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Research Article

Comparison Between Insecticidal Activity of *Lantana camara* Extract and its Synthesized Nanoparticles Against Anopheline mosquitoes

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Abstract

Background and Objective: Malaria is still a severe health problem especially in developing countries which occur and young children are the most affected. The present study was designed to compare the insecticidal potential of *Lantana camara* leaves extract alone and its synthesized nanoparticles against *Anopheles multicolor*. **Materials and Methods:** Copper nanoparticles CuNPs were synthesized by green bio-reduction method using aqueous extract of leaves of *Lantana camara* plant. The CuNPs formation was confirmed by ultraviolet visible spectrophotometer (UV-VIS) and Transitional Electron Microscopy (TEM). The application of *L. camara* extract and its synthesized CuNPs on different stages of *A. multicolor* were adopted. **Results:** The biosynthesized CuNPs were spherical with the average sizes of 11-17.8 nm. The highest insecticidal effect (100% of larval mortality) achieved at high dose (140 ppm) of *L. camara* leaves extract alone comparing with that occur at low dose (20 ppm) of CuNPs synthesized *L. camara* leaves. The LC₅₀ and LC₉₀ for the 4 instar larvae were 63.5 and 119.9 ppm for plant extract alone compared to 12.6 and 18.4 ppm for CuNPs preparation. **Conclusion:** So, this study proved that CuNPs preparation of *L. camara* leaves is highly efficient compared to the plant extract alone and more economic as less quantities were used. Also, awareness against invasion of *Anopheles* mosquito vectors with effective preventive measures to protect from malaria infection.

Key words: Malaria, copper nanoparticles (CuNPs), *Lantana camara*, insecticidal potential, *Anopheles multicolor*, larval mortality

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malaria is the world's most important vector-borne disease. Many parts of sub-Saharan Africa, south and southeast Asia and central and south America are endemic with human malaria¹. The WHO African Region responsible for the global malaria burden outbreak declaration. In Malawi was highly endemic in almost all parts of Malawi, in 1960; infection rates reached up to 20% in some localities². A severe epidemic of falciparum malaria (76.5% positive in 2834 blood films examined in 1942) appeared in the area at that time and resulted in 180,000 death³. However, prevalence has shown a steady decrease in most of the Governorates by 1990 and focused mainly in El Fayoum with sporadic cases from Siwa oasis and some parts of the Nile Delta. No local cases were reported at the end of 1998, but the risk of subsequent localized outbreaks of malaria exists^{4,5}. Egypt is prone to the reintroduction of malaria as a result of climatic factors and environmental change, the previous history of the disease, the vast and dynamic geographic distribution of vectors, population movement, rapid urbanization and the extensive use of water. This is in addition to the fact that Egypt shares borders with malaria-endemic countries⁶.

Although the worldwide use of insecticide applications contributed to Anopheles control in different country regions, most of these applications, especially those relying on a synthetic organic compound usage as DDT, bypassed several important environmental and ecological considerations⁷. The use of whole plant extract or live plants and plant tissue for reducing metal salts to nanoparticles have favorable considerable attention within the last 30 years⁸. The biosynthesized nanomaterials carried out by different plant extracts have been reliable controlling the various endemic diseases with less adverse effect (e.g., Malaria). Where the plant extract contains various secondary metabolites, it acts as reducing and stabilizing agents for the bio reduction reaction to synthesized novel metallic nanoparticles. Moreover, the biological synthesis of metallic nanoparticles is inexpensive, need only of single step and eco-friendly methods. The plants are used successfully in the synthesis of common different greener nanoparticles such as; copper, silver, gold etc. which applicable widely in the vector control programs^{9,10}.

Lantana camara are found along roadsides, in degraded lands, in riparian zones (banks of water courses), in pastures, along fence lines and parklands, in plantations, forest edges and gaps. The young stems are usually green

and square-shaped¹¹. Some conducted studies have found that *L. camara* leaves can display antimicrobial, fungicidal and insecticidal properties¹². The *L. camara* plant has settled areas of Africa, southern Europe, such as; Spain and Portugal, the Middle East, India, tropical Asia, Australia, New Zealand and USA, as well as many Atlantic, Pacific and Indian Ocean islands¹³. This work was aimed to compare the insecticidal potential of *Lantana camara* leaves extract alone and its CuNPs against *Anopheles multicolor* collected from El Fayoum Governorate, Egypt to evaluate the effect of the nanotechnology in controlling malaria mosquito vector.

MATERIALS AND METHODS

Study design and area: This cross-section study was performed between November, 2018 and February, 2019. The study adopted at El-Fayoum Governorate and experiments in Medical Entomology Animal House, Department of Zoology, Faculty of Science, Al-Azhar and Department of Zoonotic Diseases, National Research Center, Cairo, Egypt.

Tested mosquitoes: Larvae of *Anopheles multicolor* were collected from Nazlah-Dobaar, Ebshway Center, El-Fayoum Governorate and reared for several generations in Medical Entomology insectary, Animal house under controlled conditions of temperature ($27\pm 2^\circ\text{C}$), relative humidity ($70\pm 10\%$) and light-dark regime (12-12 h). Adult mosquitoes were kept in ($30\times 30\times 30$ cm) wooden cages and were provided daily with cotton pieces soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host, which is necessary for laying eggs (autogamy). Plastic cup oviposition (15×15 cm) containing distilled water was placed in the cage for laying eggs. The resulting egg rafts picked up from the plastic dish and transferred into plastic pans ($25\times 30\times 15$ cm) containing 32 L of distilled water. The hatching larvae were provided daily with a piece of bread as a diet which was found to be the most preferable food for the larval development and a well female fecundity¹⁴.

Preparation of *Lantana camara* plant extract: Leaves of *Lantana camara* after collected from Sadat city, Cairo-Alexandria desert road, away from sun rays were left to dry at room temperature ($27-31^\circ\text{C}$) for 10 days and pulverized to powder separately in a hammer mill. One hundred grams of

powder were extracted and filtered five times using 300 mL of petroleum ether at room temperature solvent (40-60). After 24 h, the supernatants were decanted, filtrated through Whatman filter paper (No. 5) and dried in a rotary evaporator at 40°C for 40-60 min and dry extract was weighed and kept at -4°C till using for experiments¹⁵.

Experimental bioassay: The prepared dry extract of *L. camara* leaves was dissolved in 2 drops of Tween80 as emulsifier and then it was dissolved in 250 mL of distilled water contained in 350 mL plastic cups. Then, the 3rd instar larvae (25 larvae) were put immediately into plastic cups contained different concentrations of extract. Three replicates were usually used for each tested concentration. All plastic cups were incubated under the previous controlled conditions and subsequently mortality was recorded daily, dead larvae and pupae removed until adult emergence. All values calculated as Mean±SD and control larvae received 2 drops of Tween80 in 250 mL water¹⁶.

The larvae were observed daily until pupation and adult emergence to estimate the following parameters; larvicidal activity, larval mortality percentage and larval duration. Larval duration was calculated for each larva as the intervals between the commencement of first instar larvae and the commencement of pupation and then the mean value was taken, pupal duration was also calculated for each pupa as the interval between of pupation and the commencement of adult emergence. The emerged males and females adults were counted. The pupation rate, pupal mortality percentage, adult emergence percentage and the growth index were estimated according to equations mentioned by Briggs¹⁷.

Green Synthesis of Copper Nanoparticles (CuNPs): A fresh leaf of *L. camara* broth solution was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300 mL Erlenmeyer flask along with 100 mL of sterilized double-distilled water and then boiling the mixture for 5 min before finally decanting it, the extract was filtered with Whatman filter paper No. 1 and stored at -15°C until use. The filtrate was treated with 1 mM aqueous CuSO₄.5H₂O solution in a 250 mL Erlenmeyer flask and incubated at room temperature. About 88 mL aqueous solution of 1 mM of copper sulfate was reduced using 12 mL of leaf extract at room temperature overnight, resulting in a brown solution indicating the formation of CuNPs. The obtained CuNPs solution was purified by repeated centrifugation at 12,000 rpm and characterized by transmission electron microscope and UV-VIS spectrometry¹⁸.

Application of *L. camara*-synthesized CuNPs on different stages of *A. multicolor*: About 2 mL of *L. camara*-synthesized CuNPs was diluted in 100 mL distilled water for the preparation of 2% (v/v) stock solution. Then, the tested concentrations were prepared by subsequent dilution of stock solution in distilled water contained in 350 mL plastic cups¹⁰. The 3rd instar larvae (25 larvae) were put immediately into plastic cups contained different concentrations of CuNPs. Three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions at temperature 27±2°C, RH 70±10%, 12-12 light-dark regime and mortality was recorded daily, dead larvae and pupae were removed until adult emergence. All values calculated were as Mean±SD. The *L. camara*-synthesized CuNPs activity was tested against immature stages (different larval stages and pupae) the mortality was recorded after 24 h. Adulticidal bioassay was performed using the procedure described by Muthukumar *et al.*⁹, different concentrations of CuNPs (20, 40, 60, 80, 100, 120 and 140 ppm) was prepared. Tested concentrations of CuNPs were applied on Whatman No. 1 filter papers (size 12×15 cm). Control papers were treated with distilled water.

The bioassay was conducted using two cylindrical plastic tubes both measuring 125×44 mm following the method described by Polson *et al.*¹⁹. Briefly, one tube served to expose the mosquitoes to CuNPs tested concentration and another tube was used to hold the mosquitoes before and after the exposure periods. Each tube was closed at one end with a 16 mesh size wire screen. Twenty five female mosquitoes were collected and transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper, which was rolled and placed in the exposure tube for 1 h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept for 24 h recovery period. Mortality of the mosquitoes was recorded after 24 h. The above procedure was carried out in triplicate for each concentration to get a mean value.

Statistical analysis: The average adult mortality data were subjected to probit analysis for calculating LD₅₀, LD₉₀ and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit and chi-square values were calculated using the Statistical Package of Social Sciences 12.0 software. Results with p<0.05 were considered to be statistically significant.

RESULTS

Effect of *Lantana camara* plant extract alone: The biological activity of petroleum ether extract of *L. camara* (leaves) against the 3rd instar larvae of *A. multicolor* showed that the highest larval mortality (100.0%) was caused by the concentration 140 ppm and the lowest mortality percentage 18.7% was caused by the lowest concentration 20 ppm compared with 9.3% for the control group. The mean duration period of larvae was significantly ($p < 0.01$) affected by all concentrations used as compared with the untreated group, while the extract showed the highest pupal mortality percentage (22.2%) was induced at 120 ppm and there was a reduction in the adult emergence percentage from the treated larvae at all concentrations used, as it recorded 77.8 and 96.7% at the highest and lowest concentrations (120 and 20 ppm), respectively, compared with 100.0% for the control group. The growth index recorded 7.9 and 8.6 vs. 20.0 for the untreated

group (Table 1). The L_{50} and L_{90} of the *L. camara* leaves extract alone against *A. multicolor* larvae were 63.5 and 119.9 ppm, respectively (Table 2).

Effect of *L. camara*-synthesized CuNPs on the different stages of *Anopheles multicolor*: The highest larval mortality (100.0%) was caused by the concentration 20 ppm and the lowest mortality percentage 8.0% was caused by the lowest concentration 6 ppm compared with 4.0% for the untreated group. Also, the mean duration period of larvae was significantly ($p < 0.01$) affected by all concentrations used as compared with the untreated group. The highest pupal mortality percentage (86.7%) was induced at 18 ppm. The adult emergence percent recorded 13.3 and 82.7% at the highest and lowest concentrations (18 and 6 ppm), respectively, compared with 100.0% for the control group. The growth index recorded 1.2, 2.9, 4.4, 5.1, 6.9, 8.5 and 10.7 at 18, 16, 14, 12, 10, 8 and 6 ppm vs. 20.0 for the untreated group (Table 3).

Table 1: Effect of petroleum ether extract from *Lantana camara* leaves on some biological aspects of different stages of *Anopheles multicolor*

Concentration (ppm)	Mean \pm SD						
	LM (%)	LP (days)	PM (%)	PP (days)	AE (%)	DP (days)	GI
140	100.0 \pm 0.0	-	-	-	-	-	-
120	89.3 \pm 2.3	6.6 \pm 0.12 ^d	22.2 \pm 3.2	3.2 \pm 0.29 ^c	77.8 \pm 3.2	9.8 \pm 0.41 ^d	7.9
100	78.7 \pm 4.6	6.3 \pm 0.15 ^d	19.5 \pm 4.8	3.1 \pm 0.70 ^b	80.5 \pm 4.8	9.4 \pm 0.85 ^d	8.6
80	72.0 \pm 4.0	6.0 \pm 0.40 ^d	8.9 \pm 1.8	2.8 \pm 0.38 ^a	91.1 \pm 1.8	8.8 \pm 0.78 ^d	10.4
60	42.7 \pm 2.3	6.0 \pm 0.22 ^d	4.7 \pm 2.1	2.1 \pm 0.57 ^a	95.3 \pm 4.1	8.1 \pm 0.79 ^c	11.8
40	30.7 \pm 2.3	6.0 \pm 0.38 ^d	3.8 \pm 1.3	1.9 \pm 0.10 ^a	96.2 \pm 1.3	7.9 \pm 0.48 ^c	12.2
20	18.7 \pm 1.9	5.5 \pm 0.71 ^c	3.3 \pm 1.2	1.9 \pm 0.21 ^a	96.7 \pm 1.2	7.4 \pm 0.92 ^b	13.1
Control	9.3 \pm 2.3	3.3 \pm 0.96 ^a	0.0 \pm 0.0	1.7 \pm 0.26 ^a	100.0 \pm 0.0	5.0 \pm 1.22 ^d	20.0

No. of tested larvae: 25 per one replicate, LM: Larval mortality, LP: Larval period, PM: Pupal mortality, PP: Pupal period, AE: Adult emergence, DP: Development period, GI: Growth index, Concentration (PPM): Concentration of particle per million, SD: Standard deviation, ^aNon-significant ($p > 0.05$), ^bSignificant ($p < 0.05$), ^cHighly significant ($p < 0.01$), ^dVery highly significant ($p < 0.001$)

Table 2: Lethal concentrations of petroleum ether extract from *Lantana camara* leaves against *Anopheles multicolor* larvae

Concentrations	Value (ppm)	Regression equation	R ² -value
LC ₅₀	63.5	$y = 0.7091x + 5$	0.9719
LC ₉₀	119.9		

Table 3: Effect of *Lantana camara*-synthesized CuNPs on some biological aspects of *Anopheles multicolor*

Concentration (ppm)	Mean \pm SD						
	LM (%)	LP (days)	PM (%)	PP (days)	AE (%)	DP (days)	GI
20	100.0 \pm 0.0	-	-	-	-	-	-
18	89.3 \pm 8.3	7.9 \pm 0.12 ^d	86.7 \pm 6.1	3.3 \pm 0.09 ^d	13.3 \pm 6.1	11.2 \pm 0.21 ^d	1.2
16	74.7 \pm 2.3	7.5 \pm 0.21 ^d	69.8 \pm 6.8	2.9 \pm 0.12 ^d	30.2 \pm 6.8	10.4 \pm 0.33 ^d	2.9
14	58.7 \pm 6.1	7.3 \pm 0.20 ^d	56.1 \pm 12.9	2.7 \pm 0.10 ^d	43.9 \pm 12.9	10.0 \pm 0.30 ^d	4.4
12	46.7 \pm 6.1	7.1 \pm 0.53 ^d	51.3 \pm 10.2	2.5 \pm 0.07 ^d	48.7 \pm 10.3	9.6 \pm 0.60 ^d	5.1
10	28.0 \pm 4.0	6.7 \pm 0.86 ^d	37.1 \pm 4.0	2.4 \pm 0.05 ^d	62.9 \pm 4.0	9.1 \pm 0.91 ^d	6.9
8	18.7 \pm 6.1	5.8 \pm 0.36 ^d	32.1 \pm 6.2	2.2 \pm 0.15 ^c	67.9 \pm 6.2	8.0 \pm 0.51 ^d	8.5
6	8.0 \pm 4.0	5.6 \pm 0.29 ^d	17.3 \pm 0.8	2.1 \pm 0.31 ^b	82.7 \pm 0.8	7.7 \pm 0.60 ^d	10.7
Control	4.0 \pm 0.0	3.3 \pm 0.20 ^a	0.0	1.7 \pm 0.06 ^a	100.0 \pm 0.0	5.0 \pm 0.26 ^a	20.0

No. of tested larvae: 25 per one replicate, LM: Larval mortality, LP: Larval period, PM: Pupal mortality, PP: Pupal period, AE: Adult emergence, DP: Development period, GI: Growth index, Concentration (PPM): Concentration of particle per million, SD: Standard deviation, ^aNon-significant ($p > 0.05$), ^bSignificant ($p < 0.05$), ^cHighly significant ($p < 0.01$), ^dVery highly significant ($p < 0.001$)

Table 4: Lethal concentrations of *Lantana camara*-synthesized CuNPs against larvae, pupae and adult of *A. multicolor*

Stages	Lethal concentrations value (ppm)		Regression equation	R ² value
	LC ₅₀	LC ₉₀		
<i>A. multicolor</i> larvae	12.6	18.4	y = 6.8399x-35.906	0.9954
<i>A. multicolor</i> pupae	25.7	40.3	y = 2.746x-20.676	0.9848
<i>A. multicolor</i> adult	83.7	137.1	y = 0.7496x-12.743	0.9972

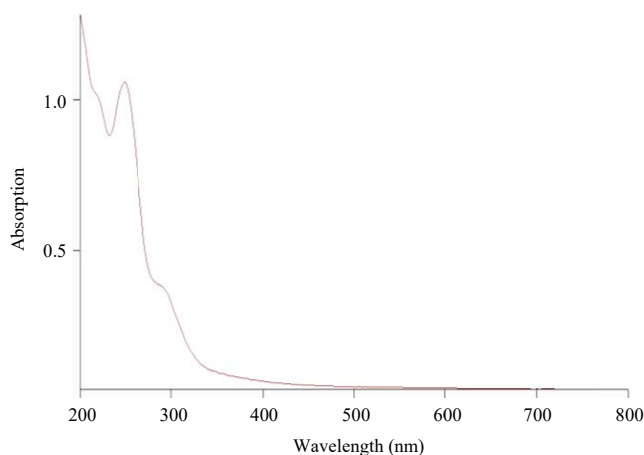


Fig. 1: UV-VIS spectra of CuNPs synthesized *Lantana camara* plant extract aqueous CuSO₄.5H₂O

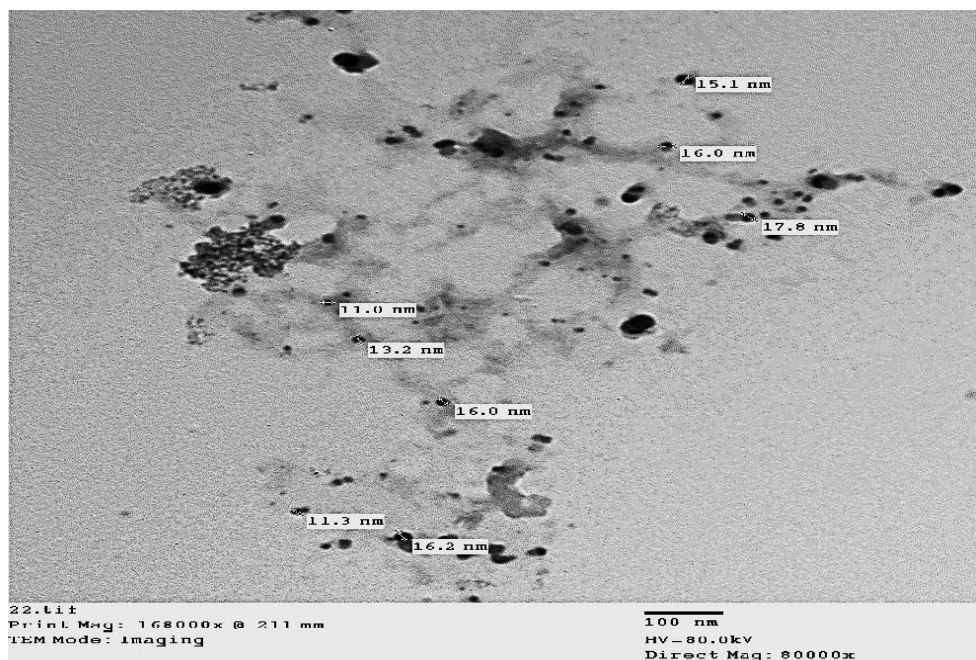


Fig. 2: TEM of CuNPs synthesized using *Lantana camara* plant aqueous extract

The averages of LC₅₀ and LC₉₀ were 12.6 and 18.4 ppm for the 4 instar larvae, 25.7 and 40.3 for pupae and 83.7 and 137.1 ppm for adults, respectively (Table 4).

Characterization of CuNPs preparation: The UV-VIS spectrometry recorded from nanoparticles solution

showed the characteristic surface plasmon resonance (SPR) spectra with absorbance at ca. 200-800 nm and peak maximum at 248 nm (Fig. 1). While the TEM of the synthesized CuNPs showed spherical, polydispersed particles and vary from 11-17.8 nm (Fig. 2).

DISCUSSION

Effective vector control tools that target malaria-carrying mosquitoes are very important in eradication of malaria from the globe². The progress has been made possible, in great part, through the wide-scale deployment of good effective vector control tools that target malaria-carrying mosquitoes²⁰. Green synthesis of CuNPs is of heavy interest because of its widespread advantages. Copper has been known to be non-toxic to mammals. In addition, Copper is highly conductive and also cheaper than silver and gold. The CuNPs are very reactive due to their surface-to-volume ratio and can easily interact with other particles. CuNPs have very wide ranges of applications like antifouling, biocidal and, catalytic activity²¹⁻²³.

The biocidal effect of tested plant (*Lantana camara*) extract alone in this study showed a high larval mortality percentage, this finding come in agreement with the previous results used extract from leaves of *Ocimum basilicum*, widely grown medicinal plant²⁴. The high concentrations of tested extract resulted significantly extended the larval and pupal durations, these finding are in agreement with that obtained results by using *Pelargonium citrosa* leaf methanol extract against *A. stephensi* larvae²⁵ and also by using the neem, *Azadirachta indica* extract against *A. stephensi* larvae²⁶. The decrease in the percentage of mosquito vector, *A. multicolor* adult emergence due to treatment with the tested *L. camara* plant extract is similar to the results obtained previously by using the petroleum ether extracts of *Artemisia annua* against *A. stephensi* and *C. quinquefasciatus* larvae²⁷. The retardation in *A. multicolor* growth recorded by the *L. camara* extract in this study was decreased as the concentration of the extract increased, such results are in consistent with previously studies using different plant extracts against some dipteran species by using *Pelargonium citrosa* leaf extracts on *A. stephensi*²⁵ and by using *Tribulus terrestris* methanolic extract against the malarial vector, *A. arabiensis*²⁸.

The biocidal activity of *L. camara*-synthesized CuNPs in this study have significant larvicidal and adulticidal activity against *A. multicolor*. These results confirmed by the previous results in which different synthesized nanoparticles were used against different species of parasites; for example, copper nanoparticles (CuNPs) synthesized by polyol process from copper acetate revealed high mortality percentage of *A. subpictus* larvae²⁹, synthesized silver nanoparticle (AgNPs) from *Heliotropium indicum* have a biocidal activity on adult *A. stephensi* and recorded LD₅₀ and LD₉₀ of 26.712 and 49.061 µg mL⁻¹, respectively³⁰ and the larvicidal activity of

AgNPs synthesized by using the stem of *Andrographis paniculata* reported that AgNPs were more effective on I instar as compare to II instars and III instars³¹.

In the present study, the highest larval mortality (100.0%) was achieved by 140 and 20 ppm of *L. camara* extract alone and its CuNPs preparation, respectively, the reduction in the adult emergence percentage among the adults developed from the treated larvae of 77.8 at 120 ppm for extract alone compared to 13.3% at 18 ppm for its CuNPs preparation and the LC₅₀ and LC₉₀ for the 4 instar larvae were 63.5 and 119.9 ppm for plant extract alone compared to 12.6 and 18.4 ppm for CuNPs preparation. These previous results obtained from this study indicated that CuNPs preparation is highly efficient compared to plant extract alone and more economic as less quantities were used. In the same context in another study, the synthesized AgNPs from *C. asiatica* plant were highly toxic on late 3rd instar larvae of *Anopheles stephensi* than crude leaf aqueous extract³².

CONCLUSION

This study evaluated and compared the effect *Lantana camara* leaf extract alone and its CuNPs preparation on the mosquito, malaria vector (*Anopheles multicolor*). The obtained results indicated that CuNPs preparation showed better biocidal activity against *A. multicolor* and is highly efficient compared to plant extract alone. Furthermore, it was concluded that the used bio reduced CuNPs is consistent and can be easily scaled up for the bulk production, more economic as less quantities were used rapid action and environment friendly without the generation of toxic by-products.

SIGNIFICANCE STATEMENT

This study introduced a good diagnostic efficacy to evaluate that CuNPs preparation of *L. camara* leaves is highly efficient compared to the plant extract alone and more economic as less quantities were used. Also, awareness and effective preventive measures against exposure to malaria infection should be enhanced with protection of Egyptian borders against invasion by *Anopheles* mosquito vectors.

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REFERENCES

- Gething, P.W., I.R. Elyazar, C.L. Moyes, D.L. Smith and K.E. Battle *et al.*, 2012. A long neglected world malaria map: *Plasmodium vivax* endemicity in 2010. PLoS Negl. Trop. Dis., Vol. 6. 10.1371/journal.pntd.0001814.
- WHO., 2017. World malaria report 2017. Global Malaria Programme, World Health Organization, Geneva, Switzerland.
- Shousha, A.T., 1948. Species-eradication: The Eradication of *Anopheles gambiae* from Upper Egypt, 1942-1945. Bull. World Health Organiz., 1: 309-352.
- El-Bahnasawy, M.M., H. Dabbous and T.A. Morsy, 2010. Imported malaria as a threat to Egypt. J. Egypt. Soc. Parasitol., 40: 773-788.
- CDC., 2013. CDC Health Information for International Travel 2014: The Yellow Book. Oxford University Press, UK, ISBN-13: 9780199948505, Pages: 640.
- WHO., 2006. Report on the fifth intrcountry meeting of national malaria programme managers-countries free of malaria or with residual transmission, Cairo, Egypt, 29-31 May, 2005. WHO-EM/MAL/317/E, World Health Organization, Regional Office for the Eastern Mediterranean, Cairo, Egypt.
- Owusu, H.F., N. Chitnis and P. Muller, 2017. Insecticide susceptibility of *Anopheles* mosquitoes changes in response to variations in the larval environment. Scient. Rep., Vol. 7. 10.1038/s41598-017-03918-z.
- Liu, Q., H. Liu, Z. Yuan, D. Wei and Y. Ye, 2012. Evaluation of antioxidant activity of chrysanthemum extracts and tea beverages by gold nanoparticles-based assay. Colloids Surf. B: Biointerfaces, 92: 348-352.
- Muthukumar, U., M. Govindarajan, M. Rajeswary, K. Veerakumar, A. Amsath and K. Muthukumaravel, 2016. Adulticidal activity of synthesized silver nanoparticles using *Chomelia asiatica* Linn. (Family: Rubiaceae) against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Int. J. Zool. Applied Biosci., 1: 118-129.
- Madhiyazhagan, P., K. Murugan, A.N. Kumar, T. Nataraj and J. Subramaniam *et al.*, 2017. One pot synthesis of silver nanocrystals using the seaweed *Gracilaria edulis*: Biophysical characterization and potential against the filariasis vector *Culex quinquefasciatus* and the midge *Chironomus circumdatus*. J. Applied Phycol., 29: 649-659.
- Thaman, R.R., 1974. *Lantana camara*: Its introduction, dispersal and impact on islands of the tropical Pacific Ocean. Micronesica J. Univ. Guam, 10: 17-39.
- Ved, A., T. Arsi, O. Prakash and A. Gupta, 2018. A review on phytochemistry and pharmacological activity of *Lantana camara* Linn. Int. J. Pharmaceut. Sci. Res., 9: 37-43.
- Meyer, J.Y., 2014. Critical issues and new challenges for research and management of invasive plants in the Pacific Islands. Pac. Conserv. Biol., 20: 146-164.
- Kasap, M. and L. Demirhan, 1992. The effect of various larval foods on the rate of adult emergence and fecundity of mosquitoes. Turkiye Parazitoloji Dergisi, 161: 87-97.
- Mukanga, M., Y. Deedat and F.S. Mwangala, 2010. Toxic effects of five plant extracts against the larger grain borer, *Prostephanus truncatus*. Afr. J. Agric. Res., 5: 3369-3378.
- WHO., 1973. Manual on larval control operations in malaria programs. WHO Division of Malaria and Other Parasitic Diseases, World Health Organization, Geneva, Switzerland.
- Harvey, T.L. and D.E. Howell, 1965. Resistance of the house fly to *Bacillus thuringiensis* Berliner. J. Invertebr. Pathol., 7: 92-100.
- Usha, S., K.T. Ramappa, S. Hiregoudar, G.D. Vasanthkumar and D.S. Aswathanarayana, 2017. Biosynthesis and characterization of copper nanoparticles from tulasi (*Ocimum sanctum* L.) leaves. Int. J. Curr. Microbiol. Applied Sci., 6: 2219-2228.
- Polson, K.A., W.G. Brogdon, S.C. Rawlins and D.D. Chadee, 2011. Characterization of insecticide resistance in Trinidadian strains of *Aedes aegypti* mosquitoes. Acta Trop., 117: 31-38.
- World Health Organization, 2018. Malaria vaccine: WHO position paper, January 2016-recommendations. Vaccine, 36: 3576-3577.
- Iakovidis, I., I. Delimaris and S.M. Piperakis, 2011. Copper and its complexes in medicine: A biochemical approach. Mol. Biol. Int., Vol. 2011. 10.4061/2011/594529.
- Nadagouda, M.N., T.F. Speth and R.S. Varma, 2011. Microwave-assisted green synthesis of silver nanostructures. Accounts Chem. Res., 44: 469-478.
- Jung, J.H., H.C. Oh, H.S. Noh, J.H. Ji and S.S. Kim, 2006. Metal nanoparticle generation using a small ceramic heater with a local heating area. J. Aerosol Sci., 37: 1662-1670.
- Maurya, P., P. Sharma, L. Mohan, L. Batabyal and C.N. Srivastava, 2009. Evaluation of the toxicity of different phytoextracts of *Ocimum basilicum* against *Anopheles stephensi* and *Culex quinquefasciatus*. J. Asia-Pac. Entomol., 12: 113-115.
- Jeyabalan, D., N. Arul and P. Thangamathi, 2003. Studies on effects of *Pelargonium citrosale* leaf extracts on malarial vector, *Anopheles stephensi* Liston. Bioresour. Technol., 89: 185-189.
- Nathan, S.S., K. Kalaivani and K. Murugan, 2005. Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Acta Trop., 96: 47-55.

27. Sharma, P., L. Mohan and C.N. Srivastava, 2006. Growth inhibitory nature of *Artemisia annua* extract against *Culex quinquefasciatus* (Say). *J. Asia-Pac. Entomol.*, 9: 389-395.
28. El-Sheikh, T.M., H.A. Bosly and N. Shalaby, 2012. Insecticidal and repellent activities of methanolic extract of *Tribulus terrestris* L. (Zygophyllaceae) against the malarial vector *Anopheles arabiensis* (Diptera: Culicidae). *Egypt. Acad. J. Biol. Sci. A, Entomol.*, 5: 13-22.
29. Ramyadevi, J., K. Jeyasubramanian, A. Marikani, G. Rajakumar and A.A. Rahuman *et al.*, 2011. Copper nanoparticles synthesized by polyol process used to control hematophagous parasites. *Parasitol. Res.*, 109: 1403-1415.
30. Veerakumar, K., M. Govindarajan and S.L. Hoti, 2014. Evaluation of plant-mediated synthesized silver nanoparticles against vector mosquitoes. *Parasitol. Res.*, 113: 4567-4577.
31. Soam, P. and G. Parisa, 2018. Biosynthesis of pyramidal nanosilver using *Andrographis paniculata* (Burm.f.) and its efficacy against *Anopheles stephensi* (Liston) larvae in laboratory. *Int. J. Zool. Anim. Biol.*, Vol. 1, No. 3.
32. Muthukumar, U., M. Govindarajan and M. Rajeswary, 2015. Mosquito larvicidal potential of silver nanoparticles synthesized using *Chomelia asiatica* (Rubiaceae) against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res.*, 114: 989-999.