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ORIGINAL ARTICLE

Insecticidal effectiveness of papaya seed ethanolic extract against adult *Aedes aegypti*

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) remains a significant public health problem in Indonesia, with *Aedes aegypti* as the primary vector. Conventional vector control relying on chemical-based electric mosquito repellents carries the risk of inducing mosquito resistance and causing adverse environmental effects. Papaya seeds (*Carica papaya* L.) contain bioactive compounds with potential as alternative botanical insecticides. This experimental study employed a completely randomized design, dividing adult *Aedes aegypti* mosquitoes into five groups: a negative control (aquadest) and treatment groups with papaya seeds ethanolic extract concentrations of 30%, 50%, 70%, and 90%, each comprising 25 mosquitoes and three replicates. Exposure was carried out using an electric liquid preparation over a 4-hour period, with mortality observed for up to 24 hours. Data were analyzed using the Kruskal–Wallis, Mann–Whitney, and probit analysis used to estimate LC_{50} and LC_{90}. Mortality increased with increasing concentration, reaching 26.7% (30%), 30.7% (50%), 58.7% (70%), and 100% (90%). LC_{50} was approximately 71.5% and LC_{90} was approximately 79–80%, with statistically significant differences among groups ($p < 0.05$). These findings indicate that the ethanolic extract of papaya seed has concentration-dependent insecticidal activity when delivered through an electric vaporizer, with the 90% concentration producing the highest mortality under laboratory conditions.

Keyword: *Aedes aegypti*, botanical insecticide, electric vaporizer, mosquito mortality, papaya seed, plant-based insecticide.

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INTRODUCTION

Dengue Hemorrhagic Fever (DHF) continues to pose a serious public health challenge in Indonesia, given its endemic nature, high risk of rapid fatality, and frequent potential to trigger outbreaks. The disease is primarily transmitted by *Aedes aegypti* mosquitoes, which thrive in hot, densely populated environments, explaining why DHF cases are far more prevalent in urban than in rural areas.[12] Every region across Indonesia is at risk of DHF transmission, as the vector is widespread in residential areas and public facilities, and all provinces have reported cases, particularly in areas with high population density and active transportation networks.[13] DHF belongs to the category of arboviral diseases transmitted through arthropods, specifically two mosquito species: *Aedes aegypti* and *Aedes albopictus*. However, only adult female mosquitoes (imago) play a role in transmission, as they bite and feed on human blood.[4] The adult stage is the final phase of the insect's life cycle, and it is during this stage that pathogen transmission occurs when an infected mosquito bites a human.[6]

According to the World Health Organization (WHO) data, DHF is among the most widely distributed diseases with the highest case counts worldwide. The two vector species differ behaviorally in ways that affect their roles in transmission: *Aedes aegypti* is endophilic and more commonly bites humans indoors, whereas *Aedes albopictus* tends to be exophagic and opportunistic, biting a range of hosts outdoors.[3,7] The close proximity of *Aedes aegypti* to human habitation makes it the primary vector of DHF and the central focus of various studies. The WHO has also documented a dramatic rise in global DHF cases, from 505,000 to 4.2 million in 2020, accompanied by a growing number of deaths. In Indonesia, DHF remains a serious public health concern, according to data from the Ministry of Health, with the number of cases and death remaining high despite most districts/cities having achieved incidence rates below the national target. Asia as a whole represents a region with a particularly high risk of DHF outbreaks.

Various efforts to prevent and control DHF have been implemented, including mosquito breeding site eradication (PSN), fogging, the use of larvicides and insecticides, and vector control through physical, chemical, and biological methods. Communities continue to favour chemical-based control due to its perceived effectiveness in rapidly reducing mosquito populations, even though prolonged use can lead to resistance and pose risks to human health and the environment.[8,9,10] Insecticides are classified by their mode of entry and physiological effects, and they have evolved into various modern formulations such as electric liquid vaporizers, which are considered more practical and generate less waste.[11,12] To reduce the adverse impacts of synthetic insecticides, plant-based natural alternatives have been explored. One promising candidate is the papaya seed (*Carica papaya* L.), which contains active compounds including alkaloids, flavonoids, saponins, tannins, the papain enzyme, and the glycoside caricin.[13,14] Together, these bioactive compounds are toxic to mosquito by disrupting their nervous system and growth, ultimately causing death, and have been demonstrated to be both effective and environmentally friendly.[14-16] Prior research has also shown that a combination of papaya seed extract and apple cider vinegar is effective as a larvicide against *Aedes aegypti*, with LT_{50} and LC_{50} values indicative of significant larval toxicity.[17]

Recent laboratory evidence also shows that *Aedes aegypti* populations may have reduced susceptibility to commonly used insecticides, while household mosquito repellents, including liquid formulations, can release fine particles and volatile organic compounds during use.[23,24] These concerns support the search for plant-based formulations, although their efficacy and

safety still require direct evaluation. Although papaya seed extract has been studied mainly as a larvicide and has also been tested against adult mosquitoes in attractive toxic sugar bait (ATSB) formulations, evidence on its application through an electric liquid vaporizer remains limited.[14-17,21] Therefore, this study evaluated the insecticidal effectiveness of papaya seed ethanol extract delivered through an electric vaporizer against adult *Aedes aegypti* mosquitoes at concentrations of 30%, 50%, 70%, and 90%.

METHODS AND SUBJECT

Study Design

This study employed an experimental method with a completely randomized design to test the effectiveness of papaya seed ethanolic extract as a natural insecticide against adult *Aedes aegypti* mosquitoes through application as an electric mosquito repellent. The study was divided into treatment groups receiving the extract and a control group using aquadest with extract concentrations of 30%, 50%, 70%, and 90%. Each treatment was replicated three times to enhance the reliability and validity of the results. The concentrations were selected by adapting the concentration range used in a previous study using an electric vaporizer containing papaya-derived extract, which tested concentrations of 50%, 60%, and 70%. The range was expanded to include 30% and 90% in the present study to provide broader low-to-high exposure levels and to allow evaluation of the concentration–mortality relationship.[21,23]

Study Sample

The study sample consisted of *Aedes aegypti* imago reared at the Parasitology Laboratory of Universitas Jenderal Achmad Yani. Inclusion criteria were adult mosquitoes that were still active and had never been exposed to insecticides. Exclusion criteria included mosquitoes other than *Aedes aegypti*, those that were inactive, or those at a life stage other than the adult stage (imago).

The study object was a self-prepared papaya seed ethanol extract applied using an electric mosquito repellent device. The papaya seeds used were sourced from ripe fruit, identifiable by their orange-yellow skin; seeds from unripe green fruit were excluded. Mosquito sampling was performed using total sampling, meaning all individuals meeting the inclusion criteria were used as the sample. The independent variable was the concentration of papaya seed ethanolic extract, while the dependent variable was the number of *Aedes aegypti* mosquitoes that died following exposure. Mosquito mortality was assessed based on the absence of response to stimuli. Results were expressed as the number of dead individuals and the percentage of mortality, which was used to determine the effective concentration capable of causing 50% mortality of the mosquito population.

Each group contained 25 adult *Aedes aegypti* mosquitoes and was tested in three replicates, resulting in a total sample of 375 mosquitoes. The number of mosquitoes and the replication scheme were adapted from the WHO guideline for efficacy testing of household insecticide products and from previous electric vaporizer bioassays that used 25 adult mosquitoes per test with repeated observations. Before exposure, the mosquitoes were acclimatized in the test cages under identical laboratory conditions. The bioassay was conducted in a closed room with limited ventilation, and all treatment and control groups were maintained under comparable environmental conditions throughout the experiment.

Equipment and Materials

The equipment used in this study included 35 × 35 × 35 cm mosquito cages constructed from wooden frames and mosquito netting, each equipped with a tube opening. Other supporting equipment consisted of pipettes, sieves, measuring cylinders, a maceration container, an aspirator, a rotary evaporator, and a stopwatch.

The materials included ripe papaya seeds, 96% ethanol, aquadest, sugar solution, adult *Aedes aegypti* mosquitoes, and a guinea pig used as a blood source for female mosquitoes during the rearing process. Papaya seed ethanol extract was administered using an electric liquid vaporizer. All measuring equipment was inspected before use to ensure that it was clean, functional, and free from residues that could interfere with the experiment. The electric vaporizer was also checked before each test to ensure stable heating and the absence of residual liquid or odor from previous use.

Research Procedure

The first stage was the preparation of papaya seed ethanol extract. Papaya seeds obtained from ripe fruits were separated, thoroughly washed, and dried until free from visible moisture. The dried seeds were then ground into simplicia powder. The powder was macerated using 96% ethanol, stirred for three hours, and allowed to stand for 24 hours to facilitate the extraction of bioactive compounds. The macerate was filtered, and the resulting filtrate was concentrated using a rotary evaporator at a low temperature until a thick ethanolic extract of the papaya seed was obtained. The concentrated extract was stored in a closed container until further use. The working solutions were subsequently prepared at concentrations of 30%, 50%, 70%, and 90%, while aquadest was used as the negative control.

The next stage involved the rearing of *Aedes aegypti*. The mosquito larvae were maintained under laboratory conditions until they developed into adults. Adult mosquitoes were reared in dedicated cages, and a guinea pig was used as a blood source for female mosquitoes to stimulate egg production. The resulting eggs were incubated and the hatched larvae were reared until they developed into adult mosquitoes. Before the experiment, 25 active adult mosquitoes were transferred into each test cage and provided with a sugar solution. The mosquitoes were maintained in a closed room with limited ventilation before exposure.

The guinea pig was used solely as a blood source during the routine maintenance of the *Aedes aegypti* colony. Blood feeding was performed by trained laboratory personnel in accordance with the standard procedures of the Parasitology Laboratory. Animal handling was kept to a minimum to reduce discomfort and stress during the blood-feeding process.

The effectiveness test was conducted by placing an electric liquid vaporizer inside each test cage. The devices contained papaya seed ethanolic extract at concentrations of 30%, 50%, 70%, and 90%, while the device in the negative control group contained aquadest. Each cage contained 25 mosquitoes, and each treatment was tested in three independent replicates.

The vaporizer was switched on, and the mosquitoes were exposed for four hours. Mosquitoes that became immobile during the exposure period were recorded as knocked down mosquitoes and were not immediately classified as dead. After the four-hour exposure, the mosquitoes were observed for up to 24 hours to confirm mortality. A mosquito was considered dead when it showed no movement after gentle mechanical stimulation and was unable to stand or fly. Before each test, the electric liquid container was cleaned using warm water and soap, soaked in a 5% vinegar solution, rinsed thoroughly, and dried. A heating test was then performed to confirm that the device functioned properly and that no chemical residue or odor remained from previous use.

Data Analysis

Prior to analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity using the Levene's test (Test of Homogeneity of Variances). If the data were normally distributed ($p > 0.05$), a one-way ANOVA followed by Tukey's post hoc test was used to identify the most effective extract concentration.

If the data were not normally distributed ($p < 0.05$), the Kruskal–Wallis and Mann–Whitney tests were used for inter-group comparisons with $\alpha < 0.05$. Probit analysis was used to estimate LC_{10}, LC_{50}, and LC_{90} with 95% confidence intervals.

RESULTS AND DISCUSSION

Table 1. Mortality of Adult *Aedes aegypti* Mosquitoes at Different Concentrations of Papaya Seed Ethanolic Extract

Extract Concentration	Trial 1	Trial 2	Trial 3	Deaths (Mean ± SD)	Mortality % (Mean ± SD)
Negative control	0	0	0	0.0 ± 0.0	0.0 ± 0.0
30%	7	7	6	6.7 ± 0.6	26.7 ± 2.3
50%	8	7	8	7.7 ± 0.6	30.7 ± 2.3
70%	14	15	15	14.7 ± 0.6	58.7 ± 2.3
90%	25	25	25	25.0 ± 0.0	100.0 ± 0.0

Mortality increased as the concentration increased, from 26.7% at a concentration of 30% to 100% at 90%, while no mosquito deaths occurred in the negative control. The largest increase was observed between the 50% and 70% concentrations, indicating a stronger insecticidal effect at medium-to-high concentrations.

Table 2. Shapiro-Wilk Test Results for Normality

Variable	Mean ± SD	Median	Min–Max	95% CI Mean	P Value
Number of Deaths	10.80 ± 8.79	8	0–25	5.93–15.67	0.043
Mortality (%)	43.20 ± 35.17	32	0–100	23.70–62.70	0.043

The Shapiro–Wilk test showed that the number of deaths and mortality percentages were not normally distributed ($p = 0.043$). Accordingly, subsequent group comparisons were performed using non-parametric tests. The concentration-dependent pattern may reflect the increasing number of bioactive compounds released from the ethanolic extract. *Carica papaya* seeds contain alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, and steroids, several of which have been associated with insecticidal activity.[18,19] When heated in an electric vaporizer, volatile or aerosolized components may enter the mosquito body through the respiratory system or through contact with the mosquito cuticle, thereby interfering with nervous and metabolic functions. The relatively small difference between the 30% and 50% concentrations suggests that these concentrations may have produced exposure below the threshold required for a marked toxic response. In contrast, the increase at the 70% and 90% concentration indicates a stronger cumulative effect. Because the airborne concentration of active compounds was not measured, the proposed mechanism should be interpreted cautiously.

Bivariate Analysis by Papaya Seed Extract Concentration

Table 3. Kruskal-Wallis Test Results

Variable	Negative Control	30%	50%	70%	90%	P Value
Deaths (Mean Rank)	2.00	5.33	7.67	11.0	14.00	0.009

As shown in Table 4, the mean rank values increased with increasing extract concentrations, from the negative control to the 90% concentration, which showed the highest mean rank. Higher extract concentrations were associated with greater mosquito mortality, supporting a dose-response relationship between concentration and mortality.

Table 4. Mann-Whitney Test Results

Concentration	Negative Control	30%	50%	70%	90%
Negative control	–	0.034	0.034	0.034	0.025
30%	0.034	–	0.099	0.043	0.034
50%	0.034	0.099	–	0.043	0.034
70%	0.034	0.043	0.043	–	0.034
90%	0.025	0.034	0.034	0.034	–

Based on Table 4, the follow-up Mann–Whitney test confirmed that the control group differed significantly from all treatment groups. This finding indicates that administering *Carica papaya* L. seed ethanolic extract at all concentrations significantly increased mosquito mortality compared to the control.

In pairwise dose comparisons, no significant difference was found between the 30% and 50% concentrations, whereas significant differences were observed between the lower doses and those of ≥70%. This suggests the existence of a biological effectiveness threshold, beyond which increasing the extract concentration resulted in a marked increase in insecticidal activity, which appears to occur between 50% and 70% concentrations. The comparison between the 70% and 90% concentrations also showed a significant difference ($p = 0.034$), indicating that the increase from 70% to 90% provided a statistically significant additional insecticidal effect. These findings suggest that while mortality at 70% was already relatively high, the extract's maximum effect was observed at 90% concentration, and the insecticidal response did not appear to have reached a plateau at the 70% concentration.

Probit Analysis of Mosquito Mortality

Probit analysis showed a significant positive association between extract concentration and the probability of adult mosquito mortality. The model had an acceptable goodness-of-fit (Pearson $p = 1.000$), and the concentration coefficient was positive and significant ($p = 0.016$). These results support the concentration-response pattern observed in the descriptive and non-parametric analyses.

Table 5. Probit Analysis of the Relationship Between *Carica papaya* L. Seed Ethanolic Extract Concentration and Adult *Aedes aegypti* Mortality

Variable	Coefficient (B)	95% CI (Lower–Upper)	P Value
Extract dose	0.167	0.031 – 0.303	0.016
Intercept	−11.945	−16.832 – −7.051	0.015

Based on Table 6, the insecticidal effect increased sharply at medium to high concentrations, indicating a non-linear concentration-response pattern. According to the probit estimates, LC_{50} was approximately 71.5% and LC_{90} was approximately 79–80%, demonstrating that high concentrations were needed to achieve maximum insecticidal activity. The model's predictions were consistent with the observed data, including mortality approaching 100% at the 90% concentration. Overall, these results strengthen the evidence that *Carica papaya* L. seed ethanolic extract possesses strong, dose-dependent insecticidal activity, consistent with the results of the preceding statistical analyses.

Table 6. LC_{10}, LC_{50}, and LC_{90} Values of *Carica papaya* L. Seed Ethanolic Extract Against *Aedes aegypti* Imago

Parameter	Estimated Dose (%)	95% Confidence Interval
LC_{10}	63.799	33.968 – 67.433
LC_{50}	71.470	68.275 – 78.238
LC_{90}	79.140	74.808 – 116.818

The results of this study demonstrate that *Carica papaya* L. seed ethanolic extract exhibits potent, concentration-dependent insecticidal activity against adult *Aedes aegypti* mosquitoes, which is in line with the phytochemical characteristics of this plant and the broader need for sustainable insecticide alternatives. These findings support and extend the existing literature that highlights the insecticidal potential of papaya seeds, which have until now been more widely recognized as larvicidal agents.

Papaya seed ethanolic extract has been reported to have an LC_{50} ranging from 800 to 3200 ppm against Aedes instar III-IV larvae following 24 hours of exposure, with higher mortality than the control group, an effect attributed to compounds such as benzyl isothiocyanate and aliphatic amides.[20,22]

Additionally, papaya seed powder has been shown to produce a stronger synergistic effect than its leaf extract, while studies on adult mosquitoes have demonstrated repellent, ovicidal, and knockdown activity, particularly in the ethanolic fraction. Papaya leaf ethanolic extract at high concentrations has been reported to achieve 100% mortality with an LC_{50} of approximately 215.96 ppm, an effect mediated by flavonoids and alkaloids. Furthermore, the development of silver nanoparticles derived from papaya latex has shown high effectiveness against both *Aedes aegypti* and *Culex quinquefasciatus*.[20,22]

Previous studies have largely focused on the larvicidal activity of papaya seed ethanolic extracts. Reported mortality has been associated with benzyl isothiocyanate, aliphatic amides, alkaloids, and other secondary metabolites that disrupt physiological processes in mosquito larvae.[20,22] These findings support the biological plausibility of the present results, although direct

comparison of LC values is limited because the target life stage, formulation, exposure route, and concentration units are different.

Evidence on adult mosquitoes is more limited. Papaya seed ethanolic extract has been evaluated as the toxic component of attractive toxic sugar baits against mature female *Aedes aegypti* and *Aedes albopictus*, while other papaya-derived preparations have shown repellent, ovicidal, or knockdown effects.[21] The present study extends this evidence by using an electric liquid vaporizer and confirming mortality after 24 hours. The lack of a significant difference between 30% and 50%, followed by higher mortality at 70% and 90%, suggests a concentration threshold; however, this interpretation should be confirmed by evaluating additional concentrations between 50% and 70%.

From a public health perspective, papaya seeds are inexpensive and widely available as agricultural waste, making them a potential raw material for botanical insecticides. Nevertheless, the 90% preparation should not yet be considered suitable for household use. Further studies are needed to characterize the vaporized compounds, assess inhalation safety, determine product stability, compare its performance with that of a commercial positive control, and evaluate effectiveness in room-scale or field conditions. Recent studies showing particulate and volatile emissions from household repellents also underline the need to assess exposure safety alongside insecticidal efficacy.[24]

This study had several limitations. Only three replicates were performed, the bioassay was conducted under controlled laboratory conditions, and no commercial electric insecticide was included as a positive control. The concentration of active compounds released into the air was not measured, and the extract was not chemically standardized. In addition, inhalation safety, product stability, residual effects, and effectiveness under room-scale conditions were not evaluated. These limitations restrict the generalizability of the findings to household use.

CONCLUSION

Papaya seed ethanolic extract delivered through an electric vaporizer demonstrated concentration-dependent insecticidal activity against adult *Aedes aegypti*. The 90% concentration produced 100% mortality and was the most effective concentration under the laboratory conditions of this study. Probit analysis estimated an LC_{50} of 71.47% and an LC_{90} of 79.14%. These findings indicate insecticidal activity based on mosquito mortality rather than repellency and required further formulation optimization, safety evaluation, and field-effectiveness studies before practical use.

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DECLARATION OF INTERESTS

The authors declare that they have no conflict of interest related to the conduct of this research or the preparation of this article.

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