Novel Biogenic Synthesis of Silver Nanoparticles Using Alstonia venenata *Leaf Extract: An Enhanced Mosquito Larvicidal Agent with Negligible Impact on Important Eco-biological Fish and Insects*

Venkattan Esan, Shahid Mahboob, Khalid A. Al-Ghanim, Chakkaravarthy Elanchezhiyan, et al.

Journal of Cluster Science
Including Nanoclusters and Nanoparticles

ISSN 1040-7278

J Clust Sci
DOI 10.1007/s10876-020-01808-5

Springer

Journal of Cluster Science
https://doi.org/10.1007/s10876-020-01808-5

ORIGINAL PAPER

Novel Biogenic Synthesis of Silver Nanoparticles Using *Alstonia venenata* Leaf Extract: An Enhanced Mosquito Larvicidal Agent with Negligible Impact on Important Eco-biological Fish and Insects

Venkattan Esan[1] · Shahid Mahboob[2] · Khalid A. Al-Ghanim[2] · Chakkaravarthy Elanchezhiyan[1] · Fahad Al-Misned[2] · Zubair Ahmed[2] · Marimuthu Govindarajan[1,3]

Received: 5 March 2020

Abstract
Bio-fabrication of metal nanocrystals can be achieved using eco-friendly and cost-effective routes with plants as reducing and capping mediators. A rapid and simple method for producing silver nanoparticles (AgNPs) using the plant *Alstonia venenata* (R. Br.) (Family: Apocynaceae) was investigated. The mosquito larvicidal potential and the effect of the *A. venenata* aqueous leaf extract and AgNPs on non-target fish and insects were evaluated. The AgNPs were studied using UV–Vis spectroscopy, FTIR spectroscopy, SEM, TEM, AFM, and XRD analysis. The larvicidal effectiveness on early third instar larvae was higher for the AgNPs than the plant extract; this was observed by testing on *Anopheles stephensi* (LC_{50} = 12.28 µg/mL), *Aedes aegypti* (LC_{50} = 13.49 µg/mL), and *Culex quinquefasciatus* (LC_{50} = 14.50 µg/mL). Furthermore, the plant extract and AgNPs were found to be safe for the environment-friendly *Gambusia affinis* fish, and *Anisops bouvieri* and *Diplonychus indicus* aquatic insects. This study confirmed that *A. venenata* is a potential bio-resource for the fabrication of nanocrystals as an effective mosquito control tool with negligible harmful on aquatic fish and insects in the environment.

Keywords Green-synthesis · Nanotechnology · XRD, TEM, AFM · Zika virus · Biological control

Introduction

Dipterans insects are important vectors of several deadly illnesses [1]. This order of insects includes mosquitoes, which spread illnesses that require large amounts of financial resources and can cause far-reaching societal issues in emerging countries [2–6]. These illnesses are often controlled using various insecticides that have been successfully improved and widely applied. However, excessive use of these insecticides in the management of *Aedes, Culex* and *Anopheles* mosquitoes has had detrimental effects, including insecticide resistance, ecological contamination and harm to non-target organisms and human beings [7–12]. Alternative insecticides must be developed that are safe, cost-effective and target-specific.

Herbs produces a multitude of biological active molecules with medicinal and insecticidal properties [13–19]. Green nanotechnology is a branch of nanomaterial science focused on the bio-based development of nano-scale materials for biological applications [20–23]. The use of bio-inspired silver nanoparticles (AgNPs) is preferable to synthetic practices, as the material is low cost and biodegradable [9, 24, 25]. An increasing number of plants have been found to be capable of efficient extracellular synthesis of AgNPs [26–30] that have demonstrated outstanding insecticidal effects with minimal ecological risk [31–34].

✉ Shahid Mahboob
mushahid@ksu.edu.sa

✉ Marimuthu Govindarajan
drgovind1979@gmail.com

[1] Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

[2] Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

[3] Department of Zoology, Government College for Women (Autonomous), Kumbakonam 612 001, Tamil Nadu, India

Published online: 08 May 2020

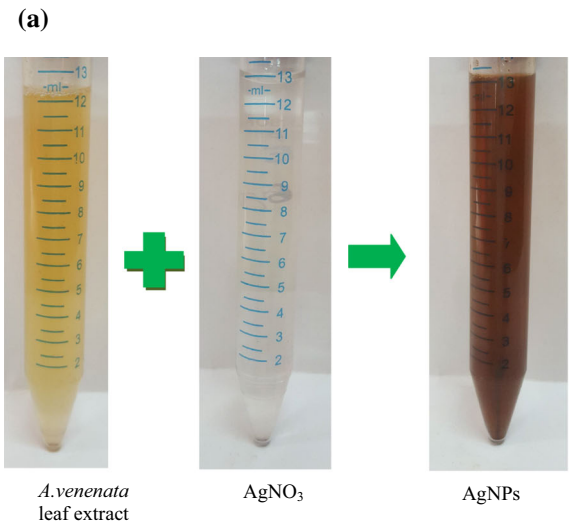

Fig. 1 The color of the solution changed from light yellow to light brown (**a**) and UV–visible spectrum (**b**) of AgNPs prepared from the *Alstonia venenata* leaf extract (Color figure online)

Alstonia venenata R. Br. (Family: Apocynaceae) is an important therapeutic herb. It grows widely in the Western Ghats, western Himalayas and southern region of India. *A.venenata* is a shrub or small tree with the vernacular name 'Sinnappalai' in Tamil [35]. Two indole alkaloids have been isolated from the bark of *A.venenata* [36]. Furthermore, this plant is used as a powerful antidote for snake bite treatment [37] and the leaf, flowers, and fruit extracts have been found to have antifungal potential [38].

This study aimed to produce AgNPs from *A.venenata* and analyze the particles using UV–Vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy TEM, atomic force microscopy (AFM) and X-ray powder diffraction (XRD) analysis. An *A. venenata*

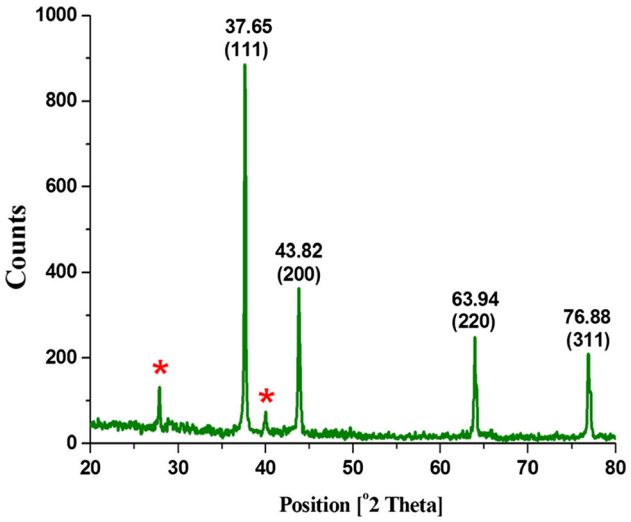

Fig. 2 XRD pattern of AgNPs prepared from the *Alstonia venenata* leaf extract

aqueous solution and the bio-synthesized AgNPs were applied to third instar mosquito larvae and the impact on non-target aquatic fish and insects was evaluated.

Materials and Methods

Collection of Chemicals and *A. venenata* Leaves

$AgNO_3$ was obtained from Fisher Scientific, India. Fresh *A. venenata* leaves were collected from the Kodiyakkarai forest (Tamilnadu, India) under the supervision of a plant taxonomist (Alagappa University, India).

Production of *A. venenata* Aqueous Extract

A. venenata leaves were dried in shade and powdered using an electric grinder. A plant leaf extract was produced by combining 100 g desiccated plant dust and 1 L distilled water. The mixture was stirred constantly at 100 °C for 1 h using a magnetic stirrer before the extract was filtered with Whatman n1 filter paper and stored [39].

Green Fabrication and Characterization of AgNPs

The leaves were rinsed quickly with double distilled water (ddH_2O) and 10 g was boiled for 5 min in 100 mL of ddH_2O. The aqueous solution was filtered and stored at -15 °C. 12 mL of the plant aqueous solution and 88 mL of 1 mM $AgNO_3$ solution were mixed at 37 °C for 10 min. Color change (light brown color) evidenced the development of Ag NPs [40].

UV–Vis spectroscopy (UV-1900, 190-110 nm) was used to evaluate the reduction of Ag^+ ions. For the

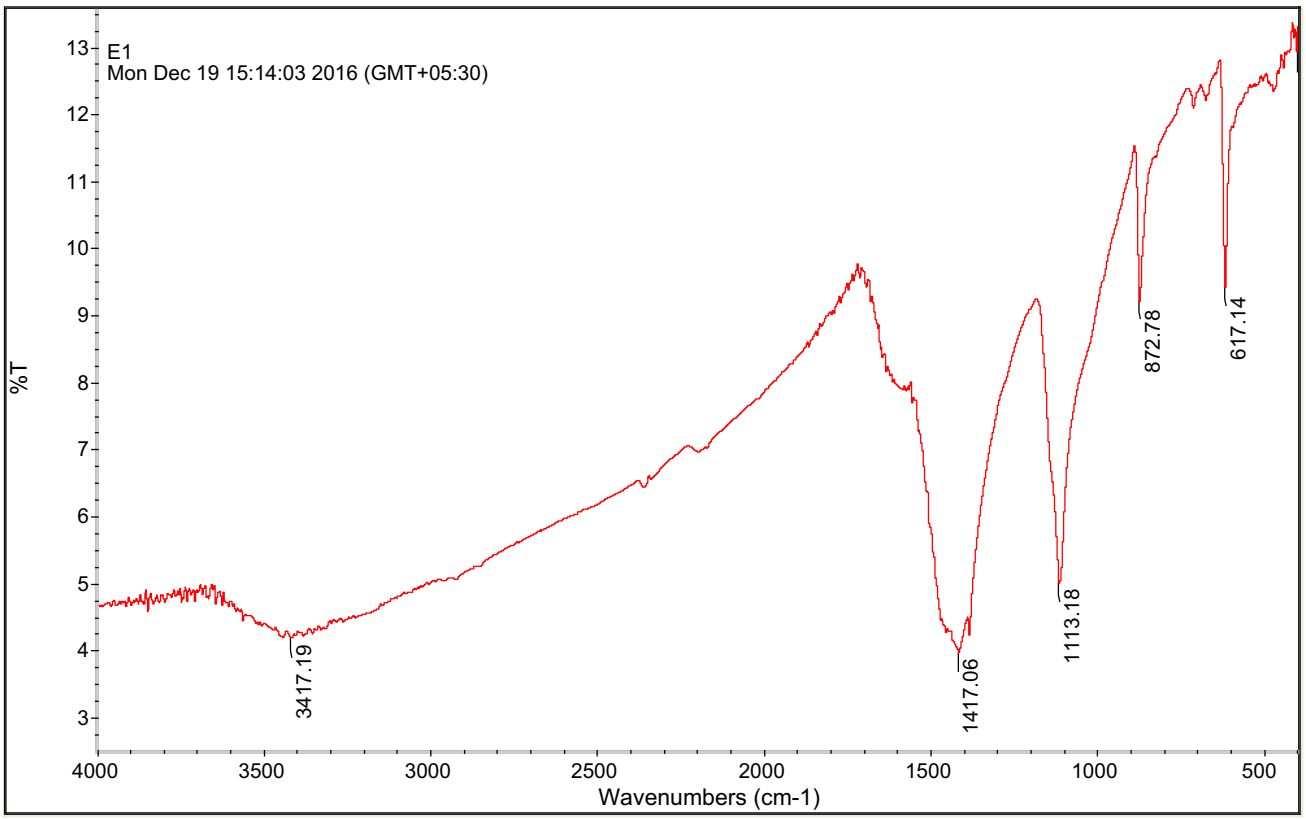

Fig. 3 FTIR spectrum of AgNPs prepared from the *Alstonia venenata* leaf extract

morphology, AFM (Park NX10), SEM (JEOL JSM-7500F SEM) with EDX and TEM (HF 3300 TEM Hitachi) was used. The biomolecules were identified using FTIR spectroscopy (Bruker Alpha II) and the XRD was used to identify the crystalline structure of the AgNPs [41].

Larvicidal Bioassay

Laboratory-reared pathogen free mosquito species were continuously cultured and the culture method described by Govindarajan and Benelli [15] was used. The aqueous extract and the AgNPs were assessed using an adapted version [15] of the World Health Organization (WHO) standard method [42]. Five concentrations of the *A. venenata* aqueous extract (50, 100, 150, 200 and 250 µg/mL) and AgNPs (6, 12, 18, 24 and 30 µg/mL) were tested. Twenty mosquito larvae (third instar) were used at test cup. Five replicates of each experiment was performed and the mosquito larval mortality was noted after 24 h. Silver nitrate and distilled water were used as a control for each concentration.

Environmentally Friendly Toxicity Assay

Field collected aquatic insects (*Diplonychus indicus, Anisops bouvieri*) and the fish (*Gambusia affinis*) was used in the environmental toxicity assay. The effects of the *A.venenata* aqueous extract and the AgNPs on insects and fish were assessed using a standard method described by Sivagnaname and Kalyanasundaram [43]. The insects and fish were held under conditions described by Govindarajan and Benelli [15]. The applied dose of the aqueous extract and AgNPs was 50 times the dose used for the target organism. The suitability index was calculated using the following equation [44]:

$$\text{Suitability Index} = \text{LC}_{50}\text{of non} - \text{target organisms} / \text{LC}_{50}\text{of target insect species}$$

Statistical Analysis

Mortality facts were evaluated by probit analysis. A method described by Finney [45] was used to establish the lethal concentration at 50 and 90% levels. All the experimental facts were scrutinized using SPSS 16.0 version.

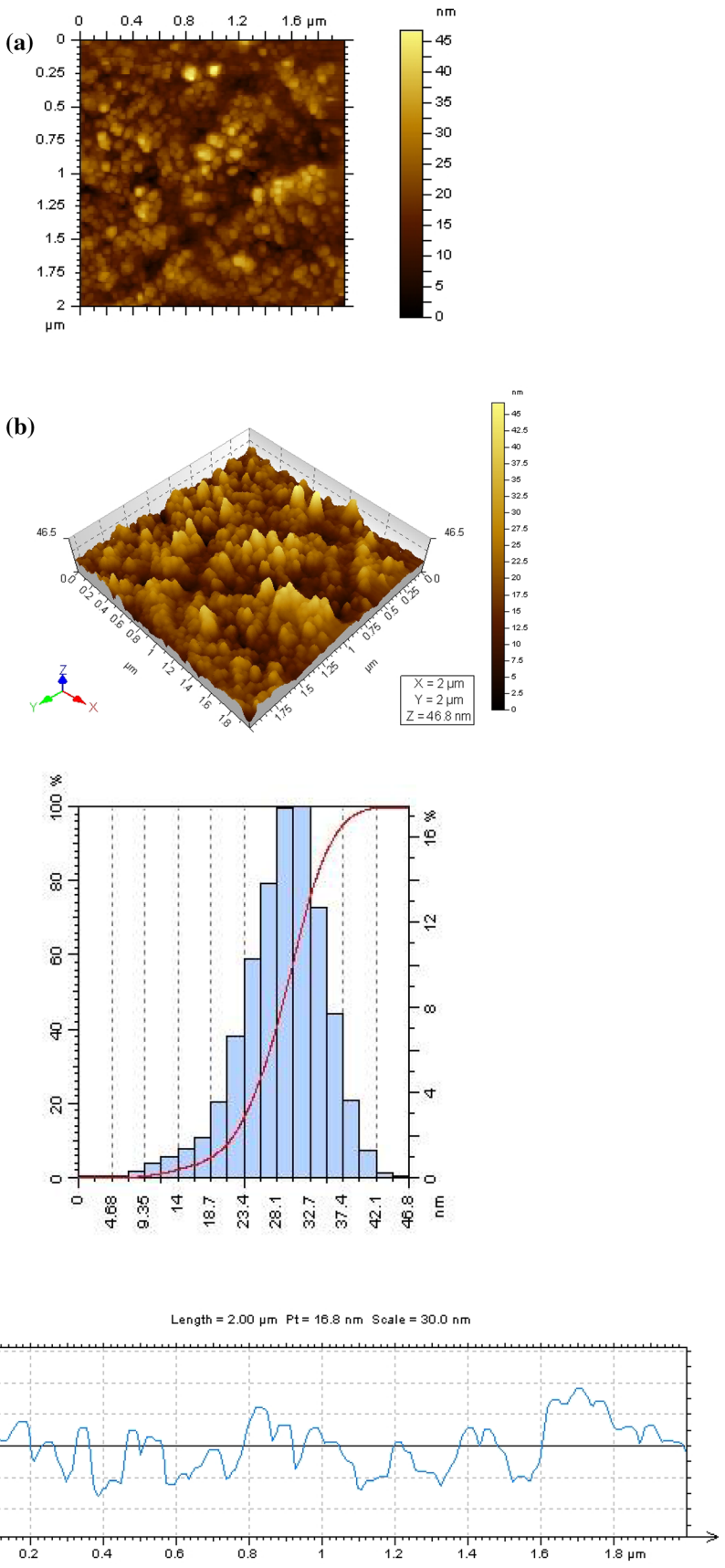

Fig. 4 AFM images of AgNPs prepared from the *Alstonia venenata* leaf extract (**a**) 2D image, (**b**) 3D image, (**c**) histogram showing the particle size distribution, (d) line graph showing the size distribution of AgNPs

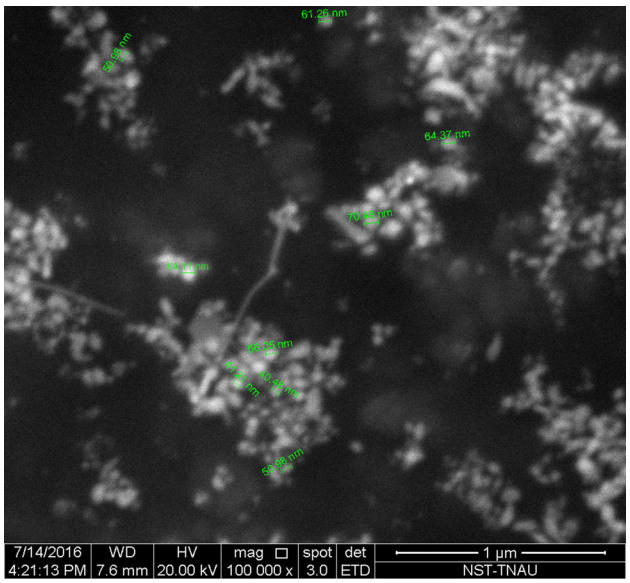

Fig. 5 Scanning electron microscopy image (×1,00,000) of AgNPs prepared from the *Alstonia venenata* leaf extract

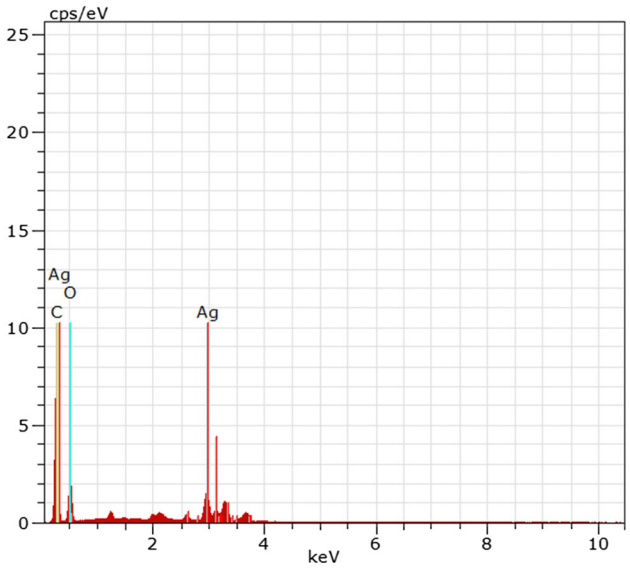

Fig. 6 Energy dispersive X-ray spectrum image of AgNPs prepared from the *Alstonia venenata* leaf extract

Results and Discussion

AgNPs Characterization

AgNPs derived from *A. venenata* leaves were produced within two hours of adding the aqueous extract to the silver nitrate ($AgNO_3$) solution. The color of the solution changed from light yellow to light brown (Fig. 1a) as the biosynthesized AgNPs exhibited surface plasmon resonance (SPR) [46] and synthesized AgNPs was definited by examining the peak at 428.5 nm in the UV–Vis spectrum

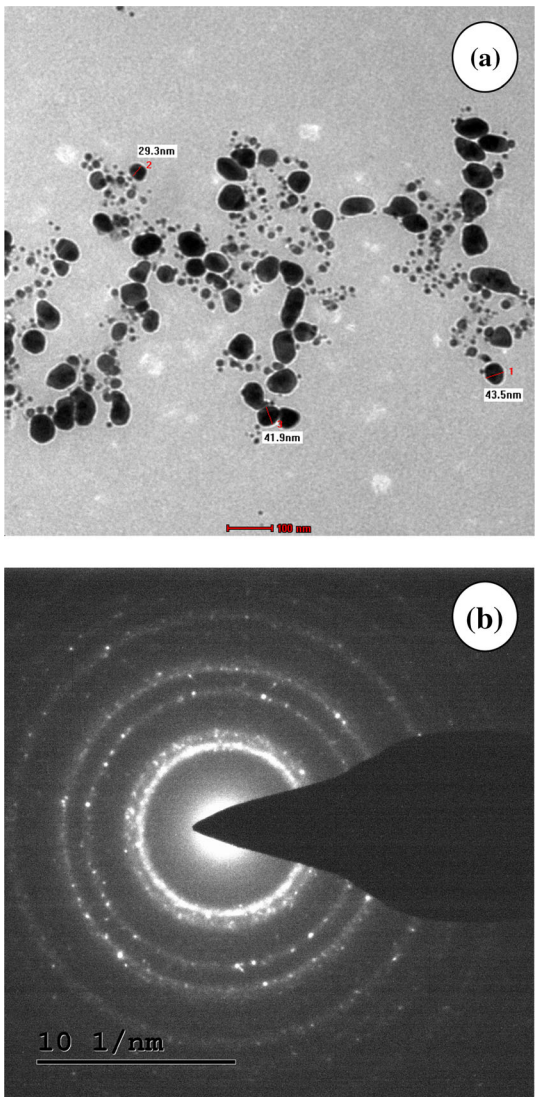

Fig. 7 **a** Transmission electron microscopy image of AgNPs prepared from the *Alstonia venenata* leaf extract; **b** SAED pattern

(Fig. 1b). A previous study by the current author investigated AgNPs synthesized from *Nicandra physalodes* that exhibited an absorbance peak at 449 nm [47].

The *A. venenata* derived AgNPs were evaluated using XRD analysis. The AgNPs diffractogram exhibited several sharp peaks (Fig. 2). The four clear reflections at 37.65° (111), 43.82° (200), 63.94° (220), and 76.88° (311) were attributed to a face centered cubic (fcc) structure, and indicated that AgNPs were crystalline in nature. Moreover, few unallocated peaks (pointed with stars) were also noticed indicate that the crystallization of bio-organic phase appear on the surface of the AgNPs. These findings were consistent with previous reports [32, 33, 48].

The FTIR spectrum of the *A. venenata* derived AgNPs gave an indication of the biological molecules present (Fig. 3). The band at 3417 cm^{-1} was attributed to

Table 1 Efficacy of *Alstonia venenata* aqueous leaf extract against mosquito larvae

Target organism	Concentration (µg/mL)	% of mortality (24 h.) ± SD	LC_{50} (µg/mL) (95% LCL-UCL)	LC_{90} (µg/mL) (95% LCL-UCL)	Slope	Regression analysis	χ^2
An. stephensi	50	27.6 ± 0.6	103.41	203.61	3.2	$y = 11.13 + 0.367x$	5.568
	100	48.9 ± 1.4	(91.65–113.82)	(188.81–223.06)			
	150	66.7 ± 1.2					
	200	87.4 ± 0.6					
	250	100.0 ± 0.0					
Ae. aegypti	50	24.4 ± 0.8	112.02	216.37	2.9	$y = 6.98 + 0.374x$	3.262
	100	45.2 ± 0.6	(100.38–122.52)	(200.73–236.98)			
	150	62.8 ± 0.8					
	200	84.6 ± 1.2					
	250	98.1 ± 1.4					
Cx. quinquefasciatus	50	20.5 ± 1.2	121.01	228.6	2.63	$y = 3.12 + 0.378x$	2.388
	100	42.8 ± 1.4	(109.55–131.58)	(212.12–250.42)			
	150	59.3 ± 0.6					
	200	80.6 ± 0.8					
	250	96.2 ± 0.6					

Control nil mortality

Table 2 Efficacy of *Alstonia venenata* derived AgNPs against mosquito larvae

Target organism	Concentration (µg/mL)	% of mortality (24 h.) ± SD	LC_{50} (µg/mL) (95% LCL-UCL)	LC_{90} (µg/mL) (95% LCL-UCL)	Slope	Regression analysis	χ^2
An. stephensi	6	29.4 ± 1.4	12.28	24.36	3.33	$y = 11.77 + 3.035x$	5.511
	12	46.7 ± 0.8	(10.86–13.54)	(22.58–26.71)			
	18	68.3 ± 1.2					
	24	87.6 ± 1.4					
	30	100.0 ± 0.0					
Ae. aegypti	6	25.9 ± 0.8	13.49	26.46	3.13	$y = 7.6 + 3.06x$	2.428
	12	42.6 ± 1.2	(12.05–14.78)	(24.51–29.05)			
	18	64.2 ± 1.4					
	24	83.4 ± 0.8					
	30	97.3 ± 1.2					
Cx. quinquefasciatus	6	22.7 ± 1.4	14.5	27.91	2.85	$y = 4.23 + 3.085x$	1.358
	12	39.5 ± 0.8	(13.07–15.81)	(25.84–30.66)			
	18	61.8 ± 1.4					
	24	79.6 ± 1.2					
	30	95.2 ± 0.8					

Control nil mortality

absorption by O–H in alcohols and phenols, 1417 cm^{-1} to N–H bending in 1° amines [15], 1113 cm^{-1} to C–H wagging in alkyl halides, and 872 cm^{-1} to –C≡C–H: C–H bending in alkynes. These findings were similar to those of a study on AgNPs bio-synthesized from *Feronia elephantum* leaf extract [33].

The size and morphology of the AgNPs were examined using AFM (Fig. 4), SEM (Fig. 5), EDX (Fig. 6) and TEM (Fig. 7). The AgNPs were spherical in shape. Similarly, a previous report by Ankana et al. [49] recorded SEM images of AgNPs that were well dispersed and varied in size from 30 to 40 nm. Another study synthesized spherical shaped

Table 3 The impact of *Alstonia venenata* aqueous leaf extract against environment-friendly aquatic insects and fish

Eco-friendly organism	Concentration (µg/mL)	% of mortality (48 h.) ± SD	LC_{50} (µg/mL) (95% LCL-UCL)	LC_{90} (µg/mL) (95% LCL-UCL)	Slope	Regression analysis	χ^2
A. bouvieri	3000	26.4 ± 1.2	6233.26	12,006	2.86	$y = 9.91 + 0.006x$	4.652
	6000	48.5 ± 0.6	(5556.09–6837.01)	(11,151.66–13,122.52)			
	9000	67.8 ± 1.4					
	12,000	88.7 ± 1.2					
	15,000	100.0 ± 0.0					
D. indicus	5000	27.3 ± 0.8	10,519.3	20,500.4	3.02	$y = 9.83 + 0.004x$	4.142
	10,000	46.7 ± 0.6	(9360.75–11,550.93)	(19,023.17–22,438.67)			
	15,000	65.9 ± 1.2					
	20,000	88.6 ± 0.8					
	25,000	99.2 ± 0.6					
G. affinis	10,000	25.7 ± 1.4	21,141	41,514.1	3.15	$y = 9.93 + 0.002x$	1.704
	20,000	47.2 ± 1.2	(18,776.33–23,239.34)	(38,513.64–45,454.36)			
	30,000	68.9 ± 0.8					
	40,000	86.5 ± 1.2					
	50,000	98.4 ± 0.8					

Control nil mortality

Table 4 The impact of *Alstonia venenata* derived AgNPs against environment-friendly aquatic insects and fish

Eco-friendly organism	Concentration (µg/mL)	% of mortality (48 h.) ± SD	LC_{50} (µg/mL) (95% LCL-UCL)	LC_{90} (µg/mL) (95% LCL-UCL)	Slope	Regression analysis	χ^2
A. bouvieri	350	27.3 ± 1.4	734.73	1418.27	2.89	$y = 9.56 + 0.053x$	5.829
	700	46.8 ± 0.8	(655.27–805.69)	(1316.82–1551.17)			
	1050	65.9 ± 0.6					
	1400	88.6 ± 1.2					
	1750	100.0 ± 0.0					
D. indicus	500	25.8 ± 0.8	1057.86	2089.53	3.25	$y = 10.2 + 0.037x$	2.144
	1000	48.6 ± 1.2	(938.29–1163.79)	(1937.41–2289.80)			
	1500	66.2 ± 1.4					
	2000	87.4 ± 0.8					
	2500	98.1 ± 0.6					
G. affinis	1000	28.4 ± 1.2	2107.07	4317.84	4.22	$y = 11.95 + 0.018x$	1.406
	2000	46.2 ± 0.8	(1850.63–2331.46)	(3991.25–4752.97)			
	3000	67.5 ± 0.6					
	4000	85.3 ± 1.2					
	5000	97.1 ± 0.8					

Control nil mortality

NPs from *Annona squamosa* extract with sizes ranging from 20 to 100 nm [50]. Govindarajan and Benelli [15] synthesized the spherical AgNPs from an extract of *Barleria cristata* (38–41 nm).

Mosquito Larvicidal Activity

Alstonia venenata extract had a moderately lethal effect on *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae with LC_{50} values of 121.01, 112.02 and 103.41 µg/mL, respectively (Table 1). Current research on botanicals as potential agents for controlling young mosquito populations is reliant on the required dose [51–55]. The *S. reticulata* derived AgNPs were extremely lethal against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae with LC_{50} values of 14.50, 13.49 and 12.28 µg/mL, respectively (Table 2). Several recent studies have focused on the biosynthesis of AgNPs for mosquito control [56–60]. For

Table 5 Suitability index of *Alstonia venenata* aqueous solution and AgNPs on three environment-friendly aquatic insects and fish in comparison with larvae of three important mosquito species

Test materials	Eco-friendly organism	*An. stephensi*	*Ae. aegypti*	*Cx. quinquefasciatus*
A. venenata aqueous solution	*A. bouvieri*	60.27	55.64	51.51
	D. indicus	101.72	93.90	86.92
	G. affinis	204.43	188.72	174.70
Silver nanoparticles	*A. bouvieri*	59.83	54.46	50.67
	D. indicus	86.14	78.41	72.95
	G. affinis	171.58	156.19	145.31

example, *Bauhinia variegata* derived AgNPs were toxic to three important vector mosquitoes, namely *An. subpictus, Ae. albopictus* and *Cx. tritaeniorhynchus* with LC_{50} values ranging from 41 to 51 µg/mL [40]. Veerakumar et al. [33] successfully used AgNPs from *F. elephantum* on *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* larvae with LC_{50} values of 21.84, 20.10 and 18.40 µg/mL, respectively. The effects of AgNPs from *Euphorbia hirta* was studied on *An. stephensi* larvae from all the four instars and established elevated larval mortality with LC_{50} values ranging from 10 to 27 µg/mL and LC_{90} values ranging from 31 to 69 µg/mL [61]. Greener silver nanoparticles from *Cassia fistula* [62], *Aquilaria sinensis* and *Pogostemon cablin* [63], *Bacillus amyloliquefaciens* and *Bacillus subtilis* [64], and phenolic acid [65] have the potential controlling agent for several mosquitoes.

Biotoxicity on Field Collected Insects and Fish

The eco-toxicology of the plant aqueous solution and synthesized AgNPs on field collected aquatic insects (*D. indicus* and *A. bouvieri*) and fish (*G. affinis*) was evaluated. The LC_{50} values ranged from 734 to 21,140 µg/mL (Tables 3 and 4) and suggested that the materials are safe and environment friendly. The suitability index value indicated that the AgNPs have low toxicity to eco-friendly insects and fish (Table 5) in contrast to the target mosquito larvae toxicity, which is supported by numerous previous studies. AgNPs derived from *Barleria cristata* was less toxic to the eco-friendly organisms viz, *A. bouvieri, D. indicus* and *G. affinis* with LC_{50} values ranging from 633 to 866 µg/mL [15]. AgNPs derived from *Clerodendrum chinense* [39], *Drypetes roxburghii* [24], *Vinca rosea* [66] had negligible toxicity on non-target insects *D. indicus, A. bouvieri* and the fish *G. affinis.*

Conclusions

AgNPs were produced from *A. venenata* leaves and the enhanced mosquito larvicidal activity was analyzed. The morphology of the AgNPs was spherical with a size of 27 to 36 nm. The *A. venenata* derived AgNPs are simple to fabricate, cost-effective, and have the potential to control mosquito populations with extremely low toxicity on environment-friendly aquatic insects and fish.

Acknowledgements The authors (SM, KAH, FAM and ZA) would like to their sincere appreciation to the Deanship of Scientific at King Saud University for its funding of this research through the Research Group Project No. RG-1435-012.

Compliance with Ethical Standards

Conflicts of Interest The authors stated that they have no conflicts of interest.

References

1. H. Mehlhorn, K. A. Al-Rasheid, S. Al-Quraishy, and F. Abdel-Ghaffar (2012). *Parasitol. Res.* **110**, 259–265.
2. P. J. Hotez, J. H. F. Remme, P. Buss, G. Alleyne, C. Morel, and J. G. Breman (2004). *Clin. Infect. Dis.* **38**, 871–878.
3. G. Benelli and H. Mehlhorn (2016). *Parasitol. Res.* **115**, 1747–1754.
4. G. Benelli (2016). *Parasitol. Res.* **115**, 23–34.
5. G. Benelli *Nanoparticles in the fight against parasites* (Springer International Publishing, Switzerland, 2016), pp. 155–172.
6. G. Benelli (2016). *Enzyme Microbial. Technol.* **95**, 58–68.
7. S. E. Lee, J. E. Kim, and H. S. Lee (2001). *Agric. Chem. Biotechnol.* **44**, 105–112.
8. M. Sarwar, N. Ahmad, and M. Toufiq (2009). *Pak. J. Bot.* **41**, 3047–3052.
9. G. Benelli (2015). *Parasitol. Res.* **114**, 2801–2805.
10. G. Benelli (2015). *Parasitol. Res.* **114**, 3201–3212.
11. P. Madhiyazhagan, K. Murugan, A. Naresh Kumar, T. Nataraj, D. Dinesh, C. Panneerselvam, J. Subramaniam, P. Mahesh Kumar, U. Suresh, M. Roni, M. Nicoletti, A. A. Alarfaj, A. Higuchi, M. A. Munusamy, and G. Benelli (2015). *Parasitol Res.* **114**, 4305–4317.
12. M. Govindarajan (2011). *Parasitol. Res.* **109**, 93–103.
13. S. Patil, S. Mahure, and A. Kale (2014). *Am. J. Ethnomed.* **1**, 174–185.
14. R. Srinivasan, M. S. Shivakumar, and D. Natarajan (2015). *J. Biol. Active. Prod. Nat.* **4**, 391–399.
15. M. Govindarajan and G. Benelli (2016). *Parasitol. Res.* **115**, 925–935.
16. M. Govindarajan and G. Benelli (2016). *RSC. Adv.* **6**, 59021–59029.
17. M. Govindarajan and G. Benelli (2016). *J. Clust. Sci.* **28**, 15–36.

18. A. T. Aziz, M. A. Alshehri, C. Panneerselvam, K. Murugan, S. Trivedi, J. A. Mahyoub, M. M. Hassan, F. Maggi, S. Sut, et al. (2018). *J. Photochem. Photobiol. B: Biol.* **180**, 225–234.
19. G. Benelli, S. Kadaikunnan, N. S. Alharbi, and M. Govindarajan (2018). *Environ. Sci. Poll. Res.* **25**, 10228–10242.
20. M. Dubey, S. Bhadauria, and B. S. Kushwah (2009). *Dig. J. Nanomater. Biostruct.* **4**, 537–543.
21. R. Sathyavathi, M. Balamurali Krishna, S. Venugopal, R. Saritha Rao, and D. Narayana Rao (2010). *Adv. Sci. Lett.* **3**, 1–6.
22. T. Ahmad, I. Wania, N. Manzoor, J. Ahmed, and A. M. Asiri (2013). *Colloids. Surf. B.* **107**, 227–234.
23. A. Rawani, A. Ghosh, and G. Chandra (2013). *Acta. Trop.* **128**, 613–622.
24. B. Haldar, G. Haldar, and G. Chandra (2013). *Parasitol. Res.* **112**, 1451–1459.
25. A. S. Hanan, M. A. Jazem, A. G. Hamed, and A. K. Sadeq (2018). *Res. J. Biotechnol.* **13**, 65–72.
26. K. Murugan, M. Aamina Labeeba, C. Panneerselvam, D. Dinesh, U. Suresh, J. Subramaniam, P. Madhiyazhagan, J. S. Hwang, L. Wang, M. Nicoletti, and G. Benelli (2015). *Res. Vet. Sci.* **102**, 127–135.
27. M. S. AlSalhi, S. Devanesan, A. A. Alfuraydi, R. Vishnubalaji, M. A. Munusamy, K. Murugan, M. Nicoletti, and G. Benelli (2016). *Int. J. Nanomedicine.* **11**, 4439–4449.
28. S. Devanesan, M. S. AlSalhi, R. V. Balaji, A. J. A. Ranjitsingh, A. Ahamed, A. A. Alfuraydi, F. Y. AlQahtani, F. S. Aleanizy, and A. H. Othman (2018). *Nanoscale Res. Lett.* **13**, 315.
29. S. Devanesan, M. S. AlSalhi, R. Vishnubalaji, A. A. Alfuraydi, N. M. Alajez, M. Alfayez, K. Murugan, R. M. S. Shaban, M. Nicoletti, and G. Benelli (2017). *J. Clust. Sci.* **28**, 595–605.
30. A. A. Alfuraydi, S. Devanesan, M. Al-Ansari, M. S. AlSalhi, and A. J. Ranjitsingh (2019). *J. Photochem. Photobiol. B.* **192**, 83–89.
31. U. Muthukumaran, M. Govindarajan, and M. Rajeswary (2015). *Parasitol. Res.* **114**, 989–999.
32. K. Veerakumar, M. Govindarajan, M. Rajeswary, and U. Muthukumaran (2014). *Parasitol. Res.* **113**, 2363–2373.
33. K. Veerakumar, M. Govindarajan, M. Rajeswary, and U. Muthukumaran (2014). *Parasitol. Res.* **113**, 1775–1785.
34. U. Suresh, K. Murugan, G. Benelli, M. Nicoletti, D. R. Barnard, C. Panneerselvam, P. Mahesh Kumar, J. Subramaniam, D. Dinesh, and B. Chandramohan (2015). *Parasitol Res.* **114**, 1551–1562.
35. K.R. Kirtikar, and B.D. Basu (1975). Indian Medicinal Plants, International Book Publishers Vol. II.
36. D. S. C. Mandal, S. M. Lakshmi, C. K. A. Kumar, T. K. Sur, and R. Boominathan (2003). *Phytother. Res.* **17**, 817–820.
37. D. K. Golwala, L. D. Patel, S. B. Bothara, P. M. Patel, S. K. Vaidya, and M. K. Raval (2009). *Int. J. Pharm. Sci. Drug. Res.* **1**, 119–120.
38. S. K. Thomas, M. Kunjumon, R. E. George, and T. Vaidyanatha Iyer (2015). *Int. J. Pharm. Sci. Res.* **6**, 1741–1745.
39. M. Govindarajan, M. Rajeswary, S. L. Hoti, K. Murugan, K. Kovendan, S. Arivoli, and G. Benelli (2016). *J. Asia. Pac. Entomol.* **19**, 51–58.
40. M. Govindarajan, M. Rajeswary, K. Veerakumar, U. Muthukumaran, S. L. Hoti, H. Mehlhorn, D. R. Barnard, and G. Benelli (2016). *Parasitol. Res.* **115**, 723–733.
41. M. Govindarajan, M. Rajeswary, K. Veerakumar, U. Muthukumaran, S. L. Hoti, and G. Benelli (2016). *Exp. Parasitol.* **161**, 40–47.
42. WHO (2005). Guidelines for laboratory and field testing of mosquito larvicides. Communicable Disease Control, Prevention and Eradication,WHO Pesticide Evaluation Scheme, Geneva, Switzerland (WHO/CDS/WHOPES/GCDPP/2005.13).
43. N. Sivagnaname and M. Kalyanasundaram (2004). *Mem. Inst. Oswaldo Cruz.* **99**, 115–118.
44. P. G. Deo, S. B. Hasan, and S. K. Majumdar (1988). *Int. Pest Control.* **30**, 118–129.
45. D. J. Finney *Probit Analysis*, 3rd ed (Cambridge University Press, UK, 1971).
46. A. Ahmad, M. Mukherjee, D. Mandal, S. Senapati, M. I. Khan, R. Kumar, and M. Sastry (2003). *Colloids. Surf. B.* **28**, 313–318.
47. M. Govindarajan, H. F. Khater, C. Panneerselvam, and G. Benelli (2016). *Res. Vet. Sci.* **107**, 95–101.
48. S. S. Shankar, A. Ahmad, and M. Sastry (2003). *Biotechnol. Prog.* **19**, 1627–1631.
49. S. Ankanna, T. N. Prasad, E. K. Elumalai, and N. Savithramma (2010). *Dig. J. Nanomater. Biostruct.* **5**, 369–372.
50. R. Vivek, R. Thangam, K. Muthuchelian, P. Gunasekaran, K. Kaveri, and S. Kannan (2012). *Process. Biochem.* **47**, 2405–2410.
51. M. Govindarajan, A. Jebanesan, and D. Reetha (2005). *Trop. Biomed.* **22**, 1–3.
52. T. Mathivanan, M. Govindarajan, K. Elumalai, K. Krishnappa, and A. Ananthan (2010). *J. Vector Borne Dis.* **47**, 178–180.
53. M. Govindarajan (2011). *Asian Pac. J Trop. Med.* **4**, 176–181.
54. M. Govindarajan and R. Sivakumar (2012). *Parasitol. Res.* **110**, 1607–1620.
55. M. Govindarajan, R. Sivakumar, M. Rajeswary, and K. Veerakumar (2013). *Parasitol. Res.* **112**, 3713–3721.
56. M. Abinaya, B. Vaseeharan, M. Divya, A. Sharmili, M. Govindarajan, N. S. Alharbi, S. Kadaikunnan, J. M. Khaled, and G. Benelli (2018). *J. Trace Elem. Med. Bio.* **45**, 93–103.
57. M. Govindarajan, M. Nicoletti, and G. Benelli (2016). *J. Cluster Sci.* **27**, 745–761.
58. M. Govindarajan, M. Rajeswary, S. Arivoli, S. Tennyson, and G. Benelli (2016). *Parasitol. Res.* **115**, 1807–1816.
59. M. Govindarajan, S. L. Hoti, M. Rajeswary, and G. Benelli (2016). *Parasitol. Res.* **115**, 2685–2695.
60. G. Benelli and M. Govindarajan (2017). *J. Cluster Sci.* **28**, 287–308.
61. A. K. Priyadarshini, K. Murugan, C. Panneerselvam, S. Ponarulselvam, J. S. Hwang, and M. Nicoletti (2012). *Parasitol. Res.* **111**, 997–1006.
62. H. Fouad, L. Hongjie, D. Hosni, J. Wei, G. Abbas, et al. (2018). *Artif Cells Nanomed. Biotechnol.* **46**, 558–567.
63. H. Ga'al, H. Fouad, J. Mao, J. Tian, and M. Jianchu (2018). *Artif Cells Nanomed. Biotechnol.* **46**, 1171–1179.
64. H. Fouad, L. Hongjie, D. Yanmei, Y. Baoting, A. El-Shakh, G. Abbas, and M. Jianchu (2017). *Artif. Cells Nanomed Biotechnol.* **45**, 1369–1378.
65. H. Ga'al, H. Fouad, J. Tian, Y. Hu, G. Abbas, and J. Mo (2018). *Pestic Biochem. Phys.* **144**, 49–56.
66. S. Subarani, S. Sabhanayakam, and C. Kamaraj (2013). *Parasitol. Res.* **112**, 487–499.