Bioinsecticide-based malaria control strategy: epidemiological implications and environmental sustainability

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Article Info

Article history:

Received Jul 12, 2025
Revised Oct 20, 2025
Accepted May 23, 2026

Keywords:

Anopheles mosquito
Bioinsecticide
Effectiveness
Malaria
Plants

ABSTRACT

Anopheles mosquitoes are the main vector of malaria, which remains a global health problem. This study aims to evaluate the effectiveness of various types of plants as bioinsecticides on the mortality of *Anopheles* larvae based on variations in extract concentration and exposure time. The experimental study design used a completely randomized factorial design. The variables were studied in bioinsecticide formula consisting of 66 levels; 3 levels of concentration and contact time to *Anopheles* larvae. The number of replications was four times following the Foreder formula. The study was conducted at the Parasitology Laboratory from January to June 2025. Findings showed the factors of plant type (p <0.001), extract concentration (p <0.001), and exposure time (p <0.001) had a significant effect on the level of larval mortality. The interaction between plant type and concentration showed a synergistic effect, where the effectiveness of the bioinsecticide increased with the right combination. Longer exposure times also significantly increased the larval mortality rate. The results of this study indicate that the use of plants as bioinsecticides can be a potential alternative in malaria vector control. The study suggests that plant-based bioinsecticides can effectively combat malaria by enhancing effectiveness through synergistic interactions between plant species, extract concentration, and exposure duration.

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1. INTRODUCTION

Malaria is an infectious disease that remains a global public health problem, particularly in tropical and subtropical regions. It is caused by the *Plasmodium* parasite, which is transmitted through the bite of an infected female *Anopheles* mosquito. According to the World Health Organization (WHO) in 2022, there were approximately 249 million cases of malaria worldwide, with a death toll reaching 608,000. Most cases and deaths from malaria occur in Sub-Saharan Africa, although parts of Southeast Asia, South America, and Oceania also have a significant malaria burden [1].

The epidemiology of malaria is influenced by a complex interaction between environmental and socioeconomic factors, as well as resistance to treatment. Adequate rainfall creates breeding sites for these vectors, while favorable temperatures accelerate both mosquito development and the growth of *Plasmodium* parasites [2]. Research shows that high temperatures can shorten the parasite's reproductive cycle in mosquitoes and accelerate malaria transmission [3], while excessive rainfall creates puddles of water which become breeding grounds for mosquitoes [4]. On the other hand, socioeconomic factors such as access to health services, population mobility, and education also determine malaria distribution patterns, with more mobile populations such as migrant workers being at higher risk of infection [5]. This mobility often leads to more interaction with vectors in endemic areas [6].

Malaria control remains a global priority, with various strategies being continuously strengthened, including the use of insecticide-treated bed nets and indoor residual spraying (IRS) [7], as well as strengthening an effective epidemiological surveillance system for case detection [8]. Efforts to develop a malaria vaccine, particularly the RTS,S/AS01 vaccine, have shown significant promise. A study in Ghana showed that this vaccine reduced incidence of malaria in children by up to 39% with appropriate dosing [8]. Discussion of vaccine delivery strategies in the context of seasonal transmission is also important, with an emphasis on implementing mass vaccination campaigns during peak malaria transmission times [9], [10].

The use of chemical insecticides to control *Anopheles* mosquitoes, the primary vector for malaria transmission, is effective in the short term. However, repeated use can trigger mosquito resistance to the chemicals, reducing the effectiveness of control [11]. Research shows that *Anopheles* species in various regions, including Africa and Asia, have developed resistance to various classes of insecticides such as pyrethroids, organophosphates, and carbamates [12]. These resistance mechanisms are often related to genetic changes, including variations in the number of copies of genes that increase insecticide metabolism [13]. A number of studies also note that environmental factors, such as the use of agricultural chemicals, exacerbate this situation [14]. Therefore, continuous monitoring and implementation of more sustainable management strategies are essential to address this challenge [15]. Long-term exposure to insecticides can cause toxic effects in humans. Some health effects that can arise from exposure to insecticide chemicals include nervous system disorders, such as tremors, headaches, and seizures, which can occur due to organophosphate insecticides [16], respiratory disorders, and skin irritation, especially in workers exposed to high amounts of insecticides [17], and carcinogenic effects, particularly in organochlorine-based insecticides such as dichlorodiphenyltrichloroethane (DDT), which have been linked to an increased risk of cancer in humans [18].

This study aimed to test the effectiveness of a mixture of plant extracts on the mortality of *Anopheles* larvae. The novelty of this study primarily lies in the bioinsecticide formulation derived from various plant types, which demonstrates significant efficacy in targeting *Anopheles* larvae. While existing literature often addresses chemical insecticides and their ecological impacts, this research emphasizes the synergistic interactions between specific plant species, extract concentration, and exposure duration in enhancing larval mortality rates. The study introduces an innovative approach to malaria vector control by using natural plant-based substances, thus addressing two critical aspects: improved effectiveness in larvae elimination and reduced environmental harm. Although the implications relate to epidemiological grounds and sustainability assessments, the core contribution is the development of an optimized bioinsecticide formulation, which holds promise for broader applications in integrated pest management strategies aimed at malaria control.

2. METHOD

This study was conducted to evaluate the combined efficacy of extracts from four types of plants, namely periwinkle (*Catharanthus roseus*), soursop (*Annona muricata*), guava (*Psidium guajava*), and starfruit (*Averrhoa bilimbi*). To test the effect of these combinations, this study designed six extract mixtures with the same ratio, namely 1:1. The details of the mixtures include: first, a combination of periwinkle leaves and soursop leaves (AB); second, a combination of periwinkle leaves and guava leaves (AC); third, periwinkle leaves and starfruit leaves (AD); fourth, soursop leaves and guava leaves (BC); fifth, soursop leaves and starfruit leaves (BD); and sixth, guava leaves and starfruit leaves (CD). This study aims to determine the therapeutic potential of each combination in the context of medical applications, paying attention to antimicrobial properties or other bioactive properties that may be present in these mixtures.

Extraction of bioactive compounds from plants can be performed through the maceration method, which is recognized as an efficient technique for harnessing the potential of natural biomolecules. The process begins with thoroughly washing the plant leaves with tap water to remove contaminants and ensure the cleanliness of the material. After washing, the leaves are cut into small pieces and dried in the shade for seven days, aiming to prevent damage to the active compounds due to direct sunlight exposure. Drying is continued in an oven at 40 °C for four hours to reduce the water content to a minimum level. Next, the dried leaves are ground into a fine powder using an electric grinder (Anex, Germany). In the extraction stage, 250 grams of leaf powder are mixed with 1 liter of 96% ethanol and left for 48 hours. The process ends with filtration of the soaking mixture and evaporation using a rotary evaporator at 60 °C, resulting in a concentrated extract with a concentration of 100%. With these steps, the final product is ready for further analysis.

Anopheles larvae were collected at a natural breeding site in a coastal pond in Hanura Village, Teluk Pandan District, Pesawaran Regency, Lampung Province. The larvae were placed in plastic containers half-filled with water from the collection site and transported to the Department of Environmental Health laboratory of the Poltekkes Kemenkes Tanjung Karang. In the laboratory, *Anopheles* larvae were sorted into developmental instars (I, II, III, and IV) based on the size of their head capsules. The larvae were fed fish pellets to ensure optimal growth and development, with third and fourth-instar larvae selected for testing due to their higher survival rates at these stages.

Bioassay testing was conducted in accordance with WHO guidelines with *Anopheles* larvae in the third and fourth instars. In this test methodology, 200 ml of well water was placed in a 250 ml Pyrex beaker, and the extract was added in various concentrations, namely 1 ml, 4 ml, and 6 ml to make the final concentrations of 0.5%, 1%, and 3%. Each treatment was filled with 20 active larvae, while the control group was only filled with water without the extract.

Bioinsecticides consist of plant extracts, which act as active agents against *Anopheles* larvae. The phytochemicals in these extracts possess insecticidal properties due to various active compounds that disrupt larval development or cause death upon exposure. Application methods include larval habitat treatment, where the plant extract is applied directly to the breeding site, and residual spraying, where the extract is applied in a formulation that remains active for a longer duration. Doses vary, with concentrations of 0.5%, 1.0%, and 3.0% reported to increase larval mortality.

The test was repeated four times to ensure accuracy. Larval mortality was observed at specified time intervals: the first hour, the second hour, and until the 48th hour. The test was conducted under laboratory conditions at a temperature of 28±2 °C and humidity maintained at 75±5%. Observational data were analyzed using one-way analysis of variance (ANOVA) to determine differences in larval mortality rates exposed to the extract at a 95% significance level. All data were analyzed using SPSS version 24.0 software to ensure the validity and reliability of the research results.

3. RESULTS AND DISCUSSION

Figure 1 shows variations in the mortality rate of *Anopheles* larvae due to exposure to various plant species. Several key findings that can be analyzed include the highest mortality rate in group AD2, with an average mortality rate of 4.9, followed by AB3 (4.4) and AD3 (4.0). This indicates that the plants or plant combinations in group AD2 have the strongest insecticidal effect on *Anopheles* larvae. The lowest value was found in group BD3 (2.9), indicating lower effectiveness in killing larvae compared to the other groups. This could be due to the active ingredients in the plants being less toxic to larvae or the lower volatility of the compounds. The majority of groups had average mortality rates between 3.0 and 3.9, indicating that most plants or plant combinations had a moderate effect in killing *Anopheles* larvae. Differences in values between groups indicate that plant effectiveness varies depending on the species or combination used.

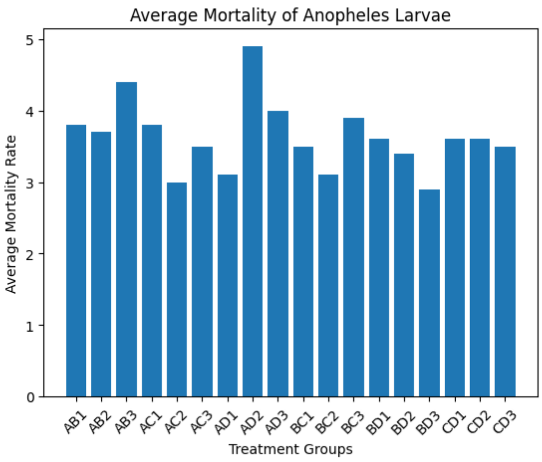

Figure 1. Average mortality of *Anopheles* larvae based on variations in plant extract types

Figure 2 shows that at a concentration of 0.5%, the average larval mortality rate reached 3.6 individuals. At a concentration of 1.0%, the average mortality rate was slightly lower, remaining at 3.6 individuals. At a concentration of 3.0%, the average mortality rate increased to 3.7 individuals. These data indicate that increasing the concentration of the plant extract tends to increase the larval mortality rate, although the difference is relatively small.

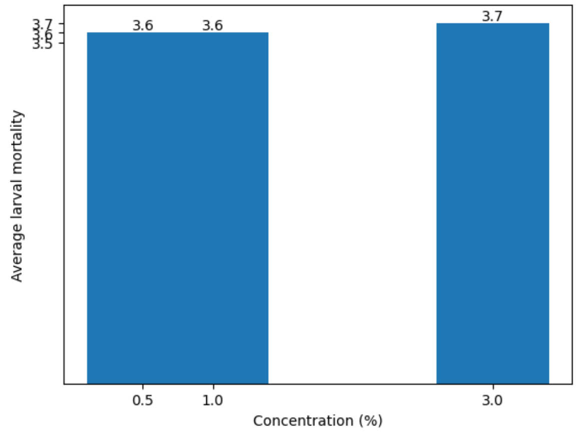

Figure 2. Average mortality of *Anopheles* larvae based on bioinsecticide concentration

Figure 3 shows the average mortality of *Anopheles* larvae based on contact time with the plant extract. A trend is evident: the average mortality of *Anopheles* larvae increases with increasing contact time with the plant extract. At the beginning of exposure, larval mortality is still low, but it gradually increases until it reaches a maximum value approaching 100% at the end of the observation. There are points of change in the mortality rate, indicating the existence of an effective time threshold. Figure 4 shows that the higher the concentration of extract used, the higher the mortality rate of *Anopheles* larvae. This is clearly evident in the trend of red (3.0%) being higher than orange (1.0%) and blue (0.5%). Figure 5 shows the *Anopheles* mortality based on variations in extract combination, concentration, and contact time.

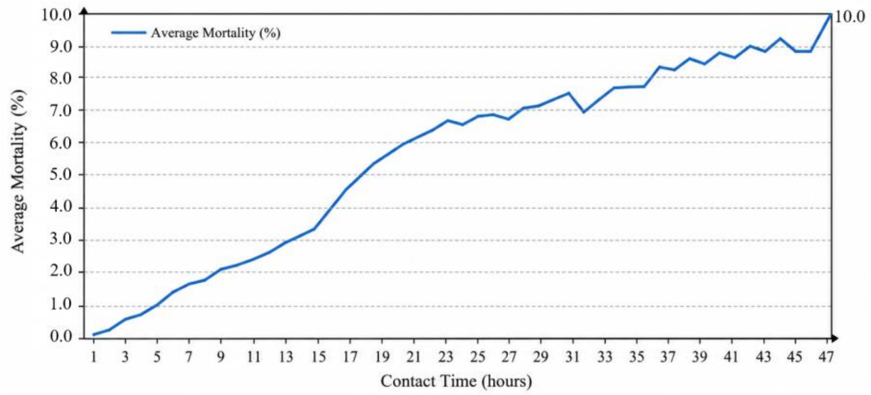

Figure 3. Average mortality of *Anopheles* larvae based on bioinsecticide contact time

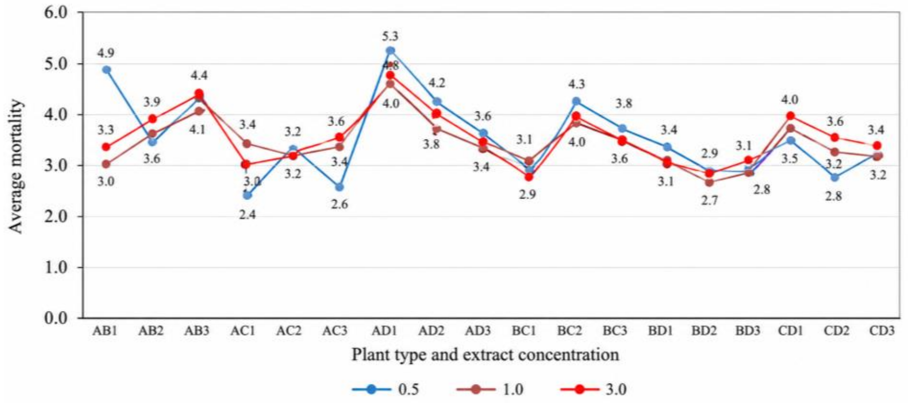

Figure 4. Average mortality of *Anopheles* larvae based on variations in plant type and extract concentration

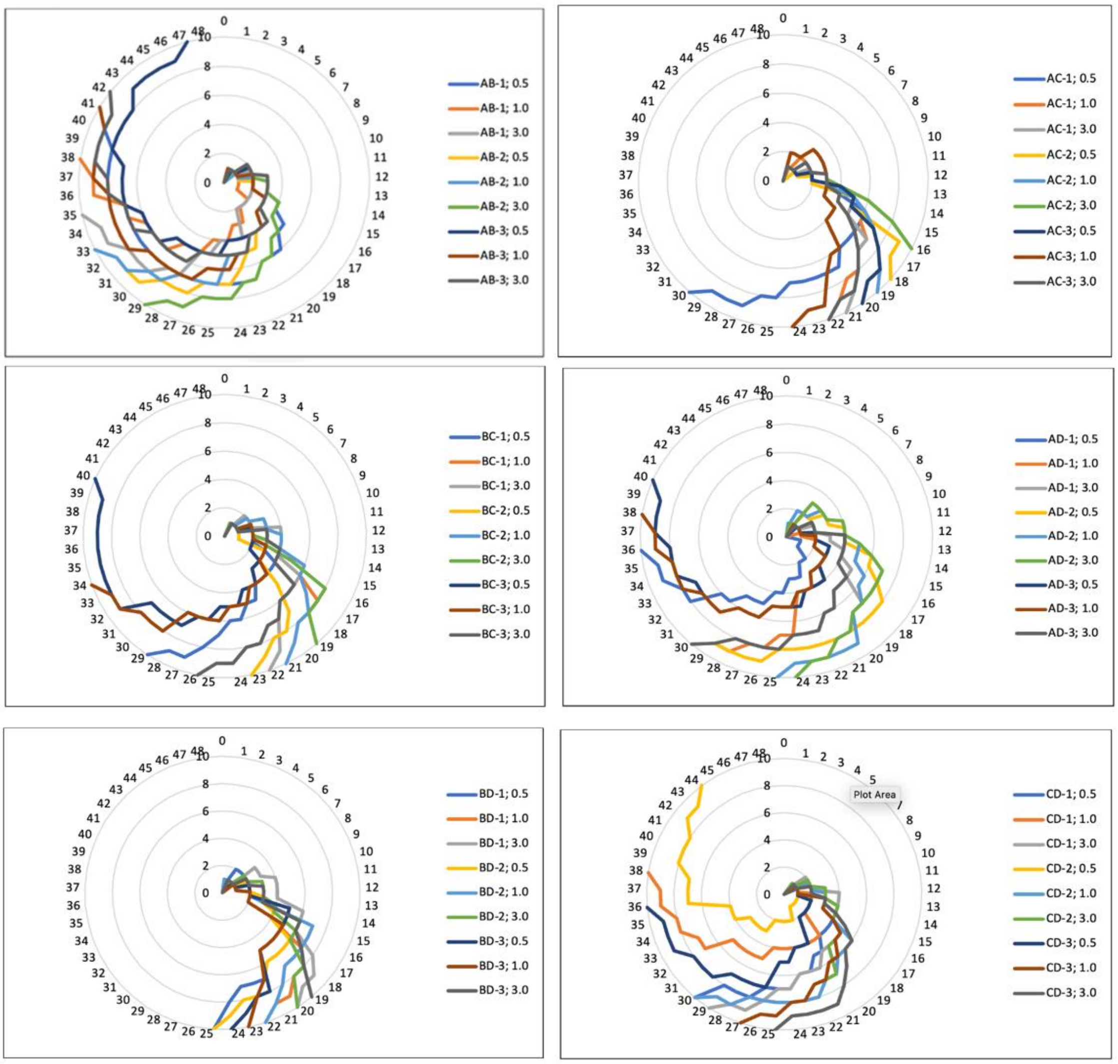

Figure 5. *Anopheles* mortality based on variations in extract combination, concentration, and contact time

Table 1 shows that the analysis using ANOVA test, corrected model value was obtained =13723.747 with F =26.525 and p-value (Sig.) =0.000. This indicates that statistical model used is statistically significant in explaining data variability. The R-squared value =0.957 (adjusted R-squared =0.921) indicates that 95.7% of the variability in the data can be explained by this model, while the remainder (4.3%) is likely due to other factors not included in the model.

Table 1. Effect of plant type, concentration, and contact time of bioinsecticide on *Anopheles* larvae mortality

Source	Type III sum of squares	Degrees of freedom	Mean square	F	Sig.
Corrected model	13723.747a	701	19,577	26,525	<0.001
Intercept	11705.528	1	1,1705.528	15,859.452	<0.001
plant	1151,079	17	67,711	91,739	<0.001
consent	192.166	2	96,083	130,180	<0.001
time	9896.126	46	215,133	291,477	<0.001
plant * consent	527,864	34	15,525	21,035	<0.001
plant * time	986,112	521	1,893	2,564	<0.001
consent * time	180,637	81	2,230	3,021	<0.001
Error	611,129	828	0.738		
Total	37653.000	1530			
Corrected total	14334.876	1529			

a. R-squared =.957 (adjusted R-squared =.921)

Table 2 shows the variability in three parameters related to vector epidemiology: the number of bites per day, the infection rate (I), and the entomological inoculation rate (EIR) across the different variants (AB, AC, AD, BC, BD, and CD). Variant AC1, with 6.0 bites per day and an infection rate of 0.25, exhibited the highest EIR of 1.50, indicating significant transmission potential. In contrast, variant AB3 recorded 3.5 bites per day with an infection rate of 0.20 and the lowest EIR of 0.70, indicating a lower transmission risk. Increasing the number of bites is often associated with higher infection rates and more significant EIRs.

Table 2. The EIR

Variation	Bite per day (L)	Infection rate (I)	EIR
AB1	5.0	0.15	0.75
AB2	4.5	0.18	0.81
AB3	3.5	0.20	0.70
AC1	6.0	0.25	1.50
AC2	4.0	0.20	0.80
AC3	5.0	0.18	0.90
AD1	4.5	0.20	0.90
AD2	3.5	0.25	0.88
AD3	6.0	0.20	1.20
BC1	4.0	0.20	0.80
BC2	5.0	0.25	1.25
BC3	4.5	0.20	0.90
BD1	3.5	0.25	0.88
BD2	6.0	0.20	1.20
BD3	4.0	0.20	0.80
CD1	5.0	0.20	1.00
CD2	4.5	0.25	1.13
CD3	3.5	0.20	0.70

Controlling *Anopheles* larvae through the use of certain plants can increase the effectiveness of reducing malaria vector populations. Some plants contain secondary metabolites such as alkaloids, flavonoids, and saponins, which have insecticidal properties. Research shows that essential oils from plants such as *Ocimum gratissimum* and *Citrus sinensis* have significant larvicidal potential against *Anopheles* larvae [19]. Furthermore, the use of silver nanoparticles synthesized from these plant extracts also increases toxicity to larvae. This combination of plants could provide a more effective control strategy by harnessing their natural insecticidal properties, while reducing reliance on chemical insecticides that can lead to resistance [20].

Plant extracts, particularly from species such as *Carica papaya* and *Cymbopogon citratus*, have shown significant effectiveness as bioinsecticides against mosquito larvae, including *Aedes aegypti* and *Anopheles gambiae*. Active compounds such as saponins and flavonoids in these extracts can increase larval mortality, providing a safer alternative to synthetic insecticides [21], [22]. With increasing resistance to synthetic insecticides, approaches using plant-based bioinsecticides are becoming increasingly relevant in the management of disease vectors such as dengue fever and malaria [22], [23].

Plant-based bioinsecticides, such as *Aegle marmelos* extract, exhibit significant larvicidal activity against *Aedes aegypti* larvae, as indicated by damage to the larval midgut. Exposure to these bioactives causes severe histopathological changes, including damage to epithelial cells, which ultimately affect larval health and increase mortality [24], [25]. Active compounds such as flavonoids and saponins have been shown to play an important role in reducing larval mortality, where damage to the midgut epithelial structure reflects a direct interaction between these compounds and the larvae [24], [25]. Therefore, further understanding of the concentration and formulation of bioinsecticides is needed for future development, in order to improve the effectiveness and instructions for use in pest control [26].

Figure 2 shows that at a concentration of 3.0%, the mortality rate of *Anopheles* larvae tends to be higher compared to lower concentrations (0.5% and 1.0%). This indicates that increasing the extract concentration is directly proportional to the toxicity to the larvae. However, there are several points where the effectiveness does not differ significantly between the 1.0% and 3.0% concentrations. This may be due to several factors, such as the resistance of the larvae to the active compounds in the extract, variations in the active ingredient content in the extracts from each plant type, the duration of exposure, and the environmental conditions of the test.

Increasing the concentration of the extract allows for higher levels of active compounds, thereby increasing toxicity to larvae. Bioinsecticide compounds work through various mechanisms, such as inhibiting the larval respiratory system (respiratory inhibition), disrupting the larval central nervous system, causing paralysis and death, damaging the larval cuticle structure, and causing dehydration that leads to death.

The effectiveness of plant-based bioinsecticides can vary depending on the larval species, environmental conditions, and application method. For example, temperature and humidity can affect the degradation of active compounds in plant extracts. Although plant-based bioinsecticides are more environmentally friendly than synthetic insecticides, their effectiveness is often lower and requires higher doses to achieve significant mortality [27].

A trend is evident in the increase in average *Anopheles* larval mortality with increasing contact time with the plant extract (Figure 3). At the beginning of exposure, larval mortality was still low, but gradually increased, reaching a maximum value approaching 100% at the end of the observation. These results indicate that the plant extract has a toxic effect on *Anopheles* larvae, causing a progressive increase in mortality. This effect likely stems from active compounds contained in the extract, such as alkaloids, flavonoids, saponins, or tannins, which are known to have larvicidal and insecticidal properties. There are change points in the mortality rate indicating a threshold time of effectiveness. In the initial phase, mortality increases slowly, likely because the concentration of the toxin is not yet high enough in the larvae's bodies. However, after passing a certain time threshold, mortality spikes, indicating that the extract's toxicity has reached a lethal level. The increase in larval mortality with increasing contact time suggests that the application of plant-based bioinsecticides could be an alternative in vector control programs. However, its effectiveness depends on the concentration and duration of exposure. Therefore, further research is needed to determine the optimal dose and the most effective application method in natural environments. Some plant species showed greater effectiveness than others. For example, there were points where the average mortality was consistently higher for all extract concentrations, indicating that these plants contained active compounds with stronger insecticidal effects against *Anopheles* larvae. Plants containing alkaloids, flavonoids, saponins, and tannins are known to have insecticidal activity that can disrupt the larvae's nervous system or damage their body cuticles, ultimately leading to death.

The deployment of plant-based bioinsecticides, particularly those derived from species like *Ocimum gratissimum*, *Citrus sinensis*, *Carica papaya*, and *Cymbopogon citratus*, demonstrates potential in controlling *Anopheles* larvae, which are critical in malaria vector management. Compounds such as flavonoids and saponins found in these plants exhibit larvicidal properties, contributing to larval mortality while reducing dependence on synthetic insecticides known to cause resistance [28]. Moreover, the integration of nanotechnology, such as silver nanoparticles synthesized from these plant extracts, can enhance their efficacy [29].

The effectiveness of plant extracts is influenced by factors like concentration and exposure duration, with higher concentrations linked to increased larval mortality rates [30]. Furthermore, bioactive compounds can disrupt the larval midgut, causing negative histopathological effects. Community-led initiatives employing these plant-based strategies show promise in pest management policies, especially considering the environmental and health concerns associated with synthetic insecticides [31].

Differences in effectiveness between groups indicate that some plants have higher insecticidal potential than others (Table 1). Previous studies have shown that plants such as *Azadirachta indica* (neem), *Cymbopogon citratus* (lemongrass), and *Ocimum basilicum* (basil) have high insecticidal effects against larvae [27]. The synergy between active compounds such as flavonoids and tannins in guava leaves and alkaloids in periwinkle can increase the effectiveness of bioinsecticides. The CD-2 plant variety (*Psidium guajava* and *Averrhoa bilimbi*) showed the lowest mortality (2.99 individuals). It is possible that antagonistic properties between the active compounds reduce the effectiveness of the insecticide [32].

Heightened concentrations of extracts influence the mortality of *Culex* larvae, indicating a concentration-dependent relationship [33]. Supporting this, research on various plant extracts shows that larvicidal efficacy is generally concentration-dependent, with higher concentrations leading to greater larval mortality rates [34]. Thus, the relationship between concentration and larval mortality appears consistent across different species and studies.

Flavonoids, commonly found in various plant species, serve as natural insecticides with evidence supporting their role in inhibiting insect feeding behavior, resulting in antifeeding effects that can lead to pest mortality. Studies indicate that flavonoids can reduce the digestibility of plant tissues, impacting insect growth and behavior by acting as deterrents or toxins [35]. These compounds affect critical digestive enzymes and disrupt feeding patterns, leading to reduced larval activity and potential death of the insects [36]. The mechanisms attributed to flavonoids involve their ability to penetrate the insect cuticle, causing respiratory failure, thereby confirming their effectiveness in pest control strategies [37]. Furthermore, enhanced flavonoid accumulation in plants has been noted to correlate with increased insect resistance, highlighting their ecological significance as protective agents against herbivory [38], [39].

This study also demonstrated an interaction between plant variation and concentration, as well as variations in plant type and concentration, which also influenced larval mortality. The statistical test model showed an adjusted R-square of 0.792, indicating that variations in plant type, concentration, exposure time, and their interaction accounted for 79.2% of larval mortality, with other factors contributing to the remainder. In *Anopheles* larvae, this occurred after 47 hours, with an average mortality of 10 larvae. This suggests that longer contact times allow the active compounds in bioinsecticides to work more effectively, particularly on the insect's central nervous system or metabolism [40]. As seen in Figure 4, the combination of plant type and concentration variations (AD-2) resulted in the highest mortality rate for *Anopheles* larvae (5.3 individuals). The statistical test results in Table 1 show that the variables of concentration, contact time, and plant type variation, both individually and collectively, significantly influenced the mortality rate of larvae ($p < 0.05$). The interaction between concentration and time, as well as plant type and concentration variations, was also significant, indicating that optimizing bioinsecticide plant type variations requires consideration of variable interactions to achieve maximum effectiveness. The R-squared value of 0.921 indicates that 92.1% of the variability in *Anopheles* larval mortality can be explained by the model used, indicating a very strong relationship between the variables.

Transmission parameters are guided by the concept of vectorial capacity (Vc), which provides a quantitative measure of the potential for a vector to transmit a pathogen to humans. This parameter is defined by several components: the average daily biting rate of the vector (a), the probability of vector to human transmission per bite (bh), the probability of human-to-vector infection per bite (bm), the duration of extrinsic incubation period (n), vector mortality rate (μ), and vector density in relation to humans (m) [41]. Each of these elements plays a crucial role in driving the dynamics of vector-borne disease transmission, and modifications in any of these parameters can significantly affect the overall transmission potential.

Furthermore, specific vector management strategies can lead to significant reductions in vector populations, thereby influencing the overall incidence of vector-borne diseases. For instance, the use of microbial larvicides has demonstrated high efficacy in controlling malaria vectors, leading to reduced transmission rates [42]. Similarly, the integration of long-lasting insecticidal nets (LLINs) and IRS has been empirically shown to alter the density and species composition of *Anopheles* mosquitoes, subsequently impacting malaria transmission dynamics [43].

Human behaviors and environmental factors also play pivotal roles in shaping infection rates. During the COVID-19 pandemic, movement restrictions significantly altered the contact dynamics between humans and vectors, which resulted in reduced transmission rates for diseases like dengue [44]. However, environmental changes and urbanization continue to present challenges, allowing vectors such as *Aedes* and *Anopheles* mosquitoes to thrive near human populations [45], [46]. Moreover, changing climate conditions may extend the active periods of vectors, possibly leading to increased disease transmission as seen in various scenarios across the globe [46].

Bioinsecticides showed higher effectiveness against *Anopheles* compared to *Aedes*. This may be due to differences in the morphological, physiological, or metabolic structures of the two larval species. Bioactive compounds appear to more readily induce mortality in *Anopheles*. Plant-based bioinsecticides were effective against *Aedes aegypti* and *Anopheles*, with the best results at a concentration of 3% for *Aedes* and a concentration of 0.5% for the AD2 plant variety on *Anopheles*. The optimal contact time was 47 hours, and the interaction between concentration, plant variety, and contact time significantly affected larval mortality. The R-squared value indicates that this model is very good at explaining the relationship between variables and larval mortality, especially in *Anopheles*.

Flavonoids are recognized for their role as antifeedants across various insect species, primarily by impairing the insects' taste perception, leading to starvation [47]. They inhibit taste receptors in larval mouths, causing a lack of food recognition and an energy deficit that stunts growth and development [47]. As described by El Shafiey and Abdelaziz [48], the entry of toxic substances into the larva's body necessitates significant energy for detoxification processes, further exacerbating metabolic strain and ultimately contributing to their demise. This biotransformation process demands energy, which, combined with the energy loss from reduced feeding, results in an unsustainable metabolic state for the larvae [48]. Thus, the integration of flavonoids in pest control strategies can effectively hinder insect development and reduce infestation impacts on crops [49].

Flavonoids exhibit insecticidal properties, particularly against *Aedes aegypti* larvae, functioning as respiratory toxins that disrupt metabolic processes. They induce larval mortality by inhibiting crucial respiratory enzymes, although the specific enzymes inhibited need further elucidation [50], [51]. One notable flavonoid derivative recognized for its insecticidal activity is rotenone. However, its use may be more complex as it is derived from other sources rather than being a flavonoid itself [52]. While flavonoids act through multiple mechanisms, including their role as stomach and respiratory poisons, they represent a potential eco-friendly alternative to synthetic insecticides [53]. Studies have highlighted various plant extracts rich in flavonoids—such as those from jackfruit and papaya—demonstrating significant larvicidal effects, thereby supporting the role of flavonoids in controlling mosquito populations [54].

Flavonoids can act on the insect nervous system by affecting key enzymes such as AChE, but flavonoids can also affect nerve pathways, although this has not been widely studied. For example, Ren *et al.* [55] showed that the mechanism underlying the insecticidal activity of three biflavones is related to their capacity to inhibit voltage-gated potassium channels of the ventral nerve cord. Variations in certain plant species showed a significant effect on larval mortality. The AD-2 plant species (0.5% concentration) produced the highest mortality rate at the lowest concentration, indicating a possible synergistic effect of the bioactive plant species variations [56].

The exploration of plant-based bioinsecticides is integral to advancing vector control strategies aligned with the WHO's global vector control response (GVCR) and sustainable development goal 3 (good health and well-being). Various studies indicate that extracts from plants like *Carica papaya* and *Cymbopogon citratus* exhibit significant larvicidal effects against malaria vectors such as *Anopheles arabiensis*, attributed to bioactive compounds like flavonoids and saponins. These natural products not only mitigate the environmental impacts associated with synthetic insecticides but also address the challenge of insecticide resistance, which is critical for sustaining malaria control efforts [57].

Vector control is a key component in disease management. Vector control is also crucial for diseases like filariasis, which is controlled primarily through preventive mass drug administration (MDA). Current strategies for eradicating lymphatic filariasis are unlikely to achieve complete eradication of the infection unless MDA is supplemented by transmission control interventions in some areas. The national vector control program strategy is based on the use of insecticides for IRS and insecticide-treated bed nets containing synthetic pyrethroids. Due to intense selection pressure, larvae are developing resistance to the main insecticides used in public health programs [58], [59].

The use of plant-based bioinsecticides presents a promising approach to enhancing malaria vector control strategies, particularly concerning *Anopheles* larvae. Research indicates that various plants, especially those rich in alkaloids, flavonoids, and saponins, exhibit significant larvicidal effects. For instance, extracts from *Citrus aurantiifolia* and *Citrus sinensis* have been shown to effectively reduce *Anopheles* populations [60]. Additionally, increased concentrations of these extracts correlate positively with larval mortality.

4. CONCLUSION

This research indicates that the use of plants as bioinsecticides has the potential to be a more environmentally friendly and sustainable alternative for controlling larval vectors. Selecting the appropriate plant species and concentration can increase the effectiveness of bioinsecticides. Some plants are more effective at certain concentrations. Exposure time plays a significant role in bioinsecticide effectiveness, with results showing that the longer the exposure time, the higher the larval mortality rate. The interaction between extract concentration and exposure time indicates that insecticide effectiveness can vary over time, necessitating the determination of the optimal duration of application in the field. The success of plant-based bioinsecticides can be enhanced by considering the optimal combination of plant species, extract concentration, and exposure time. Further research is needed to understand the specific mechanisms of active compounds in plants against *Anopheles* larvae and their effectiveness under natural environmental conditions. The advancement of plant-based bioinsecticides offers an innovative approach to enhancing vector control strategies against malaria. Future research could benefit from exploring climate-resilient vector control methods that account for the impacts of climate change on mosquito populations and resistance patterns. The integration of artificial intelligence (AI) for habitat mapping could facilitate targeted interventions, identifying high-risk areas for *Anopheles* proliferation. Moreover, establishing participatory monitoring systems involving local communities may enhance the efficacy of such bioinsecticides by fostering awareness and adaptive management. This approach aligns with the WHO's GVCR, emphasizing sustainable health solutions. Further investigation into the specific mechanisms behind the larvicidal activity of bioactive compounds in plants, alongside field studies assessing their effectiveness in varied environmental conditions, will be essential for optimizing these natural products' roles in vector control.

ACKNOWLEDGMENTS

We extend our sincere gratitude to the Indonesian Ministry of Health for the financial support provided through a collaborative program with the Poltekkes Kemenkes Tanjung Karang.

FUNDING INFORMATION

This research was funded by the Ministry of Health through a collaborative program with the Poltekkes Kemenkes Tanjung Karang with the number: HK/11/2112/2025/KKPT.

AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
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Prayudhy Yushananta	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

ETHICAL APPROVAL

This research was conducted in accordance with applicable ethical considerations and received approval from the Ethics Committee of the Poltekkes Kemenkes Tanjung Karang. The ethics approval number obtained is 259/KEPK-TJK/IV/2025, indicating that this research adhered to strict ethical standards in the treatment of subjects and the methodology used.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, [MA], upon reasonable request.

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