Original Article

Evaluation of the Larvicidal and Repellency of Extracts and Silver Nanoparticles Biosynthesized with *Zataria multiflora* (Shirazi Thyme) against *Anopheles stephensi*

Asghar Yadollahi[1], Aboozar Soltani[1], Ahmad Gholami[2], Azim Paksa[1], *Kourosh Azizi[1]

[1]Department of Biology and Vector Control, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

***Corresponding author:** Dr Kourosh Azizi, E-mail: azizik@sums.ac.ir

(Received 06 Feb 2024; accepted 26 Feb 2025)

Abstract

Introduction: Mosquitoes transmit several diseases to humans. New and environmentally safe methods are needed to control mosquito populations effectively. This study aimed to evaluate the larvicidal and repellent properties of extracts and silver nanoparticles synthesized from *Zataria multiflora* (Shirazi thyme).
Methods: To determine the larvicidal and repellent properties of *Z. multiflora*, we tested different concentrations of synthesized silver nanoparticles and crude extract against this malaria vector.
Results: The crude extract exhibited weak larvicidal activity, with an LC_{50} of 616.52 μg/mL and an LC_{90} of 1109.74 μg/mL. However, the synthesized silver nanoparticles are highly active on the larvae of *Anopheles stephensi*, with an LC_{50} at 51.07 μg/mL and an LC_{90} at 119.04 μg/mL. Both the crude extract (ED_{50}: 0.00031 μg/cm²; ED_{90}: 0.0018 μg/cm²) and silver nanoparticles (ED_{50}: 0.000072 μg/cm²; ED_{90}: 0.00048 μg/cm²) demonstrated significant repellent activity. Particle size analysis revealed a range of 24.56 to 63.76 nanometers, with an average size of 52.7 nanometers.
Conclusion: This study showed that *Z. multiflora* silver nanoparticles have significant larvicidal properties, so they are suggested as a nature-friendly larvicide.

Keywords: Larvicidal; Repellency; *Zataria multiflora; Anopheles stephensi*; Silver nanoparticles

Introduction

Mosquitoes transmit serious human parasitic and arboviral diseases such as malaria and dengue fever in tropical and subtropical regions (1). *Anopheles stephensi* transmits malaria in many regions of the world (2). *Plasmodium* parasites cause malaria. Among the different species, *P. falciparum* causes more deaths (2, 3). Vector control is a cornerstone preventing vector-borne diseases. The immature stages of mosquitoes are typically controlled using pyrethroids and organophosphates, while insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are widely employed in tropical regions to curb malaria transmission (4, 5). While significant progress has been made in controlling mosquito populations globally, the scarcity of effective insecticides remains a critical challenge. A promising approach to mosquito control involves combining insecticides with phytochemicals (6).

Nanoparticle biosynthesis offers an economical and environmentally friendly alternative for mosquito control, with advantages such as lower energy consumption, reduced chemical use, and minimal environmental impact (7). Currently, plants and microorganisms are used to synthesize nanoparticles (8). Silver nanoparticles (AgNPs) exhibit potent larvicidal and repellent activity against *Anopheles stephensi* (9). Advances in nanotechnology have enabled the

http://jad.tums.ac.ir
Published Online: March 31, 2025

widespread production of silver nanoparticles (AgNPs) in laboratories globally (9). Nanotechnology advancements have led many laboratories worldwide to produce silver nanoparticles (AgNP). Various botanical combinations are currently used to control mosquitoes during outbreaks of mosquito-borne diseases. Therefore, plants possess excellent insect-repellent properties and low ecotoxicity (10–21). The repeated and widespread use of pesticides against mosquitoes has created and increased resistance in these health-threatened creatures. Therefore, it is necessary to search for effective and efficient control strategies (22–27). Nowadays, many studies have been conducted to investigate the insecticidal properties of plants. Several substances have been identified as effective insecticides, and some of them are currently under investigation for the creation of metal nanoparticles (28,29). Botanical nanoparticles exhibit mosquito-repellent and insecticidal properties even under challenging conditions (30–33).

Over 100 studies on metal nanoparticles have demonstrated their high effectiveness as repellents and mosquito-killing toxicants (2, 34, 35). *Zataria multiflora*, commonly known as Shirazi thyme or Iranian oregano, is an aromatic herb native to Iran and neighboring regions. It is widely used in traditional cuisine and herbal medicine due to its unique flavor and potential health benefits, including antimicrobial and antioxidant properties (35). In the current project, we surveyed the larvicidal and repellency characteristics of AgNP synthesized with *Z. multiflora* against the larvae and adults of *An. stephensi* respectively.

Materials and Methods

Plant sample

In the present study, fresh leaves of *Z. multiflora* (Shirazi Thyme) were collected from the mountains of Nurabad District with latitudes 30°6'25"N and longitudes 51°32'50"E, Fars Province, southern Iran, and subjected to taxonomic identification in the Herbarium, Department of Pharmacognosy, and School of Pharmacy, Shiraz University of Medical Sciences (SUMS).

Rearing mosquitoes

Anopheles stephensi larvae were reared in the Department of Biology and Vector Control of Diseases insectary at Shiraz University of Medical Sciences at a temperature of 27 ± 2 °C, 80% humidity, and 10:14 photoperiod. Mosquito larvae were fed on fish flakes, and adult mosquitoes were fed on rabbit blood and 10% sucrose. Repellent experiments were conducted using 100 non-blood-fed female mosquitoes, aged 5–7 days.

Extracting production, preparation of the nano solution

Shirazi thyme leaves were washed with water and dried at room temperature away from sunlight. To prepare the plant leaf broth, 12 grams of thyme leaves were mixed with 100 ml of water, boiled for 7 minutes, and filtered through filter paper. The filter was kept at -12 °C and used for testing within 7 days. The filter was treated with $AgNO_3$ (Aburaihan Pharmaceutical Company) aqueous solution (1–5 µm) and kept at room temperature. The final product was a yellow-brown, which showed the formation of Silver nanoparticles (AgNPs) with high stability in water.

Characterization of Synthesized Silver Nanoparticles (AgNPs) Using SEM, EDS, FTIR, XRD, and UV-Vis Spectroscopy

Scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), Fourier-transformed infrared (FTIR) spectroscopy, and X-ray diffraction (XRD) were used to analyze the attributes of synthesized AgNPs. A UV-Vis spectrophotometer was used to investigate the biological reduction of silver ions in solution. Scanning electron microscopy (SEM), mapping, Wavelength Dispersive Spectroscopy (WDS), and Electron Backscatter Diffraction (EBSD), equipped with Oxford Instruments©) FESEM /ZEISS /SigmaVP was used for nanoparticle size, morphology, and composition studies. X-ray diffraction (XRD) is primarily used to determine the crystal structure, phase identification, and crystallite size of materials. It is widely used in mineralogy, metallurgy, geology, and materials science to characterize crystalline materials such as metals, ceramics, minerals, and pharmaceuticals. Fourier-transformed infrared (FTIR) spectroscopy is used for qualitative and quantitative analysis of organic and inorganic materials. It is widely applied in chemistry, biochemistry, polymer science, pharmaceuticals, and forensic science for identifying compounds, characterizing molecular structures, and studying chemical reactions. In the analysis of synthesized AgNPs, EDS can confirm the presence of silver (Ag) in the nanoparticles and provide information about the elemental composition of any impurities or stabilizing agents present in the sample (3, 36).

Testing the larvicidal effect

The larvicidal effect of *Z. multiflora* extract and its silver nanoparticles (AgNPs) was evaluated according to the WHO protocol (34). *Zataria multiflora* extracts were tested at concentrations of 240, 480, 720, 960, and 1200 μg/mL, and AgNPs synthesized using *Z. multiflora* extracts were tested at 24, 48, 72, 96, and 120 μg/mL concentrations. 249 ml of water and 1 ml of *Z. multiflora* leaf extract were mixed in a half-liter beaker, and then 24, 48, 72, 96, and 120 μg/mL of silver nanoparticles were added to this mixture. In the last step, four replications were performed for each concentration along with a control test. 20 larvae were used in each replication. Mortality of larvae was recorded after 24 hours of contact with different concentrations of crude extract and nanoparticles (38). The lethal concentration was calculated using probit regression analysis (39).

Testing the repellency effect

To determine the effective repellency dose (ED), the Klun and Debboun model was used (40). In this method, a standard plastic cage consisting of a Plexiglas cube measuring 4x5x18 cm with four rectangular holes measuring 3 x 4 cm was used. To calculate the effective dose of each repellent, it was applied directly to volunteer human skin. The consent form was filled out for each volunteer. For the control test, N, N-diethyl-meta-toluamide (DEET) repellent was used as a positive control. Five circles were drawn on the skin, and 50 microliters of the diluted repellent were placed on them. The treated area was exposed to air to dry, and then an experimental apparatus containing five starved 3–5-day-old female mosquitoes per cell was attached to the treated skin, and the cell door was opened (41). The number of mosquito bites and landings was recorded every 5 minutes. To perform the test, each dose was repeated four times to estimate ED_{50} and ED_{90} (42). The concentrations used in *Z. multiflora* extract were 0.125, 0.25, 0.5, and 1 mg/cm^2, and 0.125, 0.25, 0.5, and 1 mg/cm^2 for silver nanoparticles (AgNPs) synthesized using *Z. multiflora* extract. Protection time was evaluated for both compounds. All repellency tests were carried out in a standard laboratory setting. Mortality and repellency data were analyzed using probit analysis to calculate LC_{50}, LC_{90}, ED_{50}, and ED_{90} with 95% confidence limits.

Results

During this study, a color change of *Z. multiflora* extract was observed when incubated with silver nitrate. After 70 minutes of incubation, the color of the extract changed from light brown to dark brown. The EDS plot was used for elemental identification, which showed that 18% of the compound was silver, confirming synthesis. Additionally, the EDS plot showed that the compound contains C (35.4%), O (21.5%), Mo (9%), K (5.3%), Cl (5.2%), Ca

(4.8%), and Mg (1.1%) (Fig. 1). X-ray showed different peaks of silver nanoparticles synthesized from aqueous extracts of *Z. multiflora* leaves (Fig. 2). SEM revealed that the shape of AgNPs was spherical. This study showed that some particles were medium in size (52.7 nm) and some particles were small (24.56 nm), (zeta potential, -25.2 mV) (Fig. 3).

In this study, LC_{50} and LC_{90} for *Z. multiflora* extract were recorded as 616.52 µg/mL and 1109.74 µg/mL, respectively, while for synthesized silver nanoparticles, LC_{50} and LC_{90} were 51.07 and 119.04. µg/mL, respectively (Fig. 4 and Table 1). Using the K and D method, the results indicated that *Z. multiflora* extract exhibited significant repellent activity (ED_{50}: 0.00031 µg/cm²; ED_{90}: 0.0018 µg/cm²). Additionally, the repellent activity of silver nanoparticles (AgNPs) synthesized using *Z. multiflora* extract was recorded as ED_{50}: 0.000072 µg/cm² and ED_{90}: 0.00048 µg/cm². Therefore, the results suggest that silver nanoparticles (AgNPs) are more effective in repelling compared to *Z. multiflora* extract. The protection time for the silver nanoparticle formulation varied between 20 and 115 minutes (Table 2).

Table 1. Larvicide rate of aqueous crude and synthesized AgNPs of *Zataria multiflora* extract against larvae of *Anopheles stephensi*, Shiraz City, Fars Province, Iran

Compound	Concentrations (µg/mL)	Mortality percentage %±SD	LC_{50} (mg/L) (µg/mL) ±SD	LC_{90} (mg/L) ±95 % CL	Heterogeneity x^2	Df
Crude aqueous extract	240	17.5±2.1%				
	480	27.5±2.8%				
	720	45.0±3.2%	616.52±0.75	1109.74±45.23	1.31	2
	960	86.25±2.5%				
	1200	100±0.0%				
	24	13.75±1.9%				
AgNPs	48	46.25±3.5%				
Zataria multiflora	72	63.75±2.7%	51.07±0.26	119.04±12.75	2.20	2
	96	87.5±2.2%				
	120	100±0.0%				

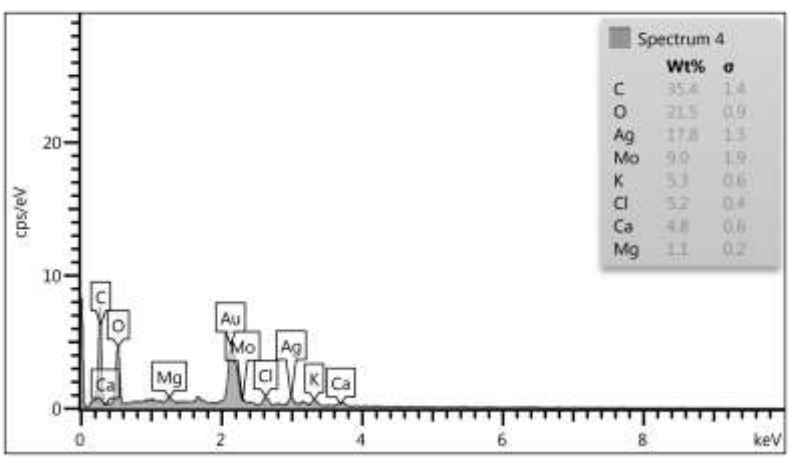

Fig. 1. Energy dispersive spectroscopy (EDS) analysis showing elemental composition of synthesized silver nanoparticles using *Zataria multiflora*. 5 µl of the sample was used for analysis. The results show that 18% of the compounds are silver, confirming the synthesis. Shiraz city, Fars province, Iran. X-axis: Energy (keV). Y-axis: Intensity (counts). Device: FESEM / ZEISS / Sigma VP equipped with Oxford Instruments EDS

http://jad.tums.ac.ir
Published Online: March 31, 2025

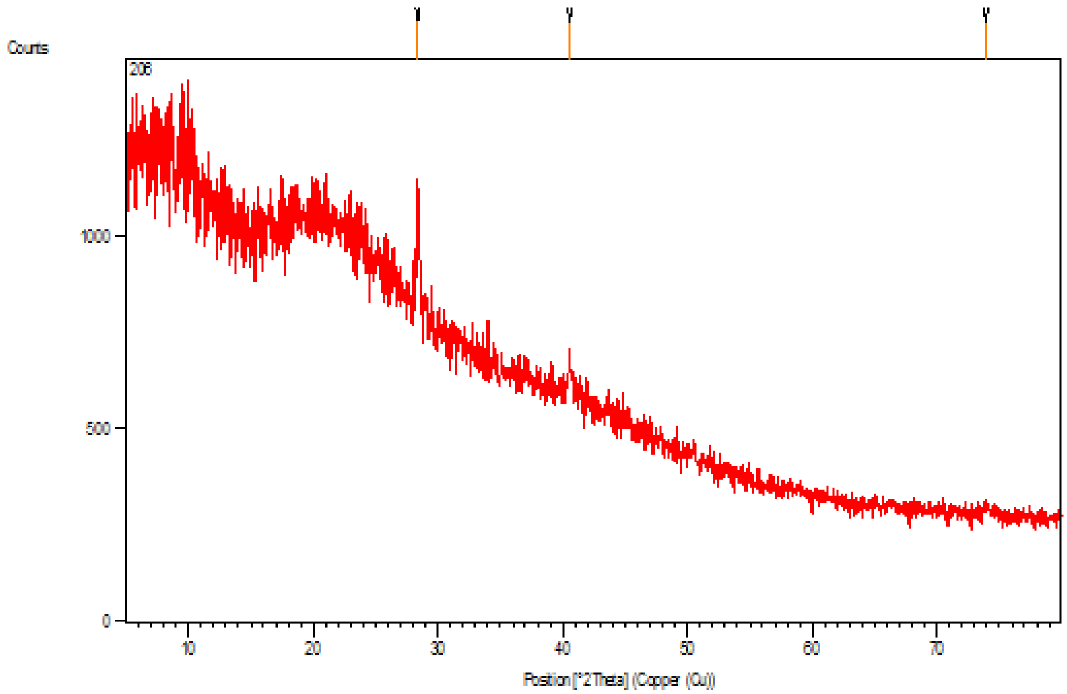

Fig. 2. X-ray diffraction (XRD) pattern of silver nanoparticles synthesized from *Zataria multiflora* extract. Red line represents the fitted diffraction peaks indicating the crystalline structure of silver nanoparticles. No baseline control sample was used for comparison in this pattern. X-axis: 2θ degrees. Y-axis: Intensity (arbitrary units). Device: PANalytical X'Pert PRO XRD system

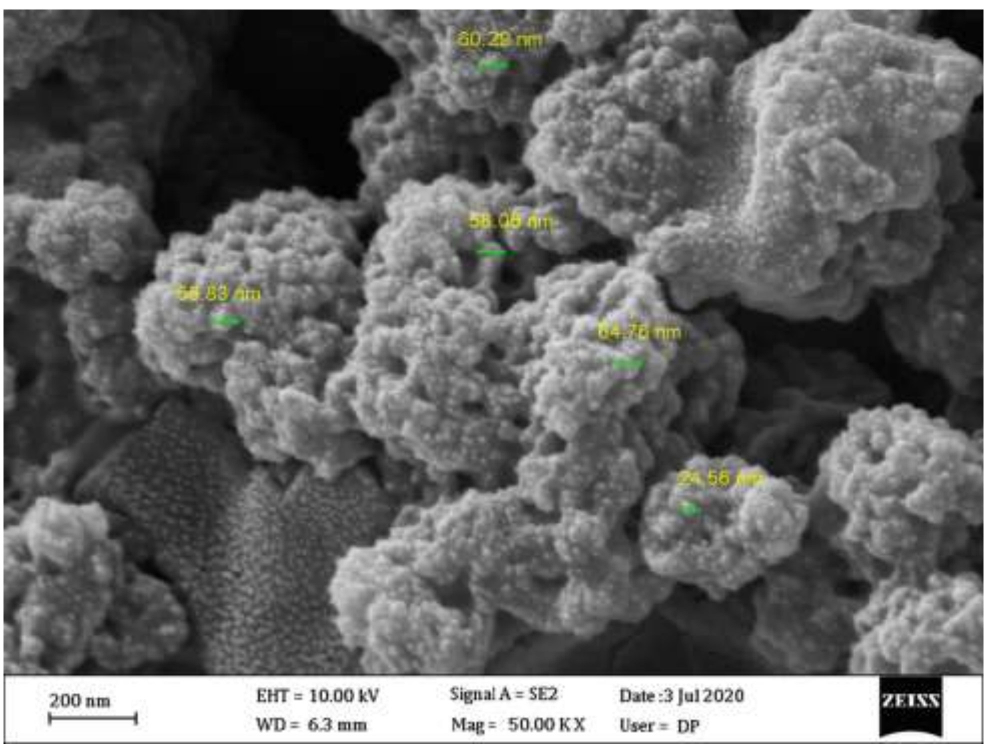

Fig. 3. Scanning electron microscope (SEM) image of silver nanoparticles synthesized using *Zataria multiflora* extract. Sample: Dried suspension of AgNPs coated on SEM stub. Particle shape: Mostly spherical, average diameter 24–63 nm. Device: FESEM / ZEISS / Sigma VP

Table 2. The repellency effect of *Zataria multiflora* extract and AgNPs against *Anopheles stephensi*, Shiraz City, Fars Province, Iran

Compound	B±SE	ED_{50} (μg/cm^2)±95 % CL	ED_{90} (μg/cm^2)±95 % CL	Df
extract	2.84±0.32	0.00031±0.00005	0.0018±0.0002	2
AgNPs	1.76±0.21	0.000072±0.00001	0.00048±0.00006	2

B: Regression Coefficient, **SE:** Standard Error, **ED_{50}:** Effective Dose to repel 50% of mosquitoes, **ED_{90}:** Effective Dose to repel 90% of mosquitoes, **CL**: Confidence Limits, **DF:** Degrees of Freedom

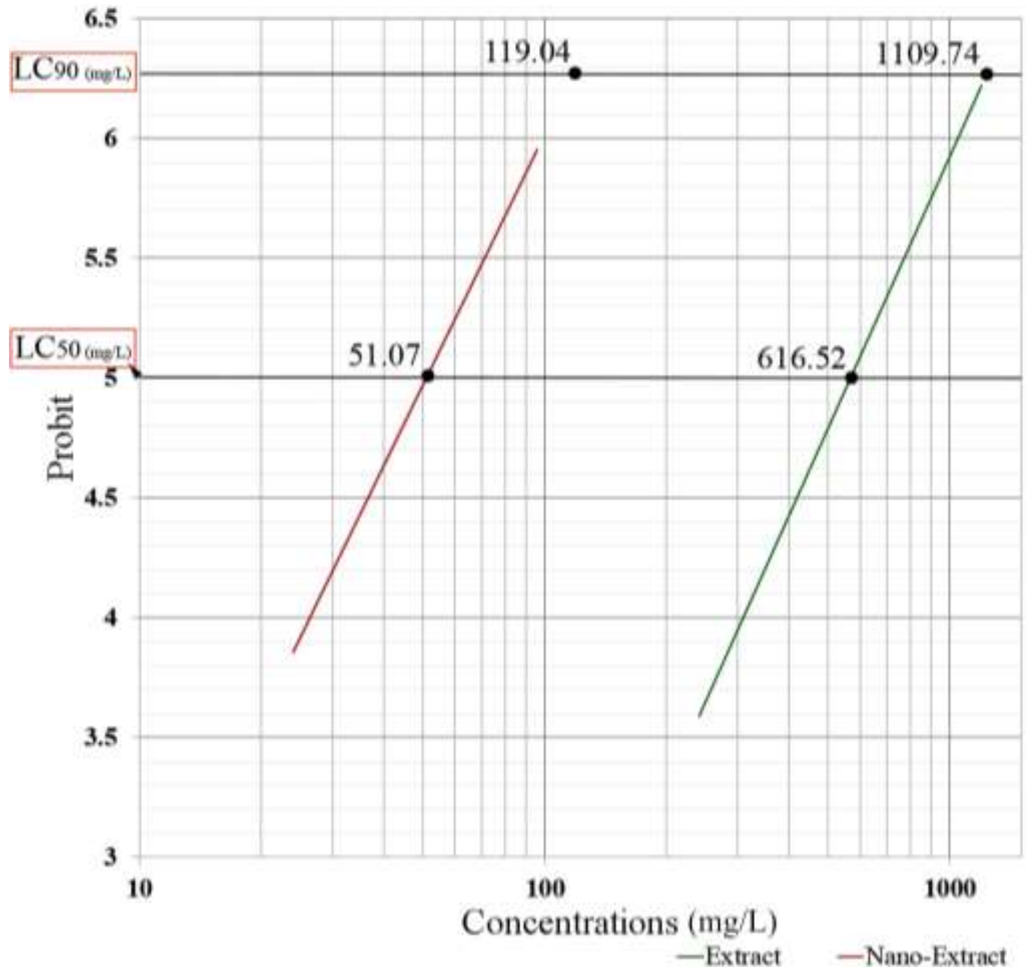

Fig. 4. Probit regression lines of *Anopheles stephensi* larvae exposed to *Zataria multiflora* extract and silver nanoparticles, Shiraz City, Fars Province, Iran

Discussion

Larvicidal activity of plant extracts and AgNPs

Our study showed that the plant crude extract had weak larvicidal activity (LC_{50} at 616.52 μg/mL and LC_{90} at 1109.74 μg/mL). In contrast, the synthesized AgNPs showed significantly greater efficacy (>10 folds lower values), with LC_{50} and LC_{90} values of 51.07 μg/mL and 119.04 μg/mL, respectively, demonstrating stronger activity against mosquito larvae.

Marimuthu et al. (43) found the synthetic AgNPs of *Mimosa pudica* had the highest mortality rate among the synthetic silver nanoparticles against *Anopheles subpictus* larvae. Govindarajan et al. (44) investigated the effect of the methanolic extract of *Cassia fistul* against *An. stephensi* and showed that the LC_{50} value of this substance is 17.97 μg/mL. A study on *Nucifera* leaves reported that the maximum potency larvicidal of *An. subpictus* was observed with synthetic AgNPs. The LC_{50} value was 0.69 ppm, while the LC_{90} value was 2.15 (45). Salunkhe found that the synthetic AgNPs with the fungus *Cochliobolus lunatus* were effective against *An. stephensi* (31). Another study showed that *Acacia cassia* using synthesized silver nanoparticles has a high larvicidal effect on *Ae. albopictus* (24). Muthukumaran et al. (30) synthesized AgNPs using crude aqueous extracts of *Cassia roxburghii* and tested their efficacy against *An. stephensi*. The LC_{50} value of 26.35 ppm, was calculated. The results of Patil et al. (31)'s study with AgNPs synthesized using *Plumeria Rubra* showed that the LC_{50} value was 1.82 ppm against *Aedes aegypti*. Elumalai et al. (33) synthesized AgNPs using *Leucas aspera* and tested their efficacy against Culicidae mosquitoes. The most lethal effect was observed on *Ae. aegypti* and the least lethal effect was observed on *Cx. quinquefasciatus*. A study was conducted using crude aqueous extracts and synthesized AgNPs of *Gmelina asiatica* on *Cx. quinquefasciatus* and the results indicated that the lethal effect of AgNPs is greater than the crude extract (21). In general, the results of the conducted studies confirm the results of the present study. AgNPs were synthesized from *Z. multiflora* against the malaria vector *An. stephensi* for the first time in Iran and showed comparable activity against malaria vectors compared to crude extracts. However, the lethal dose was higher compared to other plants.

Repellent activity of plant extracts and AgNPs

The study also evaluated the repellent activity of *Z. multiflora* extract and its AgNP formulation. The extract showed an ED_{50} of 0.00031 μg/cm² and ED_{90} of 0.0018 μg/cm². These results suggest considerable repellent capacity, though comparisons with commercial repellents such as DEET remain necessary.

The use of repellents represents a practical and cost-effective approach to mitigate the transmission of arthropod-borne diseases to humans. Among the various formulations available, those containing DEET (N, N-diethyl-3-methylbenzamide) are predominant due to their proven efficacy against mosquitoes and other biting insects (46). Despite its effectiveness, DEET application can result in a spectrum of human toxicity reactions, ranging from mild to severe (47). Consequently, there has been substantial research interest in developing plant-derived repellents as safer alternatives to DEET (48–49).

For instance, a study by Kelidari et al. (50) evaluated the repellent effect of a *Z. multiflora* nano formulation, which provided significantly extended protection (93±5 minutes) compared to an unformulated essential oil (29±2 minutes). This finding underscores the potential of botanical-based formulations as effective repellents. *Citronella* oil extracted from *Cymbopogon nardus* has also been extensively researched for its mosquito-repellent properties, demonstrating efficacy across various concentrations (49, 51). It is used either alone or in combination with other natural or commercial products to enhance efficacy against mosquitoes (52).

Beyond repellents, biological control measures for mosquitoes include plant extracts and essential oils, which serve as repellents, ovicides, larvicides, and adulticides. However, their effectiveness varies depending on the species and parts of the plant used (53). Our study demonstrated repellent activity against the malaria vector *An. stephensi* using extracts and silver nanoparticles synthesized from *Z. multiflora*.

In summary, while DEET remains a standard in mosquito repellents despite associated risks, ongoing research into botanical alternatives highlights promising avenues for safer and effective repellent strategies against arthropod-borne diseases.

Characterization of synthesized AgNPs

The SEM analysis showed that the synthesized nanoparticles were small and spherical, consistent with prior findings (54), where plant-mediated AgNPs typically assume this morphology. Particle shape and size significantly influence bioactivity, with smaller particles often offering greater surface area and interaction with target organisms. XRD analysis confirmed the crystalline nature of the AgNPs, revealing prominent peaks at 2θ 28.35°, 40.56°, and 73.88°, indicating a globular structure (55). The sharp diffraction lines reflect successful nanoparticle formation and the presence of a bioorganic phase, which can enhance stability and biocompatibility.

Environmental and practical implications

This study reinforces the potential of eco-friendly nanoparticle synthesis for vector control. Unlike conventional chemical pesticides, plant-mediated synthesis is cost-effective, requires less energy, and minimizes environmental pollution. However, deploying AgNPs at scale requires assessment of environmental risks, including toxicity to non-target organisms and accumulation in aquatic systems. Future research should address these risks and investigate biodegradable or hybrid formulations that retain efficacy while ensuring environmental safety.

Study limitations

Some limitations of the study include: A lack of field validation for repellent or larvicidal efficacy under natural conditions. Limited comparison with standard chemical agents beyond DEET. The need for more detailed formulation and stability testing of AgNP-based repellents.

Implications and future directions

The findings support the integration of botanical-based nanoparticle formulations into vector control strategies. Future studies should explore: Broader-spectrum activity against multiple mosquito species. The identification of active compounds responsible for larvicidal and repellent effects. Scalable production models suitable for public health implementation.

Conclusion

This study demonstrated that silver nanoparticles synthesized using *Z. multiflora* possess significant larvicidal and repellent activities against *An. stephensi*. While not as potent as some AgNPs derived from other botanicals, their eco-friendly synthesis and biological efficacy highlight their potential as alternatives to conventional pesticides and synthetic repellents. These findings contribute to the growing interest in plant-based nanotechnology for sustainable vector control and warrant further exploration in real-world settings.

Acknowledgements

This study was derived from the MSc thesis written by Mr Asghar Yadollahi at the School of Health, Shiraz University of Medical Sciences, Iran. The authors are grateful for the financial support of the Shiraz University of Medical Sciences to carry out this thesis.

Ethical considerations

This study was approved by the research ethics committee of the School of Health, Shiraz University of Medical Sciences, and the ethical code number is IR.SUMS.REC.1398. 978.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

References

1. Solomon R, Mallewa M (2001) Dengue and other emerging flaviviruses. J Infect. 42: 104–115.
2. Benelli G (2016) Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer. Enzyme Microb Technol. 95: 58-68.
3. Dinesh D, Murugan K, Madhiyazhagan P, Panneerselvam C, Mahesh Kumar P, Nicoletti M (2015) Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from *Aloe vera* extracts: towards an effective tool against the malaria vector *Anopheles stephensi*? Parasitol Res. 114(4): 1519–1529.
4. Amarasinghe L, Wickramarachchi P, Aberathna A, Sithara W, De Silva C (2020) Comparative study on larvicidal activity of green synthesized silver nanoparticles and Annona glabra (*Annonaceae*) *aqueous* extract to control *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). Heliyon. 6(6): e04322.
5. Madpathi S, Bannoth RN (2021) The efficacy of *Ximenia americana* plant mediated silver nanoparticles against dengue vector mosquito larvae [*Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762)(Diptera: Culicidae)]. Int J Mosq Res. 8(5): 48–56.
6. Şengül Demirak MŞ, Canpolat E (2022) Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. Insects. 13(2): 162.
7. Stampoulis D, Sinha SK, White JC (2009) Assay-dependent phytotoxicity of nanoparticles to plants. Environ Sci Technol. 43(24): 9473–9479.
8. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X (2007) Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. J Nanotechnol. 18(10): 105104.
9. Osanloo M, Amini SM, Sedaghat MM, Amani A (2019) Larvicidal activity of chemically synthesized silver nanoparticles against *Anopheles stephensi*. J Pharm Negat Results. 10(1): 69–72.
10. Deletre E, Martin T, Campagne P, Bourguet D, Cadin A, Menut C (2013) Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* mosquito. PLoS One. 8(12): e82103.
11. Pålsson K, Jaenson TG (1999) Plant products used as mosquito repellents in Guinea Bissau, West Africa. Acta Trop. 72 (1): 39–52.
12. Shanmugasundaram R, Jeyalakshmi T, Dutt MS, Murthy PB (2008) Larvicidal activity of neem and karanja oil cakes against mosquito vectors, *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.). J Environ Biol. 29 (1): 43–45.
13. Norris EJ, Johnson JB, Gross AD, Bartholomay LC, Coats JR (2018) Plant essential oils enhance diverse pyrethroids against multiple strains of mosquitoes and inhibit detoxification enzyme processes. Insects. 9(4): 132–152.
14. Sanei-Dehkordi A, Soleimani-Ahmadi M, Akbarzadeh K, Salim Abadi Y, Paksa A, Gorouhi MA (2016) Chemical composition and mosquito larvicidal properties of essential oil from leaves of an Iranian indigenous plant *Zhumeria majdae*. J Essent Oil Bear. 19(6): 1454–1461.
15. Soleimani-Ahmadi M, Gorouhi MA, Mohammadi Azani S, SalimAbadi Y, Paksa A, Rashid G (2017) Larvicidial Effects of essential oil and methanol extract of *Achilleawilhelmsii* (Asteraceae) against *Anopheles stephensi* Liston (Diptera: Culicidae), a malaria vector. J Kerman Univ Med Sci. 24(1): 58–67.
16. Soleimani-Ahmadi M, Abtahi SM, Madani

A, Paksa A, Abadi YS, Gorouhi MA (2017) Phytochemical profile and mosquito larvicidal activity of the essential oil from aerial parts of *Satureja bachtiarica Bunge* against malaria and lymphatic filariasis vectors. J Essent Oil Bear. 20(2): 328–336.

17. Soleimani-Ahmadi M, Sanei-Dehkordi A, Turki H, Madani A, Abadi YS, Paksa A (2017) Phytochemical properties and insecticidal potential of volatile oils from *Tanacetum persicum* and *Achillea kellalensis* against two medically important mosquitoes. J Essent Oil Bear. 20(5): 1254–1265.
18. Sanei-Dehkordi A, Soleimani-Ahmadi M, Abadi YS, Paksa A (2019) Wild chive oil is an extremely effective larvicide against malaria mosquito vector *Anopheles stephensi*. Asian Pac J Trop Med. 12(4): 170–174.
19. Vatandoost H, Nikpour F, Hanafi-Bojd AA, Abai MR, Khanavi M, Hajiiakhondi A, Raesi A, Nejati J (2019) Efficacy of extractions of Iranian native plants against main malaria vector, *Anopheles stephensi* in Iran for making appropriate formulation for disease control. J Arthropod Borne Dis. 13(4): 344–352.
20. Torabi Pour H, Shayeghi M, Vatandoost H, Abai MR (2016) Study on larvicidal effects of essential oils of three Iranian native plants against larvae of *Anopheles stephensi* (Liston). Vector Biol J. 1 (2): 2–6.
21. MA Oshaghi, R Ghalandari, H Vatandoost, M Shayeghi, M Kamali-nejad, H Tourabi-Khaledi, M Abolhassani, M Hashemzadeh (2003) Repellent effect of extracts and essential oils of *Citrus limon* (Rutaceae) and *Melissa officinalis* (Labiatae) against main malaria vector, *Anopheles stephensi* (Diptera: Culicidae). Iran J Public Health. 32(4): 47–52.
22. Muthukumaran U, Govindarajan M, Rajeswary M, Hoti S (2015) Synthesis and characterization of silver nanoparticles using *Gmelina asiatica* leaf extract against filariasis, dengue, and malaria vector mosquitoes. Parasitol Res. 114(5): 1817–1827.
23. Osanloo M, Amini SM, Sedaghat MM, Amani A (2019) Larvicidal activity of chemically synthesized silver nanoparticles against *Anopheles stephensi*. J Pharm Negat Results. 10(1): 69–72.
24. Benelli G, Kadaikunnan S, Alharbi NS, Govindarajan M (2018) Biophysical characterization of *Acacia caesia-fabricated* silver nanoparticles: effectiveness on mosquito vectors of public health relevance and impact on non-target aquatic biocontrol agents. Environ Sci Pollut Res Int. 25(11): 10228–10242.
25. Salim-Abadi Y, Asadpour M, Sharifi I, Sanei-Dehkordi A, Gorouhi MA, Paksa A, Tayyebi Z, Aghaei-Afshar A (2017) Baseline susceptibility of filarial vector *Culex quinquefasciatus* (Diptera: Culicidae) to five insecticides with different modes of action in southeast of Iran. J Arthropod Borne Dis. 11(4): 453–462.
26. Abadi YS, Sanei-Dehkordi A, Paksa A, Gorouhi MA, Vatandoost H (2021) Monitoring and mapping of insecticide resistance in medically important mosquitoes (Diptera: Culicidae) in Iran (2000–2020): A review. J Arthropod Borne Dis. 15(1): 21–40.
27. Hazratian T, Paksa A, Sedaghat MM, Vatandoost H, Moosa-Kazemi SH, Sanei-Dehkordi A, Salim-Abadi Y, Pirmohammadi M, Yousefi S, Amin M, Oshaghi M (2019) Baseline susceptibility of *Culiseta longiareolata* (Diptera: Culicidae) to different imagicides, in eastern Azerbaijan, Iran. J Arthropod Borne Dis. 13 (4): 407–415.
28. Dasgupta N, Ranjan S, Mundekkad D, Ramalingam C, Shanker R, Kumar A (2015) Nanotechnology in agro-food: from field to plate. Food Res Int. 69: 381–400.
29. Sorbiun M, Shayegan Mehr E, Ramazani

A, Mashhadi Malekzadeh A (2018) Biosynthesis of metallic nanoparticles using plant extracts and evaluation of their antibacterial properties. Nanochemistry Res. 3(1): 1–16.
30. Muthukumaran U, Govindarajan M, Rajeswary M (2015) Green synthesis of silver nanoparticles from *Cassia roxburghii*-a most potent power for mosquito control. Parasitol Res. 114(12): 4385–4395.
31. Salunkhe RB, Patil SV, Patil CD, Salunke BK (2011) Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedes aegypti* and *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res. 109(3): 823–831.
32. Dua VK, Pandey A, Alam M, Dash A (2006) Larvicidal activity of *Hibiscus abelmoschus Linn* (Malvaceae) against mosquitoes. J Am Mosq Control Assoc. 22(1): 155–157.
33. Elumalai D, Hemavathi M, Deepaa CV, Kaleena PK (2017) Evaluation of phytosynthesised silver nanoparticles from leaf extracts of *Leucas aspera* and *Hyptis suaveolens* and their larvicidal activity against malaria, dengue and filariasis vectors. Parasite Epidemiol Control. 2(4): 15–26.
34. Geetha R, Roy A (2014) Essential oil repellents-a short review. Int J Drug Dev Res. 6(2): 12–17.
35. Sharififar F (2007) Antibacterial activity of the crude extract and fractions of *Zataria multiflora* Boiss. Pharm Biol. 45 (7): 491–495.
36. Benelli G (2015) The best time to have sex: mating behaviour and effect of daylight time on male sexual competitiveness in the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae). Parasitol Res. 114(3): 887–894.
37. World Health Organization (1970) Insecticide resistance and vector control. 17th Repot of WHO Expert Committee on Insecticides. WHO Tech Rep Ser. 443: 47–71.
38. Murugan K, Vahitha R, Baruah I, Das S (2003) Integration of botanical and microbial pesticides for the control of filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Ann Emerg Med. 12(1/2): 12–23.
39. Finney D (1947) The estimation from individual records of the relationship between dose and quantal response. Biometrika. 34(3): 320–334.
40. Malengier B, Goessens T, Mafo FF, De Vrieze M, Van Langenhove L, Wanji S (2015) Model-based determination of the influence of textile fabric on bioassay analysis and the effectiveness of a textile slow-release system of DEET in mosquito control. Pest Manag Sci. 71(8): 1165–1174.
41. Kim D-Y, Leepasert T, Bangs MJ, Chareonviriyaphap T (2021) Evaluation of mosquito attractant candidates using a high-throughput screening system for *Aedes aegypti* (L.), *Culex quinquefasciatus* and *Anopheles minimus* Theobald (Diptera: Culicidae). Insects. 12(6): 528–542.
42. Badolo A, Ilboudo-Sanogo E, Ouédraogo AP, Costantini C (2004) Evaluation of the sensitivity of *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes to two insect repellents: DEET and KBR 3023. Trop Med Int Health. 9(3): 330–334.
43. Marimuthu S, Rahuman AA, Rajakumar G, Santhoshkumar T, Kirthi AV, Jayaseelan C (2011) Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Res. 108(6): 1541–1549.
44. Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K (2008) Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Dip-

tera: Culicidae). Parasitol Res. 103(3): 691–695.

45. Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C (2011) Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol Res. 108(3): 693–702.
46. Qiu H, McCall JW, Wonjun H (1998) Formulation of topical insect repellent N Ndiethyl-m-toluamide (DEET): vehicle effects on DEET in vitro skin permeation. Int J Pharm. 163(4): 167–176.
47. Qiu H, Jun W, McCall JW (1998) Pharmacokinetics, formulation, and safety of insect repellent N N-diethyl-3-methylbenzamide (DEET): a review. J Am Mosq Control Assoc. 14(2): 12–27.
48. Chokechaijaroenporn O, Bunyapraphatsara N, Kongchuensin S (1994) Mosquito repellent activities of *Ocimum volatile* oils. Phytomedicine. 1(2): 135–139.
49. Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C (2005) Comparative repellency of 38 essential oils against mosquito bites. Phytother Res. 19(4): 303–309.
50. Kelidari HR, Moemenbellah-Fard MD, Morteza-Semnani K, Amoozegar F, Shahriari-Namadi M, Saeedi M (2021) Solid-lipid nanoparticles (SLN) containing *Zataria multiflora* essential oil with no-cytotoxicity and potent repellent activity against *Anopheles stephensi*. J Parasit Dis. 45: 101–108.
51. Yang P, Ma Y (2005) Repellent effect of plant essential oils against *Aedes albopictus*. J Vector Ecol. 30(2): 231–234.
52. Fradin MS (1998) Mosquitoes and mosquito repellents: a clinician's guide. Ann Intern Med. 128(11): 931–940.
53. Shaalan EAS, Canyonb D, Younesc MWF, Abdel-Wahaba H, Mansoura AH (2005) A review of botanical phytochemicals with mosquitocidal potential. Environ Int. 31(8): 1149–1166.
54. Shankar SS, Rai A, Ahmad A, Sastry M (2004) Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J Colloid Interface Sci. 275 (2): 496–502.
55. Balaji D, Basavaraja S, Deshpande R, Mahesh DB, Prabhakar B, Venkataraman A (2009) Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. J Colloids Surf B Biointerfaces. 68(1): 88–92.