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RESEARCH PAPER

Evaluation of larvicidal efficacy of Andrograpanin formulation against mosquito vectors

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Abstract
This study evaluates the larvicidal efficacy of an andrograpanin formulation against two vector mosquito species, *Aedes aegypti* and *Culex quinquefasciatus*. The active compound, andrograpanin, was isolated from fraction 4 of the methanol extract of the leaves of *Andrographis paniculata* (family: Acanthaceae), a well-known medicinal plant traditionally used in Ayurvedic and Chinese medicine for its anti-inflammatory, antibacterial, and antiviral properties. Recognized for its diverse bioactive compounds, *A. paniculata* has recently gained attention for its pesticidal potential. In this study, andrograpanin was formulated in mineral oil and tested at concentrations of 0.5, 1.0, 1.5, and 2.0 ppm under laboratory conditions. The larvicidal activity was compared to an azadirachtin-based formulation as a positive control. Results showed that andrograpanin exhibited significant larvicidal effects, with LC_{50} and LC_{90} values for *Aedes aegypti* at 1.27 ppm and 3.35 ppm, respectively, and for *Culex quinquefasciatus* at 1.26 ppm and 3.03 ppm. The azadirachtin formulation demonstrated greater potency with lower LC_{50} and LC_{90} values. This study underscores the potential of andrograpanin-based formulations as eco-friendly alternatives to synthetic pesticides for controlling mosquito-borne diseases.

Keywords Andrograpanin · *Andrographis paniculata* leaves · Larvicidal efficacy · Mosquito vectors

Introduction

Historically, plants containing bioactive compounds have been employed with considerable efficacy in controlling various crop pests and combating human infections (Karpagam and Devaraj 2011; Thiruppathi et al. 2010). Pyrethrum flowers and sweet wormwood are prime examples of plants utilized as natural sources for insecticides. They have proven effective in controlling insect pests and malaria vectors, respectively (Sarwar 2015). Over time, pest management has evolved from traditional plant-based remedies to the widespread adoption of synthetic pesticides (Mahmood et al. 2016). However, the overreliance on synthetic pesticides has resulted in detrimental consequences such as pollution, health risks, and biodiversity loss (Nikkhah et al. 2017).

Recently, there has been a renewed interest in botanical pesticides due to growing concerns over the negative impacts of synthetic alternatives on human health and the environment (Shabana et al. 2017). Botanical pesticides offer a sustainable solution, characterized by efficacy, biodegradability, diverse modes of action, low toxicity, and readily available source materials (Neeraj et al. 2017). Examples of plants serving as sources for botanical pesticides available on the market include pyrethrum (*Tanacetum cinerariifolium*), neem (*Azadirachta indica*), sabadilla (*Schoenocaulon officinale*), tobacco (*Nicotiana tabacum*), and ryania (*Ryania speciosa*) (Arnason et al. 2012). Additionally, plants like garlic (*Allium sativum*), turmeric (*Curcuma longa*), rosemary (*Rosmarinus officinalis*), ginger (*Zingiber officinale*), and thyme (*Thymus vulgaris*) also exhibit pesticidal properties.

Botanical pesticides, derived from various plant families, are obtained as extracts or essential oils (Mizubuti et al. 2007). These plant parts are processed by drying and grinding into a fine powder, followed by extraction using organic solvents to maximize compound yield (Chougule and Andoji 2016). The resulting extracts undergo concentration, formulation, and

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evaluation for efficacy under laboratory, controlled, or field conditions (Zarubova et al. 2014). Botanical pesticides contain a variety of bioactive compounds, primarily secondary metabolites like steroids, alkaloids, tannins, terpenes, phenols, flavonoids, and resins. These compounds exhibit properties such as antifungal, antibacterial, antioxidant, or insecticidal effects (Ahmad et al. 2017). Bioactive compounds found in botanical pesticides exhibit diverse modes of action against various pests, including insects, fungi, bacteria, nematodes, and viral pathogens infecting plant host cells. These modes of action encompass repellence, inhibition, protein denaturation, and other effects, contingent upon the type of botanical compound and the targeted pest. For example, pyrethrum-based pesticides disrupt nerve cells in insects, leading to paralysis and eventual demise, while neem-derived pesticides possess anti-feedant and repellent properties, induce abnormalities in molting, inhibit oviposition, and disrupt the endocrine system (Grdiša and Gršić, 2013). Synthetic pesticides, though more targeted in their approach, often share neurotoxic properties with botanical pesticides, yielding similar outcomes (Stevenson et al. 2017). Understanding the complex interactions between pests and pesticides, encompassing physical, biological, and chemical aspects, is crucial in devising effective pest management strategies (Lee and Lee 2015; Wink 2015).

Several studies have explored the efficacy of botanical formulations against vector mosquitoes. Azadirachtin-based formulations have been extensively investigated for their larvicidal effects (Maheswaran and Ignacimuth 2012; Su and Mulla 1998). Additionally, plant-based essential oils such as *Eucalyptus maculate citriodon*, *Cymbopogon* spp., *Peleargonium citrosum*, *Mentha piperita*, *Ocimum* spp., *Cymbopogon citratus*, *Plectranthus marrubioides*, *Conyza newii*, *Tetradenia riparia*, *Lippia javanica*, *Lippia ukambensis*, and *Tarchonanthus camphoratus* have shown repellent efficacy against adult vector mosquitoes (Collins et al. 1993; Matsuda et al. 1996; Ansari et al. 2000; Tawatsin et al. 2001; Oyedele et al. 2002; Omolo et al. 2004). However, research on andrograpanin based formulations targeting various larval stages of vector mosquitoes remains limited. The study aimed to develop a pesticidal formulation using Andrograpanin, an active compound extracted from the leaves of *A. paniculata*. This formulation was tailored to effectively target the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. In addition, its impact on non-target species, including *Daphnia indicus* and *Gambusia affinis*, was evaluated to ensure the formulation's environmental safety.

Materials and methods

Botanical sourcing

The leaves of *Andrographis paniculata* were gathered from the Tirunelveli district in Tamil Nadu, India, based on ethnobotanical studies. The plant's identity was confirmed by specialists at the Siddha Central Research Institute (SCRI), Chennai, and a voucher specimen (A24012304P) was preserved in the institute's herbarium.

Extraction and fractionation

The leaves were shade-dried and ground into a coarse powder (1.5 kg). Methanol extraction was performed using a Soxhlet apparatus at 68–69 °C for 6–8 h. The resulting extract was concentrated using a rotary vacuum evaporator, yielding 65 g of dry extract, which was stored at 4 °C in airtight containers. For fractionation, silica gel column chromatography (100–200 mesh) was employed with a stepwise elution using mixtures of hexane and ethyl acetate, followed by pure methanol. A total of 80 fractions were collected and grouped into 10 fractions based on their thin-layer chromatography (TLC) profiles. These fractions were then tested for ovicidal, larvicidal, and pupicidal activities at concentrations of 10.0, 7.5, 5.5, and 2.5 ppm (Vilvest et al. 2023, 2024).

Isolation of andrograpanin

Andrograpanin, a labdane diterpenoid, was isolated from the third fraction obtained during column chromatography of the methanol extract of *A. paniculata* leaves. This fraction underwent further purification by rechromatography on a silica gel column, using a chloroform–ethyl acetate (3:1) solvent system as the eluent, yielding a colourless crystalline solid from acetone. The purity of andrograpanin was confirmed through thin-layer chromatography (TLC), where it exhibited a single spot with an Rf value of 0.50 on TLC over silica gel using toluene-ethyl acetate–methanol (4:6:1) as the developing system. Its molecular formula ($C_{20}H_{30}O_3$) and molecular weight (318) were established by EI-MS, while IR, ^{1}H-NMR, and ^{13}C-NMR spectra provided detailed structural information, matching known literature values and confirming the successful isolation and purity of andrograpanin (Fujita et al. 1984; Han et al. 1998; Cangiano et al. 2001; Wang et al. 2014; Vilvest et al. 2025).

Preparation of formulations

The formulation was crafted using andrograpanin in mineral oil. A stock solution was made by dissolving 5 mg of

andrograpanin in 5 ml of mineral oil, resulting in a concentration of 1000 ppm (parts per million). To aid in dissolving, an emulsifier was added (1 mL). The solution underwent thorough mixing with a magnetic stirrer for a duration of 30 min. Similarly, a formulation containing azadirachtin was employed as a positive control. This was prepared using isopropyl alcohol, wherein 5 mg of azadirachtin was dissolved in 5 ml of mineral oil, yielding a concentration of 1000 ppm. Both formulations were meticulously prepared to ensure uniform dispersion and effective utilization of their active ingredients.

Test mosquitoes

The mosquito life stages used in this study were maintained at the Animal Husbandry Unit within the Department of Advanced Zoology and Biotechnology at Loyola College, Chennai. They were reared in lacquer trays filled with toxin-free tap water, free from pests and repellents. The larvae were fed a mixture of Brewer's yeast and dog biscuits in a 2:3 ratio, with environmental conditions maintained at 27 ± 1 °C, 75–85% relative humidity, and a 13:11 light–dark cycle. For the experiments, only third instar larvae of the laboratory-reared *Ae. aegypti* and *Cx. quinquefasciatus* strains were selected, following protocols established in prior toxicology research (Yagoo et al. 2024).

Larvicidal assay at laboratory condition

The andrograpanin formulation was evaluated at concentrations of 0.5, 1.0, 1.5, and 2.0 ppm. Control groups included water and mineral oil, while a positive control was set up with an azadirachtin formulation at the same concentrations. For each test, 100 ml of the solution was prepared in 150-mL plastic containers, and twenty third-instar mosquito larvae were introduced to each container. Each concentration was tested in five replicates to ensure result reliability. Additional control setups included untreated samples and samples with only emulsifier and mineral oil to monitor any potential effects from formulation components. After 24 h of exposure, mortality was assessed by gently prodding the larvae with a glass rod to check for movement. Mortality rates were calculated using a standard formula (I), with corrections for control mortality made according to Abbott's (1925) formula (II) as needed. The results were then compared to the positive control, azadirachtin.

$$Percentage\ mortality\ (I)\ :\ \frac{No.\ of\ dead\ larvae}{No.\ of\ larvae\ exposed} \times 100$$

$$Corrected\ percentage\ mortality\ (II)\ :\ \left[1 - nT/nC\right] \times 100$$

Where n is the number of larvae alive, T represents Treated larvae and C represents Control larvae.

Statistical analysis

The larvicidal activity observed in the laboratory study was corrected using Abbott's formula, as outlined by Abbott in 1925. Additionally, the US EPA Probit Analysis software version 1.5 was employed to compute the lethal concentrations (LC_{50} and LC_{90}). These analytical tools were utilized to accurately determine the concentrations at which 50% and 90% of the tested larvae were killed, respectively (Finney, 1927). This approach ensured precise estimation of the potency of the larvicidal agents under investigation, contributing to a thorough knowledge of their effectiveness and potential applications.

Results

The laboratory study revealed the remarkable efficacy of the andrograpanin-based pesticidal formulation against larvae of two prominent vector mosquito species, *Aedes aegypti* and *Culex quinquefasciatus*. The LC_{50} and LC_{90} values for the andrograpanin-based formulation were 1.27 ppm and 3.35 ppm, respectively, for *Ae. aegypti* larvae, and 1.26 ppm and 3.03 ppm, respectively, for *Cx. quinquefasciatus* larvae, as shown in Tables 1 and 2. Both mosquito species displayed considerable susceptibility to the andrograpanin and azadirachtin formulations. Specifically, the azadirachtin-based formulations exhibited even greater efficacy, with

Table 1 Lethal concentration (in ppm) of andrograpanin based formulation against *Ae. aegypti* larvae in comparison with azadirachtin

Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	χ^2
		LL	UL		LL	UL			
Andrograpanin formulation	1.27	1.10	1.49	3.35	2.55	5.42	3.0 ± 0.4	4.6 ± 0.4	5.7*
Azadirachtin formulation	0.41	0.33	0.46	0.67	0.60	0.82	7.3 ± 0.3	6.0 ± 1.3	0.0*

LC_{50} represents the concentration lethal to 50% of larvae, while LC_{90} denotes the concentration lethal to 90% of larvae

LL and UL denote the lower and upper limits, respectively, of the 95% confidence interval

*$p < 0.05$ indicates the significance level for chi-square values

Table 2 Lethal concentration (in ppm) of andrograpanin based formulation against *Cx. quinquefasciatus* larvae in comparison with azadirachtin

Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	χ^2
		LL	UL		LL	UL			
Andrograpanin formulation	1.26	1.09	1.45	3.03	2.39	4.53	3.3 ± 0.4	4.6 ± 0.1	3.1*
Azadirachtin formulation	0.33	0.25	0.38	0.58	0.50	0.64	5.1 ± 0.1	5.0 ± 1.2	0.1*

LC_{50} represents the concentration lethal to 50% of larvae, while LC_{90} denotes the concentration lethal to 90% of larvae

LL and UL denote the lower and upper limits, respectively, of the 95% confidence interval

*$p < 0.05$ indicates the significance level for chi-square values

LC_{50} and LC_{90} values of 0.41 ppm and 0.67 ppm, respectively, against *Ae. aegypti* larvae, and 0.33 ppm and 0.58 ppm, respectively, against *Cx. quinquefasciatus* larvae. Importantly, no larvicidal activity or developmental abnormalities were observed in the negative control group (emulsifier and mineral oil), confirming that the formulation base components did not contribute to larval mortality or interfere with normal development. These findings highlight the potential of both andrograpanin and azadirachtin formulations as highly effective larvicides for controlling key vector mosquito populations, thus offering promising solutions for mosquito-borne disease management.

Discussion

The utilization of plant-derived products as insecticides, repellents, or larvicides represents a highly effective and environmentally friendly alternative for mosquito control, offering diverse solutions to combat mosquito populations and reduce the risk of mosquito-borne diseases (Brown 1986; Sukumar, 1991). Traditional pesticide formulations come in solid, gas, liquid, and gel forms, each requiring appropriate carriers, synergists, additives, and other components to optimize effectiveness, prolong residual effects, and enhance safety for humans, non-target organisms, and the environment (Stejskal et al. 2021). The emphasis on using natural products for mosquito control has escalated due to the risks associated with insecticides (Murty et al., 1987). Various plant-derived substances were tested to determine their effectiveness in killing mosquito larvae (Sujatha et al. 1988; Sukumar et al. 1991).

The methanolic extract of *Annona squamosa* leaves demonstrated dose-dependent mosquitocidal activity against *Cx. quinquefasciatus*, with the formulated insecticide at 1%, 3%, and 5% w/w concentrations in deodorized kerosene showing significantly shorter knockdown (KD) and 24-h mortality times, indicating notable efficacy (Jaswanth et al. 2002). A neem oil formulation, consisting of 32% neem seed oil (equivalent to 0.03% azadirachtin), an emulsifier (5%), and 63% isopropanol (as a solvent), exhibited larvicidal activity against *An. gambiae* with an LC_{50} value of 11 ppm, and was also noted to possess insect growth regulators (Okumu et al. 2007). Azadirachtin, present in neem oil, functions as an anti-ecdysteroid, leading to larval mortality through growth inhibition effects (Zebit 1984). In a study, the larvicidal effectiveness of an emulsified neem oil concentration was assessed against *Culex*, *Aedes*, and *Anopheles* mosquitoes. The neem oil formulation demonstrated LC_{50} values of 1.8 ppm for *Cx. quinquefasciatus*, 1.7 ppm for *Ae. aegypti*, and 1.6 ppm for *An. stephensi* under both field and laboratory conditions (Dua et al. 2009).

A formulation containing 32% neem seed oil, 63% iso propanol, and 5% emulsifier showed an LC_{50} of 11 ppm and growth inhibitory efficacy against *An. gambiae* (Okumu et al. 2007). A nonoemulsion of neem oil and Tween 20 showed an LC_{50} of 11.75 mg/L against *Cx. quinquefasciatus* (Anjali et al. 2012). The botanical formulation PONNEEM exhibited ovicidal activity against *Spodoptera litura* and *Helicoverpa armigera* eggs at 20 µL/L concentration with 76.74% and 67.39%, and also showed promising larvicidal and ovicidal activities against *Ae. albopictus* and *Ae. aegypti* (Maria Packiam et al. 2012; Maheswaran and Ignacimuthu 2012). In combating vector-borne diseases, controlling the population of vector mosquitoes is crucial. Many countries, especially rural households, rely on pyrethrin-based mosquito liquid formulations for protection from mosquito bites due to repellent properties and insecticidal effects. Synthetic pyrethroids like D-allethrin and D-trans allethrin are increasingly utilized as substitutes for natural pyrethrins in various mosquito control products. While effective with low toxicity to mammals, prolonged exposure to these chemicals can cause local irritation, severe allergic dermatitis, and systemic allergic reactions (Reynolds 1996). Managing vector mosquitoes presents a significant challenge as they develop resistance to pesticides and larvicides, while excessive chemical use exacerbates human health risks.

In our study, the andrograpanin-based formulation demonstrated significant larvicidal activity against both *Ae. aegypti* and *Cx. quinquefasciatus*, with LC_{50} values of 1.27 ppm and 1.26 ppm, respectively. These values indicate a high level of potency, especially considering that

andrograpanin is a novel active component formulated in mineral oil. While the azadirachtin-based formulation exhibited comparatively lower LC_{50} and LC_{90} values, the results for andrograpanin are promising and demonstrate the compound's potential as a botanical larvicide. The consistent susceptibility observed in both mosquito species suggests that andrograpanin has broad-spectrum larvicidal efficacy.

Importantly, no mortality or developmental abnormalities were observed in the negative control (emulsifier and mineral oil), confirming that the base formulation itself did not contribute to the observed effects. This underscores the larvicidal action as being solely attributable to the active compound. Compared to previous botanical studies, the LC_{50} values of andrograpanin fall within an effective range and are competitive with other natural larvicidal agents. This supports its potential use in integrated mosquito management programs. Additionally, the use of *A. paniculata*, a medicinal plant with a well-established safety profile in traditional medicine, further enhances the formulation's appeal for eco-friendly vector control.

In a prior investigation, botanical formulations displayed efficacy lasting approximately 4–8 days in open field conditions (Mulla and Su 1999), while crude neem oil extracts exhibited effectiveness for up to 5 days in laboratory settings against *Ae. aegypti* and *Cx. quinquefasciatus* (Sinniah et al. 1994). In conclusion, this study reinforces the potential of andrograpanin formulation, a major bioactive compound from *A. paniculata*, as an effective mosquitocidal agent against *Ae. aegypti* and *Cx. quinquefasciatus*. These results support the ongoing exploration of plant-derived insecticides as safe, sustainable, and potent alternatives for vector control and pest management strategies. However, it is important to acknowledge the limitations of this study, particularly the lack of assessment on non-target organisms and human cell lines. Further research is necessary to evaluate the environmental safety and cytotoxicity of these formulations to ensure their broader applicability and ecological compatibility. Additionally, while ovicidal, larvicidal, and pupicidal activities have been previously assessed for fractions and the pure compound andrograpanin, the present study focused solely on larvicidal action in the formulated product. We recognize the importance of evaluating all developmental stages and will consider including ovicidal and pupicidal assessments in future work to provide a more comprehensive understanding of the formulation's full mosquitocidal potential. Future studies should also assess the formulation's efficacy under semi-field and field conditions to better understand its stability, residual activity, and practical applicability in real-world vector control scenarios.

Conclusion

The findings emphasize the significant effectiveness of the andrograpanin-based formulation in controlling the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* under controlled laboratory conditions. The formulation showed considerable larvicidal activity against these mosquito species. This suggests that the andrograpanin-based formulation holds promise as a potent tool in mosquito vector management, offering a balance between efficacy and environmental safety. The observed effectiveness against these key mosquito species positions this formulation as a valuable addition to mosquito control efforts, potentially contributing to reducing the burden of mosquito-borne diseases while minimizing adverse impacts on non-target organisms and ecosystems.

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Data availability The datasets are available from the corresponding author on reasonable request.

Declarations

Conflict of interest There are no competing interests to declare with respect to this manuscript.

Ethical approval Not applicable for this work.

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