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RESEARCH PAPER

Mosquito Larvicidal Activity of ZnO Nanoparticles against Dengue Causing Vector *Aedes Albopictus* Using Leaf Extract of *Lavandula Angustifolia*

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ABSTRACT

The employment of greener-reducing extracts for ZnONPs biosynthesis is a facile, simple, and eco-friendly approach than physical and chemical synthesis. The present study was designed to the synthesis of ZnONPs for the first time using the Lavandula angustifolia leaf extract. The techniques like UV-Visible spectroscopy, PXRD, FESEM, EDAX, and FTIR were used to characterize the ZnONPs. In dose dependent manner from 80mg/L to 160mg/L, the ZnONPs were exposed to dengue-causing vector A. albopictus for 24 hours. The UV-Vis absorption peak was found at 346 nm confirmed the biosynthesis of ZnONPs. FESEM results showed the ZnONPs were formed in aggregates with truncated octahedron morphology. The average particle size was found to be 74.58 nm. The PXRD analysis showed the ZnONPs were crystalline in nature. FTIR analysis showed the presence of different functional groups like phenolics, alcohols, and amines were involved in ZnONPs synthesis. The ZnONPs showed significant mosquito larvicidal activity after being treated with fourth instar larvae of A. albopictus. After 24h exposure the ZnONPs showed 100% mortality at a concentration of 160mg/L with LC50 value at 118mg/L and LC90 at 135mg/L respectively. Based on these results, we strongly recommend the truncated octahedron-shaped L. angustifolia ZnONPs could act as a potent biomedical agent against mosquito-borne diseases and pest management.

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INTRODUCTION

Mosquitoes are medically life threatened arthropod insect that carries many types of pathogens among people worldwide [1]. In various mosquito groups, *Aedes aegypti* and *Aedes albopictus* are causing diseases like dengue fever, chikungunya, zika fever, and yellow fever [2]. They make their breeding sites widely almost at all stagnant clear water sites except sewage water [3]. Dengue is the most lethal mosquito-borne disease that has attracted worldwide attention, it is a very challenging task to control it due to the absence of an effective vaccine. According to WHO data, an estimated 390 million infections occur annually in 129 countries, putting the

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whole world's population at risk.[4]. Thus, it is an important task to control the mosquito-borne diseases causing mosquito vectors at effective methods. The use of nanotechnology in mosquito control has shown a lot of interest recently. Currently, nanotechnology has been attracted the attention of many researchers due to its wide range of applications in many fields like medicine, agriculture, and electronics [5]. Nanoparticles can be synthesized by using a variety of traditional methods, including physical, chemical, and biological approaches. Nowadays, the synthesis of nanoparticles by a greener route utilizing plant extracts, roots, flowers, and the stem is considered to be safer due to its cost-effectiveness, low toxicity, and environmentally friendly than traditional methods [6]. Over the last years, various types of nanoparticles such as metallic silver, gold, copper, and iron as well as metal oxides such as magnesium oxide, iron oxide, zinc oxide, and titanium oxide have been synthesized [7]. Among the metal oxide nanoparticles, ZnONPs have shown outstanding properties like semiconducting, photocatalytic, Uv blocking, large binding energy, and high bandgap [8]. Its exceptional qualities allow it to be used in a variety of applications, including biomedical engineering, drug delivery, bio-imaging, and cancer research. It's also used in cosmetics, sunscreens, food packaging, and paintings and among other things [9]. Zinc oxide nanoparticles possess excellent antibacterial, anticancer, antioxidant, wound healing, and larvicidal properties. It has been considered a safer metal oxide nanoparticle by US FDA [8]. Moreover, it possesses excellent anti-diabetic properties. It has been deemed safer for humans and animals due to its non-toxic nature and environmentally friendly [10]. Various forms of Zinc have been previously studied, in the form of ZnO films, ZnO nano wires and ZnO nanotubes for structural, optical, chemical and morphological applications [11-13].

The previous studies on mosquito control using nanoparticles have been performed against many mosquito vectors including dengue and Zika virus vector *A. albopictus* due to having environmental friendly and selective toxicity to the mosquito vector by interrupting ZnONPs into mosquito gut membrane leading to damage all physiological functions of the mosquito behaviors but the precise mechanisms of selected nanoparticles on mosquito larvae are still examined [14]. The ZnONPs can be prepared through various methods like sol-gel, direct precipitation, solvothermal, hydrothermal, and microwave irradiation. Due to the limitations of these methods like involving toxic chemicals, time-consuming, and requiring huge set-up, biosynthesis of ZnONPs employing greener route is considered to be safer and eco-friendly [9]. Green synthesis of ZnONPs using plant extracts is non-toxic, safer, one-step approach, and less expensive. Secondary metabolites found in plants, such as alkaloids, tannins, and flavonoids function as capping and reducing agents in the bio-reduction of metal oxides into metal oxide nanoparticles. There are various reports by using plant extracts for the synthesis of ZnONPs like *Bauhinia tomentosa* [15], *Cassia alata* [16], *Deverra tortuosa*, [17] *Cynara scolymus* [18] and *Bergenia ciliata* [8] *Lavandula angustifolia* belongs to the family of *Lamiacea* and is a perennial evergreen plant. It is abundantly found in Mediterranean regions, and it has a wide range of biological and therapeutic properties, including antibacterial, anti-inflammatory, antioxidant, and anxiolytic properties. [19]. The aim of the present study is the biosynthesis of ZnONPs using the leaf extract of *L. angustifola* and to investigate its mosquito larvicidal activity against Dengue causing vector *Aedes albopictus.*

MATERIALS AND METHODS

Collection and authentication of plant material

The *Lavandula angustifolia* plant material were procured from the Botanical Garden Department of the Botany University of Kashmir. The plant was recognized and authenticated by a taxonomist at the Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir herbarium, with accession number 2721- (KASH).

Preparation of plant extract and Synthesis of ZnONPs

The fresh leaves of *L. angustifolia* were surface sterilized with tap water before being washed twice with distilled water and followed by saline solution. The leaves were left in the shade for seven days to dry and then blended into a fine powder using a mixer grinder. The 5 g of *L. angustifolia* leaf powder was added into the 100 ml of distilled water and boiled in a water bath for 20 minutes at 60°C. Afterwards the resulting solution was filtered by using Whatman's no.1 filter paper. The biosynthesis of *L. angustifolia* mediated ZnONPs were carried as per our already

reported work with slight modifications [8]. One ml of leaf extract was added into 60 ml of 0.01 M zinc acetate dehydrate solution and stirred continuously till it changed into white suspension. By adding 2 M NaOH solution, the *p*H of the solution was adjusted to 12. The solution was then centrifuged at 7000 rpm. The white -colored pellet was kept in hot air oven at 80ºC for 12 hours. The dried pellet was crushed into a fine white powder for further analysis.

Characterization of ZnO-NPs

The green synthesized ZnO-NPs were characterized by UV-Visible spectroscopy (Model SHIMADZU UV-1800 Japan) in the UV range of 200-800nm to determine the lambda max, which indicates synthesis of ZnO-NPs. The crystalline nature of ZnO-NPs was analyzed through Powder X-ray diffraction (Malvern Pan analytical Ltd., Malvern, UK). Fourier transform-infra red spectroscopy (FTIR) model ALPHA BRUCKER was used to detect the presence of functional groups in the range of 400 400 cm^{-1}. The size and the morphology of ZnO-NPs were analyzed through FESEM model (FEI Quanta). The elemental composition of ZnO-NPs was studied through EDAX analysis.

Mosquito Larvae Collection and rearing

The different larval instars (I-III$^{rd)}$ of *Aedes albopictus* were collected from Korukkupet, Chennai, Tamil Nadu, India. The larvae were taken to the laboratory after collection and reared under optimal conditions, such as 28±2º C temperature, 55-60 % relative humidity and 12:12 h (light: dark) photoperiod. Until the fourth instar, the larvae were fed with dog biscuit and yeast (1:3) solution. After that the larvicidal activity of ZnONPs were evaluated against fourth instar larvae of *Aedes albopictus* for 24 h.

Larvicidal Bioassay

For the larvicidal bioassay, *A. albopictus* larvae in their early fourth instar were used. The larvicidal assay was performed according to the WHO standard procedures with slight modifications, Parthiban *et al*. [3]. In each assay 10 larvae were placed in a bowl containing different concentrations of ZnONPs starting from (80, 100, 120,140 and 160 mg/L), with tap water were used as a control. Mortality was measured after 24 h, and the experiments were carried out in triplicates.

Statistical analysis

To check the larval percent mortality probit analysis was used to calculate LC_{50} and LC_{90} statistics at 95% confidence limits of upper confidence limit (UCL), and lower confidence limit (LCL) values was calculated using Statplus (V.5.00).

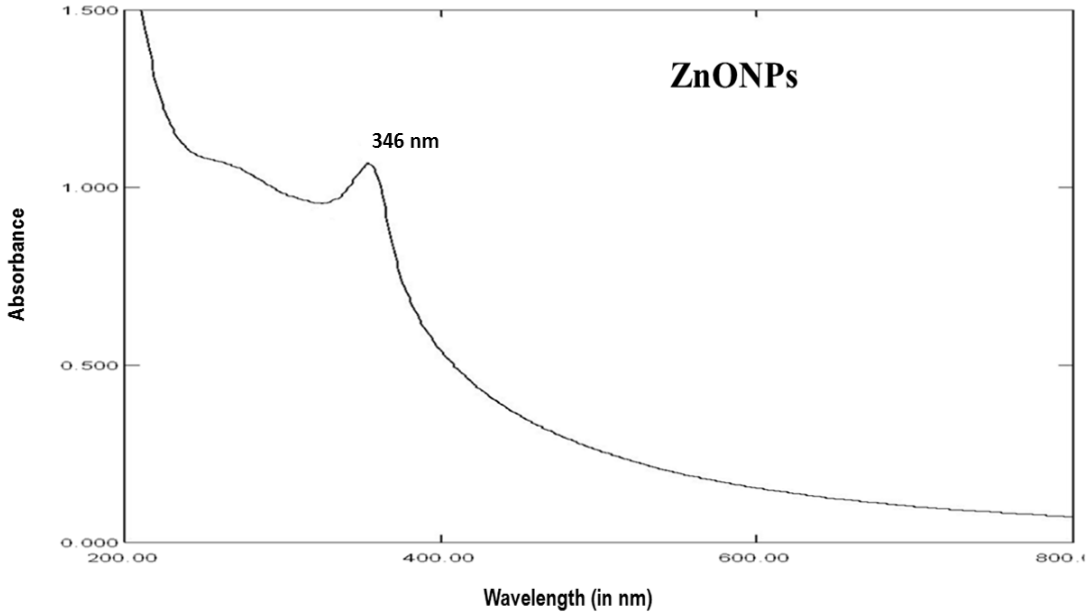

Fig. 1. UV-Vis spectra analysis of ZnONPs

RESULTS AND DISCUSSION

UV spectra analysis

The bio-synthesis of ZnONPs was first confirmed by UV-Vis spectroscopy. Upon increasing the *p*H, the formation of white color from the pale yellow indicates the formation of ZnONPs. The peak was

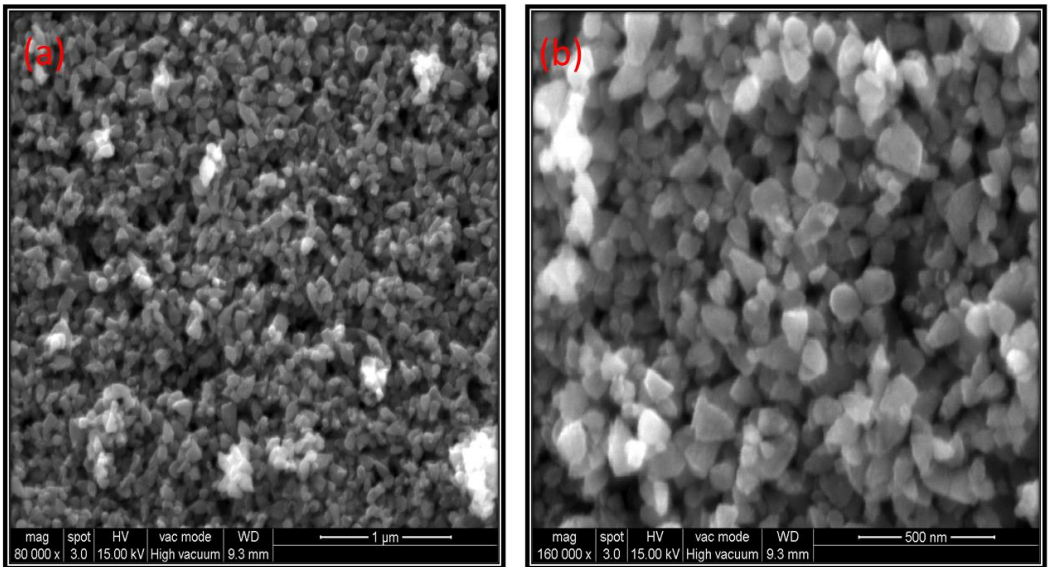

Fig. 2. Low (a) and High (b) magnification FESEM images of ZnONPs

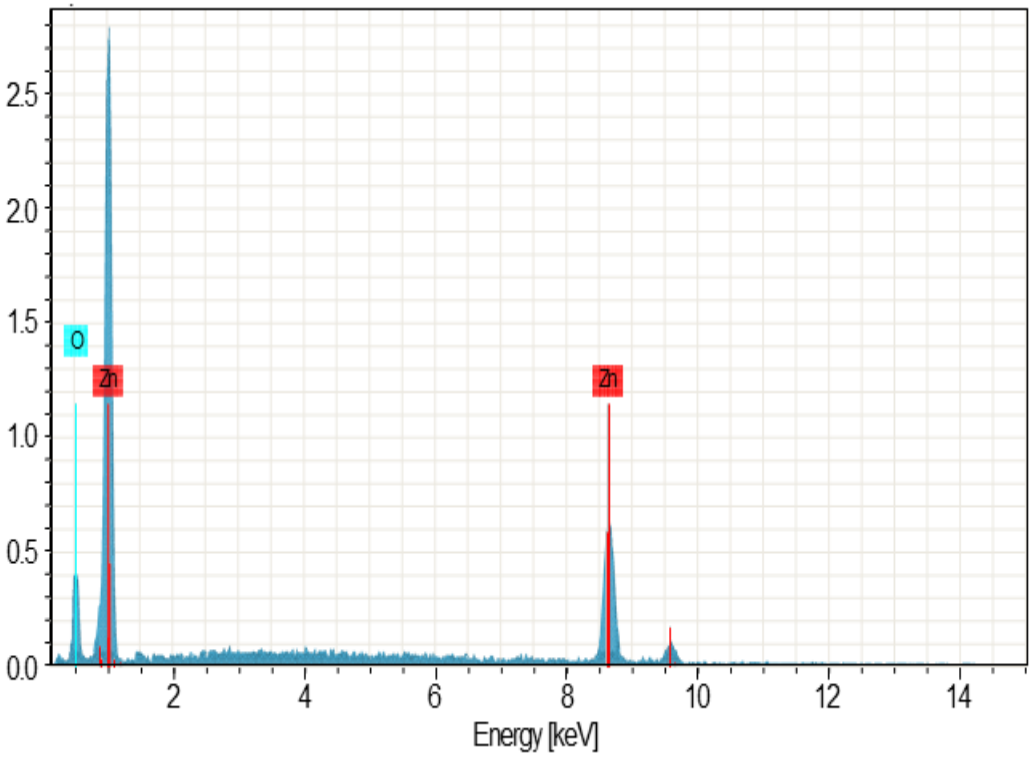

Fig. 3. EDAX analysis of ZnONPS

observed at 346 nm in the UV range from 200-800 nm as shown in Fig. 1. The formation of UV peak at 346 nm is consistent with recent research that showed a peak at 341, confirming the synthesis of ZnONPs [20].

FESEM and EDAX analysis

The size and the morphology of ZnONPs were determined by using FESEM analysis. It was observed from the FESEM analysis the size of ZnONPs were ranges from 64-83 nm with average particle size 74.58 nm. The ZnONPs were formed in aggregates with truncated octahedron morphology as shown in Fig. 2 [8]. The phase purity and the elemental composition of ZnONPs were determined by EDAX analysis. From the EDAX results, elemental composition of zinc was found to be (75.31%) and oxygen (24.69 %) in ZnONPs as shown in Fig. 3. Similar kinds of atomic compositions were found when garlic skin extract was used to synthesize ZnONPs, the composition of Zn was found (78%) and O (22%) which confirms the purity of ZnONPs [21].

PXRD analysis

The crystalline nature and structural properties of *L. angustifolia* mediated ZnONPs were studied by using PXRD analysis. From 2Θ, the values of diffraction signals as 31.8°,34.5°,36.6°,57.2° and 63.2° corresponds to (100), (002), (101), (110) and (103) respectively as shown in Fig. 4. The PXRD results shows the ZnONPs were formed with hexagonal wurtzite phase and matching with the patterns of JCPDS card no.36-1451. The average crystalline size was determined by using Scherrer formula which was found to 19 nm. Similarly, Vinayagam *et al*. [22], reported that the average crystalline size of ZnONPs were 17.79 nm by using *Peltophorum pterocarpum* pod extract.

FT-IR analysis

The presence of different functional groups involved in ZnONPs synthesis were analyzed by Fourier transform infra-red Spectroscopy (FTIR) in the range from 400-4000 cm^{-1} (Fig. 5). The peaks at 441cm^{-1} and 590 cm^{-1} indicates the Zn-O bonding in ZnONPs formation [12]. The presence of peaks at 906 cm^{-1},1402 cm^{-1} and 1490 cm^{-1} corresponds to the C-N stretching of amines and alkene groups. The peak at 1667 cm^{-1} indicates to the C=O stretching due to primary amines [23]. The presence of broader peak at 3370 cm^{-1}

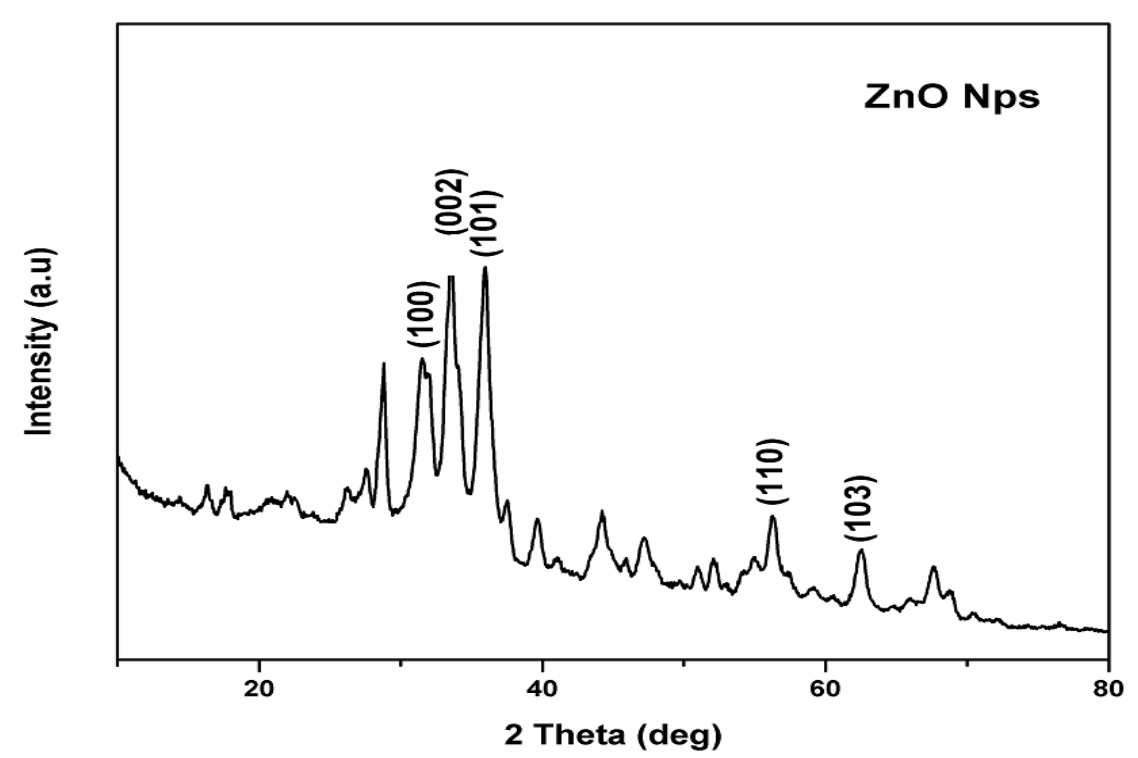

Fig. 4. PXRD analysis of ZnONPs

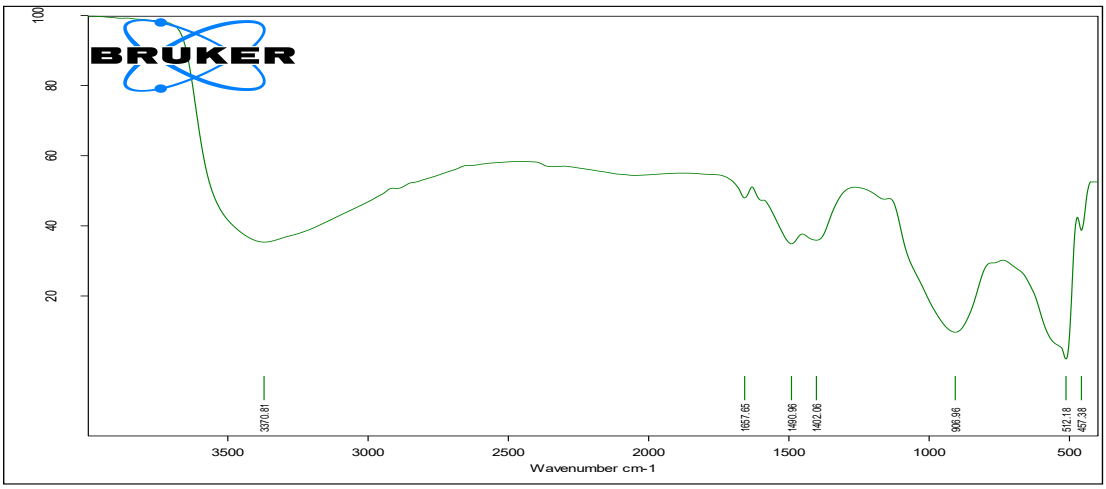

Fig. 5. FTIR spectrum of *L. angustifolia* mediated ZnONPs

corresponds to the O-H stretching of phenolics and alcohols present in the leaf extract of *L. angustifolia* involved in ZnONPs formation [24].

Larvicidal activity of ZnONPs

Many investigators are continuously involved in the prevention of mosquito borne disease by testing the various kinds of silver nanoparticles including ZnONPs against major dengue causing vectors *Aedes albopictus* and *Aedes aegypti* [25-26], both have ability to transmit such a disease among the populations. In this study, the larvicidal activity was performed against fourth instar larvae of *A. albopictus* using ZnONPs synthesized from *L. angustifolia* to study effects of ZnONPs nanoparticles on the tested mosquito vector. In this accordance, the ZnONPs shows a dose dependent larval mortality as shown in Table. 1 and its lethal concentration were as 118 mg/L (LC_{50}) and 135 mg/mL (LC_{90}) for 24 h exposure. The larvicidal activity of metal nanoparticles mode of action on mosquito are still unknown. The common proposed mechanisms of metal nanoparticles behind this mode of action are to believed that these nanoparticles have an ability to penetrate through the insect gut cell wall membrane where they bind with such a macromolecule of proteins and DNA, consequentially by altering their structures lead to the disruption of whole metabolic functions and leads to the death of bacteria [27].Therefore, in the present investigation the ZnONPs shows a better larvicidal property against tested mosquito species with the unknown proposed mechanism behind. Therefore, the mode of action of nanoparticles in the mosquito larvae finding are help to promote the ZnONPs production in the

Table 1. Mosquito larvicidal property of ZnO-NPS

Concentration mg/l	*Aedes albopictus (4th instar)*	LC_{50} (LCL-UCL)$	LC_{90} (LCL-UCL) $
	Percent mortality (24 h)@		
80	26.6 ± 0.5		
100	43.3 ± 0.5		
120	76.6 ± 1.15	118 (105.1-125.08)	135 (127.9-151.98)
140	93.3 ± 0.57		
160	100 ± 0.0		

@ Data represent mean values ± SD (n=3) from three replicates using sample from exposure larvae for 24 h. $$LC_{50}$ and $_{90}$represents concentration required for 50 and 90 % mortality of larvae to ZnONPs exposure and (LCL-UCL) Lower confidence limit-Upper confidence limit.

integrated pest management system to control the mosquito borne diseases.

CONCLUSION

The green and facile biosynthesis of ZnONPs from *L. angustifolia* was successfully carried out. The ZnONPs were characterized by different microscopic techniques as discussed *vide-supra*. The average size of ZnONPs was found to be 74.58 nm with truncated octahedron morphology. The different functional groups like phenolics, alcohols and amines were found in ZnONPs by FTIR analysis. On dose dependent manner the ZnONPs showed excellent larvicidal activity against fourth instar larvae of *A. albopictus.* The *L. angustifolia* mediated ZnONPs showed the 100 % mortality at 160 mg/L with LC_{50} and LC_{90} values at 118mg/L and 135 mg. Overall our study showed that the *L. angustifolia* mediated ZnONPs synthesis is safer and non-toxic approach than traditional ways, and could be used to combat mosquito borne diseases and the production of novel pesticides.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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