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Larvicidal and Pupicidal Activity of Green Synthesized Silver Nanoparticles Using Lawsonia Inermis against Culex Quinquefasciatus

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Abstract:

The use of chemical insecticides for mosquito control often leads to issues such as resistance development, highlighting the need for alternative control methods using plant-based products. In this context, the present study investigates the green synthesis of silver nanoparticles (AgNPs) using *Lawsonia inermis* leaf extract and evaluates their larvicidal effects on the II, III, IV instars, and pupa of *Culex quinquefasciatus*. The synthesized AgNPs were characterized using UV-Vis spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). Larvae from the II, III, IV instar stages and pupa were exposed to varying concentrations of silver nanoparticles (ranging from 5 ppm to 50 ppm) for 24 hours. The LC50 values recorded after 24 hours were 13.14 ppm for II instar, 16.52 ppm for III instar, 33.40 ppm for IV instar, and 43.21 ppm for the pupa. The biosynthesis of silver nanoparticles using *Lawsonia inermis* leaf extract offers a promising and eco-friendly approach for controlling *Culex quinquefasciatus* larvae.

Keywords — Larvicidal activity, pupicidal activity, *Lawsonia inermis*, *Culex quinquefasciatus,* green synthesized silver nanoparticles

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I. INTRODUCTION

Mosquito-borne diseases are a significant health threat in tropical and subtropical regions, with mosquito species from the genera *Anopheles*, *Culex*, and *Aedes* serving as vectors for diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, hemorrhagic fever, yellow fever, chikungunya, and Zika virus (ICMR, 2003; Lixin *et al*., 2006). Efforts to control mosquitoes have targeted both their larval and adult stages (Liu *et al*., 2005). While synthetic chemical pesticides are widely used to control mosquito populations, they contribute to pesticidal pollution and can harm non-target organisms, in addition to being non-biodegradable (Brown, 1986). In response, bio-pesticides derived from plants have shown promising results (Parashar, 2009). To further enhance their effectiveness, nanotechnology is being applied to plant-based products for mosquito larval control. Biosynthesized nanoparticles are not only environmentally safe and biodegradable but also non-toxic to non-target organisms (Subarani *et al*., 2013).

Lawsonia inermis (Lythraceae), commonly known as henna, is a plant found in India and is widely used for dyeing skin, hair, fingernails, leather, silk, and wool, especially during traditional festivals and celebrations. Previous studies have reported its larvicidal activity against *Anopheles stephensi* (Bakhshi *et al*., 2014). Additionally, *Lawsonia inermis* has been shown to possess a range of bioactive properties, including antibacterial and antifungal activities, analgesic effects, anthelmintic activity, antiparasitic properties, anti-inflammatory, antioxidant, anticancer, hepatoprotective, protein glycation inhibitory,

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tuberculostatic, antiviral, and antifertility activities. It has also demonstrated antinematodal, enzyme inhibitory, immunomodulatory, anti-diabetic, antitrypanosomal, molluscicidal, antidermatophytic, wound healing, ROS inhibition, and antisickling activities. The current study aims to evaluate the mosquito larvicidal activity of lawsone, a compound isolated from *Lawsonia inermis*, and perform in silico docking with the detoxifying enzyme acetylcholinesterase (AChE1) (Babili *et al*., 2013).

2. Materials and Methods

2.1 Collection of Plant Materials

The leaves of *Lawsonia inermis* were collected from Sulur village (11°01′31″N 77°07′29″E), Coimbatore district, Tamil Nadu, India.

2.2 Preparation of Plant Extract

Fresh leaves were collected and thoroughly washed with distilled water. A total of 25g of leaves was taken and crushed with 100ml of sterile distilled water. The extract was filtered through Whatman No.1 filter paper (pore size 25µm). The filtrate was then further filtered using a 0.6 µm filter (Suganya *et al*., 2013).

2.3 Synthesis of Silver Nanoparticles

A 2.0 mM aqueous solution of silver nitrate (AgNO3) was prepared for the synthesis of silver nanoparticles. To this, 10 ml of leaf extract was added to 90 ml of the 2 mM silver nitrate solution to reduce the Ag+ ions. This mixture was kept at room temperature for 5 hours, during which a brown solution formed, indicating the successful synthesis of silver nanoparticles. The sample was then centrifuged at 5000 rpm for 20 minutes. The resulting pellet was redispersed in 10 ml of sterile distilled water, and the centrifugation and redispersion process was repeated three times. The purified suspension was freeze-dried to obtain dried powder, which was used for further analysis (Suganya *et al*., 2011).

2.4. Characterization of Silver Nanoparticles

1.0 mg of nanoparticles was dissolved in 1.0 ml of distilled water, and the particle size distribution was measured at a light scattering angle of 90°. The intensity-weighted mean value was recorded as the average of three measurements.

2.5. UV-Vis Spectra Analysis

UV-Vis spectroscopy was used to examine the morphology and stability of the nanoparticles. The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium 5 hours after the synthesis process. A small aliquot of the sample was diluted in distilled water for analysis, and the UV-Vis spectra were recorded using a UV-Vis spectrometer (UV-3024).

2.6. Preparation of Stock Solution and Test Concentrations

50 mg of silver nanoparticles was dissolved in 10 ml of distilled water to prepare a stock solution. From this stock solution, test concentrations were prepared at 5 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm, respectively.

2.7. Culture of Test Organism

Egg rafts were collected from a stagnant water body, and the hatched larvae were cultured and maintained in the laboratory at 27 ± 2°C and 85% relative humidity. The larvae were fed a mixture of dog biscuit and yeast powder in a 3:1 ratio (Kuppusamy et al., 2009).

2.8. Larvicidal Bioassay

Mosquito larvicidal bioassays were conducted following the standard WHO procedure (WHO, 1992), with slight modifications. 200 ml of tap water was added to a series of 250 ml beakers. Test concentrations of silver nanoparticles from *Lawsonia inermis* were prepared at concentrations ranging from 5 ppm to 50 ppm. A control group was also maintained by adding 2 ml of distilled

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water to 200 ml of water. Ten larvae were introduced per concentration for all the experiments. The number of dead larvae was recorded after 24 hours, and the mortality rate (%) was calculated using Abbott's formula (Abbott, 1925).

2.9. Pupicidal Bioassay

The pupicidal activity of the green-synthesized silver nanoparticles from *Lawsonia inermis* was evaluated against *Culex quinquefasciatus* pupae, and the LC50 and LC90 values were determined.

2.10. Statistical Analysis

LC50 and LC90 values for mortality were determined using SPSS 16 (SPSS, 2007) version. The relationship between concentration and mortality was subjected to regression analysis, and the 95% confidence limits were calculated using Probit analysis. ANOVA was performed to evaluate the relationship between the green-synthesized silver nanoparticles and larval and pupal mortality.

Fig 1: *Lawsonia inermis* (Henna, Marudaani (Tamil) leaf

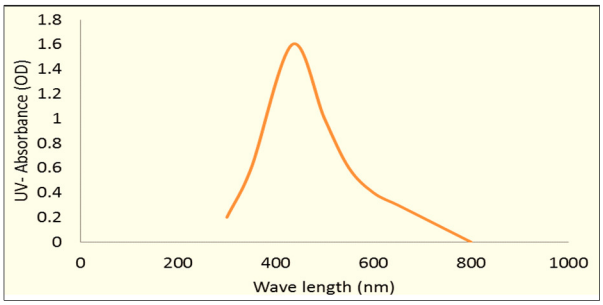

Fig 2: UV-Vis absorption spectra of silver nano-particles synthesized by *Lawsonia inermis* leaf extract

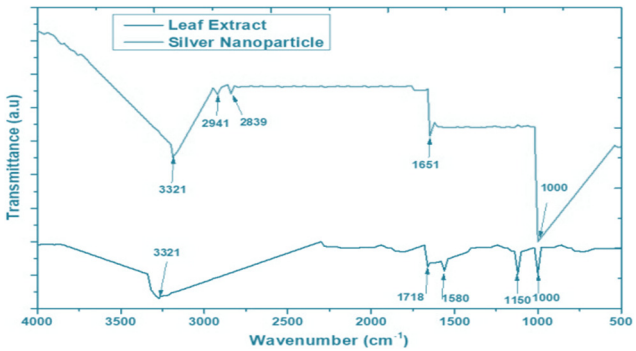

Fig 3: FTIR spectrum of silver nano-particles synthesized by *Lawsonia inermis* leaf extract

Table 1: The LC50 and LC90 values of synthesis of *Lawsonia inermis* silver nanoparticles against the II, III, IV instar and pupa of *Culex quinquefasciatus* under 24 h exposure.

Plant species	Larval stages	LC50 (ppm) (UCL-LCL)	LC90 (ppm) (UCL-LCL)	$\chi 2$	Regression equation
Lawsonia insemis	II instar	13.14 (17.31-11.48)	36.18 (40.35-34.52)	1.005	Y= 1.78 + 0.18 X
	III instar	16.52 (20.15-15.21)	54.54 (58.16-53.22)	.468	Y= 1.35 + 0.16 X
	IV instar	33.40 (39.36 - 31.75)	72.47 (78.44-70.82)	1.237	Y= -1.05 + 0.17 X
	Pupa	43.21 (52.83 - 41.27)	71.98 (81.60-70.04)	.520	Y= -1.26 + 0.11 X

Table 2: Two-way ANOVA to test the validity if relationship in mortality (LC50) as a function of two plants green silver nanoparticles and larval and pupa

Source of variation	SS	*df*	F	P-Value	F-crit
Green synthesized silver nanoparticles	3.836	1	3.83	1.67	0.2865
Larval stages	1397.63	3	465.87	203.04	0.0058
Error	6.88	3	2.29		
Total	1408.35	7			

Significant at $P<0.05$ level

3.Results

The aqueous extract of *Lawsonia inermis* leaves was filtered and mixed with a 2mM silver nitrate ($AgNO_3$) solution, which was then incubated at room temperature for 5 hours. During this period, the solution changed to a dark brown color, signaling the formation of silver nanoparticles. To confirm the synthesis of these nanoparticles, UV-Visible absorption spectroscopy was conducted, showing a peak at 438 nm, indicating the successful formation of silver nanoparticles from *Lawsonia inermis* (Figure 2). In this study, the toxicity of silver nanoparticles synthesized from

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Lawsonia inermis was evaluated against various developmental stages of *Culex quinquefasciatus*, including the II instar, III instar, IV instar, and pupa. Lethal concentration values (LC50 and LC90) were determined after a 24-hour exposure for each developmental stage, and regression equations were derived to describe the relationship between nanoparticle concentration and mortality rates.

Fourier Transform Infrared Spectroscopy (FTIR) analysis (Figure 3) was used to identify functional compounds and macromolecules involved in the synthesis of *Lawsonia inermis* silver nanoparticles by reducing Ag+ ions. The strong peak at 3321 cm^{-1} was attributed to the stretching vibrations of -NH_2 and -OH groups, representing the water and protein components in the leaf extract.

Table 1: II Instar: The LC50 for II instar larvae was found to be 13.14 ppm (95% CI: 17.31–11.48 ppm), while the LC90 was 36.18 ppm (95% CI: 40.35–34.52 ppm). The regression equation for the relationship between concentration (X) and mortality (Y) was **Y = 1.78 + 0.18X**, with a chi-squared value (χ^2) of 1.005, indicating a good fit of the data to the model. **III Instar:** For III instar larvae, the LC50 was 16.52 ppm (95% CI: 20.15–15.21 ppm) and the LC90 was 54.54 ppm (95% CI: 58.16–53.22 ppm). The regression equation was **Y = 1.35 + 0.16X,** with a chi-squared value of 0.468, indicating a more precise fit of the data. **IV Instar:** In IV instar larvae, the LC50 value was 33.40 ppm (95% CI: 39.36–31.75 ppm), and the LC90 was 72.47 ppm (95% CI: 78.44–70.82 ppm). The regression equation for this stage was **Y = -1.05 + 0.17X,** with a chi-squared value of 1.237, indicating a reasonably good fit, though with some variability in the data. **Pupa:** For the pupa, the LC50 was 43.21 ppm (95% CI: 52.83–41.27 ppm), and the LC90 was 71.98 ppm (95% CI: 81.60–70.04 ppm). The regression equation for the pupa was **Y = -1.26 + 0.11X,** with a chi-squared value of 0.520, suggesting an adequate fit to the model.

4.Discussion

The results indicate that *Lawsonia inermis*-synthesized silver nanoparticles exhibit considerable toxicity against all stages of *Culex quinquefasciatus*. The II instar larvae were the most susceptible, with the lowest LC50 value, whereas the pupal stage exhibited the highest LC50, indicating a reduced susceptibility to the nanoparticles in comparison to earlier larval stages. The relationship between the nanoparticle concentration and mortality followed a linear pattern across all stages, albeit with different slopes, as shown by the regression equations. These findings highlight the potential use of *Lawsonia inermis*-synthesized silver nanoparticles as an environmentally friendly and effective approach for controlling *Culex quinquefasciatus* populations at various stages of development.

The detection of carbonyl straining in the proteins' amide connections could be attributed to the spectrum at 1718 cm^{-1}, while the linked doubly bonded vibratory stretching at 1580 cm^{-1} suggested the existence of likely terpenoids or additional heterocyclic phytoconstituents (Anand *et al.* 2023).

A comparative study was conducted on the larvicidal activity of *Nymphaea nucifera* leaf crude extract and silver nanoparticles against malaria and filaria vectors. The results demonstrated that the green-synthesized silver nanoparticles exhibited higher mortality rates in the malarial and filarial vectors compared to the crude extract (Finney *et al*., 1971). Gnanadesigan *et al*. (2011) also reported the effectiveness of *Rhizophora mucronata* nanoparticles in controlling *Aedes aegypti* and *Culex quinquefasciatus* larvae, with the nanoparticles proving to be more toxic to *Culex quinquefasciatus* than to *Aedes aegypti*. Additionally, biogenic nanoparticle synthesis using fungi and bacteria, such as *Agaricus bisporus*, *Penicillium* spp., *E. coli*, and *Vibrio* sp., has been explored. Among these, *Agaricus bisporus*-synthesized nanoparticles showed promising results in controlling *Culex quinquefasciatus* (Dhanasekaran *et al*., 2013). Phytosynthesized nanoparticles from *Jatropha gossypifolia*, *Euphorbia tirucalli*, *Poinsettia tithymaloides*, and *Acalypha macrophylla* were

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tested against the IV instar larvae of *Aedes aegypti* and *Anopheles stephensi*. Of these, *Jatropha gossypifolia* nanoparticles were most effective, with LC50 values of 4.44 ppm for *Aedes aegypti* and 4.90 ppm for *Anopheles stephensi* (Borase *et al*., 2013). Silver nanoparticles synthesized using *Euphorbia hirta* leaf extract also showed larvicidal activity against the malarial vector *Anopheles stephensi* (Priyadarshini *et al*., 2012). *Cocos nucifera* coir-mediated AgNPs were studied against the IV instar larvae of *Anopheles stephensi* and *Culex quinquefasciatus* (Roopan *et al*., 2013). Recent studies have reported the use of methanolic crude extracts from *Colocasia esculenta*, *Eclipta prostrata*, and *Wrightia tinctoria* against *Culex quinquefasciatus*. The *Colocasia esculenta* extract showed an LC50 value of 165.69 ppm for IV instar larvae. The insecticidal activity of these plants is enhanced by the favorable surface area to volume ratio of the nanoparticles, which typically range in size from 1–100 nm, increasing their efficiency (Nalini *et al*., 2017). This effect is further amplified by the synthesis of silver nanoparticles, as demonstrated in the report.

The data suggests that the synthesis of silver nanoparticles using *Lawsonia inermis* may have a broad spectrum of insecticidal activity, making it a viable alternative to conventional chemical insecticides, with implications for integrated pest management strategies. Further studies should focus on optimizing the synthesis process for enhanced toxicity and examining the long-term environmental impact of using these nanoparticles for vector control.

CONCLUSION

The regression equations developed for each stage also demonstrate a clear linear relationship between the nanoparticle concentration and mortality, supporting the potential for effective application of these nanoparticles in pest management strategies, particularly in the IV instar and pupa, where the observed mortality rates showed slightly higher variability. Overall, Lawsonia inermis silver nanoparticles represent a viable and eco-friendly alternative to conventional insecticides for controlling Culex quinquefasciatus, particularly during its early developmental stages. Further studies are recommended to investigate the underlying mechanisms of toxicity, optimize the nanoparticle synthesis for improved efficacy, and assess the long-term environmental impact of these nanoparticles in vector control programs.

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REFERENCES

1. ICMR Bulletin. Prospects of using herbal, products in the control of mosquito vectors 2003;33(1):1-10.
2. Lixin S, Huiquin D at al., Larvicidal activity of extracts of Ginko biloba Exocarp for three different strains of Culex pipiens pallens. J Med. Entomol 2006;43(2):258- 261.
3. Liu HQ, Xu L et al., Chlorpyrifos resistance in Mosquito Culex quinquefasciatus. J Med. Entomol 2005;42(5):815- 820.
4. Brown AWA. Insecticide resistance in mosquito's pragmatic review. J Am. Mosq. Control. Assoc 1986;2:123-140.
5. Parashar UK, Saxenaa PS et al., Bioinspired synthesis of silver nanoparticles. Dig. J. Nanomater. Biostruct 2009;4:159-166.
6. Subarani S, Sabhanayakam et al., Studies on the impact of biosynthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against Anopheles stepensi Liston and Culex quinquefasiatus Say (Diptera:Culicidae). Paracitol res 2013;112(2):487-499.
7. Dhankhar S, Ruhil S, Balhara M, Dhankhar S, Chillar AK. Aegle marmelos (Linn.) Correa: A potential source of phytomedicine. J Med Plant Res 2011;5:1497-1507.

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8. Arul V, Miyazaki S, Dhananjayan R. Studies on the anti- inflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos Corr. J Ethnopharmacol 2005;96:159-163.
9. Chatterjee A, Pakrashi SC. The Treatise of Indian Medicinal Plants. (Council of Industrial and Scientific Research, New Delhi) 2001.
10. Suganya A, Murugan K et al., Green synthesis of silver nanoparticles using Murraya koenigii leaf extract against Anopheles stephensi and Aedes aegypti. Parasitol. Res 2013;112(4):1385-1397.
11. Suganya G, Karthi S et al., Larvicidal potential of silver nanoparticles synthesized using fungus Cochliobolus lunatus against Aedes aegypti (Linnaeus, 1762) and Anopheles stephensi Liston (Diptera; Culicidae). Parasitol. Res 2011;109(3):823-831.
12. Rajkumar G, Rahuman AA. Larvicidal activity of synthesized silver nanoparticles using Eclipta prostrata leaf extract against filariasis and malaria vector. Acta. Trop 2011;188(3):196-203.
13. Kuppusamy C, Murugan K. Mosquitocidal effect of Andrographis paniculatanees against the malaria vector, Anopheles stephensi Liston (Diptera: Culicidae). Inter. J Integrative. Biol 2009;5:75-81.
14. Arivoli S, John Ravindran K et al., S. Larvicidal activity of botanicals against the filarial vector Culex quinquefasciatus say (Diptera: culicidae). Inter. J Res. Zoo 2012;2(1):13-17.
15. WHO. Vector resistance to pesticides, Fifteenth report of the WHO Expert Committee of Vector Biology and Control. WHO. Tech. Rep. Ser 1992;818:1-62.
16. Abbott WS. A method of computing the effectiveness of insecticides. J. Econ. Entomol 1925;(18):267-269.
17. SPSS. SPSS for windows, version 11.5.SPSS Chicago, IL 2007.
18. Finney DJ. In Probit Analysis, Cambridge University Press, London 1971,68-78.
19. Santhoskumar T, Rahuman AA et al., Synthesis f silver nanoparticles using N.nucifera leaf Extract and its larvicidal activity against Malaria and Filariasis vectors. Parasitol. Res 2011;108(3):693-702.
20. Gnanadesigan M, Ravikumar S et al., Biosynthesis of silver nanoparticles by using mangrove plant extract and their potential mosquito larvicidal property. Asian. Pac. J Trop. Med 2011,799-803
21. Dhanasekaran D, Thangaraj R. Evaluation of larvicidal activity of biogenic nanoparticles against filariasis causing Culex mosquito vector. Asian. Pac. J. Trop. Dis 2013;3(3):174-179.
22. Borase HP, Patil CD et al., Phyto-Synthesized silver nanoparticles: A Potent mosquito biolarvicidal agent nanomedicine and biotherapeutic discovery. J Nanomedine Biotherapeutic Discov 2013;3(1):1-7.
23. Priyadarshini KA, Murugan K et al., Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using Euphorbia hirta against Anopheles stephensi Liston (Diptera: Culicidae). Parasitol. Res 2012;111(3):997- 1006.
24. Roopan SM, Rohit G et al., Low coast and eco-friendly Phyto synthesis of silver nanoparticles using Cocus nucifera coir extract and larvicidal activity. Ind. Crop. Prod 2013;43:631-635.
25. Dass K, Mariappan P. Larvicidal activity of Colocasia esculendum, Eclipta prostrata and Wrightia tinctoria leaf extract against Culex quinquefasciatus. Proc.Natil. Acad Sci India Sect. B. Biol Sci 2016;86:139-143.
26. Muthukumaran U, Govindarajan M, Rajeswary M, Hoti S. Synthesis and characterization of silver nanoparticles using Gmelina asiatica leaf extract against filariasis, dengue, and malaria vector mosquitoes," Parasitology Research 2015;114(5):1817-1827.
27. Nalini M, Lena M, Sumathi P, Sundaravadivelan C. Effect of phyto-synthesized silver nanoparticles on developmental stages of malaria vector, Anopheles stephensi and dengue vector, Aedes aegypti, Egyptian Journal of Basic and Applied Sciences 2017;4(3):212- 218.
28. Anand S, Lamba J, Sharma PK, et al. (2023). Azolla microphylla extract ZnO nanoparticles and antibacterial activity: eco-friendly wastewater treatment, Nanotechnology for Environmental Engineering, 8, 183–195

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29. H. Bakhshi et al. Larvicidal properties of botanical extracts of Lawsonia inermis against Anopheles stephensiAdv. Infect. Dis. (2014)
30. F.E. Babili et al. Lawsonia inermis: its Anatomy and its Antimalarial, Antioxidant and Human Breast Cancer Cells MCF7 ActivitiesPharm. Anal. Acta (2013)

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