

ORIGINAL ARTICLE

Effectiveness of kenanga flower infusion (*Cananga odorata*) against larval mortality of

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ABSTRACT

Dengue fever remains a major public health problem in Indonesia. Vector control commonly relies on chemical larvicides, which may lead to resistance, highlighting the need for safe and environmentally friendly alternatives. Kenanga flowers (*Cananga odorata*) contain secondary metabolites with potential larvicidal activity. This study aimed to evaluate the effectiveness of kenanga flower infusion on the mortality of *Aedes aegypti* larvae at the instar III–IV stage. An experimental study with a completely randomized post-test-only design was conducted. Larvae were divided into six treatment groups with three replicates: kenanga flower infusion at concentrations of 5%, 10%, 15%, and 20%, a positive control (1% temephos), and a negative control (distilled water). Larval mortality was observed after 24 hours and analyzed using the Shapiro–Wilk test, Kruskal–Wallis test, and Mann–Whitney post hoc test. Results showed that the 5% concentration and the negative control caused no larval mortality, while the 1% temephos positive control produced 100% mortality. The 10% concentration resulted in 31% mortality, the 15% concentration resulted in 96% mortality, and the 20% concentration achieved the highest mortality (100%) within 24 hours. The Kruskal–Wallis test indicated significant differences among the treatment groups ($p = 0.008$). Post hoc Mann–Whitney analysis demonstrated that the 20% concentration differed significantly from the 5%, 10%, and negative control groups ($p < 0.05$). Kenanga flower infusion contains flavonoids, tannins, and saponins, suggesting potential as a natural larvicide under laboratory conditions.

Keyword: *Aedes aegypti*, botanical larvicide, *Cananga odorata*, kenanga flower infusion, larval mortality

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INTRODUCTION

Mosquitoes act as vectors for various infectious diseases, especially dengue fever (DF), as well as other diseases such as yellow fever, Zika, and Chikungunya, with DF receiving the most attention due to its high incidence and impact on public health. Mosquito larvae are generally found in water-holding containers in household environments, such as bathtubs, flower pots, used cans, vehicle tires, and water drums, whereas adult mosquitoes tend to rest in dark and humid areas inside the house, such as under cabinets, clothing hangers, and enclosed corners of rooms.¹

Globally, dengue fever remains an infectious disease with hundreds of millions of cases each year. In Indonesia, data from the Ministry of Health of the Republic of Indonesia show a significant increase. In 2022, more than 43,000 cases were recorded with hundreds of deaths, which increased in 2023 to 114,720 cases and 894 deaths, and reached 210,644 cases with 1,239 deaths up to the 43rd epidemiological week of 2024, distributed across 259 regencies/cities in 32 provinces. Entering early 2025, there was a decrease, with around 10,752 cases recorded until February and 48 deaths. In Cimahi City, there was a significant surge in dengue fever in 2024, with the number of cases increasing by more than 100 % compared to the previous year. In 2023, 363 dengue cases were recorded, while in 2024 the number of cases reached 822. At the beginning of 2025, four dengue cases were recorded in Cimahi City.⁵ Dengue fever cases are closely related to climate changes that cause water accumulation to serve as breeding grounds for *Aedes aegypti*, as well as to the low level of public knowledge about prevention. Dengue fever is a significant public health problem in children in Indonesia due to their higher vulnerability compared to adults, mainly because their immune system is not yet fully developed, according to data from the Ministry of Health of the Republic of Indonesia. In addition, children's habits of being active outdoors during the day when vector mosquitoes are actively biting further

increase the risk of dengue virus exposure. According to SKI 2023 by the Ministry of Health, the 5-14 age category has the highest prevalence (0.80%), followed by 15-24 age category (0.73%), and the 25-34 age category (0.65%).^{6,7} Dengue virus transmission occurs through the bites of female mosquitoes, especially *Aedes aegypti*. Control of the larvae of this species generally utilizes chemical larvicides such as temephos.⁸ However, continuous use has the potential to trigger resistance in mosquito populations.⁹ Therefore, the development of larvicides made from natural materials becomes a relevant alternative, considering that Indonesia's biodiversity provides many plants with potential as botanical insecticides. Research by Rahma Triyana (2022) showed that star anise flowers contain essential oils, tannins, flavonoids, and saponins that are toxic to larvae.¹⁰ In addition, kenanga flowers also have potential as a natural larvicide because they contain secondary metabolites such as flavonoids, tannins, and saponins which have been proven through phytochemical tests to have insecticidal activity against mosquito larvae, thus presenting an opportunity to be used as a natural alternative for vector control.¹¹ This study aimed to evaluate the effectiveness of kenanga flower (*Cananga odorata*) infusion on the mortality of *Aedes aegypti* larvae and to assess its potential as a safer natural alternative to chemical pesticides.

Previous studies have reported the larvicidal activity of kenanga flower extract against *Aedes aegypti* larvae, but evidence regarding the effectiveness of water-based kenanga flower infusion remains limited.¹¹ Unlike solvent extracts, an infusion is prepared using water through a simpler procedure; however, its effective concentration against instar III–IV *Aedes aegypti* larvae has not been clearly established. Therefore, this study evaluated kenanga flower infusion at concentrations of 5%, 10%, 15%, and 20% to determine its larvicidal effectiveness and compare its effects with those of the positive and negative controls.

METHODS AND SUBJECT

Research Design and Sample

This research was a laboratory experiment with a completely randomized design using a post-test only group design, which means that measurements were carried out after the treatment without a pre-test.^{12,13} The research subjects were *Aedes aegypti* larvae at the instar III–IV stage, bred in the Parasitology Laboratory of the Faculty of Medicine, Universitas Jenderal Ahmad Yani.

The inclusion criteria included larvae aged 3–4 days, while larvae that died before treatment were excluded. The research object was an infusion of kenanga flowers (*Cananga odorata*) prepared in the Biochemistry Laboratory of the same faculty, using fresh greenish-yellow flowers.

A total of 25 larvae were used in each treatment group with six groups in total (5%, 10%, 15%, 20% kenanga flower infusion concentration, a positive control, and a negative control), with three replicates, resulting in a total sample of 450 larvae. Sampling was carried out using simple random sampling. The independent variable was the concentration of kenanga flower infusion, while the dependent variable was the number of larvae that died after exposure. The effectiveness of the larvicide was defined as the ability of the infusion to kill $\geq 50\%$ of larvae within 24 hours. The observation parameters including the number of living and dead larvae, which were assessed observationally and expressed as a percentage.¹⁴

The use of 25 larvae per group was based on the larvicide bioassay approach cited in this study.¹⁶ Eligible instar III–IV larvae were allocated to the six treatment groups using simple random sampling.

Research Tools and Materials

The tools used included plastic containers for larval breeding, mosquito cages, digital scales, filter paper, gloves,

pipettes, measuring tapes, label paper, observation containers, and six 100 mL beakers. The research materials included kenanga flower (*Cananga odorata*), distilled water, 1% temephos as a positive control, *Aedes aegypti* larvae at the instar III–IV stage, sugar solution for male mosquitoes, and guinea pigs as the blood source for female mosquitoes.

Research Procedure

Larval Rearing and Identification

Aedes aegypti larvae were obtained from the Parasitology Laboratory of the Faculty of Medicine, Universitas Jenderal Achmad Yani. Larvae were identified under a microscope based on body size and siphon characteristics to confirm the instar III–IV stage. Adult mosquitoes were maintained in cages; guinea pigs were used as a blood source for female mosquitoes, and sugar solution was provided for males. Eggs were collected on filter paper placed in a water-filled tray and allowed to hatch. The larvae were reared for 5–8 days until they reached the instar III–IV stage and were then used as test samples.

Preparation and Dilution of Kenanga Flower Infusion

Kenanga flower (*Cananga odorata*) infusion was prepared according to the Indonesian Pharmacopoeia by heating 50 g of fresh flowers in 100 mL of distilled water at 90°C for 15 minutes. The preparation was filtered to obtain a 50% stock infusion and stored in a cool, dark place to maintain the stability of the active compounds. The temperature was maintained at or below 90°C to reduce damage to secondary metabolites.¹⁵ Test solutions of 5%, 10%, 15%, and 20% were prepared by serial dilution of the 50% stock infusion using distilled water.

Larvicidal Bioassay and Mortality Assessment

The larvae were divided into six groups consisting of four infusion concentrations, a positive control, and a negative control. Each group contained 25 larvae. Each test solution was placed in a labeled glass beaker. The positive control contained 1% temephos, while the negative control contained aquades. Larvae were exposed for 24 hours in accordance with the larvicide testing guidelines cited in this study.¹⁶ The experiment was conducted in three replicates on different days to assess the consistency of the results.¹⁷ A larva was considered dead when it showed no response to gentle stimulation. Mortality was calculated as follows: Mortality (%) = (Number of dead larvae/Initial number of larvae) × 100.

Data Analysis

Larval mortality data were analyzed using SPSS. Normality was tested using the Shapiro–Wilk test, and homogeneity was assessed using the Levene’s test. If the data were normally distributed, the analysis was

continued with a one-way ANOVA to examine differences between groups, followed by a post hoc test to determine which groups were significantly different. If the data were not normally distributed, the Kruskal–Wallis test followed by the Mann–Whitney test was used. Furthermore, a probit test was conducted to determine the effective infusion concentration for causing larval mortality.

Ethical Approval

This research was conducted after obtaining approval from the Ethics Committee under approval number 015/UH1.07/2025 on the date 17 July 2025.

RESULTS AND DISCUSSION

This research was conducted using 25 *Aedes aegypti* larvae in each treatment group with an observation duration period of 24 hours and three replicates. This study consisted of six groups receiving kenanga flower infusion at concentrations of 5%, 10%, 15%, 20%, together with a negative control, and a positive control.

Table 1. Mortality of *Aedes aegypti* larvae after receiving kenanga flower infusion

Active Concentration	Initial Number of Larvae	Number of Dead Larvae in			Median	(%)
		Replicate				
		1	2	3		
5%	25	0	0	0	0	0%
10%	25	0	1	22	1	31%
15%	25	25	22	25	25	96%
20%	25	25	25	25	25	100%
Temefos 1%	25	25	25	25	25	100%
Akuades	25	0	0	0	0	0%

Table 1 shows that no *Aedes aegypti* larval mortality was observed within 24 hours in the negative control group (aquades). The positive control group, treated with 1% temephos, had an average

mortality of 100%, while the 15% kenangan flower infusion concentration showed 96% mortality, and the 20% concentration showed an average mortality of 100%.

Increasing the concentration of kenanga (*Cananga odorata*) flower infusion was associated with increased *Aedes aegypti* larval mortality. The 20% concentration demonstrated optimal larvicidal activity due to its high content of active secondary metabolites. The higher the infusion concentration, the greater the larvicidal effect, which is thought to be due to disrupted respiration because larvae have difficulty obtaining air from the water surface, resulting in insufficient oxygen supply.¹¹ These findings support the hypothesis that kenanga flower infusion is effective in increasing larval mortality. The results of phytochemical tests by Frida Husna Musofi and colleagues showed that kenanga flowers contain flavonoids,

tannins, and saponins, which are secondary metabolites with larvicidal activity through different mechanisms.¹⁸ Flavonoids work by inhibiting endocrine enzymes and the release of digestive enzymes, tannins suppress feeding, inhibit growth, and reduce the activity of protease and amylase enzymes and disrupt intestinal proteins, thereby preventing optimal nutrient absorption.^{19,20,21} Meanwhile, saponins are bitter and toxic, causing irritation of the mucous membranes, damaging cell membranes and the epicuticle layers, and facilitating the entry of toxic compounds into the larval body, causing death.^{22,20,21} The combination of these mechanisms results in metabolic disorders, decreased resistance, and ultimately larval death.

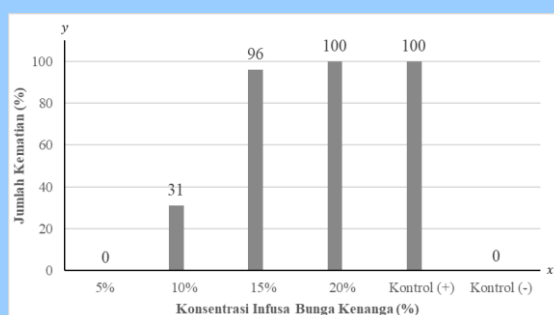


Figure 1. Percentage of *Aedes aegypti* larval mortality after 24 hours

Figure 1 shows the difference in the percentage of *Aedes aegypti* larval mortality after treatment with kenanga (*Cananga odorata*) flower infusion at various concentrations. In general, the results indicate a concentration-dependent effect, where the higher the infusion

concentration, the greater the larvicidal effect. Kenanga (*Cananga odorata*) flower infusion demonstrated larvicidal activity against *Aedes aegypti* larvae, with the 20% concentration being the most effective, resulting in 100% mortality within 24 hours.

Table 2. Shapiro-Wilks normality test results

Variable	Concentration	p-value	Interpretation
Mortality of <i>Aedes aegypti</i> larvae	5%	–	–
	10%	0.077	Normal
	15%	0.000	Not normally distributed
	20%	0.000	Not normally distributed
Temephos 1%		–	–
Aquades		–	–

The Shapiro-Wilk test for data normality in Table 2 indicates that only the 10% concentration group was normally distributed ($p > 0.05$). The

normality test did not show a non-normal distribution at 15% ($p = 0.000$) and 20% ($p = 0.000$) concentrations due to p values < 0.05 .

Table 3. Homogeneity test

Levene's Test	p-Value	Interpretation
Mortality of <i>Aedes aegypti</i> larvae	0.000	Not homogeneous

The homogeneity test using Levene's test in Table 3 shows that the mortality data of *Aedes aegypti* larvae treated with

kenanga flower (*Cananga odorata*) infusion had a significance level of 0.000 ($p < 0.05$). Therefore, the data were not homogeneous.

Table 4. Kruskal-Wallis test results

Treatment Group	Mean Larval Mortality (Mean $\pm$ SD)	p-value
Kenanga flower infusion 5%	0 $\pm$ 0	0.008
Kenanga flower infusion 10%	7.67 $\pm$ 12.42	
Kenanga flower infusion 15%	24.0 $\pm$ 1.73	
Kenanga flower infusion 20%	25 $\pm$ 0.00	
Positive control (Temephos 1%)	25 $\pm$ 0.00	
Negative control (Aquades)	0 $\pm$ 0	

Table 4 shows that the Kruskal-Wallis test performed on the *Aedes aegypti* larval mortality data treated with kenanga (*Cananga odorata*) flower infusion yielded a p -value of 0.008 ($p \leq 0.05$), indicating a significant difference among the treatment groups. Average larval mortality increased with increasing infusion concentration, starting at 5% 7.67 $\pm$ 12.42 at 10%, 24.0 $\pm$ 1.73 at 15%, and 100% at 20% concentration, equivalent to the positive control. Meanwhile, the negative control showed no larval mortality (0 $\pm$ 0).

This significant difference between concentrations indicates that the

effectiveness of kenanga (*Cananga odorata*) flower infusion against *Aedes aegypti* larvae is concentration-dependent, with increasing concentration resulting in increased *Aedes aegypti* larval mortality. The 5% concentration did not demonstrate a larvicidal effect, whereas the 10% concentration produced 31% larval mortality. The 15% concentration resulted in 96% larval mortality, and the 20% concentration achieved 100%. Therefore, it 20% concentration can be considered the most effective concentration, with larvicidal activity comparable to that of the positive control (temephos 1%).^{11,23}

Table 5. Mann-Whitney post hoc test results

Concentration	5%	10%	15%	20%	Control (+)	Control (-)
5%		0.121	0.034*	0.025*	0.025*	1
10%	0.121		0.072	0.037*	0.037*	0.121
15%	0.034*	0.072		0.317	0.317	0.034*
20%	0.025*	0.037*	0.317		1	0.025*
Control (+)	0.025*	0.037*	0.317	1		0.025*
Control (-)	1	0.121	0.034*	0.025*	0.025*	

The results of the Mann–Whitney post hoc test in Table 5 show variations in mortality responses between treatment groups, reflecting the effect of the concentration of kenanga flower infusion against *Aedes aegypti* larvae. The 5% concentration showed a significant difference compared to the 15%, 20%, and positive control groups ($p < 0.05$), but was not significantly different from the 10% and negative control groups, indicating that this low concentration was not yet effective enough in inducing larval mortality. The 10% concentration only showed a significant difference compared to the 20% and positive control groups, while no significant differences were found between the 5%, 15%, and negative control groups ($p > 0.05$). At the 15% concentration, a significant difference was observed compared to the 5% and negative control groups, but it was not significantly different from the 10%, 20%, or positive control groups, indicating that the larvicidal effect began to increase at this concentration, although it was not yet completely consistent. The positive control differed significantly from the 5%, 10%, and negative control groups, but not from the 15% and 20% groups, indicating that the two highest concentrations had a larvicidal activity comparable to that of the standard chemical larvicide. In contrast, the negative control only differed significantly from the 15%, 20%, and positive control groups, confirming that from the 5% and 10% groups, confirming that the low concentrations still resembled the untreated condition. Overall, this pattern confirms a clear dose–response relationship, where increasing concentrations of kenanga flower infusion were followed by increasing larval mortality. These findings statistically demonstrate that this flower infusion significantly affected *Aedes aegypti* larval mortality, while also indicating that high concentrations have the potential to serve

as an effective alternative natural larvicide in dengue vector control.

Study Limitations and Practical Implications

The marked variation in the 10% group, where mortality ranged from 0 to 22 larvae across the three replicates, suggests biological or experimental variation and should be interpreted cautiously. Although the 20% infusion produced 100% mortality under laboratory conditions, its use as a household larvicide cannot yet be recommended because the stability of the infusion, its safety for non-target organisms, its cost, and its field effectiveness were not evaluated. This study was also limited by the small number of replicates and the absence of quantitative analysis of the active compounds.

CONCLUSION

Kenanga flower infusion (*Cananga odorata*) effectively induced mortality of *Aedes aegypti* larvae. A 10% concentration of kenanga flower infusion (*Cananga odorata*) produced 31% larval mortality, whereas a 15% concentration produced 96% larval mortality. At a 20% concentration, the this flower infusion achieved 100% larval mortality, which was comparable to that of the 1% temephos positive control. Therefore, a 20% kenanga flower infusion demonstrated larvicidal effectiveness comparable to that of 1% temephos.

DECLARATION OF INTERESTS

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

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