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Effectiveness Test of The Combined Extract of Papaya Seeds (Carica Papaya L.) and Sweet Orange Peel (Citrus Sinesis L.) as A Larvicide Against Aedes Aegypti Mosquitoes

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ABSTRACT

Demam Berdarah Dengue (DBD) masih menjadi salah satu masalah kesehatan masyarakat yang paling signifikan di Indonesia. Penelitian ini bertujuan untuk menentukan efektivitas campuran ekstrak biji pepaya (Carica papaya L.) dan kulit jeruk manis (Citrus sinensis L.) sebagai larvasida botani terhadap larva nyamuk Aedes aegypti. Penelitian kuasi-eksperimental dilakukan menggunakan Rancangan Acak Lengkap (RAL) dengan lima perlakuan dan empat ulangan. Tiga konsentrasi (1%, 1,5%, dan 2%) ekstrak campuran diuji dengan pengamatan selama 12 jam. Tingkat kematian larva meningkat seiring dengan peningkatan konsentrasi: 1% (30%), 1,5% (67,5%), dan 2% (100%). Uji ANOVA dan uji Duncan menunjukkan perbedaan yang signifikan ($p<0,05$). Tindakan sinergis flavonoid, saponin, tanin, papain, dan limonoid meningkatkan kematian larva, yang menunjukkan potensi larvasida berbasis tanaman sebagai alternatif ramah lingkungan untuk bahan kimia sintetis.

Dengue Hemorrhagic Fever (DHF) remains one of the most significant public health problems in Indonesia. This study aims to determine the effectiveness of a mixture of papaya seed (Carica papaya L.) and sweet orange peel (Citrus sinensis L.) extracts as a botanical larvicide against Aedes aegypti mosquito larvae. A quasi-experimental study was conducted using a Completely Randomized Design with five treatments and four replications. Three concentrations (1%, 1.5%, and 2%) of the mixed extract were tested with observation for 12 hours. The mortality rate of larvae increased with the concentration: 1% (30%), 1.5% (67.5%), and 2% (100%). ANOVA and Duncan tests confirmed significant differences ($p<0.05$). The synergistic action of flavonoids, saponins, tannins, papain, and limonoids enhanced larval mortality, indicating the potential of plant-based larvicides as an eco-friendly alternative to synthetic chemicals.

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INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus and transmitted through the bite of Aedes aegypti mosquitoes. This disease remains one of the major global health problems, particularly in tropical countries such as Indonesia. Data from the Ministry of Health indicate that dengue cases in Indonesia remain high, with thousands of deaths reported each year. Vector control efforts are generally carried out using chemical larvicides or insecticides; however, continuous use of these chemicals can lead to resistance, environmental pollution, and toxic effects on non-target organisms.

As a solution to this problem, an alternative vector control method using plant-based larvicides has been developed. One potential plant is papaya seeds (Carica papaya L.), which are known to contain

active compounds such as the alkaloid carpain, flavonoids, tannins, and the enzyme papain. These compounds have toxic activity against insect larvae by inhibiting the digestive and metabolic systems of the larvae. In addition, sweet orange peel (Citrus sinensis L.) also contains limonoids, saponins, and flavonoids that can disrupt the nervous and respiratory systems of larvae.

The combination of the two extracts is expected to produce a synergistic effect that increases the effectiveness of killing Aedes aegypti mosquito larvae. This study was conducted to test the effectiveness of a mixture of papaya seed and sweet orange peel extracts at three different concentrations on the mortality rate of mosquito larvae. The results of this study are expected to contribute to the development of natural larvicides that are safe, economical, and environmentally friendly.

METHOD

This study used a quasi-experimental method with a completely randomized design (CRD) consisting of five treatments and four replicates. The treatments consisted of a negative control (distilled water), a positive control (Abate), and three concentrations of a mixture of papaya seed and sweet orange peel extracts, namely 1%, 1.5%, and 2%. Each treatment used 40 Aedes aegypti instar II–III larvae.

The extract was prepared using the maceration method with 96% ethanol as the solvent. Papaya seeds and sweet orange peel were dried, ground, and then soaked for three days with regular stirring. The filtrate obtained was evaporated to a thick extract, then mixed in a 1:1 ratio and diluted according to the treatment concentration. Mosquito larvae were placed in the treatment solution and observed for 12 hours to count the number of dead larvae every two hours. The data obtained were analyzed using a one-way ANOVA test to determine the differences between treatments, followed by a Duncan test to see which groups had significant differences.

RESULTS AND DISCUSSION

The results of this study indicate a strong relationship between increased concentrations of papaya seed (Carica papaya L.) and sweet orange peel (Citrus sinensis L.) extracts and the mortality rate of Aedes aegypti mosquito larvae. The study was conducted over 12 hours, with the number of dead larvae recorded every two hours. The observations showed that the higher the concentration of extract used, the greater the number of larvae deaths.

At the beginning of exposure (hours 0 to 2), larvae in all treatment groups showed normal activity, such as swimming and moving actively on the water surface. However, from hours 4 to 6, significant behavioral changes were observed in larvae in the treatment groups with concentrations of 1.5% and 2%. The larvae began to lose their balance, moved slowly, and appeared to rise and fall frequently to the water surface. By hour 8, most of the larvae in the 2% concentration were no longer moving actively and began to float on the water surface.

Overall, at the end of the observation (12 hours), the results showed that the group with a concentration of 2% experienced 100% mortality, while the group with a concentration of 1.5% experienced an average mortality of 67.5%, and the 1% group experienced 30% mortality. No mortality was observed in the negative control group that used only water, while the positive control (Abate) showed 100% mortality.

Table 1. Mortality of Aedes aegypti Larvae Based on Concentration of Extract Mixtures

Concentration	Number of Larvae Tested	Mean Number of Dead Larvae	Mortality Percentage (%)
Negative Control	40	0	0
1%	40	12	30
1,5%	40	27	67,5
2%	40	40	100
Positive Control (Abate)	40	40	100

From the table above, it can be seen that the mortality rate increased with the rising concentration of the extract. This indicates a strong dose–response relationship between the concentration and larval

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mortality. The higher the concentration of active compounds contained in the extract, the faster the physiological damage process occurred in the larvae.

In the treatment with a 1% concentration, the toxic effect produced was relatively mild. Most larvae only showed slight behavioral changes, such as irregular movement and difficulty reaching the water surface. However, at a concentration of 1.5%, the toxic effects became more apparent — most larvae exhibited loss of movement coordination, their bodies turned whitish, and some began to die after 8 hours of observation. At the 2% concentration, total mortality occurred more rapidly, even before the observation period was completed.

Table 2. Mean Time of Larval Mortality at Various Extract Concentrations

Extract Concentration (%)	Mean Mortality Time (hours)	Notes
1	10,5	Larval mortality occurred gradually over time
1,5	8,0	A significant increase in mortality was observed beginning at the 6th hour
2	5,5	Complete larval mortality occurred before 12 hours of exposure

These results indicate that the mean time of larval mortality is inversely proportional to the concentration of the solution. Higher concentrations resulted in shorter larval mortality times. This is due to the increased amount of active compounds acting on the larval physiological systems. Flavonoids and limonoids play a role in inhibiting vital enzyme functions and the respiratory system, while papain and tannins cause damage to the lining of the digestive tract. As a result, the larvae are unable to digest food properly and eventually die due to a lack of metabolic energy.

Furthermore, the toxic effect of saponins reduces the surface tension of cell membranes, leading to leakage of larval body fluids and internal dehydration, which ultimately results in mortality. These mechanisms occur simultaneously and enhance the synergistic effect of both plant extracts.

Table 3. Effectiveness of Mixed Extract Concentrations on Larval Mortality

Concentration (%)	Effectiveness (%)	Category
1	30	Low effectiveness
1,5	67,5	Effective
2	100	Highly effective

Based on the effectiveness categories above, it can be concluded that the 1% concentration was only sublethal, as it was not sufficient to kill the majority of the larval population. The 1.5% concentration began to show a clear toxic effect and could be categorized as effective, whereas the 2% concentration produced a complete lethal effect (100% mortality). This indicates that the active components of papaya seeds and sweet orange peels act optimally at this concentration.

Discussion

Morphologically, the dead larvae exhibited changes in body color, becoming whitish and rigid. Larval movement ceased completely, and some individuals floated on the water surface. These changes indicate tissue damage resulting from biochemical processes induced by the active compounds. Observations also showed that larvae exposed to higher concentrations experienced a faster loss of coordination and exhibited wriggling movements before death occurred.

Papain contained in papaya seeds functions as a proteolytic enzyme capable of breaking down proteins in the larval body cell walls. When papain acts synergistically with saponins and flavonoids, the resulting effect becomes stronger. Saponins reduce membrane permeability, thereby allowing other compounds to penetrate larval tissues more rapidly. Limonoids from sweet orange peel play a role in disrupting the larval nervous system, inhibiting impulse transmission, and ultimately causing paralysis.

The statistical analysis using ANOVA showed a significant difference among the treatments ($p < 0.05$), indicating that variations in extract concentration had a significant effect on larval mortality. The Duncan's multiple range test further revealed that the differences between each concentration level were also significant, with the 2% concentration showing a highly significant difference compared to the other treatments.

These findings reinforce the hypothesis that higher concentrations of active compounds in the solution result in greater toxicity toward mosquito larvae. The observed effect is not solely attributed to a single compound but rather to the combined action of multiple components working synergistically. This synergistic interaction constitutes the main strength of the mixed extracts of papaya seeds and sweet orange peels.

In addition to its high effectiveness, another advantage of this plant-based larvicide is its environmental safety. Unlike chemical insecticides, which may cause resistance, toxic residues, and water pollution, this botanical larvicide is naturally biodegradable. Therefore, its application does not pose negative impacts on humans or other non-target organisms living around water storage areas.

From an economic perspective, the raw materials—papaya seeds and sweet orange peels—are easily obtainable and originate from household waste that is often discarded. This makes the plant-based larvicide not only effective but also potentially developable as an environmentally friendly, community-based product.

Visually, the observational results supported the quantitative data obtained. Larvae exposed to higher concentrations exhibited physical symptoms such as body shrinkage, the formation of air bubbles around the thorax, and a duller body coloration. These conditions indicate disruptions in the respiratory and circulatory systems due to internal tissue damage.

Based on these findings, this study demonstrates that the combined extract of papaya seeds and sweet orange peel has strong potential to be further developed as an active ingredient in plant-based larvicides. For future research, it is recommended to conduct stability tests related to storage duration and to evaluate the influence of water pH on extract effectiveness, so that the formulation can be optimized for wider community application.

CONCLUSIONS

The combined extract of papaya seeds (Carica papaya L.) and sweet orange peel (Citrus sinensis L.) proved to be effective as a plant-based larvicide against Aedes aegypti larvae. The effectiveness increased with higher concentrations, with the best result observed at a 2% concentration, which caused 100% larval mortality within 12 hours. The active compounds in both extracts act synergistically to disrupt the larval digestive, respiratory, and nervous systems. The findings of this study are expected to serve as a foundation for the development of safer, more economical, and environmentally friendly natural larvicides to support dengue fever vector control programs.

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