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Larvicidal Activity of Biosynthesized Zinc Nanoparticles (ZnO) against Dengue Fever Vector, *Aedes aegypti* Linnaeus (Diptera: Culicidae)

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ABSTRACT

Aedes aegypti is responsible for the transmission of several viruses to many vertebrates and humans such as yellow fever virus, chikungunya virus, and dengue fever virus. Controlling of *Ae. aegypti* is considered a paramount strategy in eliminating the spread of diseases. The present study was performed to investigate the larvicidal activity of zinc nanoparticles (ZnO-NPs) synthesized using an aqueous extract of *Lantana camara* leaves against dengue fever vector, *Ae. aegypti.* Synthesized ZnO-NPs were examined by transmission electron microscopy (TEM) and UV-Vis spectroscopy. Obtained results of TEM showed the occurrence of ZnO-NPs with sizes ranging between 12.2 and 25.3 nm. Results of LSPR showed the occurrence of a single absorption peak at the range of 356-360 nm indicating the presence of spherical-shaped ZnO-NPs. Aqueous extract from leaves of *L. camara* recorded 100.0 and 28.0% larval mortality at 400 and 100 ppm, respectively. While100.0 and 29.33% larval mortality were recorded at 35 and 5 ppm of synthesized ZnO-NPs, respectively. Treatment of larvae with *L. camara* synthesized ZnO-NPs induced a significant ($P<0.05$) prolongation in both larval and pupal durations at all concentrations used, as compared with control group. Generally, synthesized ZnO-NPs have a significant efficacy against *Ae. aegypti* larvae than *L. camara* leaves aqueous extract with LC_{50} and LC_{90} values of 14.85 and 30.41 ppm vs. 182.39 and 356.69 ppm for leaves aqueous extract, respectively. Overall, synthesized ZnO-NPs have a significant efficacy against the dengue fever vector, *Ae. aegypti* different stages than leaves aqueous extract.

INTRODUCTION

Among several mosquito species that have medical importance, *Aedes* species is becoming the most important one worldwide (Alikhan *et al.,* 2014). *Aedes* species, many animals and humans get arboviruses through mosquitoes, mainly *Ae. aegypti*. This includes diseases like yellow fever, chikungunya, and dengue (Al Ahmad *et al.,* 2011; Alikhan *et al.,* 2014), however, dengue fever virus is considered a prime arbovirus disease in KSA (Khater *et al.,* 2013).

The aquatic breeding habits of mosquitoes make their embryonic stages (eggs, larvae, and pupae) particularly vulnerable to synthetic chemical pesticides. Although these chemical insecticides are very efficient and have a wide range of action against a variety of mosquito species, their use in mosquito control is threatened by the emergence of resistance to these chemicals (Liu *et al.,* 2006), in addition to the risks it poses to ecosystems, non-target creatures, and human health (Hemingway and Ranson, 2000; Gold *et al.,* 2001). From

this vantage point, it is critical that a new environmentally friendly, effective, and cheap substance be developed to stop the proliferation of various mosquito species. The biosynthesis of nanoparticles and their potential uses in fields as diverse as medicine, environmental protection, disease prevention, and biotechnology have recently attracted a lot of attention (Elemike *et al.,* 2017; Hassanain *et al.,* 2019). Due to the lack of harmful chemicals used in their manufacture, synthetic nanoparticles offer various environmental benefits (Bhosale *et al.,* 2014). Therefore, continuing research into green-synthesized nanoparticles as potential novel mosquito control agents is crucial.

One of the plants used to cure TB is *Lantana camara,* which was employed in the current research (Kirimuhuzya *et al.,*2009), applications outside of herbal medicine include: mulch, hedges, firewood, and pest control (through microbicides, fungicides, nematicides, and insecticides) (Jimenez-Arellanes *et al.,* 2003; Kirimuhuzya *et al.,* 2009).

The present study was performed to investigate the larvicidal activity of ZnO-NPs synthesized using an aqueous extract of *L. camara* leaves against the dengue fever vector, *Ae. aegypti.*

MATERIALS AND METHODS

Aedes aegypti Colonization:

Aedes aegypti larvae were collected and kept for many generations at regulated temperatures (27±2^{o}C), relative humidities (75±5%), and photoperiods (11 light:13 dark) at AbbrAl_Wady Co. For Maintenance laboratory, Jizan city, Jazan Governorate, Kingdom of Saudi Arabia. Adult mosquitoes were housed in 30x30x30 cm wooden cages and fed cotton soaked in a 10% sucrose solution twice a day. In order to get the third instar larvae required for the experiment, the usual rearing protocol of Shehata *et al*., (2020) was used.

Preparation and Characterization of Zinc Nanoparticles (ZnONPs):

1.Preparation of *Lantana camara* Aqueous Extracts:

For five days, *Lantana camara* leaves were air-dried inside, out of direct sunlight. A stainless-steel electric blender was used to turn dried leaves into powder (Philips, HR2058). The powdered leaves of *L. camara*, amounting to around 50 grammes, were cooked with 100 milliliters of distilled water in a water bath for 10 minutes. After that, we filtered the solution and stored it in the fridge at 4^{o}C until use (Mondal *et al*., 2014).

2. Preparation of Zinc nanoparticles (ZnO-NPs):

About 5 grams of zinc nitrate (purchased from Algomhuria company for pharmaceuticals, chemicals, and medical supplies) was added to aqueous leaves extract at 60°C. The next step is to cook the mixture until it becomes a thick, golden paste. Overnight, the paste was heated in hot air at a temperature of less than 100 $^{\circ}$C, and collected in a ceramic crucible. We were able to collect and characterize a powder with a pale-yellow hue. (Poovizhi and Krishnaveni, 2015).

3. Characterization of Zinc nanoparticles (ZnO-NPs):

ZnO-NPs were analyzed for their size, shape, and optical characteristics using transmission electron microscopy (TEM) and ultraviolet-visible spectroscopy (UV-Vis).

3.1. Transmission Electron Microscopy (TEM): To generate a thin film of the sample solution, only a little bit of the sample was spread over a copper grid. After about a minute, the absorbent paper was used to soak up the remaining solution, and the grid was left to air dry before being analyzed. ZnO-NPs were analyzed by TEM using a JXA 840A electron probe microanalyzer to establish their shape and size (JEOL, Japan).

3.2. UV-Vis Spectroscopy:UV-Vis spectroscopy (T80+ UV/VIS Spectrometer, PG Instruments Ltd., UK) was used to examine the nanoparticles' optical characteristics from a wavelength range of 190 to 1,000 nm.

Experimental Bioassay:

The larvicidal activity of the ZnO-NPs was investigated using Hassanain *et al*. standard protocol (2019). To recap, in 500 ml plastic cups filled with dechlorinated tap water, various quantities of L. camara aqueous extract or ZnO-NPs were created. Twenty-five *Ae. aegypti* third-instar larvae were placed in plastic cups with *L. camara* aqueous extract or ZnO-NPs at varying concentrations. Each plastic cup was incubated with a mosquito colony until adulthood, at which point daily fatality rates were recorded. In most cases, three duplicates would be employed. The data is presented as a Mean±SD. The development of the larvae into pupae and adults was tracked every day. The growth index was computed as the ratio of the percentage of successful larval development to the average number of days it took to complete its metamorphosis into an adult (Hassanain*et al*., 2019).

Statistical Analysis:

The results were tabulated using Mean±SD. According to Bailey's recommendations, we used analysis of variance (ANOVA) to examine the data (1981). SPSS V.22 was used for data encoding and entry. Mean and standard deviation was used to statistically characterize quantitative data, whereas frequency was used to characterize categorical data. The threshold for statistical significance was set at P 0.05. Multiple linear regressions were used to determine the half- and ninetieth-lethal concentrations (LC50 and LC90) (Finney 1971).

RESULTS

Characterization of Synthesized Zinc Nanoparticles (ZnO-NPs):

1-Transmission Electron Microscopy (TEM) Analysis:

The appearance and size of zinc nanoparticles (ZnO-NPs) produced using aqueous extracts from *Lantana camara* leaves was studied using transmission electron microscopy (TEM). ZnO-NPs between 12.2 and 25.3 nm in size were seen in the TEM pictures (Fig. 1).

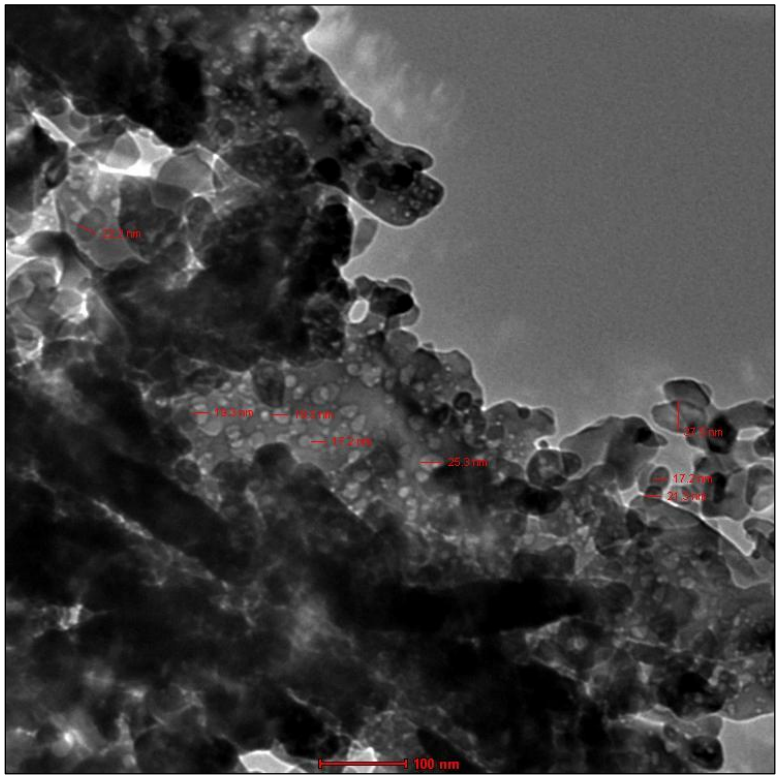

Fig. 1: TEM image of ZnO-NPs synthesized using *Lantana camara.*

2-Ultra-Violet (UV) - Visible:

ZnO-NPs produced using aqueous extracts from *L. camara* leaves were examined for the LSPR phenomena. A single absorption peak in the region of 356-360 nm was observed by LSPR, suggesting the existence of spherical ZnO-NPs (Fig. 2). The excitonic nature at room temperature was inferred from the absorption wavelength in the measured range.

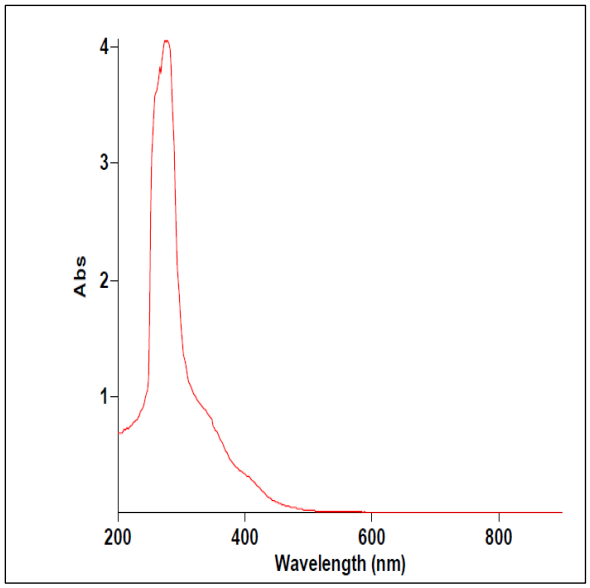

Fig. 2: Ultra-Violet (UV) - Visible curve of *L. camara*-ZnO-NPs.

3-The activity of biosynthesized zinc nanoparticles (ZnO-NPs) against *Aedes aegypti* larvae:

In Table 1, it is obvious that the aqueous extract of *L. camara* leaves is effective against *Ae. aegypti* during their third instar of larval development. The findings showed that the greatest dosage (400 ppm) resulted in 100% larval death, whereas the lowest concentration (100 ppm) resulted in only 28% mortality compared to 0% mortality in the control group. The highest two doses (350 and 300 ppm) also substantially ($P \leq 0.001$) increased the mean duration of larvae when compared to the control group.

In addition, no mortality for pupal was recorded by all concentrations used. The pupal duration recorded 3.14±0.11, 3.09±0.13, 3.02±0.09, 2.96±0.02, 2.83±0.07 and 2.72±0.16 days at 350, 300, 250, 200, 150 and 100 ppm, respectively, vs. 2.34±0.23 days for the control group. The growth index values recorded 11.48, 11.92, 12.76, 13.05, 13.42 and 14.23 at 350, 300, 250, 200, 150 and 100 ppm, vs. 15.74 for the untreated group (Table 1).

Table 1: Activity of *Lantana camara* leaves aqueous against *Aedes aegypti* different stages.

Conc. (ppm)	Larval mort. (%)	Larval Duration	Pupal Mort. (%)	Pupal Duration	Adult Emergence (%)	Development Duration	Growth Index
400	100.0±0.00	----	----	----	----	----	----
350	88.0±4.0	5.56 ± 01^{d}	0.0	3.14 ± 0.11^{d}	100.0±0.0	8.72 ± 0.10^{d}	11.48 ± 0.34^{d}
300	77.33±2.33	5.30 ± 0.30^{d}	0.0	3.09 ± 0.13^{d}	100.0±0.0	8.40 ± 0.38^{d}	11.92 ± 0.19^{d}
250	62.67±4.62	4.81 ± 0.16^{c}	0.0	3.02 ± 0.09^{d}	100.0±0.0	7.83 ± 0.22^{d}	12.76 ± 0.82^{d}
200	57.33±2.31	4.70 ± 0.12^{b}	0.0	2.96 ± 0.02^{c}	100.0±0.0	7.67 ± 0.15^{d}	13.05 ± 0.02^{d}
150	45.33±2.33	4.62 ± 0.17^{b}	0.0	2.83 ± 0.07^{b}	100.0±0.0	7.46 ± 0.26^{d}	13.42 ± 0.09^{d}
100	28.0±4.0	4.31 ± 0.12^{a}	0.0	2.72 ± 0.16^{b}	100.0±0.0	7.03 ± 0.22^{d}	14.23 ± 0.83^{d}
Control	0.0	4.01 ± 0.06^{a}	0.0	2.34 ± 0.23^{a}	100.0±0.0	6.36 ± 0.13^{a}	15.74 ± 1.12^{a}

No. of tested larvae = 25 per replicate; Conc. = Concentration; ppm = particle per million; mort. = mortality; a = non-significant ($P>0.05$); b = significant ($P<0.05$); c = highly significant ($P<0.01$); d = very highly significant ($P<0.001$). Means followed by the same letter in the same column are not statistically significant. All data is represented as Mean±SD.

L. camara-produced ZnO-NPs were effective against *Ae. aegypti* pupae, as indicated in Table 2. The findings showed that the greatest dose (35 ppm) killed 100 percent of the larvae, the lowest concentration (5 ppm) killed 29.33 percent, and untreated larvae had a zero percent mortality rate.

When compared to the control group, the larval and pupal durations of larvae treated with *L. camara*-synthesized ZnO-NPs were substantially ($P \leq 0.05$) increased across all doses tested. As a result of this, the growth index for larvae and pupae was 9.75 at 30 ppm, 10.01 at 25 ppm, 11.09 at 10 ppm, 11.36 at 5 ppm, and 11.91 at 5 ppm, compared to 14.89 for the untreated group (Table 2).

Table 2: Activity of *Lantana camara* synthesized ZnO-NPsagainst*Aedes aegypti* different stages.

Conc. (ppm)	Larval mort. (%)	Larval Duration	Pupal Mort. (%)	Pupal Duration	Adult Emergence (%)	Development Duration	Growth Index
35	100.00±0.0	----	----	----	----	----	----
30	92.0±4.0	6.82 ± 0.19^{d}	0.0	3.44 ± 0.15^{d}	100.0±0.0	10.26 ± 0.18^{d}	9.75 ± 0.14^{d}
25	77.33±6.12	6.61 ± 0.26^{d}	0.0	3.36 ± 0.21^{d}	100.0±0.0	9.99 ± 0.33^{d}	10.01 ± 0.31^{d}
20	65.33±6.11	6.19 ± 0.18^{d}	0.0	3.15 ± 0.16^{d}	100.0±0.0	9.35 ± 0.16^{d}	10.70 ± 0.22^{d}
15	44.0±4.0	5.92 ± 0.14^{d}	0.0	3.09 ± 0.11^{d}	100.0±0.0	9.01 ± 0.29^{d}	11.09 ± 0.18^{d}
10	34.67±2.31	5.88 ± 0.21^{d}	0.0	2.91 ± 0.09^{c}	100.0±0.0	8.80 ± 0.22^{d}	11.36 ± 0.45^{d}
5	29.33±2.31	5.67 ± 0.23^{d}	0.0	2.72 ± 0.12^{b}	100.0±0.0	8.40 ± 0.14^{d}	11.91 ± 0.39^{d}
Control	0.0	4.41 ± 0.29^{a}	0.0	2.31 ± 0.11^{a}	100.0±0.0	6.72 ± 0.36^{a}	14.89 ± 0.40^{a}

See footnote of Table 1.

The results show that zinc nanoparticles (ZnO-NPs) synthesized using aqueous extracts from the leaves of *L. camara* are significantly more effective than the leaves' aqueous extract against all stages of *Ae. aegypti*, with LC_{50} and LC_{90} values of 14.85 and 30.41 ppm, respectively, compared to 182.39 and 356.69 ppm.

DISCUSSION

The TEM pictures revealed that the produced ZnO-NPs had diameters between 12.2 and 25.3 nm. The TEM images of the synthesized ZnO-NPs agree with those reported by Singh *et al*. (2011), who used Maddar (*Calotropis procera*) latex to prepare ZnO-NPs; by Ramesh *et al*. (2014), who used *Citrus aurantifolia*; by Poovizhi and Krishnaveni (2015), who used an extract of *Calotropis procera* leaves; and by these authors. UV-Visible curves confirmed the existence of spherical ZnO-NPs, as predicted by Singh *et al*. (2011), by exhibiting a single absorption peak in the region of 356-360 nm.

The findings showed that the concentration of the aqueous extracts from *Lantana camara* leaves used to produce ZnO-NPs improved their activity against *Ae. aegypti*. The LC50 and LC90 figures show that *L. camara* -synthesized ZnO-NPs are more effective than leaves aqueous extract against *Ae. aegypti* larvae. Synthesized nanoparticles have a high level of activity because they can pass through the exoskeleton and into the cells of insects, where they can bind to macromolecules like proteins and DNA and alter their structure and function (Subramaniam *et al*., 2015). These findings are in line with those found by Jayaseelan *et al*. (2011) for silver nanoparticles (AgNPs) synthesised using *Tinospora cordifolia* against *Culex quinquefasciatus*, Roni *et al*. (2013) for AgNPs synthesised using *Nerium oleander* aqueous extract against *Anopheles stephensi*, and Morej'on *et al*. (2018) for AgNPs synthesized using *Ambrosia arborescens* against *Ae aegypti* and Shehata and Mahmoud (2019) for AgNPs synthesized using *Lagenaria siceraria* leaves aqueous extract against *An. pharoensis* and *Culex pipiens* larvae .

Conclusion:

In summary, Zinc nanoparticles (ZnO-NPs) synthesized using aqueous extracts from leaves of *Lantana camara* have a significant efficacy against dengue fever vector, *Aedes aegypti* different stages than leaves aqueous extract. Further studies on the activity of biosynthesized nanoparticles against other mosquito species are needed.

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