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ORIGINAL ARTICLE

Gomphrena celosioides-Wrapped Silver Nanoparticles Against Mosquito Vectors: Synthesis, Characterization and Eco-Friendly Assessment

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ABSTRACT

Mosquitoes transmit deadly diseases around the world. Sending mosquitoes to their death with insecticides can also harm the ecosystem and give rise to resistant mosquitoes. Using natural insulators to reduce, stabilise and cap the biogenic nanoparticles is greener than the chemical or physical methods. This research set out to examine the effectiveness of different doses of biosynthesised silver nanoparticles (AgNPs) and an aqueous leaf extract from Gomphrena celosioides against mosquito larvae of three different species: Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus. The synthesised AgNPs were examined using a range of analytical techniques, such as UV-Vis, FTIR, XRD, SEM, and HR-TEM. Plant aqueous extracts and synthesised AgNPs were tested for larvicidal activity after 24 h at different doses. At the highest concentration, all larvae died, but larval mortality increased with concentration, the study found. AgNPs showed higher activity against third-instar larvae of An. stephensi, Ae. aegypti, and Cx. quinquefasciatus than the aqueous extract with LC_{50} of 6.23, 6.46, and 7.02 µg/mL, respectively. Furthermore, plant extracts and synthesised AgNPs were tested against Gambusia affinis at 50-fold concentrations used for target mosquito larvae. The negative effects of target mosquito species compared to Biogenic nanoparticles were confirmed as non-fatal to G. affinis, which was well within selective and harmless profiles. The development of green plant extract and nanoparticles from G. celosioides with such characteristics indicates that they may serve as an environment-friendly alternative to the traditional control measures against mosquitoes. This study represents the first example of green nanoparticle synthesis in the context of medical entomology and parasitology and demonstrates that G. celosioides-assisted silver nanoparticles possess a promising control agent against mosquitoes effectively.

KEYWORDS: *Nanotechnology, Green synthesis, Silver nanoparticles, Gomphrena celosioides, Larvicidal activity.*

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INTRODUCTION

Among all vector species, mosquitoes are the most ubiquitous that facilitate the transmission of diseases worldwide, including malaria, dengue fever, yellow fever, filariasis, and chikungunya [1]. In people, they also induce cutaneous and systemic allergic reactions such as angioedema [2]. Malaria, dengue, and filariasis collectively account for significant global annual morbidity and mortality [3]. In India, these diseases remain significant, with two to three million new cases reported each year [4]. In India, *Aedes aegypti* represents the highest species (86.89%) that is available all over the year. *Anopheles* species are major malaria vectors. Another one important vector and nuisance species is *Culex quinquefasciatus*, the

vector of lymphatic filariasis, which prefers to bite humans [5]. Nanoparticles can be synthesized through chemical, physical and biological methods. Physical and chemical procedures can be expensive, results in harmful components, and employ harsh synthesis conditions [6,7]. The above-mentioned drawbacks have led to the development of green or biological synthetic technique using biological entities in the form of plant and microorganisms (bacteria, fungus, actinomycetes, yeasts and algae) [8,9] as well as the preparation of nanomaterials from the extracts of plant, bacterial and fungal species [10]. In contrast, the generation and preservation of liilacious and germlike cultures are laborious and require an aseptic environment and a great of dexterity, whereas the microplant mediated of nanosynthesis is economic and productive [11].

Due to the environmental advantages, quick action, cost-effectiveness, prolonged stability, and reduced public health hazards compared to classic chemical insecticides, green methods-synthesised nanoparticles are being increasingly popularised for mosquito vector control [12]. Silver nanoparticles have been extensively studied for their pest control potential, with various studies demonstrating efficacious pest management with limited impact on non-target species, including fish and beneficial insects [13]. Previous research suggested that high levels of silver nanoparticles showed toxicological effects on different species of plants and animals [14]. Sharma et al. [15] and using Achyranthes aspera stem extract mediated silver-nanocomposite to control the dengue vector, *A. aegypti* L. Furthermore, it has been reported that silver nanoparticles have more effective mosquito larvicidal activity compared to some other biosynthesised nanoparticles according to Mondal et al. [16]. Apart from this application, a pertinent observation is the toxicological effects exhibited by these synthesised nanoparticles on aquatic ecosystems [17]. This is due to the use of any mosquito larvicidal product applied to the water body in natural habitats [18].

Jayaseelan et al. [19] investigated the larvicidal potential of AgNPs synthesized using the aqueous leaf extract of *Musa paradisiaca* (banana). The study showed that these AgNPs were effective against *Hippobosca maculata* larvae, demonstrating the potential of plant-derived AgNPs in pest control. Rai et al. [20] demonstrated that AgNPs effectively controlled *Culex quinquefasciatus*, the vector of filariasis, with promising results in reducing larvae populations cost-effectively and eco-friendly. Thirumurugan et al. [21] reviewed the potential of biosynthesized silver nanoparticles in controlling mosquito larvae, concluding that AgNPs possess strong larvicidal properties against various mosquito species, with minimal toxicity to non-target species.

A perennial species of the Amaranthaceae family, *Gomphrena celosioides* Mart. Despite its origin in South America, the plant has disseminated over the majority of Asia and Africa. Efficacy of *G. celosioides* has been reported in various publications for treatment of diabetes, dysmenorrhea, coughs, colds, bronchitis, hay fever, asthma, helminthiasis, kidney infection, sexually transmitted infections, vulvovaginitis and dermatological infections [23,24]. Many phytochemicals were identified from *G. celosioides* and reported to exhibit hepatoprotective efficacy in carbon tetrachloride-intoxicated Wistar rats [26,27]. The herb's traditional use for urinary tract infections (UTIs) [28] has been documented by ethnobotanical investigations. According to the literature review, this study focuses on the effectiveness of an aqueous leaf extract and green synthesised silver nanoparticles produced by *G. celosioides* against larvae. An environmentally friendly evaluation of the leaf extract and a green synthesis of AgNPs were conducted for the non-target organism, *Gambusia affinis*.

MATERIAL AND METHODS

Plant material

Plant leaves (*G. celosioides)* were naturally collected from in and around Thanjavur District, Tamil Nadu, India. The plant materials were authenticated and herbarium where a specimen voucher (BDUGC-201) is deposited for reference. Soil debris was removed using tap water, leaves were separated from the plant, and shade was allowed at room temperature for two weeks. After trying, they were milled into powder using an electronic blender.

Test organism

The eggs of *An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus* species were sourced from the Vector Control Research Laboratory in Pondicherry, India, and subsequently reared. Following the standard method established by Govindarajan and Benelli [29], larvae were fed a mixture of powdered dog biscuits and yeast tablets in a 3:1 ratio after hatching. The female adult mosquitoes were fed a blood meal via a membrane feeder, while the male adult mosquitoes were fed honey and a 5.0% glucose solution.

Silver nanoparticles fabrication

In a 300 mL Erlenmeyer flask, 10 g of cleaned and finely chopped *G. celoisioides* leaves were combined with 100 mL of sterile double-distilled water to make Ag NPs. After five minutes of heating, the liquid was

poured into a new container. Using Whatman filter paper number one to filter the colloidal extract and tested within a week. The combination of 88 mL 1 mM $AgNO_3$ solution and 12 mL aqueous filtrate was incubated at room temperature in an Erlenmeyer flask for 10 min [30].

Silver nanoparticles characterization

Initially, the bio-reduction of $AgNO_3$ to AgNPs fabricated by *G. celoisioides* was examined with the help of a UV-vis spectrophotometer and the spectra were recorded between 200-800 nanometer. The existence of biomolecules or functional groups in *G. celoisioides* and synthesized nanoparticles was carried out using FTIR spectroscopy (wavenumber between 500-4000 cm^{-1}). Using X-ray diffraction analytical studies to investigate the crystal form of Ag NPs. The elemental materials of the synthesised Ag NPs from *G. celoisioides* extract were examined using EDX. The aggregation pattern, scattered nature, size, and morphology of the *G. celoisioides* AgNPs were investigated using the SEM and HR-TEM analytical techniques.

Larvicidal bioassay

The larvicidal activity of *G. celoisioides* was evaluated according to the methods described by the World Health Organisation [31]. Larvae were treated with various concentrations of silver nanoparticles (3-15 µg/mL) and aqueous extract (20-100 µg/mL). Twenty-five late third instar *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were placed in 250 mL water cups with 200 mL of dechlorinated water, with an appropriate amount of test substances, aqueous extract and synthesized AgNPs. Each concentration was tested five times, with a corresponding number of control groups using tap water for the same time. The test procedure was carried out at a controlled temperature 27°C ± 3°C in laboratory conditions.

Biotoxicity test on non-targeted species

The methods of Sivagnaname and Kalyanasundaram [32] were used to assess the effects of biotoxicity against non-target species. Plant aqueous extracts and AgNPs from *G. celoisioides* were evaluated for their biotoxicity on the aquatic non-target species *Gambusia affinis.* The specimens of this species were kept in cement tanks. At 50 times the mosquito larvae LC_{50} dosages, *G. celoisioides* extracts and AgNPs were tested. Tests will comprise 10 replicates of each concentration and 4 untreated control samples. After 48 hours, non-target species have been examined for lower activity, swimming ability, and death.

Statistical analysis

In order to determine LC_{50} and LC_{90} values, we used probit statistical analysis on mortality data [33]. To examine all of the data, the SPSS software (version 26) was used. To calculate the Suitability Index (SI) for each non-target organism, we used the following formula [34] to evaluate the biotoxicity to that organism. $P < 0.05$ was established as the significant criterion.

$$SI = \frac{LC_{50} \text{ of non-target organisms}}{LC_{50} \text{ of target vector species}}$$

RESULTS AND DISCUSSION

Characterization of nanoparticles

The bio-reduction of green AgNPs was achieved through the combination of *G. celoisioides* plant extract and a 1 mM silver nitrate solution. The emergence of a reddish-brown hue signifies the formation of Ag nanoparticles (Fig. 1a). Upon the complete synthesis, the stability of AgNPs in solution transitioned to a dark brown colour, which was subsequently analysed using UV–vis spectroscopy for further confirmation (Fig. 1b). UV-vis spectral study revealed Surface Plasmon Resonance peak at 443 nm. Similarly, previous study reported *Andrographis paniculata* plant leaf extract synthesised AgNPs exhibited broad peak at 430 nm in UV–vis spectrum analysis [35]. *Passiflora subpeltata* mediated AgNPs showed absorbance peak at 456 nm [36]. Previous studies indicated that the optimal SPR absorption peak for biologically synthesised Ag-NPs falls within the range of 400–460 nm [37]. In alignment with our study, Wang and colleagues reported that the optimal SPR peak for Ag-NPs synthesised using metabolites of Aspergillus sydowii occurred at 420 nm [38]. The phytochemicals in A. sativum bulb extract facilitated the reduction of silver ions, resulting in the formation of AgNPs, as confirmed by UV–Vis spectroscopy. This outcome demonstrates the effective application of the protocol previously employed with various other plant species [39].

FTIR spectroscopy was conducted on the leaf extract to analyse potential biological components in *G. celosioides* that could be involved in the synthesis and stabilisation of AgNPs. Additionally, FTIR analysis was performed on the synthesised AgNPs, with the spectra of both the leaf extract and nanoparticles presented in Fig. 2a,b. The FTIR spectrum of the leaf extract (Fig. 2a) reveals a broad peak around 3322

cm^{-1}, indicative of the stretching vibration of the O-H group. The peak identified at 2869 cm^{-1} corresponds to the symmetric and asymmetric C-H stretching vibrations of the aliphatic group. The absorption band identified at around 1072 cm^{-1} is associated with the C-O stretching vibration. For *G. celosioides*-mediated AgNPs (Fig. 2b), O-H stretching and bending vibrations were observed at 3316 cm^{-1} and 1694cm^{-1}, respectively. The absorption peak identified at 2955cm^{-1} is associated with C-H stretching. The absorption band identified at 1074cm^{-1} serves as evidence for the presence of the C-O stretching vibration. Furthermore, the absorption band observed at 605cm^{-1} was associated with the stretching and deformation vibrations of Ag, thereby validating the synthesis of AgNPs. Similar to our finding, Mondal et al. [17] have demonstrated FTIR spectrum of *Digiteria sanguinallis* seaweed extract showed a broad peak at 3433cm^{-1} indicated N-H stretching of primary amine, 2056cm^{-1} peak representing the N=C=S strong bend and 1639 cm^{-1} corresponding to medium C=C stretching vibration. Amarasinghe et al. [40] have denoted *Annona glabra* plant mediated Ag NPs revealed O-H, CH_3/CH_2, C-N and C=O stretching vibrations.

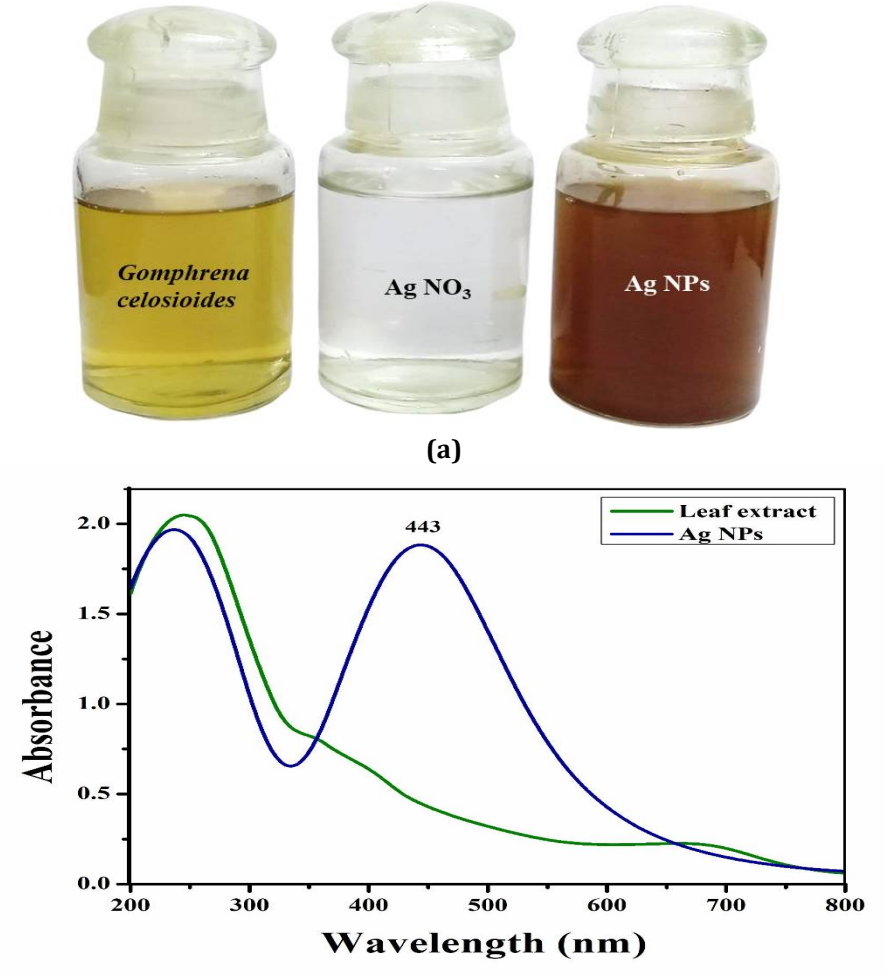
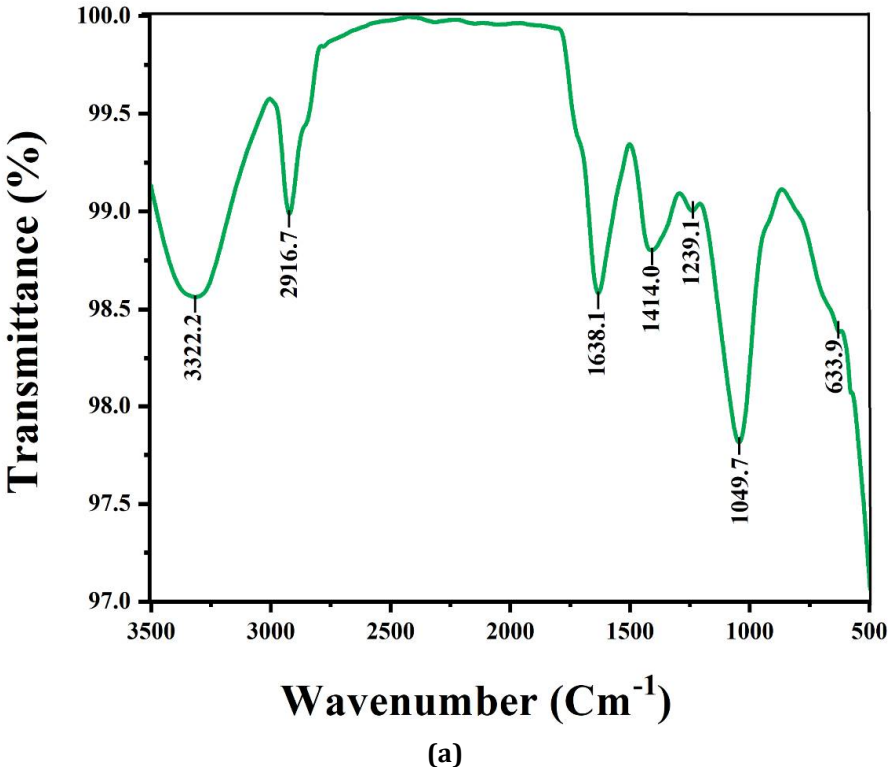
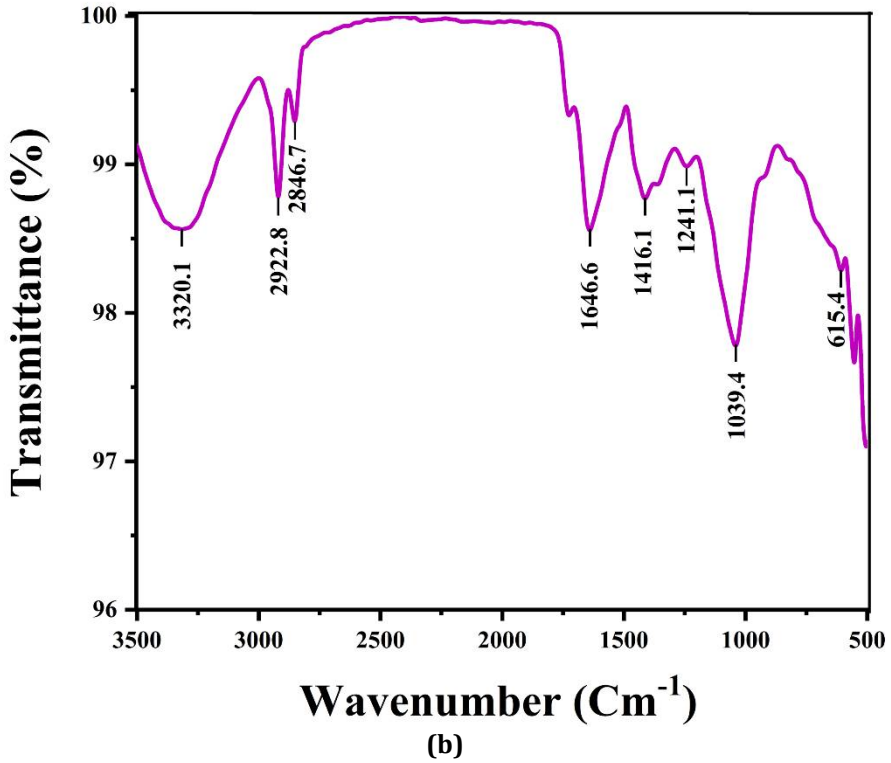


Fig. 1 (a) Colour intensity of *Gomphrena celosioides* aqueous extract and Ag NPs, (b) UV-visible spectrum of Ag NPs after 180 min from the reaction.



(a)



(b)

Fig. 2 FTIR spectrum of *Gomphrena celosioides* aqueous extract (a) and synthesized Ag NPs (b).

Fig. 3 shows the XRD pattern of Ag nanoparticles, the peaks at the diffraction angles of 38.22, 44.41, 64.52 and 77.29 ascribed to (111), (200), (220) and (311) planes, respectively. Thus, strong planes indicated the Ag nanoparticles as a major element in the bio fabrication and crystalline in nature [41,42]. SEM images (Fig 4a-f) showing the spherical morphology and agglomeration pattern of *G. celoisioides*-fabricated AgNPs [43]. EDX study resulted (Fig. 4g) in the major elements (Oxygen, Corbon and silver) found in the bio fabricated Ag NPs [44]. *G. celoisioides*-synthesized AgNPs morphology was further characterized using HR-TEM analysis. Synthesized AgNPs are mostly spherical in shape and distributed in

different sizes 5 to 100 nm (Fig. 5 a-e), along with SAED analysis of AgNPs shown in Fig. 5f. Our study is related to the previous report of Ramesh et al. [45]. *Pisum sativum* plant-mediated silver nanoparticles are 30 nm in size. The XRD results obtained align with the findings of Wang et al. [46], who adeptly demonstrated the crystalline characteristics of AgNPs at 2θ values of 38.2°, 44.4°, 64.6°, and 77.8° through the utilisation of a biomass filtrate derived from *Aspergillus sydowii*. This exemplified the significance of various functional groups in the synthesis and stabilization of AgNPs [39,9].

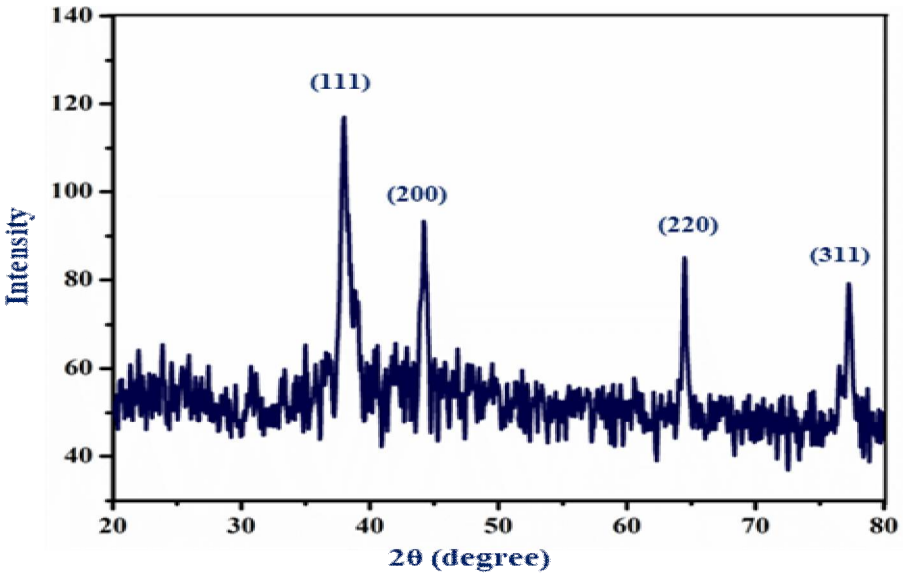


Fig. 3 XRD illustration of *Gomphrena celosioides* mediated Ag NPs

Larvicidal activity

At 24 h treatment, excellent larval toxicity was achieved by exposure to *G. celoisioides* mediated AgNPs against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} values were 6.23, 6.46 and 7.02 µg/mL, respectively (Table 1). The lowest toxicity achieved by *G. celoisioides* leaf aqueous extract against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} values were 41.02, 44.26 and 48.19 µg/mL, respectively (Table 2). Supporting our study, Loganathan et al. [36] have demonstrated *Passiflora subpeltata* synthesized AgNPs showed excellent larvicidal toxicity against *Ae. aegypti* and *An. stephensi* than plant extract after 24 h post treatment with LC_{50} values were 10.25 and 11.55 mg/mL (AgNPs), 13.88 and 15.43 µg/mL (Leaf extract), respectively. Mosquito larvicidal efficacy of methanol extract from *Rosmarinus officinalis*, *Melissa officinalis* medicinal plants revealed excellent larvicidal toxicity on third instar larvae of *Cx. pipiens* with LC_{50} values were 9.795 and 26.505 µg/mL, respectively [47]. *Solanum torvum* plant aqueous extract exhibited natural mosquito control agent [48].

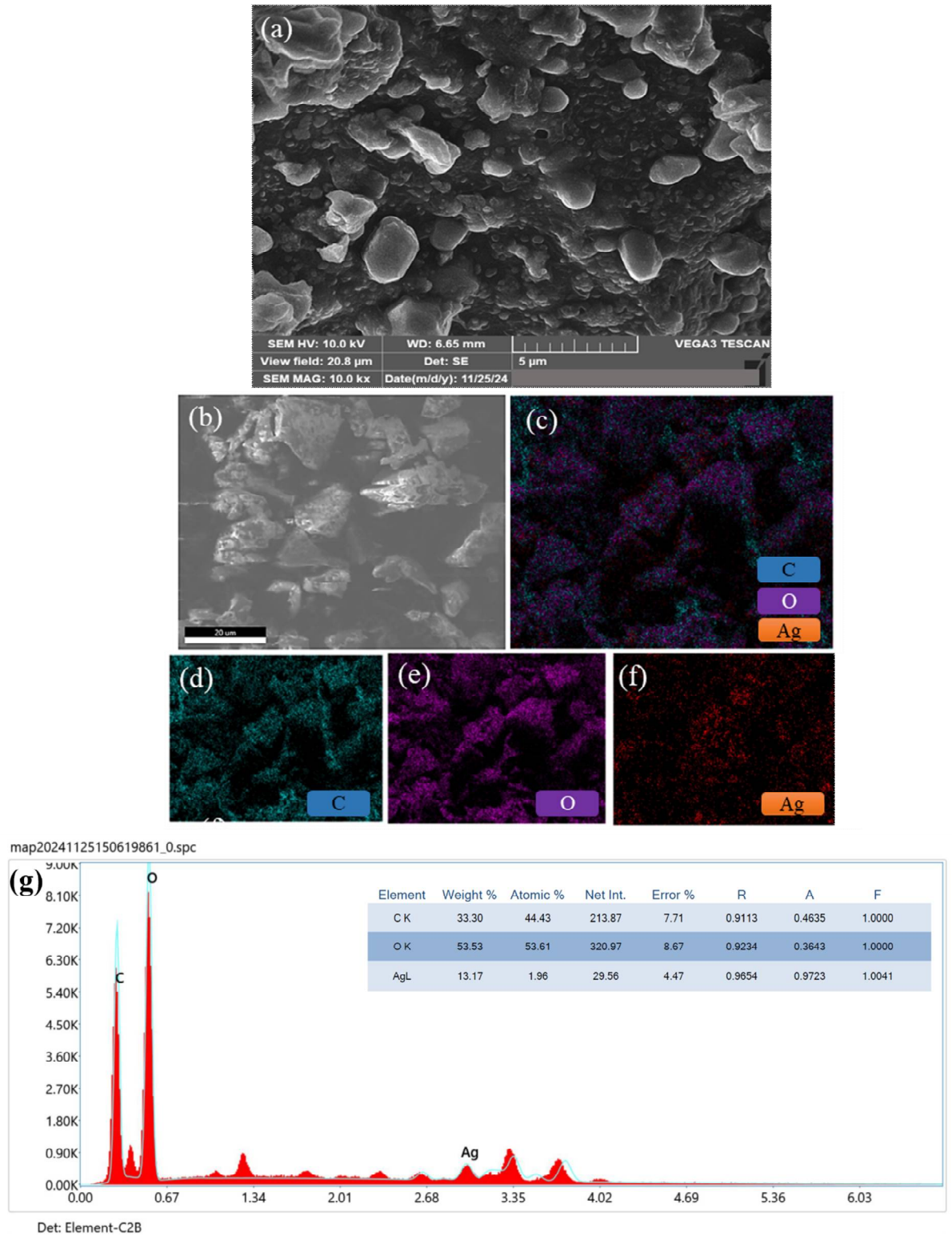


Fig. 4 (a) SEM image of *Gomphrena celosioides* synthesized Ag NPs, (b) elements scan area, (c) Elements scan merged image, (d-f) element distribution of C, O and Ag, (g) EDX profile of synthesized Ag NPs.

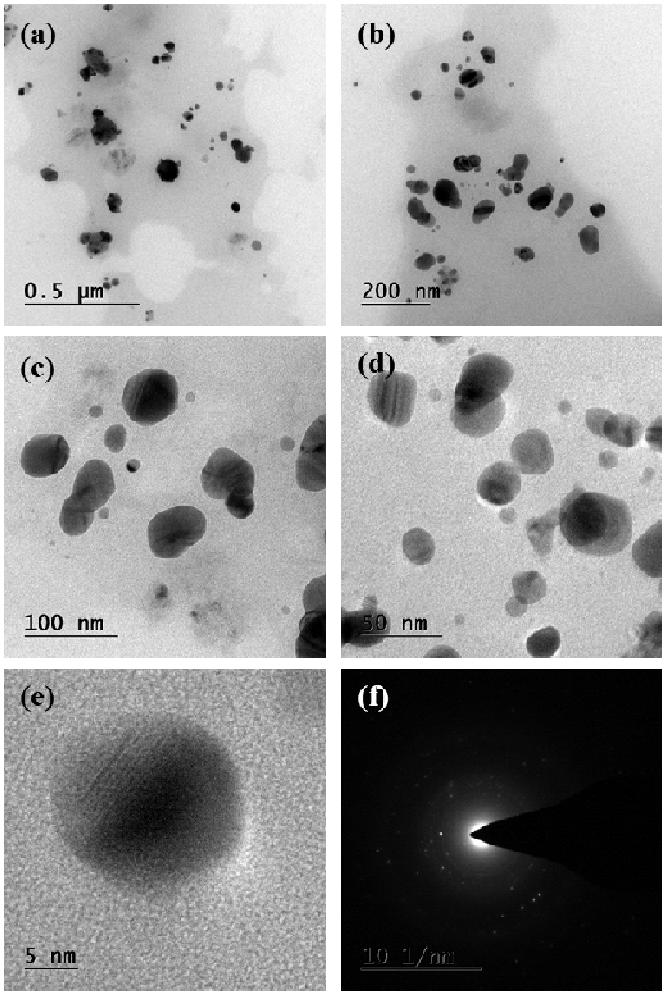


Fig. 5 (a-e) FE-TEM images with different magnifications (f) SAED analysis of *Gomphrena celosioides* synthesized Ag NPs.

Table 1. Effect of *Gomphrena celosioides* leaves aqueous extract against larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*.

Vector species	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	Regression equation	χ^2 (d.f)*
An. stephensi	41.02 (36.22 - 45.24)	81.58 (75.64 - 89.39)	y=11.68+0.912x	1.670
Ae. aegypti	44.26 (39.43 - 48.57)	87.21 (80.8 - 95.7)	y=8.40+0.916x	1.846
Cx. quinquefasciatus	48.19 (43.18 - 52.75)	95.36 (88.04 - 105.24)	y=6.16+0.892x	1.509

Table 2. Effect of synthesized silver nanoparticles using *Gomphrena celosioides* leaves against larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*

Vector species	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	Regression equation	χ^2 (d.f)*
An. stephensi	6.23 (5.54 - 6.84)	12.12 (11.24 - 13.26)	y=10.40+6.187x	6.017
Ae. aegypti	6.46 (5.71 - 7.12)	12.99 (12.02 - 14.29)	y=10.56+5.973x	2.850
Cx. quinquefasciatus	7.02 (6.27 - 7.69)	13.96 (12.89 - 15.39)	y=7.28+5.947x	4.164

Biotoxicity assessment on non-target species

The non-target aquatic mosquito predator *G. affinis* was used in this investigation to evaluate the biotoxicity of *G. celoisioides* leaf extracts and synthesized AgNPs. Aqueous leaf extract and AgNPs of *G.*

celoisioides revealed biotoxicity impact on *G. affinis* showed in Table 3, along with LC_{50} values of 18099.2 and 2172.51 µg/mL, respectively. The Suitability Index (SI) revealed that comparing the aquatic non-target organisms studied with the targeted vector mosquito larval populations, *G. celoisioides* AgNPs showed extremely low toxicity (Table 4). In an earlier report, the bio-toxicity effect of *Merremia emarginata* plant-synthesized AgNPs revealed very low toxicity against non-target organism *A. bouvieri, D. indicus* and *G. affinis* with LC_{50} values were 415.61, 633.51 and 1056.04 µg/mL, respectively [49]. *Couroupita guianensis* plant crude extract revealed no toxicity against non-target fish *Cyprinus carpio* [50]. Vasantha-Srinivasan et al. [51] demonstrated that the *Swietenia mahagoni* plant methanolic extract exhibits a low bio-toxicity effect on the non-target mosquito predator *Toxorhynchites splendens*. Moreover, *Pimenta dioica*-fabricated AgNPs exhibited no toxic impact on non-target species *Mesocyclops thermocyclopoides* [52].

Table 3. Biotoxicity of *Gomphrena celosioides* aqueous leaf extract and synthesized Ag NPs against non-target organism *Gambusia affinis* sharing the same ecological niche of *Anopheles, Aedes* and *Culex* mosquito vectors.

Test materials	Concentration (µg/ml)	Mortality (%)±SD[a]	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	Regression equation	χ^2 (d.f)*
Aqueous extract	Control	0.0±0.0	18099.2 (16357.28 - 19690.33)	33708.18 (31375.65 - 36743.57)	y=4.45+0.002x	1.463
	8000	22.0±0.48				
	16000	43.3±0.84				
	24000	65.3±0.63				
	32000	86.6±0.66				
	40000	98.0±0.48				
Ag NPs	Control	0.0±0.0	2172.51 (1949.26 - 2373.61)	4123.35 (3834.23 - 4500.33)	y=7.23+0.019x	2.225
	1000	22.6±0.84				
	2000	48.0±0.63				
	3000	66.6±0.66				
	4000	87.3±0.87				
	5000	98.6±0.42				

Table 4. Suitability index of non- target organism *Gambusia affinis* over young instars of mosquitoes exposed to *Gomphrena celosioides* leaf aqueous extracts and green synthesized silver nanoparticles.

Treatment	*An. stephensi*	*Ae. aegypti*	*Cx. quinquefasciatus*
Aqueous extract	441.22	408.92	375.57
Ag NPs	348.71	336.30	309.47

CONCLUSION

Insecticide resistance remains a major challenge in mosquito control programs and indicates that an alternative safe and effective biocontrol agent is needed. The silver nanoparticles were synthesized using *G. celosioides*. The synthesised AgNPs were extensively characterised using various analytical methods including UV-Vis Spectrophotometer (peak at 443 nm), FTIR, XRD, SEM with EDX, and HR-TEM. *G. celosioides*-mediated AgNPs and leaf extracts considerably inhibit the larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* mosquitoes without toxicity to non-target species. This underscores the potency of green synthesized silver nanoparticles from *G. celosioides* as eco-friendly and sustainable alternatives to chemical pesticides in mosquito control.

CONFLICTS OF INTEREST

The authors disclose no conflicts of interest.

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