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New Bioinsecticide Granules Toxin from Extract of Papaya (*Carica papaya*) Seed and leaf Modified Against *Aedes aegypti* larvae

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**Abstract**

The epidemic of dengue hemorrhagic fever (DBD) in Indonesia, which occurs every year, has become a problem for the Indonesian society. *Aedes aegypti* mosquito is a vector of this disease. During this time the larvae of *Aedes aegypti* only has one exterminator is abate. In the long time exposure of single type insecticide will cause resistance. Therefore, it is necessary to find new bioinsecticide which is expected to have effects such as abate. The aims of this study are to offer technology, dosage, formulation and toxicity of new bioinsecticide granules toxin from extract of papaya (*Carica papaya*) seed and leaf modified against *Aedes aegypti* larvae. Method: preparation of the extract by percolation method with 70% ethanol for seeds, and with water-ethanol 70% for leaf. Bioassay with active ingredients with thin layer chromatography. Formulation for 300 g Granules are: active substances papaya seed and leaf modified: 90%, seed flour 5%, filler: 5%. This was an experiment using a completely randomized design with 6 treatment groups. Each group contained 20 third instar larvae of *Aedes aegypti*. Concentrations of granules are 0, 30, 60, 90, 120, 150 ppm, with Temephos 1% as a positive control. Data was analyzed using Probit analysis. Results: The result of phytochemical analysis contains secondary metabolites compounds of saponin, flavonoid and triterpenoid. Toxicity (LC₅₀) in this study is reported 107 ppm; 48 hours and LC₉₀: 150 ppm, 48 hours. Conclusion: New Bioinsecticide Granules Toxin from extract of Papaya (*Carica papaya*) Seed and leaf Modified Against *Aedes aegypti* larvae possess technology, dosage, formulation, and larvicidal remarkable. Further investigations are needed to elucidate active ingredient responsible for larvacidal activity should be identified and utilized in preparing a commercial product.

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**Keywords:** granule, *Carica papaya*, seed extract, leaf extract, bioinsecticide, *Aedes aegypti*

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1. Introduction

_Aedes aegypti_ (Diptera: Culicidae) is an important vector of dengue hemorrhagic fever (DHF). It has spread with the growth of towns and poor sanitation [1]. The epidemic of dengue hemorrhagic fever (DBD) in Indonesia which occurs every year, has become a problem for the Indonesian society. During this time the larvae of _Aedes aegypti_ only has one exterminator is _abate_. It has caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans and other non-target organisms [2]. To alleviate these problems, major emphasis on the use of natural plant-based products as larvicides because they constitute a rich source of bioactive chemicals. They may also greatly reduce the risk of adverse ecological effects.

_Carica papaya_, the sole species in the genus Carica from the plant family Caricaceae, extracts of seed and leaf have were investigated larvicidal to _Aedes aegypti_ [1]. The hypoglycemic effect of ethanolic extract of _Carica papaya_ Seed and leaf to _Aedes aegypti_ larvae has been reported [3]. The larvicidal potentials extract of _Carica papaya_ Seed and leaf against larvae of _Aedes aegypti_ were investigated in the laboratory. Extract of papaya leaf and seeds are effective in killing mosquito larvae _Aedes aegypti_, indicated by the percentage of larval mortality, the observation to 48 h, the highest larval mortality in LC50: 54 ppm and LC90: 111 ppm [4]. Insecticidal activity from content of secondary metabolites in the leaves and seeds of papaya its nature is toxic such as saponin, flavonoid and triterpenoid. Flavonoids works as a stomach poison that lowers appetite larvae because larvae fail to recognize food stimulus, so that over time the larvae will die of starvation [5]. Saponin is a toxin that is polar, soluble in water, and when it enters the body in larvae can result in hemolysis in the blood vessels. Saponin contained in papaya seed extract and inhibit the process of metamorphosis, inhibit the formation of the larval skin, thus resulting in the death of the larvae [2]. Triterpenes is acute toxic compounds when were applied topically and/or incorporated into the water. Triterpenoid causing reduced feeding and increased mortality. The use of leaf extracts and papaya seed extract as larvicides relatively safer for the environment because it is a natural substance and its nature is not toxic, but extract of Papaya leaf into a body of water will affect the color and flavor. The use of papaya seed extract relatively no effect on the color, but it can change the taste of water, because papaya seed extract has a brighter color than the color. of papaya leaf extract but very bitter taste [6]. To alleviate these problems is needed the formulation of granules.

The prominence of granule extracts has given rise to a number of new trends in terms of formula composition and dosage. The development of concentrated extract granule has consistent and easily quantifiable nature. The portability and convenience of granules dramatically increases toxicity [7]. Research question in this study were: a) How are the technology for producing of manufacturing industrial-scale new bioinsecticide granules extract papaya seed and leaf modified against _Aedes aegypti_ larvae. b) How are formulas and toxicity of new bioinsecticide granules of extract from papaya (_Carica papaya_) seed and leaf modified against _Aedes aegypti_ larvae. This study offers to technology, dosage, formulation, and the prominence toxicity of new bioinsecticide granules of extract from papaya seed and leaf modified against _Aedes aegypti_ larvae.

2. Materials and Methods

2.1 Preparation of Granule

Preparation of extract by percolation method with 70% ethanol. Formulation Granules of condensed extract of papaya (_Carica papaya_ L.) seed flour substituted with modified seed granule weighing 300 g: Active substances papaya seed extract: 90% modified seed flour: 5% filler: 5% (consists of dry starch 1%, citric acid 1%, Sodium bicarbonate 3%). _Aedes aegypti_ larvae were collected from Jember-Indonesia. The larvae were kept at 28 ± 2 °C temperature and 65±5% relative humidity in the laboratory.
2.2 Method

Larvacidal bioassay experiments were conducted according to standard [8]. During experiments with granula of extract from papaya seeds and leaf modified were used third instar larvae of *Aedes aegypti*. Formulation for 300 g Granules are: active substances papaya seed and leaf modified: 90%, seed flour 5%, Filler: 5%. Each experiment was carried out triplicate. The larvae were put in glass Petri-dishes (9 cm diameter/150 mL capacity) containing 100 mL of tap water. Concentrations of granules are 0, 30, 60, 90, 120 and 150 ppm respectively with Temephos 1% as a positive control treatment. Larval mortalities were recorded after 48 h of exposure. Preparative thin layer chromatography was done to separate the compounds of identified region of definite Rf values. Clear solutions were taken in conical flasks discarding the precipitate containing silica gel. The alcohol was evaporated and the solid mass present at the bottom of the conical flask was scrapped and weighed. The fractions were dissolved in distilled water to prepare different concentrations. Then third instar larvae of *Aedes aegypti* larvae mosquito species were introduced separately to different graded concentrations and the larvae death rates determined after 48 h. The photochemical analysis was carried out using extract ethanol 70% seeds and leaf of *C. papaya* using the standard methods of Harbone. Chromatographe using silica gel 'G' TLC plates. The plates (thickness 0.5 mm) were prepared with silicagel G (Sigma, USA) and a thin-layer coating apparatus (Unoplan-Shandon, London). The mobile phase was petroleum ether. The thin layer chromatography (TLC) plates (50 in numbers) were sprayed with different spraying reagents for identification of active photochemical and the Rf value of positive spot was measured. Mortality data was corrected by Abbot’s formula [9] and the data was analyzed by Probit analysis [10], using POLO-PC software for dose and time mortality regression lines. Significant differences were concluded of 95% fiducial limits. Data on percent mortalities were analyzed with Statistix version 8.1 [11] and means were compared with least significant difference (LSD) test at 5% level of significance [12].

3. Result and Discussion

Acute toxic effects that occur after exposure from content of secondary metabolites in the leaves and seeds of papaya as saponin, flavonoid and triterpenoid is typically expressed as the concentration that will be lethal to fifty percent of the test population (LC$_{50}$). The LC$_{50}$ in this study is reported time period 48 hours 107 ppm (107 ppm; 48 hours). The lower the value of LC$_{50}$ the more toxic the substance. Although the lethal concentration varies between deference species, the relative toxicity of substances is usually constant. The lower the value LC$_{50}$ will the more toxic. LC$_{50}$ measurement is micrograms or milligrams of material per liter, or parts per million (ppm), of water. The lower the amount, the more toxic. The result of phytochemical analysis of granules of extract from papaya (*Carica papaya*) seed and leaf modified by maceration with ethanol contains secondary metabolites compounds of saponin, flavonoid and triterpenoid are represented in Figure 1.

![Figure 1](image)

Fig 1. The extract was chromatographed using silica gel 'G' TLC plates, indicate secondary metabolites compounds.
The LC$_{50}$ values of papaya (Carica papaya) seed and leaf modified granules extract against A. aegypti larvae as determined by log-probit analysis were LC$_{50}$ : 107 ppm and LC$_{90}$: 150 ppm. The results of log probit analysis 95% confidence level.

![Fig 2. Larvicidal efficacy of papaya (Carica papaya L.) seed and leaf modified granules extract against A. aegypti larvae.](image)

The larvicidal activities of different concentrations of papaya (Carica papaya) seed and leaf modified granules extract against A. aegypti larvae are represented in Table 1. The high larvicidal mortality at higher concentrations; 95 % larval mortality was achieved with oil and extract at concentrations 150 ppm.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Log Concentration</th>
<th>Dead/total/%</th>
<th>Corrected %</th>
<th>Probit</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0/20/0</td>
<td>0</td>
<td>2,915</td>
</tr>
<tr>
<td>30</td>
<td>0,751</td>
<td>5/20/25</td>
<td>25</td>
<td>3,52</td>
</tr>
<tr>
<td>60</td>
<td>1,204</td>
<td>7/20/35</td>
<td>35</td>
<td>5,14</td>
</tr>
<tr>
<td>90</td>
<td>1,903</td>
<td>8/20/40</td>
<td>60</td>
<td>7,30</td>
</tr>
<tr>
<td>120</td>
<td>2,011</td>
<td>12/20/60</td>
<td>90</td>
<td>8,41</td>
</tr>
<tr>
<td>150</td>
<td>2,061</td>
<td>19/20/95</td>
<td>95</td>
<td>9,17</td>
</tr>
</tbody>
</table>

In the present study produce of the new bioinsecticide granules of extract from papaya (Carica papaya) seed and leaf modified very effectively to Aedes aegypti larvae. The 100% mortality was not achieved in the bioassay at the tested concentrations, the hourly observations of the behaviour of the larvae before 48 hours test period revealed that most larvae were restless few minutes after their introduction to different concentrations of papaya (Carica papaya) seed and leaf modified granules extract as compared with control. This behaviour was displayed attributed to toxicity secondary metabolites compounds of saponin, flavonoid and triterpenoid in the papaya (Carica papaya) seed and leaf modified granule extract, usually affect the digestive and nervous systems of the larvae before death. Gas Chromatography-mass spectroscopy profiles for the elucidation of phytochemical. Effect studies and field trials are recommended for further studies the required concentrations above 150 ppm as shown in this study, if 100% mortality is targeted within 48 hours. This is because the mortality increases as concentration increases, depend and
mostly on the phytochemistry of such plant [5].

Concentration above 150 ppm, granule of extract from papaya (Carica papaya) seed and leaf modified was not evaluated in this study, relatively safe to non-target organisms but toxic to Aedes aegypti larvae. The phytochemical studies have shown that the leaves of papaya contain saponin and phenolic compounds which have been known to exhibit insecticidal properties can therefore be used as part of the constituents in the manufacture of insecticide [13]. However, the phytochemical properties of the seeds still remain scanty in the literature. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from plants have been reported previously for insecticidal activities. [14]. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. [15]. The results phytochemical analysis granule of extract from papaya (Carica papaya) seed and leaf modified by maceration with ethanol contains secondary metabolites: flavonoid, triterpenoid and saponin. Flavonoids, work as a stomach poison that lowers appetite is a toxin that is polar, soluble in water, and when it enters the body in larvae can result in hemolysis in the blood vessels. Organic fatty acids contained in papaya seed extract and inhibit the process of metamorphosis, inhibit the formation of the larval skin, thus resulting in the death of the larvae [16]. Saponins give rise to increased mortality levels, low-ered food intake, weight reduction, retardation in development, disturbances in development and decreased reproduction larvae. saponins could either make the food less attractive to eat (repellent/deterrent activity), bear digestive problems, cause moulting defects or have toxic effects on cells [17]. Triterpenes act as acute toxic compounds when were applied topically and/or incorporated into the water. The triterpenoid (causing reduced feeding and increased mortality [18].

LC₅₀ Aedes aegypti larvae were tested to determine the value of the effective concentrations of granules of extract for papaya seed and leaf at 0, 30, 60, 90, 120 and 150 ppm respectively under simulated conditions. The time persistence of to kill tested larvae in simulated conditions were analysed was performed to determine the extent of the pattern (shape) of the mortality rates of Aedes aegypti larvae by administering increasing concentrations (0 ppm, 10 ppm, 30 ppm, 60 ppm, 90 ppm, 120 ppm, 150 ppm). Mortality data was corrected by Abbot’s formula [9] and the data was analyzed by Probit analysis [10], using POLO-PC software for dose and time mortality regression lines. Significant differences were concluded of 95% fiducial limits. Data on percent mortalities were analyzed with Statistix version 8.1 [19] and means were compared with least significant difference (LSD) test at 5% level of significance. In the present investigation, the papaya (Carica papaya) seed and leaf extract showed excellent larvicidal activity against Aedes aegypti and this is the first report on biological control of Aedes aegypti larvae using granule of extract from papaya. The papaya (Carica papaya ), is one of the most commonly studied plants for the control of mosquitoes; it contains several biologically active principles, and papain being the predominant insecticide [20]. Granule of extract from papaya (Carica papaya ) seed and leaf modified showed lethal effects against Aedes aegypti larvae. The toxicity of Aedes aegypti LC₅₀ : 107 ppm and LC₉₀ : 150 ppm. The results of log probit analysis 95% confidence level. very effectively to Aedes aegypti larvae. The lower the value of LC₅₀ the more toxic the substance. Toxicity of substances is usually constant. The lower the value LC₅₀ will the more toxic. LC₅₀ measurement is micrograms or milligrams of material per liter, or parts per million (ppm), of water. The lower the amount, the more toxic.

4. Conclusion

Conclusion the present investigation revealed that the papaya (Carica papaya) seed and leaf modified granule extract possess technology, dosage, formulation, and remarkable larvicidal Aedes aegypti LC₅₀ (107 ppm:48 h) and LC₉₀ : (150 ppm: 48 h) Further investigations are needed to elucidate active ingredient of papaya (Carica papaya) seed and leaf modified granule extract responsible for larvicidal activity should be identified and utilized, if possible, in preparing a commercial product.

Aknowledgement

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References


