentration extract: put 25 mL extract + 1 mL alcohol + 74 mL aquadest.
- To make 50% concentration extract: put 50 mL extract + 1 mL alcohol + 49 mL aquadest.
- To make 75% concentration extract: put 75 mL extract + 1 mL alcohol + 24 mL aquadest.
- Control: aquadest only without Srikaya seeds extract.

The observation was repeated three times on each trays contained 30 larvae. The parameter was observed are mortality of larvae, growth of larvae to pupa, and fecundity of pupa to imago. The power of resistances of larvae observed everyday until all of the larvae showed mortality or growth becoming imago stages. The mortality data were analyzed by probit analysis. The data of larvae growth into pupa and pupa into imago were analyzed by descriptive methods.

RESULTS AND DISCUSSIONS

A. The Effects of Srikaya Seeds Extract to Moratlity of *Ae. aegypti* Larvae

The mortality of *Ae.aegypti* increased significantly on each concentration test, they are 25%, 50% and 75%. This result shows that Srikaya seeds extract have a potency as larvicide (causing mortality of larvae). The result value of significancy analysis was 0.365 (>0.005), indicating that there is no significant differences for three concentration tests either 25%, 50% or 75%. All concentrations have same effects to *Ae. aegypti* larvae mortality.

The result of CI analysis with 95% confident limit showed the value of LD 50 for 25% concentration is 63.490 on normal distribution tolerance. The value of ED 50 is 4.151 with under-upper limits are 3.828-4.316. And then, the value of LD 90 is 97.738 on normal distribution tolerance. Nilai ED 50 is 4.582 with under-upper limits are 4.401-5.150.

The value of LD 50 for 50% concentration is 65.441 on normal distribution tolerance. The value of ED 50 is 4.181 with under-upper limits are 3.797-4.313. And then, the value of LD 90 is 100.741 on normal distribution tolerance. Nilai ED 50 is 4.613 with under-upper limits are 4.474-5.043.

The value of LD 50 for 75% concentration is 65.099 on normal distribution tolerance. The value of ED 50 is 4.176 with under-upper limits are 3.764-4.320. And then, the value of LD 90 is 100.214 on normal distribution tolerance. Nilai ED 50 is 4.607 with under-upper limits are 4.462-5.028.

B. The Growth of Larvae to Pupa Stages

The results revealed that for 25% concentration test on first tray, there were four larvae that still alive, and can grow into pupa (on 3rd day observation). Pupa that can grow into imago stages only two mosquitos, and two of them have died or not growth. The second tray of 25% concentration shows only one larvae that can growth into pupa but can not growth into imago. And the 3rd tray shows mortatlity of all larvae.The total mortality of larvae on 6th day.

The result of 50% concentration showed that only one larvae can grow into pupa and died (first tray on 4th day). Second tray showed two larvae grow into pupa and only one pupa can grow into imago (3rd day). The 3rd tray showed two larvae grow into pupa and get successfull growth into imago on 6th day. All larvae died on day 5.

Figures 1. *Ae.aegypti* larvae mortality of after effication test of Srikaya seeds extract

The result of 75% concentration showed five larvae can grow into pupa and get success growth into imago (first tray on 5th day). Second tray also showed five larvae can grow into pupa and only two pupa can grow into imago (3rd day). The 3rd tray showed one larvae can grow into pupa and then died. All larvae died on day 5.

Control tray shows there is no mortality afer observed 1 x 24 hours. All of larvae growth into pupa on 4th day. Imago stages totally 22 mosquitos until 6th day.

DISCUSSIONS

The of research results show that Srikaya seeds extracts have effect as contact toxin and effect the gastrointestinal system of larvae (stomach toxin effects). Active material that obstruct the growth of larvae are annonain and squamosin as acetogenines.

The mechanisms of this active material have been detected on moleculer level[10]. This materials are cytotoxic and neurotoxic that causing cells lysis. If this material contact or enter the body will makes barrier for compounding off NADH enzymes with cytochrom c-reductase and sub-unit I cytochrom complex in mitochondria of insect cells. Annonain and squamosin can obstruct electron transfer on sub-unit I that makes production of energy metabolic by celluler respiration.

This mechanisms also was studied on cow liver cells, they are obstruction to sub unit I complex of sytochrom enzymes that makes cell respiration stoped and causing lysis of cells. Continues research is isolating the acetogenin as active material of Srikaya seeds[13]. This research also revealed that acetogenines mostly effects the chain of respiration cells and have cytotoxic characteristics (toxic to cells).

Insecticides absorption that have stomach toxin is in mesenteron (medial digestives system). The wall of mecenteron cells consist of epithelium cells. This epithelium consist of two kinds of layers, they are lipida material and proteins material that spreaded in some parts of those lipid layers. Generally, this layers have lipofilic characteristics[6].

The Srikaya seeds extracts in this research is likes oily (containing lipids). This perform of extract revealed that mortality of *Aedes*larvae causing by toxic effect of Srikaya seeds. Active material of Srikaya seeds extract that solved on lipid makes easily absorp by epithelium cells so lysis of cell happens.

Srikaya seeds extract was many reported containing insecticides material and have efication test to some pests for examples *Callobruchusanalis* (pest of soya bean), *Pediculushumanus* (head louse), *Plutullaxylotella* L. (pest of cabbages), dog louse, fish toxin, *Boophilusmicroplus*, *Nilaparvatalugens* dan *Crocidolomia binotalis*[7,14,9,8,15,16].

This research also revealed that Srikaya seeds extracts as contact toxin with clinical symptons of larvae mortality, they are movement of larvae become slowly, the body of larvae is dried up and died. Larvae was not showed convulcions likes neurotoxin cases. The symptons revealed that larvae have lost of energy (ATP) and this mechanisms according to mechanisms systems of bioactive materials of Srikaya seeds extract on moleculer levels[10]. The continues research is needed to apply the extract to *Ae. aegypti* larvae in the field.

CONCLUCIONS

Srikaya seeds extract have a potency as botanical insecticides, not only to pest control on farm and poultry but also in vector diseases control.

ACKNOWLEDGEMENTS

The authors wish to thank the Ministry of Education and Culture of Indonesia that have supported this work research by grant of Ditjen-Dikti (Program Hibah Penguatan Politeknik Kerjasama Pemda (PHP-PKP) and wish to thank the Entomology Laboratory of Balai P2B2 Banjarnegara, Central Java for the eggs colony of *Aedes aegypti* for this research.

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Tropical Medicine Journal

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Scientific Journal

1. *Standard journal article*

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We thank to the reviewers of this edition:

dr. Abu Tholib, M.Sc., Ph.D., Sp.MK
dr. Ahmad Hamim Sadewa,Ph.D
dr. Arta Farmawati, Ph.D
dr. Elizabeth Henny Herningtyas, M.Si, Ph.D
Dr. Dra. Erna Kristin, Apt., M.Si.
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