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Laboratory efficacy of green-synthesized silver and copper nanoparticles against malaria vector, *Anopheles sergentii* (Theobald, 1907) (Diptera: Culicidae)

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ABSTRACT

Background: *Anopheles sergentii* is regarded as a significant vector-borne insect such as malaria.
Aim: This work sought to investigate the efficacy of silver and copper nanoparticles (AgNPs and CuNPs) produced from aqueous extracts of *Cestrum nocturnum* and *Salvia malticaulis* against various immature stages of *An. sergentii.*
Methods: The transmission electron microscopy (TEM) pictures of generated AgNPs and CuNPs indicated that the diameters of the nanoparticles varied from 8.39 to 113.68 nm for AgNPs and from 12.05 to 66.45 nm for CuNPs, respectively. The efficacy of synthesized AgNPs and CuNPs against various immature stages of *An. sergentii* was estimated after 24 and 48 hours.
Results: The UV–vis Spectrophotometric analysis findings for both AgNPs and CuNPs exhibited a single absorption peak within the 300–400 nm region, signifying the existence of spherical AgNPs and CuNPs. The efficacy of the produced AgNPs and CuNPs against the immature stages of *An. sergentii* escalated with higher concentrations of the tested nanoparticles. Calculated LC_{50} values indicate that AgNPs and CuNPs produced from the aqueous extract of *Cestrum nocturnum* leaves were more effective against various stages of *An. sergentii* than those against *Sa. malticaulis.* In addition, the tested AgNPs were more effective against *An. sergentii* different immature stages than CuNPs. The LC_{50} values of *Ce. nocturnum*- AgNPs against immature stages of *An. sergentii* recorded 13.93 and 13.18 mg/l against first instar larvae, 15.0 and 14.22 mg/l against second instar larvae, 17.06 and 14.31 mg/l against third instar larvae, 17.24 and 14.32 mg/l against fourth instar larvae, 17.56 and 14.82 mg/l against pupae after 24- and 48-hours post-treatment, respectively. In addition, a depression in Acetylcholinesterase level in *An. sergentii* different immature stages were recorded.
Conclusion: The *Ce. nocturnum* and *Sa. malticaulis* - synthesized AgNPs and CuNPs possess a significant activity against *An. sergentii* immature stages.
Keywords: *Anopheles sergentii, Cestrum nocturnum,* Larvae, *Salvia malticaulis.*

Introduction

Vector-borne diseases in The Kingdom of Saudi Arabia (KSA) and other countries of Eastern Mediterranean region of the World Health Organization (WHO) represent more than 10% of vector-borne diseases in the whole world (WHO, 2004). *Anopheles* species members are malarial vectors to more than 10 million people worldwide, causing about 69,000 deaths between 2019 and 2020 (WHO 2021); however, the transmission of malaria in KSA recorded low and unstable levels as compared with other countries in South-East Asia and Africa (Hay *et al.*, 2009; Khater *et al.*, 2013). Within the epidemiological context of the Middle East, *Anopheles sergentii* is the main vector of *Plasmodium falciparum* malaria (Sarih *et al.*, 2019).
Despite the high efficacy of pesticide treatments against target species, vector control is jeopardized by the emergence of resistance to chemical insecticides, leading to a resurgence in vectorial capacity (Liu *et al.*, 2006). Moreover, they pose significant risks to other non-target creatures and the environment through biomagnification (El-Mehdawy *et al.*, 2021; Shehata

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et al., 2023). For many years, chemical insecticides have primarily targeted aquatic larvae of various *Anopheles* species; however, the development of novel control agents that are safer, more effective, and environmentally friendly is seen to be a suitable and essential substitute to avoid the risks associated with chemical insecticides (Shehata *et al.*, 2020; El-Tabakh *et al.*, 2023).

Biosynthesized AgNPs and CuNPs are often safer and more specialized (El-Waseif *et al.*, 2023). Biosynthesized nanoparticles possess distinctive and remarkable properties that facilitate their utilization across various disparate domains, including nanomedicine, antimicrobials, and control agents for multiple mosquito species (Agalya Priyadarshini *et al.*, 2012; Sirelkhatim *et al.*, 2015; Ahmed *et al.*, 2016; Bobo *et al.*, 2016; Chen *et al.*, 2016). Synthesized nanoparticles are environmentally benign due to the absence of hazardous substances in their production (Bhosale *et al.*, 2014; Abbas *et al.*, 2022).

Consequently, the pursuit of synthesized nanoparticles as novel agents for mosquito control remains essential, so this work examined the efficacy of AgNPs and CuNPs produced from aqueous extracts of *Cestrum nocturnum* and *Salvia malticaulis* against several immature stages of the malaria vector, *An. sergentii.*

Materials and Methods

Anopheles sergentii' culture

Larvae of *An. sergentii* were obtained from breeding sites in the Al-Aydabi region, Jazan Governorate, Kingdom of Saudi Arabia (17°14'15.1"N, 42°56'18.6"E, altitude 1693 m) utilizing a rounded net dipper (25 cm in diameter) with a stainless-steel handle (120 cm in length) (Shehata *et al.*, 2022). The gathered larvae were classified based on the prior keys of Kirkpatrick (1925), Harbach (1985), and Morsy *et al.* (1990). The larvae were cultivated for five generations using a standardized protocol in a controlled laboratory environment (27ºC ± 3ºC, 70%–80% relative humidity, and a 12:12 (dark/light) photoperiod (El-Tabakh *et al.*, 2023).

Synthesis of silver and copper nanoparticles (AgNPs and CuNPs)

Preparation of aqueous extracts

The leaves of *Ce. nocturnum* and *Sa. malticaulis,* sourced from a private garden in the Al-Aydabi district of Jazan Governorate, Kingdom of Saudi Arabia, were washed and air-dried in the shade for ten days at ambient temperature. Four grams of powdered leaves from each plant were heated with 100 ml of distilled water in a water bath for 3 minutes. The solution was filtered and stored in the refrigerator at 4ºC until utilized (Zaki *et al.*, 2024).

Biosynthesis of silver nanoparticles

The $AgNO_3$ stock solution was prepared by dissolving 0.17 g of $AgNO_3$ in 100 ml of distilled water. Aqueous extracts of *Ce. nocturnum* and *Sa. malticaulis* were individually combined with $AgNO_3$ solution in a 1:9 ratio at ambient temperature for 72 hours, resulting in a reddish-brown coloration indicative of AgNPs production **(**Shehata and Mahmoud, 2019).

Biosynthesis of CuNPs

About 50 ml (5 mM) of copper sulfate solution was mixed with 5 ml of *Ce. nocturnum* and *Sa. malticaulis* aqueous extracts separately. The pH value 7.0 adjusted for the mixture by the addition of NaOH (1 N) solution. The green color mixture was obtained. The mixture was centrifuged, pellets collected, and dried overnight in a hot air oven at 60ºC. A dark green color powder obtained was stored at room temperature for further use **(**Mali *et al.*, 2020).

Characterization of AgNPs and CuNPs

Transmission electron microscopy

The AgNPs and CuNPs suspensions were subjected to sonication for 10 minutes and subsequently diluted to produce somewhat turbid suspensions. The AgNPs and CuNPs suspensions were analyzed using a JEOL JEM-2100 high-resolution TEM at an accelerating voltage of 200 kV (Abdel-Shafy *et al.*, 2019).

Ultraviolet-Visible spectroscopy (UV/VIS)

The AgNPs and CuNPs suspensions were diluted from 1 to 10 times using distilled water derived from colloidal solutions produced during the production process. The UV/VIS spectroscopy of the suspension was conducted utilizing a UV-Vis spectrophotometer (Model: Evolution™ 300, Serial Number: EVon 10600z, from Thermo Scientific).

The bioassay

Activity of AgNPs and CuNPs

The efficacy of the evaluated AgNPs and CuNPs against various immature stages of *An. sergentii* was conducted in accordance with the established protocol of Hassanain *et al.* (2019). Various quantities of AgNPs and CuNPs were formulated in 250 ml of distilled water within 500 ml beakers. Twenty-five *An. sergentii* larvae (I-IV) and pupae were promptly allocated into beakers containing varying amounts of AgNPs and CuNPs. All beakers were incubated under regulated conditions of the *An. sergentii* colony. Mortality was documented 24- and 48-hours following therapy. Typically, three replicates were employed.

Acetylcholinesterase (AChE) activity

The activity of AChE in various immature stages of *An. sergentii* was assessed 24- and 48-hours post-treatment with half-lethal concentrations (LC_{50}) of AgNPs and CuNPs. Approximately 10 ml of 0.1 M phosphate buffer solutions at pH 7.5 (KH2PO4-NaOH), incorporating 1% Triton X-100, 1% ethanol, and 1% Triton X-100, were utilized to homogenize three distinct batches of larvae (I-IV) and pupae individually, derived from each evaluated LC_{50}. The Hereaeus Labofuge 400 R, manufactured by Kendro Laboratory Products GmbH, Germany, was utilized to centrifuge the homogenates for 60 minutes at 4ºC and 15,000 × g. Ten microliter aliquots of supernatant were utilized without further

purification for the *in vitro* inhibition test of AChE (U/l) (Ellman *et al.*, 1961).

Statistics

Data was encoded and input with the statistical software SPSS Version 22. The data were evaluated for compliance with the assumptions of parametric tests, and continuous variables underwent the Shapiro-Wilk and Kolmogorov-Smirnov tests for normality. Probability and percentile data were normalized for normalcy by Arcsine Square Root transformation. Data was given as mean and standard deviation. The Analysis of Variance (ANOVA) analyses were conducted for experimental groups with three replicates per group; post-hoc analysis was performed using Tukey pairwise comparison; *p*-values were deemed significant at < 0.05. Analysis conducted with MiniTab Version 14. Data were displayed where feasible, utilizing R Studio Version 2022.02.4.

Ethical approval

The authors confirm that the conducted research was in accordance with the ethical guidelines and international regulations.

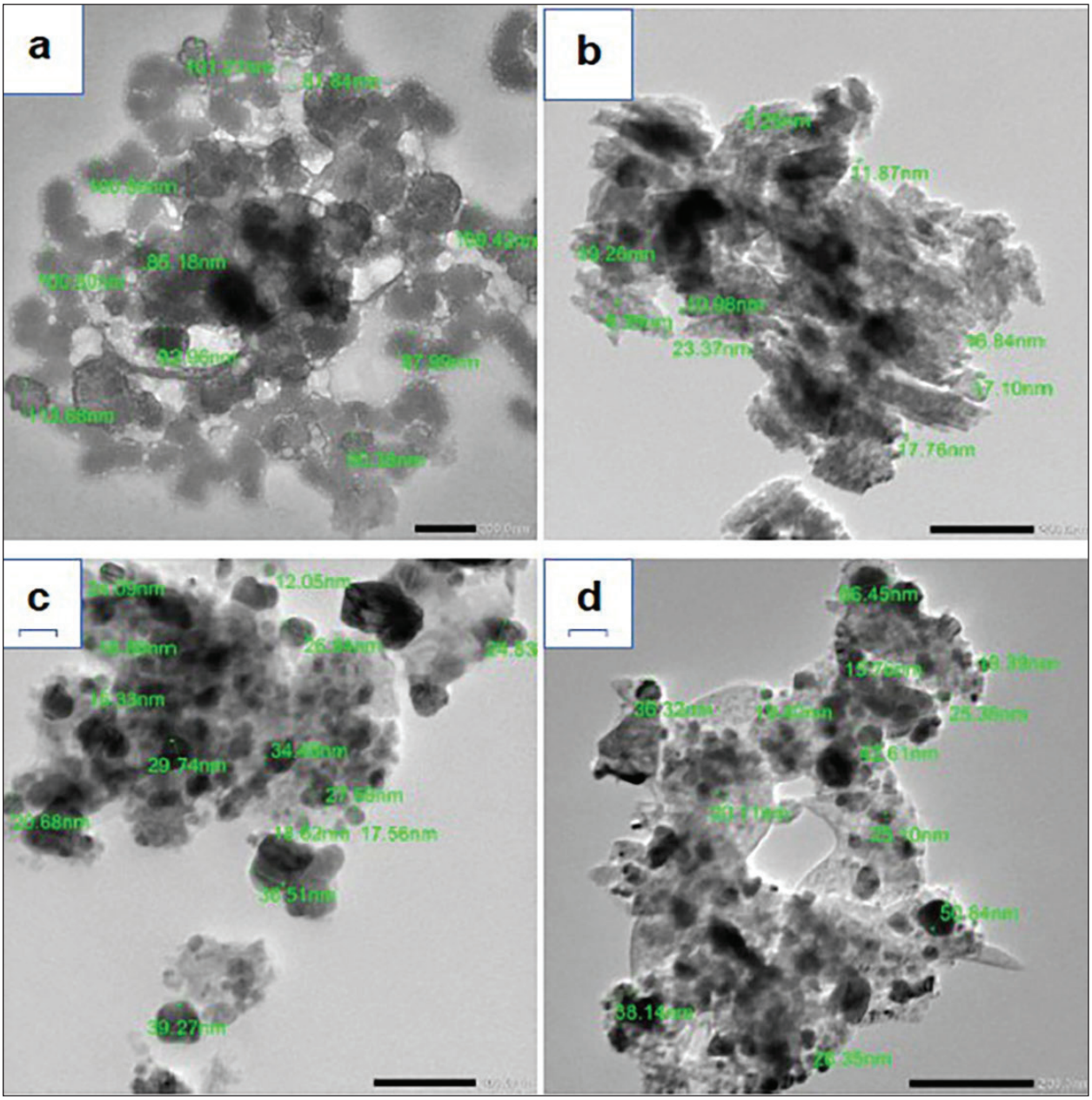

Fig. 1. The TEM images of synthesized nanoparticles. (a): *Cestrum nocturnum*- AgNPS; (b): *Salvia malticaulis*- AgNPs; (c): *Cestrum nocturnum*- CuNPS, and (d): *Salvia malticaulis*- CuNPs.

Results

Transmission Electron Microscopy

The appearance and size of silver and copper nanoparticles (AgNPs and CuNPs) produced from aqueous extracts of *Ce. nocturnum* and *Sa. malticaulis* leaves were analyzed using TEM. The TEM pictures indicated that the dimensions of the produced nanoparticles varied from 8.39 to 113.68 nm for AgNPs and from 12.05 to 66.45 nm for CuNPs, respectively (Fig. 1).

Ultraviolet-Visible spectroscopy

Localized Surface Plasmon Resonance phenomenon of AgNPs and CuNPs synthesized using *Ce. nocturnum* and *Sa. malticaulis* leaves aqueous extracts were tested. The results exhibited a singular absorption peak within the region of 300–400 nm, signifying the existence of spherical-shaped AgNPs and CuNPs (Fig. 2). The reported absorption wavelength indicated an excitonic nature at ambient temperature.

Activity of AgNPs and CuNPs

At the highest concentrations of *Ce. nocturnum*-AgNPs (20 and 18 mg/l), the mortality recorded 100.0%, 92.0% in *An. sergentii* first instar larvae (Larvae I); 92.0%, 78.67% in Larvae II, and 62.67%, 53.33% in pupae, respectively. The LC_{50} values of *Ce. nocturnum*- AgNPs against immature stages of *An. sergentii* recorded 13.93 and 13.18 mg/l against first instar larvae, 15.0 and 14.22 mg/l against second instar larvae, 17.06 and 14.31 mg/l against third instar larvae, 17.24 and 14.32 mg/l against fourth instar larvae, 17.56 and 14.82 mg/l against pupae after 24- and 48-hours post-treatment, respectively (Table 1 and Fig. 3).

Also, *Sa. malticaulis*—AgNPs recorded 100.0%, 96.0%, 90.67%, 85.33%, and 77.33% mortality in *An. sergentii* Larvae I, Larvae II, Larvae III, Larvae IV, and pupae at the highest concentration (20 mg/l) after 48 hours, respectively (Table 2 and Fig. 3).

Regarding *Ce. nocturnum* and *Sa. malticaulis* synthesized CuNPs, the LC_{50} values recorded 33.63 & 34.0, 34.28 & 34.51, 36.21 & 36.72, 36.98 & 36.98, and 37.72 & 38.39 mg/l against Larvae I, Larvae II, Larvae III, Larvae IV, and pupae after 24 hours, respectively. Meanwhile, the LC_{50} values recorded 33.71 & 33.31, 32.97 & 33.89, 34.31 & 34.64, 34.79 & 34.76, and 35.28 & 35.98 mg/l against Larvae I, Larvae II, Larvae III, Larvae IV, and pupae after 48 hours, respectively (Tables 3, 4 and Fig. 3).

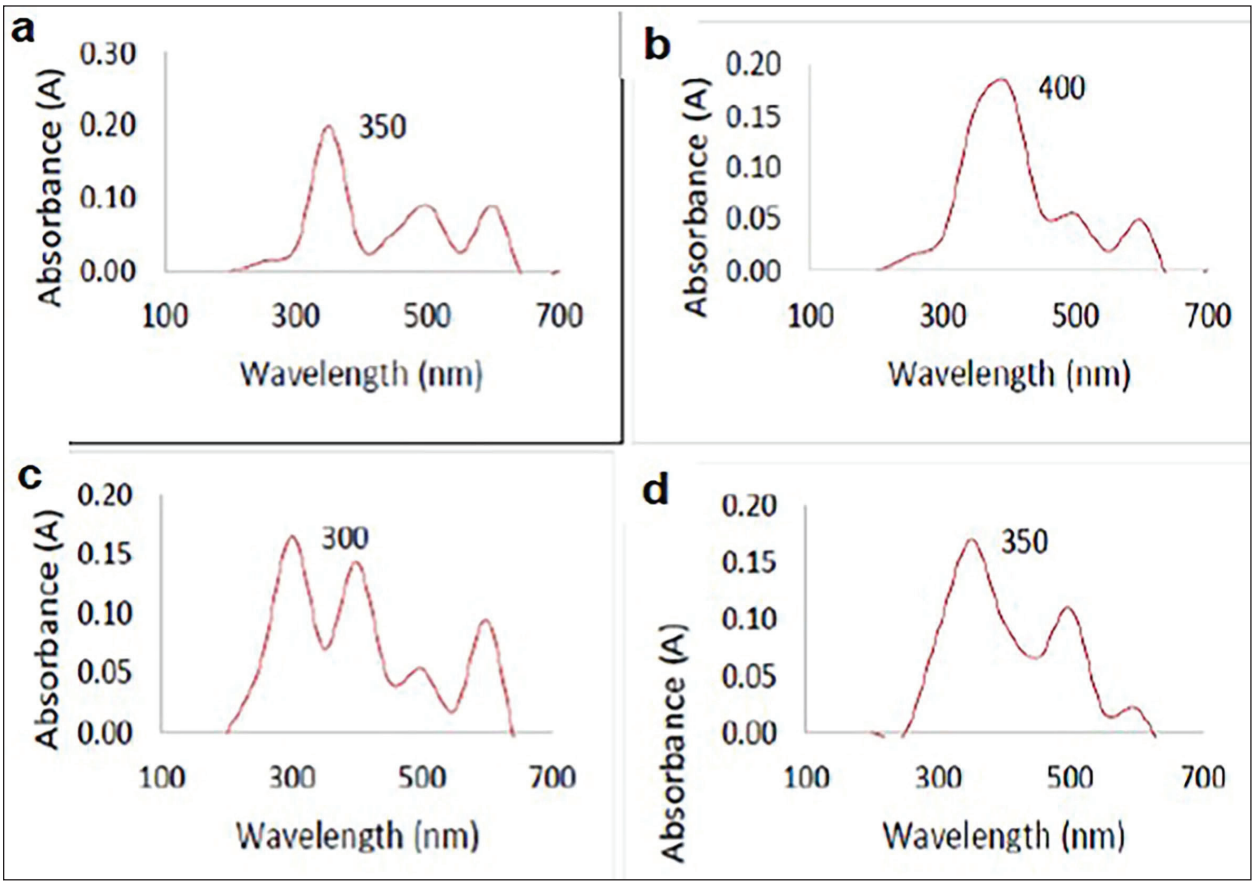

Fig. 2. Ultra-Violet (UV)—Visible curve of synthesized nanoparticles. (a): *Cestrum nocturnum*- AgNPS; (b): *Salvia malticaulis*-AgNPs; (c): *Cestrum nocturnum*- CuNPS, and (d): *Salvia malticaulis*- CuNPs.

Acetylcholinesterase activity

The results of the ANOVA and Tukey's Pairwise Comparisons reveal significant differences in AChE activity between the control group and the various nanoparticle treatments in *An. sergentii* immature stages. At 24 hours, the control group exhibited the highest AChE activity (5.83 ± 0.05 U/l in Larvae I, 6.04 ± 0.06 U/l in Larvae II, 6.34 ± 0.06 U/l in Larvae III, 6.61 ± 0.08 U/l

Table 1. Activity of *Cestrum nocturnum*-synthesized silver nanoparticles against *Anopheles sergentii* immature stages.

Target	Conc. (ppm)	24 hours			48 hours		
		Mortality %	LC_{50} (ppm) (LCL-UCL)	χ^2	Mortality %	LC_{50} (ppm) (LCL-UCL)	χ^2
Larvae I	20	100.0 ± 0.0	13.93 (13.38–14.48)	0.39^{NS}	100.0 ± 0.0	13.18 (13.04–13.31)	1.14^{NS}
	18	92.0 ± 4.0			94.67 ± 6.11		
	16	68.0 ± 4.0			73.33 ± 2.31		
	14	46.0 ± 2.0			53.33 ± 2.31		
	12	29.33 ± 2.31			38.67 ± 2.31		
	10	20.0 ± 6.93			26.67 ± 2.31		
	Control	0.0			0.0		
Larvae II	20	92.0 ± 4.0	15.0 (14.45–15.55)	0.86^{NS}	100.0 ± 0.0	14.22 (13.16–15.27)	1.0^{NS}
	18	78.67 ± 6.11			85.33 ± 4.62		
	16	50.67 ± 2.31			60.0 ± 4.0		
	14	38.67 ± 2.31			44.0 ± 4.0		
	12	26.67 ± 2.31			29.33 ± 6.11		
	10	13.33 ± 2.31			20.0 ± 4.0		
	Control	0.0			0.0		
Larvae III	20	70.67 ± 2.31	17.06 (16.04–18.08)	1.11^{NS}	100.0 ± 0.0	14.31 (13.35–15.27)	1.04^{NS}
	18	53.33 ± 2.31			76.0 ± 4.0		
	16	41.33 ± 2.31			66.67 ± 2.31		
	14	29.33 ± 2.31			49.33 ± 2.31		
	12	22.67 ± 4.62			30.67 ± 2.31		
	10	14.67 ± 2.31			21.33 ± 4.62		
	Control	0.0			0.0		
Larvae IV	20	65.33 ± 2.31	17.24 (16.60–17.88)	0.57^{NS}	98.67 ± 2.31	14.32 (13.85–14.80)	1.64^{NS}
	18	53.33 ± 2.31			78.67 ± 4.62		
	16	42.67 ± 2.31			66.67 ± 4.62		
	14	33.33 ± 2.31			46.67 ± 2.31		
	12	22.67 ± 6.11			36.0 ± 4.0		
	10	13.33 ± 2.31			20.0 ± 4.0		
	Control	0.0			0.0		
Pupae	20	62.67 ± 4.62	17.56 (16.44–18.69)	0.72^{NS}	90.67 ± 2.31	14.82 (14.01–15.63)	1.96^{NS}
	18	53.33 ± 2.31			70.67 ± 2.31		
	16	43.33 ± 3.06			61.33 ± 4.62		
	14	29.33 ± 2.31			42.67 ± 4.62		
	12	18.67 ± 4.62			29.33 ± 2.31		
	10	9.33 ± 2.31			13.33 ± 2.31		
	Control	0.0			0.0		

LC_{50} Half Lethal Concentrations; ppm part per million; LCL Lower Confidence Limit; UCL Upper Confidence Limit; χ^2 Chi square value; NS non-significant; All data calculated as Mean ± SD; Means in a column that do not share a letter are significantly different.

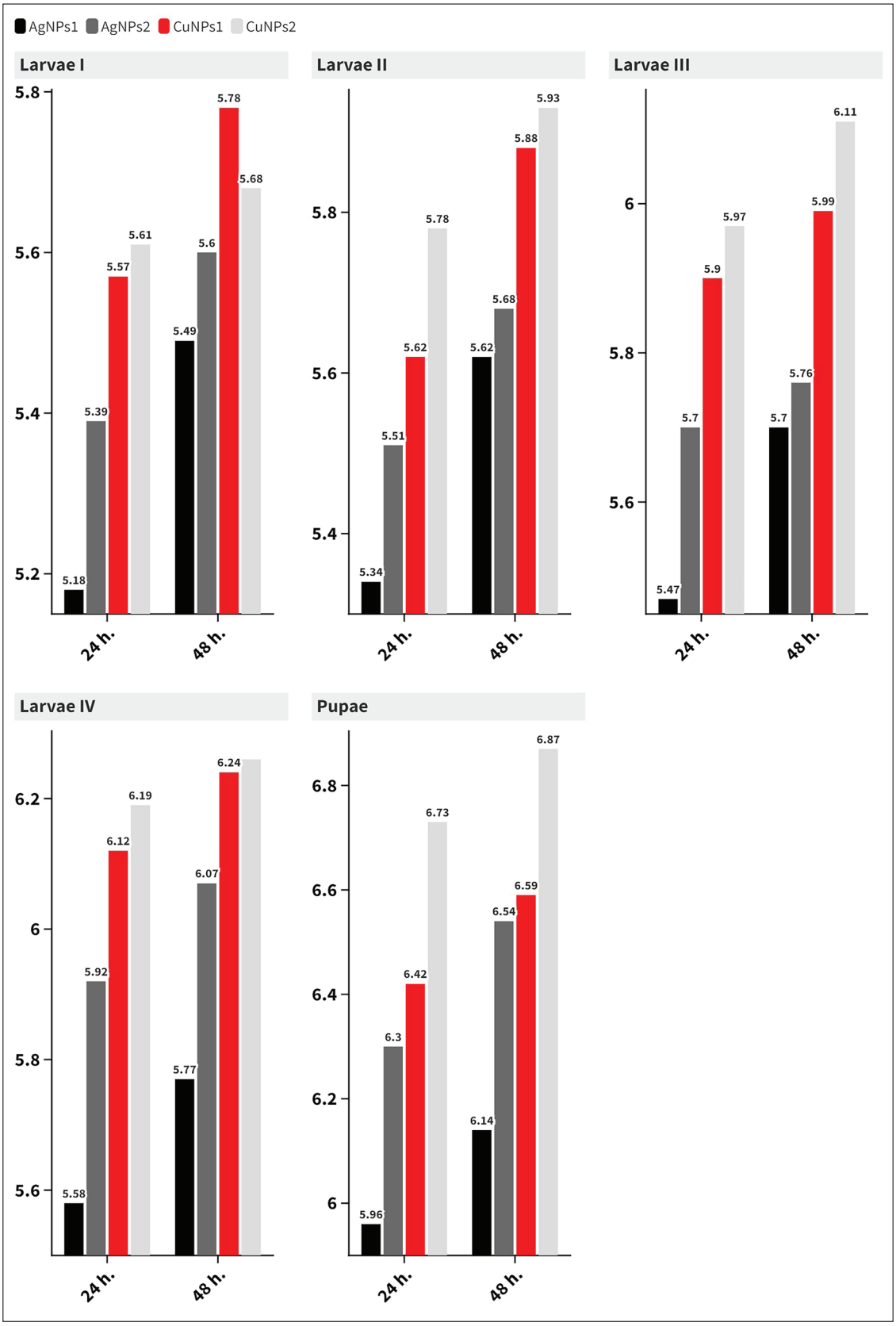

Fig. 3. Column chart represents the activity of tested silver and copper nanoparticles against *Anopheles sergentii* immature stages.

Table 2. Activity of *Salvia malticaulis* -synthesized silver nanoparticles against *Anopheles sergentii* immature stages.

Target	Conc. (ppm)	24 hours			48 hours		
		Mortality %	LC_{50} (ppm) (LCL-UCL)	χ^2	Mortality %	LC_{50} (ppm) (LCL-UCL)	χ^2
Larvae I	20	100.0 ± 0.0	14.30 (13.84–14.77)	0.89^{NS}	100.0 ± 0.0	13.46 (13.16–13.77)	1.39^{NS}
	18	89.33 ± 8.33			93.33 ± 4.62		
	16	64.0 ± 4.0			69.33 ± 2.31		
	14	43.33 ± 3.06			50.67 ± 2.31		
	12	26.67 ± 2.31			37.33 ± 2.31		
	10	14.67 ± 2.31			24.0 ± 4.0		
	Control	0.0			0.0		
Larvae II	20	90.67 ± 2.31	15.40 (15.38–15.43)	1.96^{NS}	96.0 ± 4.0	14.63 (13.99–15.27)	0.86^{NS}
	18	76.0 ± 4.0			80.0 ± 8.0		
	16	46.67 ± 2.31			56.0 ± 4.0		
	14	34.67 ± 2.31			41.33 ± 2.31		
	12	21.33 ± 2.31			26.67 ± 8.33		
	10	10.67 ± 2.31			17.33 ± 6.11		
	Control	0.0			0.0		
Larvae III	20	68.0 ± 4.0	17.56 (17.02–18.11)	0.72^{NS}	90.67 ± 4.62	14.72 (14.43–15.02)	1.04^{NS}
	18	50.67 ± 2.31			73.33 ± 2.31		
	16	38.67 ± 2.31			64.0 ± 4.0		
	14	26.67 ± 2.31			46.67 ± 6.11		
	12	18.67 ± 2.31			28.0 ± 4.0		
	10	10.67 ± 4.62			17.33 ± 6.11		
	Control	0.0			0.0		
Larvae IV	20	61.33 ± 2.31	17.82 (17.3–18.33)	0.5^{NS}	85.33 ± 2.31	14.82 (13.95–15.70)	1.0^{NS}
	18	52.0 ± 4.0			71.33 ± 9.45		
	16	38.67 ± 2.31			60.0 ± 6.93		
	14	30.67 ± 2.31			44.0 ± 4.0		
	12	21.33 ± 4.62			29.33 ± 2.31		
	10	10.67 ± 2.31			17.33 ± 2.31		
	Control	0.0			0.0		
Pupae	20	62.67 ± 6.11	17.89 (16.80–18.98)	1.11^{NS}	77.33 ± 2.31	15.64 (15.24–16.04)	0.34^{NS}
	18	50.67 ± 2.31			66.67 ± 2.31		
	16	41.33 ± 4.62			56 ± 6.93		
	14	26.67 ± 2.31			40.0 ± 4.0		
	12	16.0 ± 4.0			24.0 ± 4.0		
	10	6.67 ± 2.31			9.33±2.31		
	Control	0.0			0.0		

in Larvae IV, and 7.06 ± 0.06 U/l in Pupae), significantly higher than all treatments ($p < 0.05$). Tukey's pairwise comparison highlighted that CuNPs generally led to a slightly higher AChE activity compared to AgNPs, with *Sa. malticaulis*- CuNPs exhibiting the highest AChE activity among the treatments (6.73 ± 0.08 U/l in pupae).

At 48 hours, the control group again showed the highest activity, while copper nanoparticles, particularly *Sa. malticaulis*- CuNPs, demonstrated prolonged inhibition of AChE, with the pupae stage showing 6.87 ± 0.07 U/l, respectively. Tukey's comparisons confirmed that treatments were significantly different ($p < 0.05$),

Table 3. Activity of *Cestrum nocturnum*-synthesized copper nanoparticles against *Anopheles sergentii* immature stages.

Target	Conc. (ppm)	24 hours			48 hours		
		Mortality %	LC_{50} (ppm) (LCL-UCL)	χ^2	Mortality %	LC_{50} (ppm) (LCL-UCL)	χ^2
Larvae I	40	100.0 ± 0.0	33.63 (33.20–34.05)	1.36^{NS}	100.0 ± 0.0	33.71 (33.49–33.92)	0.86^{NS}
	38	92.0 ± 6.93			98.67 ± 2.31		
	36	73.33 ± 2.31			76.0 ± 4.0		
	34	46.67 ± 2.31			54.67 ± 2.31		
	32	36.0 ± 4.0			41.33 ± 2.31		
	30	21.33 ± 2.31			26.67 ± 2.31		
	Control	0.0			0.0		
Larvae II	40	96.0 ± 4.0	34.28 (33.74–34.82)	0.72^{NS}	100.0 ± 0.0	32.97 (32.71–33.24)	1.39^{NS}
	38	85.33 ± 4.62			93.33 ± 6.11		
	36	58.67 ± 4.62			65.33 ± 2.31		
	34	44.0 ± 4.0			48.0 ± 0.0		
	32	32.0 ± 4.0			33.33 ± 2.31		
	30	18.67 ± 2.31			24.0 ± 0.0		
	Control	0.0			0.0		
Larvae III	40	80.0 ± 4.0	36.21 (34.99–37.44)	1.11^{NS}	100.0 ± 0.0	34.31 (33.96–34.66)	0.36^{NS}
	38	56.0 ± 4.0			82.67 ± 4.62		
	36	46.67 ± 6.11			62.67 ± 2.31		
	34	34.67 ± 2.31			42.67 ± 2.31		
	32	21.33 ± 2.31			25.33 ± 2.31		
	30	17.33 ± 2.31			21.33 ± 2.31		
	Control	0.0			0.0		
Larvae IV	40	66.67 ± 2.31	36.98 (36.10–37.87)	0.36^{NS}	100.0 ± 0.0	34.79 (34.21–35.37)	1.96^{NS}
	38	54.67 ± 4.62			80.0 ± 4.0		
	36	44.67 ± 1.15			68.0 ± 4.0		
	34	34.67 ± 2.31			49.33 ± 2.31		
	32	24.0 ± 4.0			37.33 ± 2.31		
	30	13.33 ± 2.31			21.33 ± 2.31		
	Control	0.0			0.0		
Pupae	40	64.0 ± 4.0	37.72 (37.35–38.09)	1.0^{NS}	94.67 ± 2.31	35.28 (34.85–35.71)	1.39^{NS}
	38	49.33 ± 2.31			65.33 ± 6.11		
	36	41.33 ± 2.31			56.0 ± 4.0		
	34	29.33 ± 2.31			33.33 ± 4.62		
	32	18.67 ± 2.31			22.67 ± 2.31		
	30	9.33 ± 2.31			14.67 ± 4.62		
	Control	0.0			0.0		

LC_{50} Half Lethal Concentrations; ppm part per million; LCL Lower Confidence Limit; UCL Upper Confidence Limit; χ^2 Chi square value; NS non-significant; All data calculated as Mean ± SD; Means in a column that do not share a letter are significantly different.

suggesting that both AgNPs and CuNPs effectively reduced AChE activity in *An. sergentii*, with copper nanoparticles showing more persistent effects over time (Table 5 and Fig. 4).

Table 4. Activity of *Salvia malticaulis* -synthesized copper nanoparticles against *Anopheles sergentii* immature stages.

Target	Conc. (ppm)	24 hours			48 hours		
		Mortality %	LC_{50} (ppm) (LCL-UCL)	χ^2	Mortality %	LC_{50} (ppm) (LCL-UCL)	χ^2
Larvae I	40	100.0 ± 0.0	34.0 (33.75-–34.26)	1.36^{NS}	100.0 ± 0.0	33.31 (33.01–33.56)	1.0^{NS}
	38	90.67 ± 2.31			94.67 ± 2.31		
	36	70.67 ± 2.31			74.67 ± 2.31		
	34	38.67 ± 2.31			52.0 ± 0.0		
	32	33.33 ± 2.31			38.67 ± 2.31		
	30	18.67 ± 2.31			22.67 ± 2.31		
	Control	0.0			0.0		
Larvae II	40	94.67 ± 2.31	34.51 (34.34–34.67)	0.89^{NS}	100.0 ± 0.0	33.89 (33.55–34.23)	0.36^{NS}
	38	81.33 ± 4.62			89.33 ± 2.31		
	36	56.67 ± 3.06			61.33 ± 2.31		
	34	42.67 ± 2.31			44.0 ± 4.0		
	32	30.67 ± 2.31			34.67 ± 2.31		
	30	17.33 ± 2.31			24.0 ± 4.0		
	Control	0.0			0.0		
Larvae III	40	74.67 ± 2.31	36.72 (36.34–37.10)	0.68^{NS}	98.67 ± 2.31	34.64 (34.48–34.80)	1.0^{NS}
	38	53.33 ± 2.31			80.0 ± 4.0		
	36	45.33 ± 4.62			60.0 ± 0.0		
	34	32.0 ± 4.0			40.0 ± 4.0		
	32	18.67 ± 2.31			22.67 ± 2.31		
	30	13.33 ± 2.31			17.33 ± 2.31		
	Control	0.0			0.0		
Larvae IV	40	66.67 ± 2.31	36.98 (36.10–37.87)	0.72^{NS}	94.67 ± 2.31	34.76 (34.09–35.43)	0.89^{NS}
	38	54.67 ± 4.62			76.0 ± 4.0		
	36	44.67 ± 1.15			64.0 ± 4.0		
	34	34.67 ± 2.31			45.33 ± 2.31		
	32	24.0 ± 4.0			33.33 ± 2.31		
	30	13.33 ± 2.31			20.0 ± 4.0		
	Control	0.0			0.0		
Pupae	40	61.33 ± 2.31	38.39 (37.31–39.49)	0.93^{NS}	85.33 ± 2.31	35.98 (35.66–36.30)	0.89^{NS}
	38	44.0 ± 4.0			58.67 ± 2.31		
	36	40.0 ± 4.0			50.67 ± 2.31		
	34	24.0±4.0			30.67 ± 2.31		
	32	14.67 ± 2.31			20.0 ± 4.0		
	30	6.67 ± 2.31			12.0 ± 4.0		
	Control	0.0			0.0		

LC_{50} Half Lethal Concentrations; ppm part per million; LCL Lower Confidence Limit; UCL Upper Confidence Limit; χ^2 Chi square value; NS non-significant; All data calculated as Mean ± SD; Means in a column that do not share a letter are significantly different.

Discussion

The TEM images of AgNPs and CuNPs synthesized using aqueous extracts of *Ce. nocturnum* and *Sa. malticaulis* leaves revealed that the sizes of the synthesized nanoparticles ranged from 8.39 to 113.68 nm for AgNPs and from 12.05 to 66.45 nm for CuNPs, respectively. The dimensions of synthesized nanoparticles corroborate the findings previously

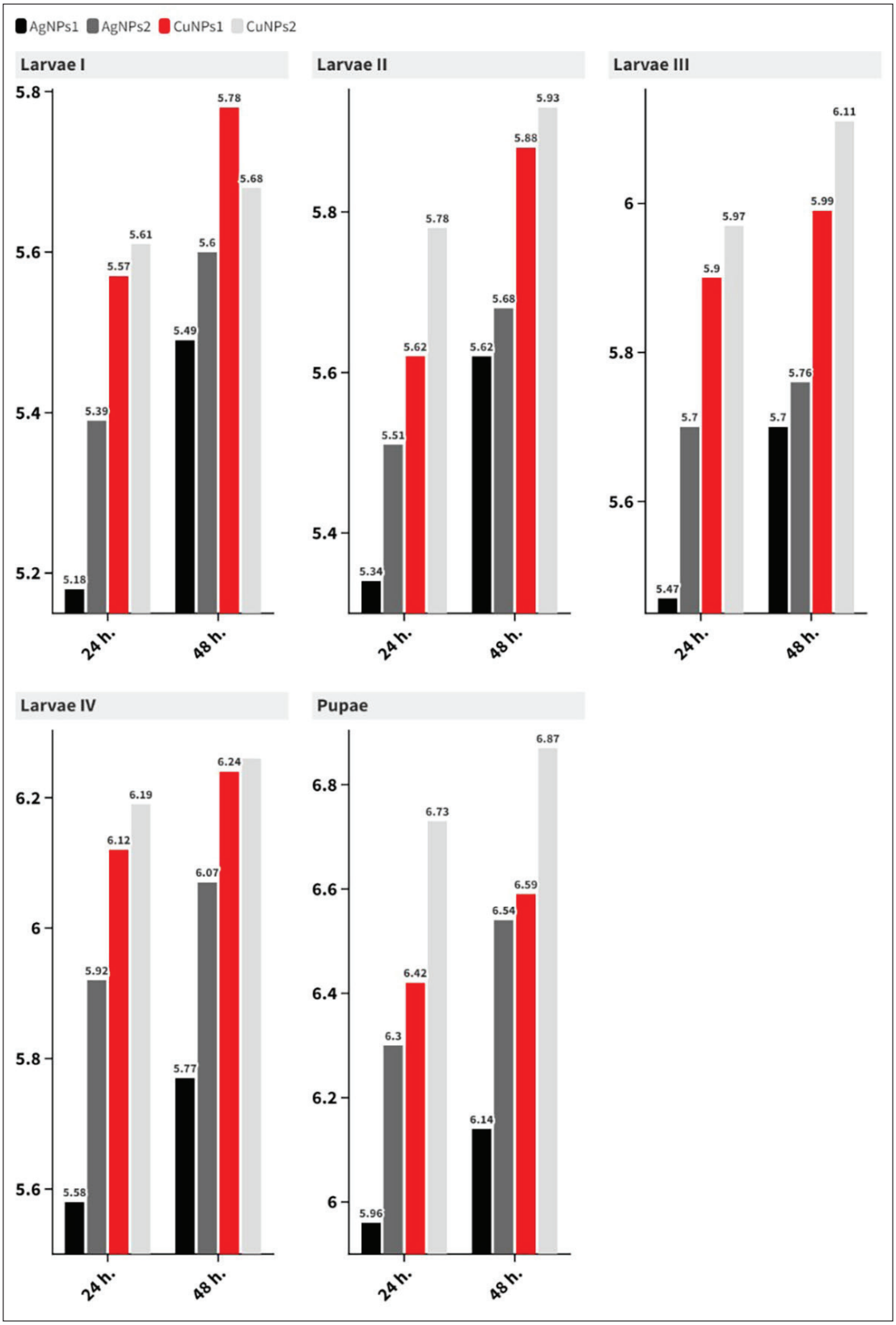

Fig. 4. Column chart represents the effect of green-synthesized silver and copper nanoparticles om Acetylcholinesterase (AChE) activity of An. sergentii immature stages.

Table 5. The effect of green-synthesized silver and copper nanoparticles om Acetylcholinesterase (AChE) activity of *Anopheles sergentii* immature stages.

Time (Hours)	Tested Nanoparticles	Larvae I	Larvae II	Larvae III	Larvae IV	Pupae
24	*Ce. nocturnum*- AgNPs	5.18 ± 0.04d	5.34 ± 0.05d	5.47 ± 0.04d	5.58 ± 0.04d	5.96 ± 0.06d
	Sa. malticaulis- AgNPs	5.39 ± 0.03c	5.51 ± 0.04c	5.70 ± 0.07c	5.92 ± 0.05c	6.30 ± 0.08c
	Ce. nocturnum- CuNPs	5.57 ± 0.09b	5.62 ± 0.03b	5.90 ± 0.04b	6.12 ± 0.05b	6.42 ± 0.04b
	Sa. malticaulis- CuNPs	5.61 ± 0.09b	5.78 ± 0.06b	5.97 ± 0.02b	6.19 ± 0.03b	6.73 ± 0.08b
	Control	5.83 ± 0.05a	6.04 ± 0.06a	6.34 ± 0.06a	6.61 ± 0.08a	7.06 ± 0.06a
48	*Ce. nocturnum*- AgNPs	5.49 ± 0.05d	5.62 ± 0.04d	5.70 ± 0.02d	5.77 ± 0.03d	6.14 ± 0.08d
	Sa. malticaulis- AgNPs	5.60 ± 0.05c	5.68 ± 0.03c	5.76 ± 0.05c	6.07 ± 0.08c	6.54 ± 0.08c
	Ce. nocturnum- CuNPs	5.78 ± 0.07b	5.88 ± 0.02b	5.99 ± 0.02b	6.24 ± 0.04b	6.59 ± 0.04b
	Sa. malticaulis- CuNPs	5.68 ± 0.02b	5.93 ± 0.03b	6.11 ± 0.04b	6.26 ± 0.05b	6.87 ± 0.07b
	Control	6.13 ± 0.06a	6.40 ± 0.03a	6.63 ± 0.03a	6.99 ± 0.06a	7.34 ± 0.10a

All data represented as U/L; All data calculated as Mean ± SD; Means in a column that do not share a letter are significantly different.

reported by Agalya Priyadarshini *et al.* (2012) regarding AgNPs prepared with Euphorbia hirta, Kumar *et al.* (2015) utilizing Morinda tinctoria leaf extract for AgNPs, Hassanain *et al.* (2019) employing petroleum ether extract from *Lantana camara* leaves for CuNPs, Shehata and Mahmoud (2019) using aqueous extract from *Lagenaria siceraria* leaves for AgNPs, and Zaki *et al.* (2024) applying aqueous extracts from *Rosa arabica* and *Eucalyptus citriodora* leaves for both AgNPs and CuNPs preparation. Furthermore, the UV–vis Spectrophotometric analysis results for both AgNPs and CuNPs exhibited a singular absorption peak within the 300–400 nm range, affirming the presence of spherical-shaped AgNPs and CuNPs, corroborating findings previously documented by El-Mehdawy *et al.* (2022); El-Waseif *et al.* (2023); and Mani *et al.* (2023).

The efficacy of AgNPs and CuNPs, produced from aqueous extracts of *Ce. nocturnum* and *Sa. malticaulis* leaves, against *An. sergentii* augmented with higher concentrations of the tested nanoparticles. Calculated LC_{50} values indicate that AgNPs and CuNPs produced from the aqueous extract of *Ce. nocturnum* leaves were more efficacious against various stages of *An. sergentii* compared to those of *Sa. malticaulis.* The studied AgNPs showed greater efficacy against various immature stages of *An. sergentii* compared to CuNPs. The significant efficacy of green-synthesized nanoparticles against *An. sergentii* and various mosquito immature stages is due to their capacity to permeate the exoskeleton and infiltrate insect cells, where they impede macromolecules such as proteins and deoxyribonucleic acid, altering their structure and consequently their function (Subramaniam *et al.*, 2015). The results obtained are consistent with those reported by Morejón *et al.* (2018) for AgNPs synthesized from Ambrosia arborescens extract against *Aedes aegypti,* Shehata and Mahmoud (2019) for AgNPs synthesized from aqueous extract of *L. siceraria* against *Culex pipiens* and An. pharoensis, Hassanain *et al.* (2019) for CuNPs synthesized from petroleum ether extract of *La. camara* leaves against *An. multicolor,* and Zaki *et al.* (2024) for AgNPs and CuNPs synthesized from aqueous extract of *R. arabica* and *E. citriodora* against *Cu. antennatus* larvae.

A decrease in AChE levels in various immature stages of *An. sergentii* was observed. AChE activity assessments have become standard as a biomarker for exposure to specific types of contaminants (Grue *et al.*, 1997). Nanoparticles may attach to and impede the function of AChE, which destroys acetylcholine, a crucial neurotransmitter in the central nervous system of insects (Nasir *et al.*, 2022).

The impact of *Ce. nocturnum* and *Sa. malticaulis*-synthesized AgNPs and CuNPs on AChE activity in various immature stages of *An. sergentii* corroborates the findings of Abdel-Gawad (2018), who noted a significant decrease in AChE activity in Musca domestica larvae after exposure to LC_{50} concentrations of *Moringa oleifera*-AgNPs and *Moringa oleifera*-ZnONPs treated diets compared to the control. Additionally, Parthiban *et al.* (2019) observed a notable reduction in the crucial esterase enzyme AChE in Ae. aegypti larval body homogenates following exposure to AgNPs relative to the control group.

Conclusion

In conclusion, the *Ce. nocturnum* and *Sa. malticaulis*—synthesized AgNPs and CuNPs scattered uniformly in water and possess a significant activity against *An. sergentii* immature stages, reflecting the importance of relying on plant-synthesized nanoparticles in *An. sergentii* control for reducing the spread of diseases. Furthermore, there is an urgent need to develop more studies concerning the activity of green-synthesized AgNPs and CuNPs against several other mosquito species.

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Conflict of interest

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Authors' contribution

All authors contributed to the experimental design, hands on work, discussions, and commented on the manuscript. The final version of the manuscript read, reviewed, and approved by all authors.

Data availability

Any datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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