153 Egypt. J. Vet. Sci. **Vol. 55,** No. 6, pp. 1685-1696 (2024)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Biosynthesis of Silver and Copper Nanoparticles Using *Rosa arabica* (Rosaceae) and *Eucalyptus citriodora* (Myrtaceae) Extracts and its Biological Activity Against *Culex antennatus* Becker (Diptera: Cuilicidae)

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Abstract

CONTROL of *Culex antennatus* is an important strategy for eliminating diseases transmition. Application of green-synthesized silver and copper nanoparticles (AgNPs and CuNPs) in mosquito' control has several advantages of eco-friendly because of the absence of deadly chemicals in their synthesis. The present study examined the biological activity of *Rosa arabica* and *Eucalyptus citriodora* leaves aqueous extract and its synthesized AgNPs and CuNPs against immature stages of *C. antennatus,* as well as the reproductive potential of females resulting from treated larvae. It was found that aqueous extract from leaves of *R. arabica* and *E. citriodora* can reduce silver and copper ions to generate AgNPs and CuNPs suspended in water. Results of Transmission Electron Microscopy (TEM) evoked the occurrence of AgNPs with sizes ranged between 14.3 and 40.70 nm, while sizes of CuNPs ranged between 19.60 and 69.40 nm, respectively. The UV-vis spectrophotometric analysis for AgNPs and CuNPs revealed the occurrence of single absorption peak at specific wavelengths ranged from 300 to 450 nm, indicating the presence of spherical-shaped NPs. Also, *R. arabica* and *E. citriodora*- synthesized AgNPs and CuNPs were more effective against *C. antennatus* than *R. arabica* and *E. citriodora* leaves aqueous extract, however the CuNPs were more effective than AgNPs. The LC_{50} recorded 38.40 and 31.26 ppm for AgNPs against *C. antennatus* third larval instar, while CuNPs recorded LC_{50} of 21.07 and 14.47 ppm, respectively. In addition, synthesized AgNPs and CuNPs significantly reduced the fecundity and fertility of *C. antennatus* females resulting from treated larvae, as compared with untreated groups.

Keywords: *Culex antennatus, Rosa arabica, Eucalyptus citriodora,* Biosynthesis, Larvicidal.

Introduction

Mosquitoes are carriers of several pathogenic agents to human and animals worldwide including dengue, malaria, filaria, Rift Valley fever (RVF) and many arboviruses causing many deaths annually and hundreds of millions of clinical cases [1-4]. Also, mosquitoes have a negative impact on livestock through weight loss and a reduction in milk production in dairy cows as a result of disease transmission [5]. *Culex antennatus* Becker one of the most common mosquito species distributed in Egypt, and plays a major role the prevalence of RVF virus in the Nile Delta, as well as Western Nile virus (WNV), and Sindbis virus [6-8].

The indiscriminate use of synthetic chemical insecticides has led to many serious problems including environmental pollution, insecticide resistance and toxic effects on human beings and non-target organisms, thus there is an urgent require to find other alternatives to these synthetic chemical insecticides to reduce the spread of diseases transmits by different mosquito species [9]. The applications of nanoparticles in different areas such as medicine, environment, controlling different diseases and biotechnology have become an area of concern

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(Received 26/01/2024, accepted 27/02/2024)
DOI: 10.21608/EJVS.2024.265721.1806

[10,11]. The green-synthesized nanoparticles such as silver and copper nanoparticles (AgNPs and CuNPs) have several advantages of being eco-friendly because of the absence of deadly chemicals in their synthesis, and efforts to use the green-synthesized nanoparticles as controlling agents for different mosquito' species remain indispensable [12, 13].

Rosa arabica and *Eucalyptus citriodora* used in the present work are medicinal and decoration plants cultivated in Egypt, as *R. arabica* is a folk medicine agent, while *E. citriodora* is a decoration plant.

From this point of view, the present study dealt with the biological activity of *R. arabica* and *E. citriodora* (leaves) aqueous extracts and their synthesized AgNPs and CuNPs against one of the most common mosquito species distributed in Egypt, *C. antennatus.*

Material and methods

Culex antennatus colony

Culex antennatus larvae were collected from Faiyum Governorate (29°18'58.3" N, 30°39'08.8" E) (Latitude: 29°18'58.3" N, Longitude: 30°39'08.8" E, Elevation: 19 m), Egypt. The collected larvae were morohologically identified using a taxonomic key [14]. Then, larvae were subjected to molecular identification. The new COI sequence (accession number: OP714215.1) was compared with those recorded in the GenBank (available at http://blast.ncbi.nlm.nih.gov/Blast.cgi) [4].

Larvae were reared for six generations in the Medical Entomology Insectary, Animal house, Department of Zoology, Faculty of Science, Al-Azhar University under controlled conditions following a standard rearing procedure [15].

Preparation of tested silver and copper nanoparticles (AgNPs and CuNPs):

Preparation of aqueous extracts:

Leaves of *Rosa arabica* were collected from Saint Catherine, South Sinai Governorate, Egypt (Latitude: 28°33'42.88" N, Longitude: 33°56'57.62" E, Elevation: 1,586 m), while leaves of *Eucalyptus citriodora* were collected from Sadat City, Cairo-Alexandria desert road (Latitude: 30º21´38.7" N, Longitude: 30º29´58.3" E, Elevation: 42 m). The collected *R. arabica* and *E. citriodora* leaves were washed and dried in the shade for 5 days at room temperature. Dried leaves were pulverized to powder separately using an electrical stainless steel blender (Philips, HR2058). Four grams of each leaf powder were boiled with 100 ml of distilled water in a water bath for three minutes. The solution was filtered and kept in the refrigerator at 4ºC until use [16].

Synthesis of AgNPs

About 0.17g of $AgNO_3$ (purchased from El-Gomhouria Co. for Trading Pharmaceuticals, Chemicals & Medical Appliances, Cairo), was dissolved in 100 ml distilled water to prepare $AgNO_3$ stock solution. The leaf extracts of *R. arabica* and *E. citriodora* were mixed separately with $AgNO_3$ solution in the ratio of 1:9 and incubated at room temperature (26±2ºC) for 72 h until the appearance of a reddish-brown color which indicated the formation of AgNPs [17].

Synthesis of CuNPs

For the synthesis of CuNPs, 50 mL (5 mM) copper sulfate solution was mixed with 5 ml of aqueous extracts from *R. arabica* and *E. citriodora* leaves. The pH value 7.0 adjusted for the mixture by the addition of NaOH (1 N) solution. Further, 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 [18].

Characterization of synthesized AgNPs and CuNPs:

Transmission Electron Microscope studies (TEM)

The AgNPs and CuNPs suspensions were sonicated for 10 min. and diluted to slight turbid suspensions. The AgNPs and CuNPs suspensions were subjected to JEOL, JEM-2100 high resolution transmission electron microscope (TEM) at an accelerating voltage of 200 kV, respectively. Studied at Nano Tech Egypt for Photo-Electronics, El-Wahaat Road, Dream Land City, Entrance 3, City of 6 October, Al Giza, Egypt.

Ultraviolet-Visible (UV/VIS) spectroscopy:

The AgNPs and CuNPs suspensions were diluted from 1 to 10 times by distilled water from colloidal solutions obtained from the synthesis process. The UV/VIS spectroscopy of suspension was carried out using UV-Vis spectrophotometer (Type: Evolution™ 300, Serial number: EVon 10600z, from Thermo Scientific). The UV/VIS spectroscopy was carried out at the General analysis room in the Department of Chemistry, Faculty of Science, Ain Shams University, Abbasia, Cairo, Egypt.

Experimental bioassay

Larvicidal activity of synthesized AgNPs and CuNPs

Larvicidal activity of AgNPs and CuNPs was carried out using a previously described procedure [11]. Briefly, different concentrations of AgNPs and CuNPs were prepared in 250 ml of dechlorinated tap water contained in 500 ml plastic cups. Twenty-five larvae of *C. antennatus* third larval instar were put immediately into plastic cups containing different concentrations. All plastic cups were incubated under controlled conditions of mosquito colony and mortality was recorded daily until adult emergence. Three replicates were usually used. All values are calculated as Mean±SD.

Reproductive potential of resulted females

Females that emerged from *C. antennatus* treated larvae were transferred with normal males from the colony to the wooden cages by electric aspirator, and fed on 10.0% sucrose solution for three days. males and females were starved for one day, and then allowed to take a blood meal from a pigeon and allowed to lay egg rafts on clean water (oviposition traps). Effect of tested AgNPs and CuNPs on the reproductive potential of resulted females carried out according to a standard method of Shehata et al. [19].

Statistics

All data were subjected to GraphPad InStat software, Inc. for the statistical analysis [20]. Data was calculated as Mean±SD. ANOVA (One-way) was used to compare the data [21]. SPSS V.22 was used for data encoding and entry. Quantitative data were reported using mean, median, standard deviation, and standard error; qualitative data were presented with frequency. The threshold for statistical significance was set at $P < 0.05$. Lethal concentrations (LC_{50}, LC_{75} and LC_{90}) were calculated using multiple linear regressions [22].

Results

Characterization of synthesized silver and copper nanoparticles (AgNPs and CuNPs)

Transmission Electron Microscopy (TEM)

Silver and copper nanoparticles (AgNPs and CuNPs) synthesized using aqueous extracts from leaves of *Rosa arabica* and *Eucalyptus citriodora* were subjected to TEM to find the information of AgNPs and CuNPs morphology and size. The TEM images showed the occurrence of individual silver nanoparticles and a few aggregates in the test suspension. The sizes of AgNPs reported by TEM ranged between 14.3 and 40.70 nm, respectively. Also, sizes of CuNPs ranged between 19.60 and 69.40 nm was attained by using aqueous extract from leaves of tested plants (Figures 1 & 2).

Ultra-Violet (UV) - Visible

The UV–vis Spectrophotometric analysis for both AgNPs and CuNPs synthesized using aqueous extract of *R. arabica* and *E. citriodora* recorded occurrence of single absorption peak at specific wavelengths (λmax) ranged from 300 to 450 nm, respectively indicating the presence of spherical-shaped NPs (Figures 3 & 4).

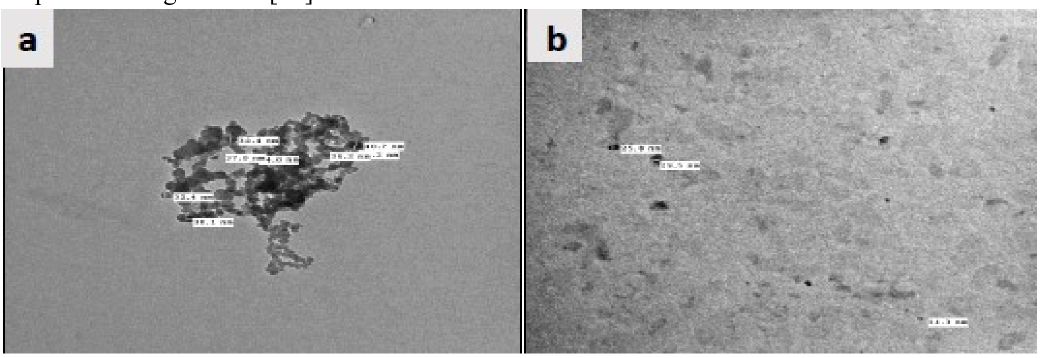

Fig. 1. TEM images of synthesized AgNPs using *Rosa arabica* (a) and using *Eucalyptus citriodora* (b) (100 nm, 60000X)

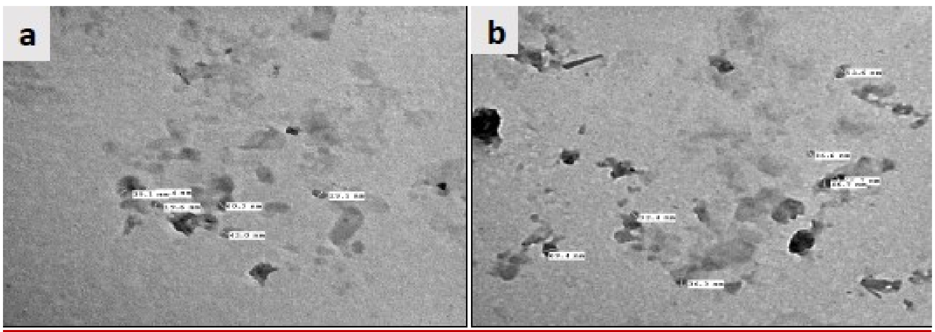

Fig. 2. TEM images of synthesized CuNPs using *Rosa arabica* (a) and using *Eucalyptus citriodora* (b) (100 nm, 60000X)

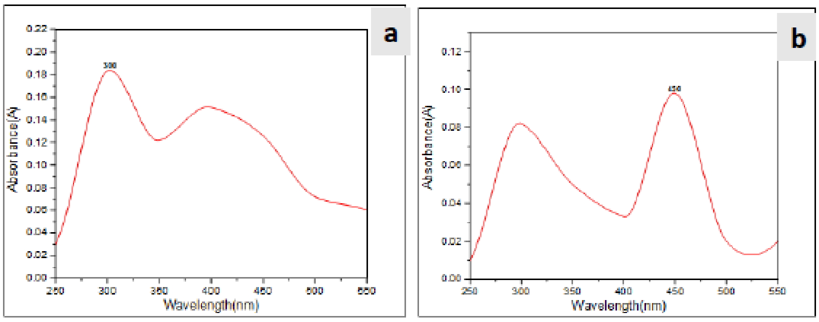

Fig. 3. Ultra-Violet (UV) - Visible curve of *Rosa arabica* -AgNPs. (a) and of *Eucalyptus citriodora* *Eucalyptus citriodora* -AgNPs. (b)

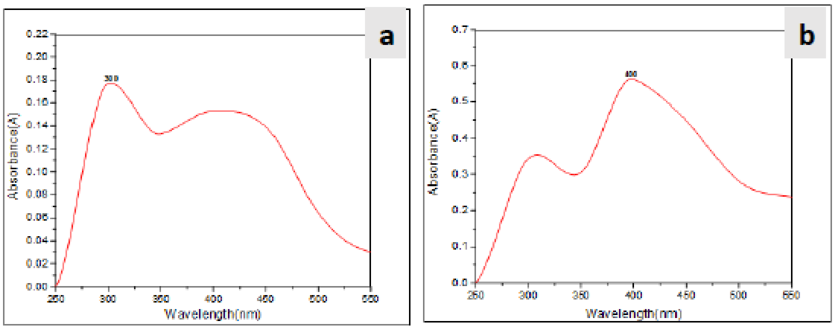

Fig. 4. Ultra-Violet (UV) - Visible curve of *Rosa arabica* -CuNPs. (a) and of *Eucalyptus citriodora* *Eucalyptus citriodora* - CuNPs.. (b)

Larvicidal activity

Larvicidal activity of aqueous extract from leaves of tested plants

The highest larval mortality (100.0%) was attained by 2000 ppm of *R. arabica* (leaves) aqueous extract, while the lowest mortality percent (9.33%) was caused by the lowest concentration (250 ppm). Also, the highest larval mortality (100.0%) was caused by *E. citriodora* (leaves) aqueous extract at 1600 ppm and the lowest mortality percent of 12.0% was caused by the lowest concentration (200 ppm), respectively.both larval and pupal durations were significantly ($P<0.05$) prolonged by the tested extracts as compared with control congers. A toxic effect of *R. arabica* and *E. citriodora* (leaves) aqueous extracts against pupae resulted from treated larvae was observed. As shown from the results, the highest pupal mortality percepts (53.18 and 61.67%) was induced by *R. arabica* and *E. citriodora* aqueous extracts at 1750 and 1400 ppm, respectively (Table 1).

The growth index of *Culex antennatus* was recorded 5.73, 6.87, 8.20, 10.09, 11.08, 11.75 and 12.53 at 1750, 1500, 1250, 1000, 750, 500 and 250 ppm of *R. arabica* leaves) aqueous extract, respectively, vs. 15.95 for the control group. Wherease, the growth index recorded 4.31, 7.67, 7.70, 9.47, 10.41, 11.29 and 11.77 at 1400, 1200, 1000, 800, 600, 400 and 200 ppm of *E. citriodora* (leaves) aqueous extract, respectively vs. 15.57 for the control group (Table 1).

TABLE 1. Effect of *Rosa arabica* and *Eucalyptus citriodora* (leaves) aqueous extract on some biological aspects of *Culex antennatus.*

Tested Extracts	Conc. (ppm)	Larval Mort. (%)	Larval Duration	Pupal Mort. (%)	Pupal Duration	Development Duration	Growth Index (a/b)
***Rosa arabica* Aqueous extract**	Control	0.0±0.0	4.25±0.15^{a}	0.0±0.0	2.02±0.07^{a}	6.27±0.22^{a}	15.95±0.56^{a}
	250	9.33±2.31	4.90±0.02^{d}	10.35±2.85	2.25±0.05^{c}	7.16±0.05^{d}	12.53±0.32^{d}
	500	13.33±2.31	4.98±0.02^{d}	13.86±0.38	2.35±0.04^{d}	7.33±0.06^{d}	11.75±0.14^{d}
	750	26.67±2.31	5.11±0.02^{d}	16.38±0.51	2.43±0.10^{d}	7.55±0.09^{d}	11.08±0.06^{d}
	1000	37.33±2.31	5.28±0.02^{d}	21.39±4.57	2.51±0.08^{d}	7.79±0.06^{d}	10.09±0.66^{d}
	1250	48.0±4.0	5.43±0.03^{d}	33.67±7.01	2.65±0.05^{d}	8.08±0.02^{d}	8.20±0.85^{d}
	1500	65.33±4.62	5.65±0.07^{d}	42.50±6.61	2.71±0.07^{d}	8.37±0.13^{d}	6.87±0.80^{d}
	1750	74.67±2.31	5.89±0.09^{d}	53.18±12.22	2.81±0.02^{d}	8.70±0.08^{d}	5.73±0.95^{d}
	2000	100.0±0.0	---	---	---	---	---
***Eucalyptus citriodora* Aqueous extract**	Control	0.0±0.0	4.30±0.02^{a}	0.0±0.0	2.13±0.05^{a}	6.42±0.03^{a}	15.57±0.08^{a}
	200	12.0±4.0	5.03±0.06^{d}	13.66±0.63	2.31±0.02^{b}	7.34±0.06^{d}	11.77±0.16^{d}
	400	21.33±6.11	5.10±0.01^{d}	15.32±1.22	2.40±0.03^{d}	7.50±0.04^{d}	11.29±0.21^{d}
	600	32.0±4.0	5.22±0.02^{d}	19.77±4.55	2.48±0.10^{d}	7.71±0.09^{d}	10.41±0.54^{d}
	800	41.33±2.31	5.36±0.12^{d}	25.08±4.50	2.55±0.04^{d}	7.91±0.14^{d}	9.47±0.68^{d}
	1000	52.00±4.00	5.48±0.16^{d}	36.52±7.84	2.75±0.06^{d}	8.24±0.21^{d}	7.70±0.79^{d}
	1200	69.33±2.31	5.73±0.04^{d}	48.21±9.94	2.86±0.04^{d}	8.59±0.04^{d}	7.67±0.82^{d}
	1400	80.0±4.0	5.96±0.03^{d}	61.67±12.58	2.93±0.06^{d}	8.89±0.04^{d}	4.31±1.43^{d}
	1600	100.0±0.0	---	---	---	---	---

Conc. = Concentration; ppm = particle per million; SD = standard deviation; mort. = mortality; Means followed by the same letter in the same column are not statistically significant. All data represented as Mean±SD.

Larvicidal activity of of synthesized AgNPs and CuNPs

The biological activity of biosynthesized silver nanoparticles using *R. arabica* and *E. citriodora* (leaves) aqueous extracts is recorded in Table (2). The 100.0% larval mortality was attained by 60 and 50 ppm, while the lowest mortality perceepts (21.33 and 24.0% was caused by the lowest concentrations 25 and 20 ppm, respectively. Also, larval and pupal durations were significantly (P<0.001) prolonged by *R. arabica E. citriodora* synthesized-AgNPs all concentrations used as compared with the control group.

The growth index recorded 0.72, 3.82, 6.46, 8.82, 10.19 and 11.48 at 45, 40, 35, 30, 25 and 20 ppm of *E. citriodora* synthesized-AgNPs, respectively, compared with 15.63 for the control group (**Table 2**).

Regarding to synthesized-CuNPs, complete larval mortality (100.0%) was attained by *R. arabica* and *E. citriodora*- synthesized-CuNPs at 40 and 35 ppm, respectively. Mean larval duration was significantly (P<0.001) affected by both *R. arabica* and *E. citriodora*- synthesized-CuNPs at all concentrations used. The pupal mortality recorded 100.0% at 35 and 30 ppm of *R. arabica* and *E. citriodora*- synthesized-CuNPs, respectively. The growth index recorded 3.14, 5.87, 7.99, 9.62 and 10.96 at 25, 20, 15, 10 and 5 ppm of *E. citriodora*- synthesized-CuNPs, respectively compared with 15.51 for the control group (Table 3).

Based on calculated lethal values, *E. citriodora*-aqueous extract and its synthesized- AgNPs and CuNPs were more effective against *C. antennatus* larvae than those of *R. arabica* (Table 4).

Reproductive potential of resulted females

Aqueous extract from leaves of tested plants

Aqueous extract from leaves of *R. arabica* and *E. citriodora* exhibited a significant effect on fecundity females resulted from treated larvae at all concentrations used, where the fecundity recorded 174.50±0.71, 177.33±2.52 and 181.25±1.71 eggs/♀ at 1750, 1500 and 1250 ppm of *R. arabica* leaves aqueous extract, vs. 217.10±1.91 eggs/♀ for the control. Also, the statistical analysis revealed a significant (P<0.001) decrease in the mean number of eggs laid by females resulted from larvae treated with *E. citriodora* leaves aqueous extract at 400, 600, 800, 1000, 1200 and 1400 ppm, where the average number was 201.11±2.03, 193.29±2.21, 187.14±2.41, 183.33±1.97, 178.40±2.07, 174.50±1.29 and 170.50±1.29 eggs/♀, respectively vs. 214.30±1.34 eggs/♀ for the control (Table 5).

TABLE 2. Effect of *Rosa arabica* and *Eucalyptus citriodora* -synthesized silver nanoparticles on some biological aspects of *Culex antennatus.*

Silver Nanoparticles	Conc. (ppm)	Larval Mort. (%)	Larval Duration	Pupal Mort. (%)	Pupal Duration	Development Duration	Growth Index (a/b)
***Rosa arabica* synthesized-AgNPs**	Control	0.0±0.0	4.29±0.06[a]	0.0±0.0	2.08±0.08[a]	6.37±0.03[a]	15.7±0.07[a]
	25	21.33±2.31	4.90±0.18[d]	10.18±0.31	2.29±0.02[c]	7.18±0.16[d]	12.51±0.32[c]
	30	32.0±4.0	5.17±0.02[d]	15.84±4.13	2.38±0.03[d]	7.55±0.05[d]	11.15±0.49[d]
	35	40.0±4.0	5.27±0.03[d]	22.44±5.35	2.45±0.03[d]	7.72±0.05[d]	10.05±0.73[d]
	40	50.67±4.62	5.41±0.02[d]	37.23±7.49	2.53±0.05[d]	7.94±0.06[d]	7.91±1.0[d]
	45	64.0±4.0	5.69±0.02[d]	56.02±6.26	2.83±0.06[d]	8.52±0.07[d]	5.16±0.70[d]
	50	78.67±4.62	5.95±0.05[d]	83.33±48.11	2.96±0.04[d]	8.90±0.08[d]	1.86±1.08[d]
	60	100.0±0.0	---	---	---	---	---
***Eucalyptus citriodora* synthesized-AgNPs**	Control	0.0±0.0	4.26±0.08[a]	0.0±0.0	2.14±0.09[a]	6.40±0.06[a]	15.63±0.13[a]
	20	24.0±4.0	5.11±0.03[d]	14.15±3.62	2.36±0.05[c]	7.48±0.02[d]	11.48±0.48[d]
	25	37.33±2.31	5.26±0.06[d]	21.39±4.57	2.46±0.04[d]	7.72±0.09[d]	10.19±0.67[d]
	30	46.67±4.62	5.35±0.05[d]	30.16±2.75	2.57±0.04[d]	7.92±0.02[d]	8.82±0.33[d]
	35	54.67±2.31	5.49±0.02[d]	47.22±6.62	2.68±0.03[d]	8.17±0.05[d]	6.46±0.83[d]
	40	68.0±4.0	5.78±0.06[d]	66.86±4.46	2.89±0.02[d]	8.66±0.06[d]	3.82±0.49[d]
	45	82.67±2.31	6.16±0.02[d]	93.33±11.55	3.08±0.08[d]	9.24±0.07[d]	0.72±1.24[d]
	50	100.0±0.0	---	---	---	---	---

See footnote of table (1).

TABLE 3. Effect of *Rosa arabica* and *Eucalyptus citriodora* -synthesized copper nanoparticles on some biological aspects of *Culex antennatus.*

Copper Nanoparticles	Conc. (ppm)	Larval Mort. (%)	Larval Duration	Pupal Mort. (%)	Pupal Duration	Development Duration	Growth Index (a/b)
***Rosa arabica* synthesized-CuNPs**	Control	0.0±0.0	4.33±0.10[a]	0.0±0.0	2.04±0.13[a]	6.36±0.03[a]	15.72±0.08[a]
	10	25.33±2.31	4.94±0.15[d]	14.33±3.32	2.33±0.02[c]	7.27±0.17[d]	11.79±0.68[c]
	15	37.33±2.31	5.21±0.07[d]	21.39±4.57	2.40±0.03[d]	7.61±0.10[d]	10.33±0.52[d]
	20	45.33±2.31	5.34±0.02[d]	31.87±5.71	2.48±0.03[d]	7.82±0.03[d]	8.71±0.73[d]
	25	56.0±4.0	5.45±0.03[d]	42.93±8.62	2.56±0.11[d]	8.02±0.09[d]	7.13±1.15[d]
	30	68.0±4.0	5.71±0.02[d]	63.16±7.96	2.86±0.03[d]	8.57±0.03[d]	4.30±.92[d]
	35	85.33±2.31	6.10±0.05[d]	100.0±0.0	---	---	---
	40	100.0±0.0	---	---	---	---	---
***Eucalyptus citriodora* synthesized-CuNPs**	Control	0.0±0.0	4.33±0.11[a]	0.0±0.0	2.12±0.12[a]	6.45±0.13[a]	15.51±0.31[a]
	5	29.33±2.31	5.16±0.02[d]	17.0±0.57	2.42±0.04[c]	7.48±0.09[d]	10.96±0.05[d]
	10	40.0±4.0	5.31±0.01[d]	24.66±5.21	2.52±0.09[d]	7.72±0.13[d]	9.62±0.56[d]
	15	50.67±4.62	5.42±0.09[d]	35.67±8.47	2.62±0.06[d]	8.04±0.12[d]	7.99±0.95[d]
	20	60.0±4.0	5.56±0.05 [d]	51.13±9.84	2.75±0.04[d]	8.31±0.07 [d]	5.87±1.15[d]
	25	72.0±4.0	5.82±0.04[d]	72.42±10.45	2.94±0.02[d]	8.76±0.05[d]	3.14±1.17[d]
	30	89.33±2.31	6.22±0.02[d]	100.0±0.0	---	---	---
	35	100.0±0.0	---	---	---	---	---

See footnote of table (1).

TABLE 4. Lethal concentrations (LC) of *Rosa arabica* and *Eucalyptus citriodora* aqueous extracts and synthesized silver and copper nanoparticles against *Culex antennatus* third larval instar.

Tested plant species	Tested Nanoparticles	LC_{50} (LCL-UCL)	LC_{75} (LCL-UCL)	LC_{90} (LCL-UCL)
Rosa arabica	Aqueous extract	1188.01 (1112.82-1263.20)	1679.87 (1592.38-1767.36)	1975.0 (1879.73-2070.27)
	Synthesized AgNPs	38.40 (35.64-41.16)	49.36 (47.30-51.41)	55.93 (54.07-57.80)
	Synthesized CuNPs	21.07 (20.24-21.90)	31.29 (30.31-32.26)	37.41 (36.37-38.46)
Eucalyptus citriodora	Aqueous extract	883.68 (863.02 -904.33)	1290.68 (1262.35-1319.0)	1534.90 (1499.77-1569.96)
	Synthesized AgNPs	31.26 (28.86-33.66)	41.56 (39.72-43.40)	47.74 (46.20-49.27)
	Synthesized CuNPs	14.47 (11.97-16.96)	25.03 (23.70-26.35)	31.36 (30.36-32.36)

LCL: 95% Lower Confidence Limits; UCL: 95% Upper Confidence Limits. All values represented as ppm, part per million.

In addition, a decrease in the average number of hatched eggs as induced by aqueous extract from leaves of *R. arabica* was recorded, where it was 146.0±1.41, 151.0±3.61 and 156.75±0.96 eggs at 1750, 1500 and 1250 ppm, respectively vs. 213.20±2.20 eggs for the control. While the mean number of eggs hatchability by females resulted from larvae treated with *E. citriodora* leaves aqueous extract recorded 173.14±2.41, 164.86±2.27, 159.00±2.19, 151.80±2.59, 146.25±1.50 and 141.75±1.71 eggs at 400, 600, 800, 1000, 1200 and 1400 ppm vs. 212.50±1.72 eggs for the control. In addition, an increase in the percentage of sterility index for all resulted from females larvae treated with *E. citriodora* leaves aqueous extract emerged from treated larvae, where it was 18.52, 22.42, 25.18, 28.57, 31.18 and 33.29% at 400, 600, 800, 1000, 1200 and 1400 ppm, respectively (**Table 5**).

Synthesized AgNPs and CuNPs:

Silver nanoparticles synthesized using *R. arabica* extract exhibited a significant effect on female fecundity at all concentrations used, where the fecundity was 169.25±1.50, 174.20±1.30 and 179.43±1.13 eggs/♀ at the concentrations of 50, 45 and 40 ppm, vs. 212.60±1.58 eggs/♀ for the control. Also, at 35, 40 and 45ppm there was significant decrease in the mean number of eggs laid by females resulted from larvae treated with *E. citriodora*-AgNPs (171.83±1.47, 167.25±1.71 and 163.25±1.26 eggs/♀, vs. 214.10±1.52 eggs/♀ for the control). A decrease in the average number of hatched eggs as induced by *R. arabica* synthesized-AgNPs was recorded, where it was 141.50±0.58, 148.80±1.79 and 156.0±1.29 eggs at 50, 45 and 40 ppm, respectively, vs. 210.10±1.37 eggs for the control. The sterility index for females resulted from larvae treated with *E. citriodora*- synthesized AgNPs was 39.79% at the highest concentration (45 ppm) decreased to 13.81% at the lowest concentration (20 ppm) (**Table 6**).

On the other hand, the fecundity of females resulted from larvae treated with *R. arabica*-synthesized CuNPs was significantly decreased from 202.20±1.40 eggs/♀ at the lowest concentration (10 ppm) to 163.33±1.53 eggs/♀ at the highest concentration (30 ppm), compared with 219.30±1.49 eggs/♀ for control. Meanwhile, at 25, 20, 15, 10 and 5 ppm of *E. citriodora*- synthesized CuNPs, there was significant ($P<0.001$) decrease in the mean number of eggs laid (156.33±0.58, 163.0±1.58, 171.86±1.35, 179.11±1.2 and 196.40±2.17 eggs/♀, vs 216.90±1.79 eggs/♀ for the control). Also, there was significant decrease in the hatchability percent of eggs laid by females resulted from treated larvae, where it was 81.84 and 90.46% at the highest and lowest concentrations (30 and 10 ppm) of *R. arabica*-synthesized CuNPs, respectively compared with 98.22% for the control group. In addition, a remarkable reduction in the hatchability percent for eggs laid by females resulted from treated larvae with *E. citriodora*- synthesized CuNPs was recorded, where the hatchability percent recorded 75.48% at 25 ppm, compared with 98.80% for the control group. The sterility index for females resulted from larvae treated with *E. citriodora*- synthesized CuNPs was 44.94% at the highest concentration (25 ppm) decreased to 17.96% at the lowest concentration (5 ppm), respectively (**Table 7**).

TABLE 5. Effect of aqueous extract from leaves of *Rosa arabica* and *Eucalyptus citriodora* on reproductive potential of *Culex antennatus* resulted female.

Tested Extracts	Con. (ppm)	No. of tested females	Eggs laid		Hatched eggs			Sterility Index (SI) %
			Total	Mean±SD	Total	Mean±SD	%	
***Rosa arabica* Aqueous extract**	Control	10	2171	217.10±1.91[a]	2132	213.20±2.20[a]	98.20±0.46[a]	0.0
	250	9	1848	205.33±1.22[d]	1721	191.22±0.97[d]	93.13±0.54[d]	10.31
	500	9	1765	196.11±1.62[d]	1599	177.67±1.58[d]	90.60±0.50[d]	16.67
	750	7	1331	190.14±1.77[d]	1187	169.57±2.51[d]	89.18±0.82[d]	20.47
	1000	6	1113	185.50±1.87[d]	979	163.17±2.32[d]	87.96±0.59[d]	23.47
	1250	4	725	181.25±1.71[d]	627	156.75±0.96[d]	86.49±0.61[d]	26.47
	1500	3	532	177.33±2.52[d]	453	151.0±3.61[d]	85.14±1.00[d]	29.18
	1750	2	349	174.50±0.71[d]	292	146.0±1.41[d]	83.67±1.15[d]	31.52
***Eucalyptus citriodora* Aqueous extract**	Control	10	2143	214.30±1.34[a]	2125	212.50±1.72[a]	99.16±0.37[a]	0.0
	200	9	1810	201.11±2.03[d]	1664	184.89±2.26[d]	91.93±0.49[d]	12.99
	400	7	1353	193.29±2.21[d]	1212	173.14±2.41[d]	89.58±0.56[d]	18.52
	600	7	1310	187.14±2.41[d]	1154	164.86±2.27[d]	88.09±0.90[d]	22.42
	800	6	1100	183.33±1.97[d]	954	159.0±2.19[d]	86.73±0.58[d]	25.18
	1000	5	892	178.40±2.07[d]	759	151.80±2.59[d]	85.09±0.87[d]	28.57
	1200	4	698	174.50±1.29[d]	585	146.25±1.50[d]	83.81±0.54[d]	31.18
	1400	4	682	170.50±1.29[d]	567	141.75±1.71[d]	83.14±0.59[d]	33.29

See footnote of table (1).

TABLE 6. Effect of *Rosa arabica* and *Eucalyptus citriodora* -synthesized silver nanoparticles on reproductive potential of *Culex antennatus* resulted female.

Silver Nanoparticles	Con. (ppm)	No. of tested females	Eggs laid		Hatched eggs			Sterility Index (SI) %
			Total	Mean±SD	Total	Mean±SD	%	
***Rosa arabica* synthesized-AgNPs**	Control	10	2126	212.60±1.58[a]	2101	210.10±1.37[a]	98.82±0.24[a]	0.0
	25	10	2073	207.30±1.16[c]	1902	190.20±1.32[d]	91.75±0.56[d]	9.47
	30	9	1703	189.22±1.48[d]	1531	170.11±1.76[d]	89.90±0.43[d]	19.03
	35	9	1651	183.44±1.01[d]	1460	162.22±2.17[d]	88.43±0.91[d]	22.79
	40	7	1256	179.43±1.13[d]	1092	156.0±1.29[d]	86.94±0.53[d]	25.75
	45	5	871	174.20±1.30[d]	744	148.80±1.79[d]	85.42±0.67[d]	29.18
	50	4	677	169.25±1.50[d]	566	141.50±0.58[d]	83.61±0.42[d]	32.65
***Eucalyptus citriodora* synthesized-AgNPs**	Control	10	2141	214.10±1.52[a]	2122	212.20±1.48[a]	99.11±0.34[a]	0.0
	20	9	1813	201.44±1.59[d]	1646	182.89±1.27[d]	90.79±0.62[d]	13.81
	25	8	1474	184.25±1.39[d]	1287	160.88±1.96[d]	87.31±0.77[d]	24.19
	30	8	1417	177.13±1.73[d]	1213	151.63±2.45[d]	85.60±0.63[d]	28.55
	35	6	1031	171.83±1.47[d]	868	144.67±0.52[d]	84.19±0.55[d]	31.82
	40	4	669	167.25±1.71[d]	547	136.75±0.96[d]	81.77±0.63[d]	35.55
	45	4	653	163.25±1.26[d]	511	127.75±0.50[d]	78.26±0.89[d]	39.79

See footnote of table (1).

TABLE 7. Effect of *Rosa arabica* and *Eucalyptus citriodora* -synthesized copper nanoparticles on reproductive potential of *Culex antennatus* resulted female.

Copper Nanoparticles	Con. (ppm)	No. of tested females	Eggs laid		Hatched eggs			Sterility Index (SI) %
			Total	Mean±SD	Total	Mean±SD	%	
***Rosa arabica* synthesized-CuNPs**	Control	10	2193	219.30±1.49^{a}	2154	215.40±1.07^{a}	98.22±0.68^{a}	0.0
	10	10	2022	202.20±1.40^{d}	1829	182.90±1.37^{d}	90.46±0.77^{d}	15.09
	15	8	1471	183.88±1.81^{d}	1300	162.50±2.0^{d}	88.38±0.88^{d}	24.56
	20	7	1248	178.29±1.80^{d}	1086	155.14±2.27^{d}	87.02±0.76^{d}	27.98
	25	6	1034	172.33±1.21^{d}	881	146.83±2.32^{d}	85.20±0.87^{d}	31.84
	30	3	490	163.33±1.53^{d}	401	133.67±1.53^{d}	81.84±0.36^{d}	37.95
***Eucalyptus citriodora* synthesized-CuNPs**	Control	10	2169	216.90±1.79^{a}	2143	214.30±1.70^{a}	98.80±0.32^{a}	0.0
	5	10	1964	196.40±2.17^{d}	1758	175.80±2.20^{d}	89.51±0.77^{d}	17.96
	10	9	1612	179.11±1.27^{d}	1389	154.33±3.28^{d}	86.16±1.41^{d}	27.99
	15	7	1203	171.86±1.35^{d}	1016	145.14±1.35^{d}	84.46±0.61^{d}	32.27
	20	5	815	163.0±1.58^{d}	666	133.20±2.39^{d}	81.72±1.19^{d}	37.85
	25	3	469	156.33±0.58^{d}	354	118.0±2.0^{d}	75.48±1.05^{d}	44.94

See footnote of table (1).

Discussion

Obtained results evoked that the Transmission Electron Microscopy (TEM) image sizes of AgNPs synthesized using aqueous extract from leaves of *Rosa arabica* and *Eucalyptus citriodora* are ranged between 14.3 and 40.70 nm, while sizes of CuNPs ranged between 19.60 and 69.40 nm, respectively. The sizes of synthesized nanoparticles confirm the previously reported using *Euphorbia hirta* in AgNPs preparation [23], *Morinda tinctoria* leaf extract in AgNPs preparation [24], petroleum ether extract from leaves of *L. camara* in CuNPs preparation [11], and aqueous extract from leaves of *L. siceraria* in AgNPs preparation [17].

Also, UV–vis Spectrophotometric analysis for both AgNPs and CuNPs showed the occurrence of a single absorption peak at specific wavelengths ranging from 300 to 450 nm, indicating the presence of spherical-shaped NPs and such results come in agreement with previously recorded reports [13, 25, 26].

As exhibited from the results, the activity of synthesized AgNPs and CuNPs using aqueous extracts from leaves of *R. arabica* and *E. citriodora* against *C. antennatus* increased as the concentration increased. Based on LC_{50}, LC_{75} and LC_{90} calculated values, *E. citriodora*- aqueous extract and its synthesized- AgNPs and CuNPs were more effective against *C. antennatus* larvae than those of *R. arabica*. In 2015, Subramaniam *et al.* attributed the high efficacy of plant-synthesized NPs against mosquito larvae to their ability to premeate the exoskeleton, penetrating into insects' cells, where they restrict macromolecules like proteins and DNA, changing their structure and therefore their function [27]. Generally, these results are in consistent with results recorded for AgNPs synthesized using *Tinospora cordifolia* against *C. quinquefasciatus* [28], AgNPs synthesized using *Nerium oleander* aqueous extract against *An. stephensi* [29], AgNPs synthesized using *Ambrosia arborescens* against *Ae. aegypti* [30], CuNPs synthesized using *L. camara* leaves aqueous extract against *An. multicolor* [11], AgNPs synthesized using *L. siceraria* leaves aqueous extract against *C. pipiens* and *An. pharoensis* larvae [17], CuNPs synthesized using *Coffee Arabica* against *Ae. aegypti* [31] and for and CuNPs synthesized using *nepeta cataria* leaves extract against *Ae. aegypti* [26].

Conclusions

In summary, the present work evaluated the efficacy of *Rosa arabica* and *Eucalyptus citriodora*-synthesized silver and copper nanoparticles (AgNPs and CuNPs) against the Rift Valley fever vector, *Culex antennatus*. The *R. arabica* and *E. citriodora*-synthesized AgNPs and CuNPs dispersed uniformly in water and had a significant efficacy against *C. antennatus* in different immature stages, as well as females resulting from treated larvae. In addition, more studies on the activity of green-synthesized AgNPs and CuNPs against other different mosquito species are desired.

Acknowledgment

The authors would like to thank all the staff of Zoology and Entomology Department, Faculty of Science, Al-Azhar University, Egypt who provided help during the research.

Conflicts of interest

The authors declare no conflict of interest.

Funding statement

This study was self-funded

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التخليق الحيوي لجسيمات الفضة والنحاس النانوية باستخدام مستخلصي نبات الورد العربي ونبات الكافور الليموني وفعاليته البيولوجية ضد بعوضة كيولكس انتيناتس (ثنائية الأجنحة: كيوليسيدى)

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تعتبر مكافحة بعوضة كيولكس انتيناتس استراتيجية مهمة للقضاء على انتقال الأمراض. تطبيق جزيئات الفضة والنحاس النانوية المخلقة خضرياً في مكافحة البعوض له العديد من المزايا الصديقة للبيئة بسبب عدم وجود مواد كيميائية مميتة في تخليقها. تناولت الدراسة الحالية النشاط البيولوجى للمستخلص المائي لأوراق نباتي الورد العربي والكافور الليموني وجزيئات الفضة والنحاس النانوية المخلقة باستخدام هذه المستخلصات ضد المراحل اليافعة المختلفة لبعوضة كيولكس انتيناتس، بالإضافة الي الكفاءة التكاثرية للإناث الناتجة من اليرقات المعاملة. وجد أن المستخلص المائي من أوراق نباتي الورد العربي والكافور الليموني يمكن أن يقلل من حجم جزيئات الفضة والنحاس لتوليد جزيئات فضة ونحاس نانوية معلقة في الماء. أظهرت نتائج المجهر الإلكتروني النافذ وجود جزيئات فضة نانوية بأحجام تتراوح بين 14.3 و40.70 نانومتر، في حين تراوحت أحجام جزيئات النحاس النانوية بين 19.60 و69.40 نانومتر تقريباً. كشف التحليل الطيفي بالأشعة فوق البنفسجية لجزيئات الفضة والنحاس النانوية المخلقة عن وجود ذروة امتصاص فردية عند أطوال موجية محددة تتراوح من 300 إلى 450 نانومتر، مما يشير إلى وجود جزيئات نانوية كروية الشكل. ايضاً، جزيئات الفضة والنحاس النانوية المخلقة باستخدام المستخلص المائي لأوراق نباتي الورد العربي والكافور الليموني كانت أكثر فعالية ضد بعوضة كيولكس انتيناتس، إلا أن جزيئات النحاس النانوية كانت أكثر فعالية من جزيئات الفضة النانوية. سجل التركيز النصفي المميت 38.40 و31.26 جزء في المليون بالنسبة لجزيئات الفضة النانوية ضد الطور اليرقي الثالث لبعوضة كيولكس انتيناتس، بينما سجلت جزيئات النحاس النانوية تركيز نصفي مميت يساوي 21.07 و14.47 جزء في المليون تقريباً. بالإضافة الي ذلك، جزيئات الفضة والنحاس النانوية المخلقة أدت إلى تقليل خصوبة اناث بعوضة كيولكس انتيناتس الناتجة من اليرقات المعاملة مقارنةً بالمجموعات غير معاملة.

الكلمات الدالة: كيولكس انتيناتس، الورد العربي، الكافور الليموني، التخليق الحيوي، جزيئات الفضة النانوية، جزيئات النحاس النانوية، مبيد اليرقات، الكفاءة التكاثرية.