OnLine Journal of Biological Sciences

Research Article

Larvicidal Activity of *Spondias purpurea* (Sineguelas) Leaf and *Hylocereus costaricensis* (Dragon Fruit) Stem Extracts Against *Aedes aegypti*

Alfredo V. Corpuz, Nigell Mikhail A. Gallardo and Ace Danielle C. Avero

College of Health Sciences, University of Northern Philippines, Vigan, Ilocos Sur, Philippines

Article history
Received: 11-02-2025
Revised: 08-07-2025
Accepted: 11-07-2025

Corresponding Author:
Ace Danielle Claro Avero
College of Health Sciences, University of Northern Philippines, Vigan, Ilocos Sur, Philippines
Email: acedanielle.avero@unp.edu.ph

Abstract: The study determined the larvicidal activity of the different concentrations of *Spondias purpurea* and *Hylocereus costaricensis* extracts by measuring percent mortality in *Aedes aegypti* larvae, a primary vector of dengue and other arboviral diseases. The hypothesis proposed that combining both extracts would yield greater larvicidal activity due to potential synergistic interactions. Ethanolic extraction was used to isolate a wide array of phytochemicals. Third- to fourth-instar larvae were exposed to the extracts using WHO standard bioassay methods, and mortality was recorded over a six-hour period. Phytochemical screening revealed active compounds such as alkaloids, saponins, flavonoids, tannins, phenolics, and glycosides. Results showed that the 100% concentrations of both individual extracts had high larvicidal activity, while their 100:100% combination exhibited mortality comparable to 1% Malathion, the positive control. Statistical analysis confirmed significant differences between treatments and control groups ($p<0.05$), though post hoc tests were not conducted. The novelty of this study lies in the combined use of native Philippine plant extracts as a natural larvicide-an approach not previously reported. The larvicidal effects may be attributed to multiple phytochemical mechanisms, including neurotoxicity, membrane disruption, and enzyme inhibition. The findings highlight the potential of these plant extracts, particularly in combination, as eco-friendly alternatives to synthetic larvicides. Further research is recommended to evaluate their environmental safety, determine LD_{50} values, assess long-term efficacy, and test performance in real breeding habitats.

Keywords: Larvicide, Instar IV Larvae, Phytochemical Screening, Bio-Agent, Synergistic Effect

Introduction

Mosquito-borne diseases remain a pervasive and escalating global health concern, particularly in tropical and subtropical countries. Among the most significant vectors is *Aedes aegypti*, the primary transmitter of debilitating and sometimes fatal arboviral diseases such as dengue fever, Zika virus, yellow fever, and chikungunya. These diseases collectively contribute to high morbidity and mortality rates, especially in low- and middle-income countries where vector control infrastructures may be insufficient.

According to the WHO (2023), more than two million cases of arboviral infections are reported annually in the Americas, with similarly alarming figures emerging from Southeast Asia. The Philippines, in particular, has recorded recurrent outbreaks of dengue and other mosquito-borne illnesses, underscoring the critical need for effective vector control interventions.

Conventional mosquito control strategies have heavily relied on chemical larvicides, especially organophosphates and pyrethroids. While effective in the short term, the extensive and prolonged use of these

synthetic agents has resulted in several adverse consequences. These include the emergence of insecticide-resistant mosquito strains, contamination of aquatic and terrestrial ecosystems, and potential toxicological impacts on human and animal health (Yameogo *et al*., 2024; Hillary *et al*., 2024). Such outcomes have diminished the long-term sustainability of chemical-based vector control and prompted an urgent search for alternative solutions that are both ecologically sound and effective.

In response, botanical insecticides have emerged as promising candidates for sustainable mosquito control. Plants naturally produce a wide array of secondary metabolites with insecticidal and larvicidal properties. These include alkaloids, flavonoids, tannins, saponins, phenolics, and glycosides, each known for distinct mechanisms of action (Ngegba *et al*., 2022).

Alkaloids, for instance, can disrupt neural transmission by targeting the insect nervous system. Saponins compromise the integrity of cell membranes, leading to lysis and death of larvae. Flavonoids interfere with mitochondrial activity and inhibit key metabolic enzymes. Tannins alter digestive processes and protein metabolism, while glycosides impair respiratory and enzymatic pathways essential to larval development (Senthil-Nathan, 2020; Priya *et al*., 2023).

These bioactive compounds may function synergistically or independently, increasing their efficacy and reducing the likelihood of resistance development-a major advantage over synthetic agents (Isman, 2020).

Despite the increasing global interest in phytochemical-based larvicides, there remains a substantial gap in locally driven, evidence-based research on the larvicidal efficacy of Philippine flora. This study addresses that gap by investigating two plant species that are both culturally relevant and botanically potent: *Spondias purpurea* (commonly known as sineguelas) and *Hylocereus costaricensis* (red-fleshed dragon fruit).

These species are widely available in the Philippines and are already used by local farmers to repel insects from livestock, suggesting their potential pesticidal value (Corpuz & Savella, 2019).

Ethnobotanical use, combined with preliminary phytochemical screenings, indicated that the leaves of *S. purpurea* and the stems of *H. costaricensis* contain significant amounts of flavonoids, alkaloids, and other secondary metabolites with potential larvicidal effects (Santos *et al.*, 2023; Ortega-Arellano *et al*., 2020; Jafri *et al*., 2022).

The plant parts selected for this study—the leaves of *S. purpurea* and the stems of *H. costaricensis*—were chosen due to their phytochemical abundance, accessibility, and the feasibility of sustainable harvesting. These parts have also been underexplored in larvicidal studies, thereby offering a novel research angle.

The use of ethanol as a solvent for extraction was deliberate and scientifically grounded. Ethanol is recognized for its ability to dissolve a broad range of polar and semi-polar compounds, making it an ideal medium for extracting a wide spectrum of bioactive constituents while preserving compound stability. Additionally, ethanol is environmentally benign and suitable for studies involving bioassays in aquatic systems (Jafri *et al*., 2022).

A particularly innovative aspect of this study is the evaluation of the combined larvicidal potential of these two plant extracts. It is hypothesized that their phytochemicals may act synergistically, enhancing larvicidal potency beyond the effect of individual treatments.

Synergy occurs when the total biological effect of the combination exceeds the sum of its individual effects, while potentiation refers to additive effects. This concept has been supported by previous studies, such as the successful larvicidal application of *Carica papaya* and *Gliricidia sepium* extract mixtures, which showed greater efficacy when used in tandem (Corpuz & Savella, 2019). However, to date, there is a scarcity of scientific literature on the larvicidal efficacy of *S. purpurea* and *H. costaricensis*, especially when used in combination. Most studies involving botanical larvicides in the Philippines focus on more commonly researched species, leaving a clear research void regarding these two locally significant plants.

This study therefore aims to address that gap by conducting a controlled laboratory assessment of their individual and combined larvicidal effects on *A. aegypti* larvae using WHO-recommended larval bioassay procedures.

The main objective of this study is to evaluate and compare the larvicidal activity of ethanolic extracts from *S. purpurea* leaves and *H. costaricensis* stems, both separately and in combination, against third- to fourth-instar larvae of *A. aegypti*. It is hypothesized that increasing concentrations of both individual and combined extracts will lead to increased larval mortality, with the combination treatment demonstrating enhanced efficacy due to possible synergistic phytochemical interactions.

This research holds practical and scientific significance. By exploring the larvicidal potential of locally sourced, plant-based materials, it contributes to the broader goal of developing effective and eco-friendly alternatives to conventional larvicides. It also supports several of the United Nations Sustainable Development Goals (SDGs), including SDG 3 (Good Health and Well-being), SDG 12 (Responsible Consumption and Production), SDG 13 (Climate Action), and SDG 15 (Life on Land). Ultimately, the findings may provide the basis for future field validation, toxicity testing in non-target organisms, LD_{50} determination, and development of safe and sustainable formulations for integrated mosquito management programs in endemic areas.

Materials and Methods

Research Design

The study utilized an experimental research design, with both treatment and control groups assessed in triplicate. Each replication consisted of 10 active third- to fourth-instar *A. aegypti* larvae. The experiment was repeated on two non-consecutive days to address inter-day variability.

This design enabled a dose-dependent evaluation utilizing doses of 25, 50, 75, and 100% for both singular and combination extracts. The LD_{50} was not determined; nonetheless, the selected concentrations conform to WHO bioassay guidelines. Subsequent research should conduct LD_{50} evaluations to enhance dosage precision.

Study Locale

All investigations were performed at the College of Health Sciences, University of Northern Philippines, Vigan City.

Plant Collection and Sample Preparation

Fresh leaves of *S. purpurea* and stems of *H. costaricensis* were gathered in Santa, Ilocos Sur. The stems and leaves were specifically chosen based on ethnobotanical practices, their availability in the locality, and reported presence of bioactive compounds such as flavonoids, saponins, and alkaloids, which are known for their larvicidal properties. The *H. costaricensis* samples were obtained from the red-fleshed variety, while the *S. purpurea* belonged to the native yellow variety. The samples were individually cleansed with tap water and subsequently rinsed with distilled water to remove surface contaminants. Both plant specimens were subjected to air-drying, pulverization, and extraction.

Authentication

The plant specimens were authenticated by the Bureau of Plant Industry, Department of Agriculture, Malate, Manila, and assigned the voucher specimen numbers PLT-ID-CRPSD-162-21 (*S. purpurea*) and PLT-ID-CRPSD-163-21 (*H. costaricensis*) respectively.

Extraction Procedure

Extraction of the pulverized plant materials was conducted using a Soxhlet's apparatus with 95% ethanol (analytical grade) as the extracting solvent. This method allowed for the efficient isolation of polar and semi-polar phytochemicals (Yadav & Agarwala, 2011). The resulting extract yield was 1.2 g (semi-dried equivalent) for *S. purpurea* and 5.2 g for *H. costaricensis* per kilogram of raw material. Extracts were stored in sterile amber bottles at 4°C to preserve their stability and prevent phytochemical degradation.

Preparation of Extract Concentrations

Extract concentrations were freshly made to preserve the activity of the bioactive components. A 100% concentration was achieved by dissolving 100mg of the semi-dried extract in 100mL of distilled water. The 75%, 50 and 25% solutions were formulated by dissolving 75mg, 50mg, and 25mg of extract in 100mL of distilled water, respectively. The concentrations were derived from conventional WHO larvicidal bioassay techniques and previous research (Filho *et al*., 2021; Chude *et al*., 2020).

Combined Extract Treatments

To examine synergistic or potentiating effects, four combinations of *S. purpurea* and *H. costaricensis* extracts were formulated: 25:75, 50:50, 75:25, 100 and 100%. Each formulation was predicated on prior findings indicating enhanced larvicidal efficacy via combinations of plant extracts (Corpuz & Savella, 2019) and a comparable investigation into the acaricidal properties of plant extracts (Corpuz & Florentino, 2025).

Positive and Negative Controls

The positive control was prepared by combining 1mL of technical-grade malathion with 99mL of distilled water to yield a 1% solution. Distilled water was used as the negative control. This study did not incorporate a solvent control. Nonetheless, it is advisable for future research to utilize distilled water with ethanol to eliminate solvent effects.

Culturing of Aedes aegypti

Eggs of *A. aegypti* were obtained from a verified reference laboratory. Two plastic basins were filled to the midpoint with dechlorinated tap water that had been allowed to stand for 48 hours. Sheets carrying mosquito eggs were immersed, and the basins were covered with mosquito nets to prevent contamination. Hatching transpired after roughly three days. Larvae were provided a 3:1 mixture of powdered dog biscuits and yeast, a conventional larval diet utilized in mosquito vector research (WHO, 2005). Cultures were maintained at 25-30°C and 70-80% relative humidity. Larvae were categorized as third- or fourth-instar according to size, morphological characteristics, and mobility observed under a compound microscope.

Larvicidal Bioassay

The bioassay adhered to WHO recommendations (WHO, 2005) with minor adjustments. Six treatment groups were evaluated:

T : Distilled water (negative control)
T2 : 25% extract
T3 : 50% extract
T4 : 75% extract
T5 : 100% extract
T6 : 1% malathion (positive control)

Each Petri dish contained 15mL of solution and 10 active larvae. The experiment was done under standardized laboratory conditions (25-30°C; 70-80% relative humidity), with three replicates per treatment group carried out over two days. Larval mortality was documented hourly for a duration of six hours. Larvae were classified as deceased if they were motionless and sunken following gentle stimulation. Deceased larvae were expeditiously removed to avert decomposition. The mortality rate was determined using: Mortality percentage = (Number of deceased larvae / Total larvae introduced) X 100.

While only 6-hour mortality was documented, subsequent research should prolong monitoring to 24-48 hours in freshwater to evaluate delayed mortality and possible recovery.

Statistical Treatment of Data

One-Way Analysis of Variance (ANOVA) was conducted to ascertain significant differences in larvicidal effects (mortality %) among treatment groups. Post hoc tests were not conducted following ANOVA to identify which specific group pairs exhibited significant differences. This is considered a limitation of the study. All analyses were conducted utilizing SPSS version 26.

Results and Discussion

Phytochemicals Present in S. purpurea and H. costaricencis Extracts

Table 1 presents the phytochemicals identified in the ethanol-based extracts of *S. purpurea* leaves and *H. costaricensis* stems. Qualitative study verified the existence of certain secondary metabolites recognized for their insecticidal characteristics. *H. costaricensis* comprised saponins, flavonoids, glycosides, tannins, and phenolics, while *S. purpurea* displayed alkaloids, saponins, flavonoids, glycosides, and triterpenes.

Table 1: Phytochemicals present in the plant extracts

Phytochemicals	*S. purpurea*	*H. costaricensis*
Tannins	(-)	(+)
Alkaloids	(+)	(-)
Saponins	(+)	(++)
Flavonoids	(+)	(++)
Phenolics	(-)	(+)
Glycosides	(+)	(+)
Carbohydrates	(+)	(-)
Proteins	(-)	(-)
Triterpenes	(+)	(-)

Despite the exclusive use of qualitative screening, the existence of bioactive metabolites exhibiting recognized larvicidal properties was apparent. The lack of quantitative data is a limitation; subsequent research should utilize methodologies such as High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS) to quantify phytochemical content and correlate it with larvicidal efficacy (Jafri *et al*., 2022; Ngegba *et al*., 2022). The discovered phytochemicals are recognized for their insecticidal properties. Alkaloids function as neurotoxins, disrupting neurotransmission (Senthil-Nathan *et al*., 2020); saponins influence molting and induce cell membrane lysis (Isman, 2020); flavonoids inhibit mitochondrial respiration and enzymatic activity (Ngegba *et al*., 2022); tannins deter feeding and denature proteins (Filho *et al*., 2021); glycosides disrupt metabolic enzymes; and phenolics provoke oxidative stress in larval tissues (Senthil-Nathan *et al*., 2022). The existence of many active chemicals indicates that larvicidal mortality may arise from both individual effects and possible synergistic combinations. The results align with other research that reported comparable phytochemical profiles in *S. purpurea* and *H. costaricensis*, with discrepancies likely attributable to extraction methods and solvent polarity (Nazmin *et al*., 2025; Silva *et al*., 2024).

Larvicidal Activity of S. purpurea and H. costaricensis Extracts and Controls

Table 2 presents the larvicidal activity of the two plant extracts and the controls against the third- to fourth-instar *A. aegypti* larvae. The ethanolic leaf extract of *S. purpurea* exhibited a dose-dependent larvicidal activity in *A. aegypti*. A 100% concentration resulted in 93.33% death at 2 hours and 100% by 3 hours. Conversely, the 25% concentration resulted in merely 21 and 32% mortality at 3 and 4 hours, respectively. The stem extract of *H. costaricensis* exhibited comparable dosage responsiveness, resulting in 87% mortality after 2 hours and 100% at 3 hours at maximum concentration. The 25% concentration showed reduced mortality rates of 20% and 53%, respectively.

Malathion (1%) achieved complete death after 1 hour, confirming the bioassay's reliability. The negative control (distilled water) exhibited a death rate of 0.00%, suggesting that the observed mortality was due to the extracts. Statistical analysis employing one-way ANOVA indicated significant differences ($p<0.05$) between treatments and controls.

The observed effects are likely attributable to the bioactivity of flavonoids, alkaloids, and saponins, which compromise the intestinal lining, impede hormonal function, or interfere with enzymatic activities (Senthil-Nathan *et al*., 2022; Isman, 2020).

Table 2: Larvicidal activity of the *Spondias purpurea* and *Hylocereus costaricensis* extracts and controls

Concentration (%)	Initial Count	Mean Count of Dead Larvae (6-hour Observation)						
		1h	2h	3h	4h	5h	6h	**Total**
Spondias purpurea								
25%	30	0	1.7 (5.7%)	6.3 (21%)	9.6 (32%)	6.7 (22.3%)	5.7 (19%)	**30**
50%	30	7.7 (25.7%	7.3 (24.3%)	7.7 (25.7%)	5.3 (17.7%)	2.0 (6.7%)	-	**30**
75%	30	20.7 (69%)	6 (20%)	3.3 (11%)	-	-	-	**30**
100%	30	23 (76.7%)	5 (16.7%)	2 (6.6%)	-	-	-	**30**
Hylocereus costaricensis								
25%	30	2.7 (9%)	3.3 (11%)	10 (33%)	8 (26.7%)	3.7 (12.3%)	2.3 (7.7%)	**30**
50%	30	10.7 (35.7%)	8 (26.7%)	3 (10%)	4.3 (14.3%)	4 (13.3%)	-	**30**
75%	30	16 (53.3%)	6 (20%)	5.7 (19%)	2.3 (7.7%)	-	-	**30**
100%	30	22 (73.4%)	4 (13.3%)	4 (13.3%)	-	-	-	**30**
Distilled water	30	0	0	0	0	0	0	**0**
1% Malathion Solution	30	30 (100%)	-	-	-	-	-	**30**

Although both extracts were efficacious, neither demonstrated the quick onset of action characteristic of malathion, underscoring the necessity for enhancing formulation distribution in future plant-derived larvicides. Compared to other extensively studied plant-based larvicides, the extracts of *S. purpurea* and *H. costaricensis* exhibited notable larvicidal efficacy. *A. indica* has been extensively researched for its effective larvicidal properties, frequently resulting in 100% mortality of *A. aegypti* larvae within 24 hours at concentrations ranging from 0.5 to 1% (Sittichok *et al*., 2024). *Citrus sinensis* peel extracts have demonstrated 80–100% mortality within 24–48 hours at doses of 2–4%, mostly due to limonoids and flavonoids that interfere with larval molting and enzymatic activity (Bhandari *et al*., 2024). Extracts from the seeds and leaves of *Annona squamosa* exhibit notable larvicidal efficacy, with mortality rates between 70 and 95%, contingent upon concentration and duration of exposure, primarily attributed to the presence of acetogenins and alkaloids (Pearlin *et al*., 2024). In contrast to these species, the 100% concentration of the combined *S. purpurea* and *H. costaricensis* extracts in our investigation. Table 3 resulted in nearly total larval mortality within 2 hours, a significantly reduced exposure duration. The elevated concentration employed surpasses that of neem or citrus-based therapies, yet the swift commencement of action suggests significant potential for practical implementation in the field. Mechanistically, neem predominantly disrupts larval hormone regulation and molting, whereas the synergistic effects of flavonoids, saponins, and alkaloids in *S. purpurea* and *H. costaricensis* may elicit a more extensive toxicological impact via neurotoxicity, membrane disruption, and oxidative stress. This indicates that the larvicidal processes of the extracts in this investigation may be both rapid and multifarious, providing an alternative to single-compound plant larvicides.

The Larvicidal Activity of the Combined Extracts of S. purpurea and H. costaricensis

The combination of *S. purpurea* and *H. costaricensis* extracts. Table 3 demonstrated increased larvicidal efficacy. The 100%:100% combination resulted in nearly complete mortality akin to malathion, although other ratios (25:75%, 50:50%, 75:25%) exhibited moderate nevertheless substantial effects. Statistical analyses validated disparities between combinations and controls ($p<0.05$). Nonetheless, post hoc pairwise comparisons were not conducted—a recognized drawback. Subsequent research should incorporate Tukey's HSD or Bonferroni test for accurate group comparisons (Field, 2024). The increased toxicity of combinations is likely due to the synergistic effects of shared phytochemicals, particularly saponins and flavonoids, which may influence several physiological targets in larvae (Ngegba *et al*., 2022; Corpuz & Savella, 2019).

Table 3: Larvicidal activity of the combined extracts of *Spondias purpurea* and *Hylocereus costaricensis*

Mixture	Initial Count	Avg. Number of Dead Larvae (6-hour observation period)						
		1 h	2h	3h	4h	5h	6h	Total
25% *S. purpurea* and 75% *H. costaricensis*	30	7.7 (25.7%)	5.4 18%	7.3 (24.3%)	6.3 (21%)	3.3 (11%)	-	30
50% *S. purpurea* and 50% *H. costaricensis*	30	15 (50%	7 (23.4%)	4 (13.3%)	4 (13.3%)	-	-	30
75% *S. purpurea* and 25% *H. costaricensis*	30	22 (73.3%)	3 (10%)	4 (13.4%)	1 (3.3%)	-	-	30
100% *S. purpurea* and 100% *H. costaricensis*	30	25 (83.3%)	5 (16.7%)	-	-	-	-	30
Distilled water (negative control)	30	-	-	-	-	-	-	0
1% Malathion Solution (positive control)	30	30 (100%)	-	-	-	-	-	30

Mortality was monitored over six hours; a prolonged exposure period of 24-48 hours is suggested for future trials to detect delayed effects. Likewise, LD_{50} values must be determined by probit analysis for toxicity assessment and comparison with synthetic larvicides.

Variations in Larvicidal Activity Across Concentrations and Extract Combination

ANOVA findings indicated significant differences ($p<0.05$) between all concentration groups and between all treatments and controls. Both extracts at concentrations of 75 and 100% resulted in the highest death rates, with the 100% extract nearly equaling the effectiveness of malathion.All concentrations of *S. purpurea* and *H. costaricencis* demonstrated significantly greater efficacy than the negative control. The lack of post hoc testing constrains the interpretation of differences among concentration levels.

The combined extracts demonstrated superior larvicidal activity compared to the individual extracts at all tested concentrations, with the 100%-100% combination yielding effects akin to the synthetic control. Subsequently, post hoc testing is necessary to statistically validate the observed trends. The absence of multiple comparison testing in the study is acknowledged as a methodological limitation. Future research should employ rigorous statistical methods to enhance analytical accuracy and determine concentration efficacy thresholds.

Conclusion

This research revealed that ethanolic extracts of *S. purpure*a and *H. costaricensis* stems possess significant larvicidal efficacy against *A. aegypti* larvae. Mortality escalated with concentration, and the combination treatment at 100-100% demonstrated efficacy comparable to malathion.

It further indicates that extracts of both plants exhibit significant larvicidal potential and could function as environmentally sustainable alternatives to chemical larvicides in integrated mosquito management strategies. Their application may diminish dependence on synthetic insecticides, lessen environmental damage, and postpone the emergence of resistance in mosquito populations.

Phytochemical analysis verified the existence of flavonoids, saponins, glycosides, alkaloids, tannins, and phenolics—bioactive compounds implicated in larvicidal effects via mechanisms including neurotoxicity, cell lysis, enzymatic disruption, and oxidative stress (Senthil-Nathan *et al*., 2022; Priya *et al*., 2023). Notwithstanding encouraging outcomes, constraints encompass a brief 6-hour exposure duration, the unavailability of LD_{50} values, and the lack of post hoc statistical analysis. To enhance future research, the following recommendations are proposed: extended observation durations (24-48 hours), LD_{50} assessment through probit analysis, implementation of a solvent control, ecotoxicity evaluations on non-target species, field trials to validate real-world effectiveness, and compound isolation utilizing HPLC. This study validates the synergistic potential of *S. purpurea* and *H. costaricensis* extracts and supports their development into environmentally sustainable botanical larvicides for integrated vector control programs.

Furthermore, in order to enable a more thorough efficacy profiling, it is advised that future research include a larger panel of synthetic and natural larvicides. This approach would help develop comprehensive vector management plans that are based on environmental safety and efficacy.

Acknowledgment

The authors extend their gratitude to the University of Northern Philippines for the financial support extended for the conduct of this study.

Funding Information

The University of Northern Philippines, under the University Research and Development Office provided funding for this study.

Author's Contributions

Alfredo Vita Corpuz: Conceived and organized the study, conducted the experiments, analyzed and interpreted the data, wrote the manuscript, and complied with the suggestions of the reviewers.

Mikhail Nigell A. Gallardo: Designed the research plan, collected the plant samples, participated in all experiments, analyzed the data, and edited the manuscript.

Ace Danielle C. Avero: Prepared the laboratory for experimentation, participated in all the experiments, proofread the manuscript and corresponded with the publishing journal.

Ethics

This article is entirely original. The corresponding author certifies that all other authors have read and accepted the work and that there are no ethical contradictions.

Biosafety Clearance

Before the conduct of the study, the research protocol was submitted to the Institutional Research Ethics Committee at the University of Northern Philippines. The committee approved ethical clearance, affirming that the work adhered to institutional and national ethical standards. The utilization of live *A. aegypti* larvae was warranted due to its public health relevance, as this species is a recognized vector for dengue, zika, and chikungunya viruses. The study did not involve any vertebrate animals or humans. To guarantee ethical management, *A. aegypti* larvae were maintained in sanitized, aerated containers under standardized laboratory settings and subjected solely to concentrations approved in the pilot tests. In accordance with bioassay procedures, deceased larvae were counted and disposed of using autoclaving, followed by disposal in compliance with biohazard safety measures. Live larvae from the negative control group were also terminated after the experiment to avert any inadvertent environmental release. This study did not use any endangered plant species. The plants were responsibly obtained from farms with permission. The researchers complied with ethical norms of care, containment, and reporting, as mandated by national and international rules.

References

Bhandari, D. P., Chaudhary, P., Upadhyaya, S. R., Ranjitkar, R., Satyal, R., Adhikari, A., Satyal, P., & Parajuli, N. (2024). Chemical Variability, Antioxidant and Larvicidal Efficacy of EOs from Citrus sinensis (L.) Osbeck Peel, Leaf, and Flower. *Horticulturae*, *10*(6), 566. https://doi.org/10.3390/horticulturae10060566

Chude, C. F., Okorie, C. J., Ayegbunam, E. S., & Aghalu, U. C. (2020). Larvicidal Activity of Leaf Extracts of Ageratum conyzoides and Hyptis Suaveolens Against Aedes Aegypti. *International Journal of Engineering Applied Sciences and Technology*, *04*(10), 304–309. https://doi.org/10.33564/ijeast.2020.v04i10.056

Corpuz, A. V., Florentino, C. M., Quilana, P. E. T., Reotutar, A. J. P., & Reyes, R. A. T. (2025). Acaricidal Activity of *Azadirachta indica* (Neem Tree) and *Carica papaya* (Papaya) Leaf Extracts against *Rhipicephalus sanguineus* (Dog Ticks) Using Spray Method. *OnLine Journal of Biological Sciences*, *25*(2), 426–436. https://doi.org/10.3844/ojbsci.2025.426.436

Field, A. P. (2024). *Discovering Statistics Using IBM SPSS Statistics*.

Filho, A. A. P., Grasielle C. D'Ávila Pessoa, Lydia F. Yamaguchi, Mariana A. Stanton, Artur M. Serravite, Rafael H. M. Pereira, Welber S. Neves & Massuo J. Kato (2021). "Larvicidal Activity of Essential Oils from Piper Species Against Strains of *Aedes aegypti* (Diptera: Culicidae) Resistant to Pyrethroids." *Frontiers in Plant Science*, 12, 685864. https://doi.org/10.3389/fpls.2021. 685864.

Hillary, V. E., Ceasar, S. A., & Ignacimuthu, S. (2024). Efficacy of plant products in controlling disease vector mosquitoes, a review. *Entomologia Experimentalis et Applicata*, *172*(3), 195–214. https://doi.org/10.1111/eea.13401

Isman, M. B. (2020). Botanical Insecticides in the Twenty-First Century—Fulfilling Their Promise? *Annual Review of Entomology*, *65*(1), 233–249. https://doi.org/10.1146/annurev-ento-011019-025010

Jafri, S. A. A., Khalid, Z. M., Khan, M. Z., & Jogezai, N. (2022). Evaluation of Phytochemical and Antioxidant Potential of Various Extracts from Traditionally Used Medicinal Plants of Pakistan. *Open Chemistry*, *20*(1), 1337–1356. https://doi.org/10.1515/chem-2022-0242

Nazmin, F., Barek, M. A., Miah, A., Hossen, Md. S., Islam, M. S., & Ahmed, J. (2025). Mosquito repellent and larvicidal activity of essential oils of aromatic plant growing in Bangladesh: a review. *Clinical*

Phytoscience, *11*(1). https://doi.org/10.1186/s40816-025-00391-4

Ngegba, P. M., Cui, G., Khalid, M. Z., & Zhong, G. (2022). Use of Botanical Pesticides in Agriculture as an Alternative to Synthetic Pesticides. *Agriculture*, *12*(5), 600. https://doi.org/10.3390/agriculture12050600

Pearlin, V. A., & Marin, E. G. (2024). Mosquito Larvicidal Activity of Leaf Extract of Annona squamosa against Dengue Vector, Aedes albopictus. *Uttar Pradesh Journal of Zoology*, *45*(9), 158–163. https://doi.org/10.56557/upjoz/2024/v45i94034

Priya, S. S., Vasantha-Srinivasan, P., Altemimi, A. B., Keerthana, R., Radhakrishnan, N., Senthil-Nathan, S., Kalaivani, K., Chandrasekar, N., Karthi, S., Ganesan, R., Alkanan, Z. T., Pal, T., Verma, O. P., & Proćków, J. (2023). Bioactive Molecules Derived from Plants in Managing Dengue Vector Aedes aegypti (Linn.). *Molecules*, *28*(5), 2386. https://doi.org/10.3390/molecules28052386

Santos, É. M. dos, Ataide, J. A., Coco, J. C., Fava, A. L. M., Silvério, L. A. L., Sueiro, A. C., Silva, J. R. A., Lopes, A. M., Paiva-Santos, A. C., & Mazzola, P. G. (2023). Spondias sp: Shedding Light on Its Vast Pharmaceutical Potential. *Molecules*, *28*(4), 1862. https://doi.org/10.3390/molecules28041862

Senthil-Nathan, S. (2020). A Review of Resistance Mechanisms of Synthetic Insecticides and Botanicals, Phytochemicals, and Essential Oils as Alternative Larvicidal Agents Against Mosquitoes. *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.01591

Silva, J. R. de A., Oliveira, A. A. de, França, L. P., da Cruz, J. D., & Amaral, A. C. F. (2024). Exploring the Larvicidal and Adulticidal Activity against Aedes aegypti of Essential Oil from Bocageopsis multiflora. *Molecules*, *29*(10), 2240. https://doi.org/10.3390/molecules29102240

Sittichok, S., Passara, H., Sinthusiri, J., Moungthipmalai, T., Puwanard, C., Murata, K., & Soonwera, M. (2024). Synergistic Larvicidal and Pupicidal Toxicity and the Morphological Impact of the Dengue Vector (Aedes aegypti) Induced by Geranial and trans-Cinnamaldehyde. *Insects*, *15*(9), 714. https://doi.org/10.3390/insects15090714

World Health Organization. (2005). *Guidelines for Laboratory and Field Testing of MosquitoLarvicides* (WHO/CDS/WHOPES/GCDPP/*(*2005.13). Geneva: World Health Organization. Published 24 May 2005.

Yameogo, F., Sombié, A., Oté, M., Saiki, E., Sakurai, T., Wangrawa, D. W., McCall, P. J., Weetman, D., Kanuka, H., & Badolo, A. (2024). Three years of insecticide resistance evolution and associated mechanisms in Aedes aegypti populations of Ouagadougou, Burkina Faso. *PLOS Neglected Tropical Diseases*, *18*(12), e0012138. https://doi.org/10.1371/journal.pntd.0012138