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Efficacy of Ethanolic Extract of Malapapaya *(Polyscias nodosa)* and Atis *(Annona squamosa)* as a Natural Larvicide Against *Culex tritaeniorhynchus* Mosquito Larvae

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Abstract. Mosquito-borne diseases remain a significant public health concern, particularly in tropical regions where environmental conditions favor mosquito breeding and rapid population growth. The increasing resistance of mosquitoes to synthetic insecticides, along with the environmental and health risks associated with chemical larvicides, has intensified the search for eco-friendly, sustainable, and locally available alternatives. This study evaluated the larvicidal efficacy of combined plant extracts from Malapapaya (Polyscias nodosa) leaves and Atis (Annona squamosa) seeds against Culex tritaeniorhynchus mosquito larvae. Ethanolic extracts of both plant materials were prepared through maceration and tested at five concentrations: 0% (control), 25%, 50%, 75%, and 100%, using a controlled larvicidal bioassay under laboratory conditions. A total of 20 larvae were exposed per treatment group, and larval mortality was recorded after 24 and 48 hours of exposure. The collected data were analyzed using descriptive statistics to determine the effectiveness of each concentration. Results showed complete (100%) larval mortality in all treatment concentrations within 24 hours, while no mortality was observed in the control group. No additional mortality was recorded at 48 hours, indicating a rapid larvicidal effect immediately upon exposure to the extracts. The strong larvicidal activity observed across all tested concentrations suggests that the bioactive compounds present in P. nodosa and A. squamosa may effectively disrupt essential physiological processes in mosquito larvae. The consistency of mortality across different concentrations further indicates that even lower concentrations may be sufficient for effective mosquito control. These findings highlight the potential of combined Malapapaya and Atis extracts as eco-friendly, sustainable, and cost-effective botanical larvicides, offering a promising alternative to conventional chemical mosquito control strategies, particularly in resource-limited communities and tropical settings.

Introduction

Mosquito-borne diseases continue to pose a serious public health threat in tropical countries such as the Philippines, where warm temperatures, high humidity, and frequent rainfall create favorable conditions for mosquito breeding (Bhatt et al., 2013). Recent reports of above-normal rainfall and flooding in several regions, including Cagayan de Oro in June 2025, have further increased stagnant water accumulation, promoting the proliferation of disease-carrying mosquitoes (PAGASA, 2025; GMA News, 2025). Among these vectors, *Culex*

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tritaeniorhynchus is of particular concern as the primary transmitter of Japanese encephalitis and other mosquito-borne diseases, commonly breeding in stagnant water environments.

Chemical larvicides have traditionally been used to control mosquito populations; however, their prolonged application has led to insecticide resistance and adverse environment effects, including contamination of water systems and harm to non-target organisms (Sengul Demirak & Canpolat, 2022; Lawler, 2017). These challenges have driven increasing interest in eco-friendly and sustainable mosquito control alternatives, particularly plant-based larvicides. (Engdahl et al., 2022).

Recent studies have increasingly explored botanical extracts as sustainable alternatives for mosquito control due to their biodegradability and lower environmental impact compared with synthetic insecticides. Various plant-derived compounds, including flavonoids, terpenoids, alkaloids, and phenolic compounds, have demonstrated significant larvicidal effects against mosquito species such as *Aedes*, *Anopheles*, and *Culex*. These bioactive substances act through multiple mechanisms, including disruption of larval metabolism, inhibition of growth and molting processes, and interference with nervous system function. Because plant extracts often contain a complex mixture of phytochemicals, they may also reduce the likelihood of insecticide resistance compared with single-compound chemical insecticides. Consequently, the investigation of locally available plant species with potential larvicidal properties has become an important area of research in applied entomology and vector control.

Botanical sources such as Malapapaya (*Polyscias nodos*a) and Atis (A*nnona squamosa)* have demonstrated insecticidal properties due to their bioactive compounds. *Annona squamosa* contains acetogenins that disrupt larval energy production, while *Polyscias nodosa* possesses flavonoids, alkaloids, and tannins that interfere with larval development and enzymatic functions (Singh et al., 2008). Despite their individual effectiveness, limited studies have examined their combined larvicidal potential against *Culex tritaeniorhynchus.*

This study aims to evaluate the larvicidal efficacy of combined Malapapaya leaf and Atis seeds extracts at varying concentrations to determine their effectiveness as a natural mosquito control agent. By exploring plant-based larvicides, the research contributes to environmentally sustainable public health strategies aligned with Sustainable Development Goals on health and ecosystem protection, offering a safer and cost-effective alternative to synthetic chemicals.

Methodology

An experimental design was employed to evaluate the larvicidal efficacy of combined ethanolic extracts of Malapapaya (*Polyscias nodosa*) leaves and Atis (*Annona squamosa*) seeds against *Culex tritaeniorhynchus* mosquito larvae. Extract concentration served as the independent variable, while larval mortality was the dependent variable. Fresh Malapapaya leaves and mature Atis seeds were collected from healthy plants, washed with distilled water, shade-dried for several days, and ground into fine powder using a blender and mortar and pestle. Ethanolic extraction was performed using the maceration method at a 1:5 (w/v) ratio, where 50 g of powdered Malapapaya leaves and 50 g of powdered Atis seeds were separately soaked in 250 mL of ethanol for 48 hours. The mixtures were then filtered using a clean cloth to obtain crude extracts, which were stored in sealed containers prior to use. The extracts were diluted with distilled water to prepare five concentrations: 0% (control), 25%, 50%, 75%, and 100%, where the 25% concentration consisted of 25 mL extract and 75 mL distilled water, the 50% concentration contained equal volumes of extract and distilled water, and the 75% concentration consisted of 75 mL extract and 25 mL distilled water, while the 100% concentration contained undiluted extract. Mosquito larvae were collected from stagnant water sources and reared under laboratory conditions until adult emergence to ensure species consistency, after which newly hatched larvae were used in the experiment. The larvicidal bioassay followed a completely randomized design consisting of five treatment concentrations and one control, with two replicates per treatment and twenty larvae per replicate, totaling 200 larvae. Larvae were exposed to the treatment solutions in labeled plastic containers without feeding, and larval mortality was recorded after 24 and 48 hours. Larvae that showed no movement when gently probed were considered dead. Data were analyzed using descriptive statistics, including frequency counts and percentage mortality.

Results and Discussion

The ethanolic extraction of Malapapaya (*Polyscias nodosa*) leaves and Atis (*Annona squamosa*) seeds successfully produced crude liquid extracts after 48 hours of maceration. The Malapapaya extract exhibited a dark green coloration, while the Atis seed extract appeared yellowish to light brown. Both extracts also produced a strong plant odor, indicating the successful recovery of plant compounds during the extraction process.

These observations suggest that ethanol effectively extracted phytochemical compounds from the plant materials. Ethanol is commonly used in plant extraction because it efficiently dissolves secondary metabolites such as alkaloids, flavonoids, and phenolic compounds that contribute to biological activity (Azwanida, 2015). The successful extraction of these compounds provided the crude extracts used for the larvicidal bioassay in this study.

Larvicidal Activity of the Combined Extract

Concentration	Mean No. Of Dead Larvae (n=20)	% Mortality
0% (Control)	0	0
25%	20	100
50%	20	100
75%	20	100
100%	20	100

Table 1. Larval Mortality of Culex tritaeniorhynchus Exposed to Combined Malapapaya and Atis Seed Extract

Table 1 presents the larval mortality of *Culex tritaeniorhynchus* exposed to different concentrations of the combined Malapapaya (*Polyscias nodosa*) leaf and Atis (*Annona squamosa*) seed extract. Complete larval mortality (100%) was observed in all treatment groups within 24 hours of exposure, while no mortality occurred in the control group. These findings indicate that the combined plant extract demonstrated strong larvicidal activity against *Culex tritaeniorhynchus* larvae.

The absence of mortality in the control group confirms that larval death resulted from exposure to the plant extract rather than environmental conditions. The rapid mortality observed suggested that the extracts contain bioactive compounds capable of disrupting essential physiological processes in mosquito larvae.
Plant-derived compounds are known to interfere with mosquito larval metabolism, digestive enzyme activity, and nervous system function, which can result in paralysis and death. Phytochemicals such as flavonoids, alkaloids, and terpenoids have been reported to exhibit strong insecticidal properties by disrupting cellular respiration and enzyme function in insect larvae (Pavela, 2015).

Studies specifically involving *Annona squamos*a have also demonstrated strong larvicidal activity against mosquito larvae. For example, Kamaraj et al. (2011) reported that extracts from *Annona squamosa* seeds produced significant larval mortality in *Aedes aegypti* and *Culex quinquefasciatus*, suggesting that the plant contains potent bioactive compounds capable of disrupting larval physiological systems. Similarly, Rawani et al. (2010) found that seed extracts of *Annona squamosa* caused high mortality rates in mosquito larvae due to the presence of acetogenins and other insecticidal phytochemicals.

The occurrence of complete larval mortality even at the lowest tested concentration (25%) suggests that the combined extract contains highly potent bioactive compounds capable of rapidly affecting larval survival. This may indicate that the lethal concentration required to cause larval mortality is lower than the minimum concentration evaluated in this study.

Duration of Larvicidal Effect

Larval mortality reached 100% within the first 24 hours of exposure in all treatment groups and remained unchanged after 48 hours. These results indicate that the larvicidal action of the combined plant extract occurred rapidly after exposure.
The fast-acting effect observed suggests that the phytochemical compounds present in Malapapaya leaves and Atis seeds produce immediate toxic effects on mosquito larvae. Rapid larvicidal responses have also been reported in other plant-based larvicide studies, where plant extracts caused significant larval mortality within short exposure periods due to their potent bioactive compounds (Pavela, 2015).

These findings are consistent with previous studies showing that botanical larvicides can produce rapid mortality by disrupting larval metabolic processes and nervous system function. The rapid mortality observed in this study demonstrates the potential of plant-based extracts to provide effective mosquito control within short exposure periods.

Effect of Extract Concentration

All tested extract concentrations (25%, 50%, 75%, and 100%) produced identical larval mortality results, with each treatment resulting in complete mortality within 24 hours. Increasing the extract concentration beyond 25% did not produce additional mortality effects.

This result indicates that the maximum larvicidal efficacy of the combined extract was already achieved at the lowest concentration tested. Such outcomes may occur when the concentration of bioactive compounds already exceeds the threshold required to produce toxic effects on mosquito larvae.

Similar results have been reported in studies involving highly potent plant extracts, where increasing the concentration does not significantly increase mortality once the effective threshold has been reached. This phenomenon is commonly described as a ceiling effect in toxicological studies.

Comparative Effectiveness Across Concentrations

The comparison of larvicidal activity across different concentrations revealed consistent effectiveness of the combined Malapapaya and Atis extracts against *Culex tritaeniorhynchus* larvae. Despite variations in extract concentration, all treatment groups produced complete larval mortality within 24 hours.

These findings demonstrate the strong and reliable larvicidal potential of the combined plant extracts. The results also highlight the potential of plant-derived larvicides as environmentally friendly alternatives to synthetic chemical insecticides used in mosquito control programs. The consistent mortality observed across all concentrations suggests that the combined extracts contain highly potent bioactive compounds capable of effectively disrupting larval physiological processes.

Conclusion and Implications

The results of this study demonstrate that the ethanolic extraction of Malapapaya (Polyscias nodosa) leaves and Atis (Annona squamosa) seeds successfully produced crude plant extracts containing bioactive compounds associated with larvicidal properties. The observable coloration and strong plant odor of the extracts suggest the presence of phytochemicals that may contribute to insecticidal activity, confirming that ethanol is an effective solvent for extracting biologically active compounds from plant materials. The combined extracts exhibited strong larvicidal effectiveness against Culex tritaeniorhynchus larvae, as all treatment concentrations resulted in 100% larval mortality within 24 hours while no mortality was observed in the control group. These findings indicate that the combined plant extracts contain potent bioactive compounds capable of rapidly disrupting larval physiological processes, highlighting their potential as environmentally friendly alternatives to synthetic mosquito control agents. The complete larval mortality observed in all treatment groups confirms the strong toxicity of the extracts against mosquito larvae and suggests their potential application as natural larvicides. Furthermore, the results showed that the larvicidal action occurred rapidly within the first 24 hours of exposure and remained unchanged at 48 hours, indicating the fast-acting nature of the phytochemical compounds present in the extracts. The identical mortality results across all tested concentrations also suggest that maximum larvicidal effectiveness was achieved even at the lowest concentration tested, indicating the presence of highly potent bioactive compounds. Based on these findings, future studies are recommended to determine the lethal concentration values (LC_{50} and LC_{90}) of the extracts in order to identify the minimum effective concentration required for mosquito larval control. Further research may also explore the use of alternative extraction techniques, evaluate the larvicidal effectiveness of the extracts under field conditions, examine their residual activity over longer exposure periods, and investigate potential synergistic effects with other botanical insecticides to support the development of practical plant-based mosquito control formulations.

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Competing Interests Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data Availability Statement

The data supporting the findings of this study are available from the authors upon reasonable request.

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Appendices

No appendices attached to this article