International Journal of Environmental Science and Technology (2019) 16:8053–8060
https://doi.org/10.1007/s13762-018-2175-z

ORIGINAL PAPER

Environmental friendly synthesis of zinc oxide nanoparticles and estimation of its larvicidal activity against *Aedes aegypti*

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Received: 26 March 2018 / Revised: 30 October 2018 / Accepted: 12 December 2018 / Published online: 18 December 2018

Abstract

Nanotechnology is a relatively new field which is making advancements each day. It is making a huge impact on our day-to-day lives. Nanoparticles possess unique properties which makes it viable to use it in a wide variety of fields such as in cosmetics, parasitology and catalysis. We used a plant-mediated, nature-friendly method which does not involve the usage of any harmful chemicals. *Syzgium cumini* seed extract was taken as the reducing agent for the preparation of zinc oxide nanoparticles. Green-synthesized zinc oxide nanoparticles were confirmed using X-ray diffraction, Fourier-transform infrared, UV–Vis spectroscopy, scanning electron microscope and transmission electron microscope. The average particle size was found to be around 50–60 nm. Additionally, larvicidal and ovicidal activity of the prepared nanoparticles against dengue causing vector was also carried out which resulted in LC_{50} and LC_{90} of 51.94 ppm and 119.99 ppm, respectively.

Editorial responsibility: Tanmoy Karak.

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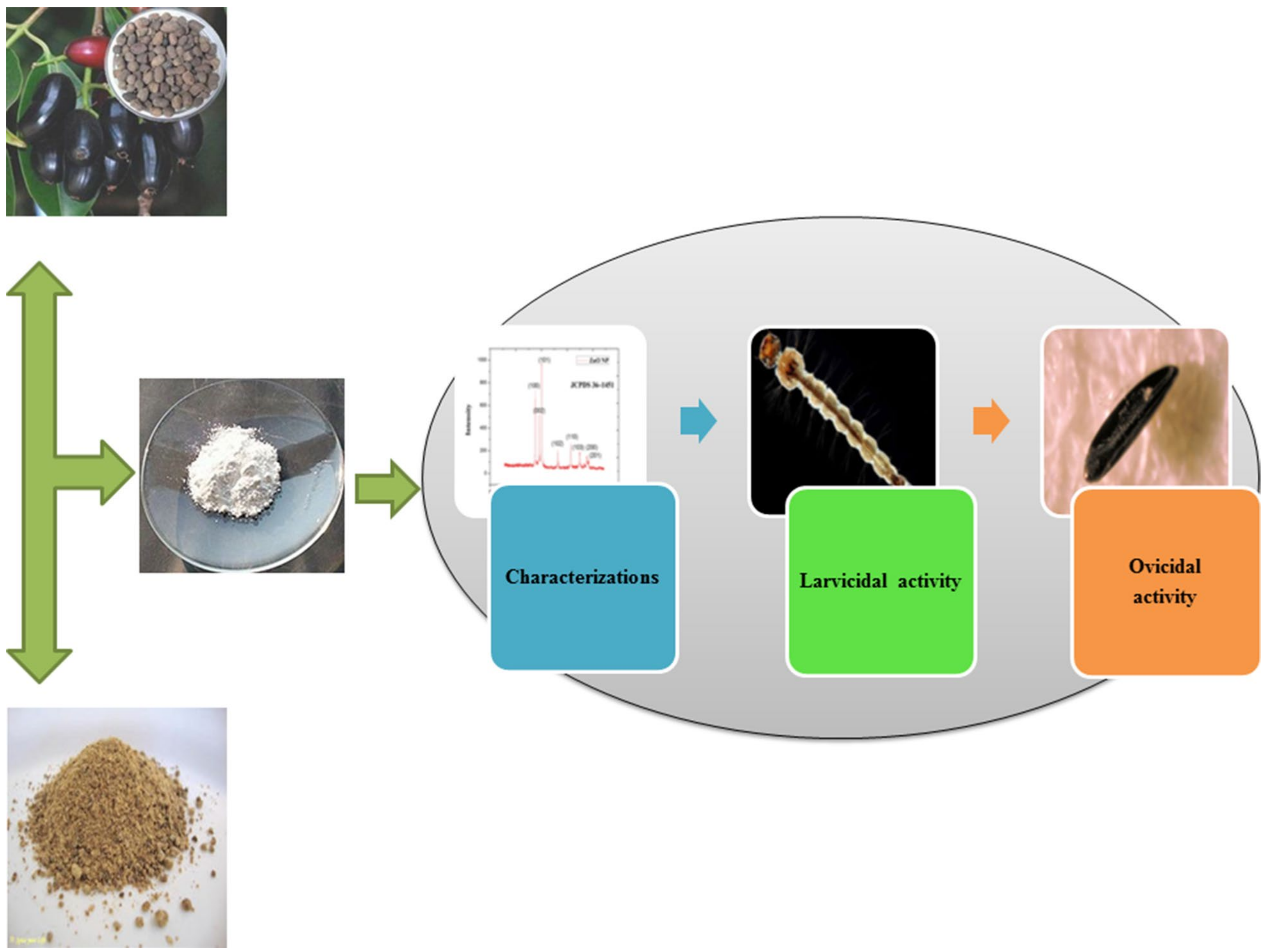
Graphical abstract



Keywords *Syzgium cumini* · Green synthesis · Zinc oxide · Nanoparticles · Larvicidal · Ovicidal activity

Introduction

Mosquito-borne diseases have caused human life miserable as over one million people die worldwide every year due to the aftermath of the disease. Dengue is one such mosquito-derived viral infection caused by the vector *Aedes aegypti* (*A. aegypti*). Transmission of dengue has enlarged recently in semi-urban and urban areas and has become a major health concern for the public (WHO 2012). To inhibit the spread of these mosquito-borne diseases, a proper mosquito control is required. New tools are being implemented for enhanced mosquito control (Benelli 2015). The application of synthetic insecticide is the major tool used, but this has not been successful as this created toxicity problems and harmful effects on environment. Additionally, continual use of synthetic insecticides for mosquito control has led to resurgences in mosquito population. Therefore, alternate control measures are needed as they are getting resistant to the biological controls (Ghosh et al. 2012).

One such alternative, gaining attention is the use of nanoparticles. Nanoparticles (NPs) are becoming inevitable in the field of science. Nanotechnology supports diverse areas such as electronics (Shipway et al. 2000), pharmaceuticals (Ramala and Manivasagam 2016; Bobo et al. 2016), imaging (Xia et al. 2016), cosmetics (Kapuscinska et al. 2015), parasitology (Kirthi et al. 2011), agriculture (Singh and Prasad 2017) and environmental remediation (Rani and Shanker 2017), thus providing a platform for collaboration. The preparatory methods include process that adheres to biosynthesis where either microorganisms or plant extract is used for the production of nanoparticles (Helan et al. 2016). Biologically synthesized nanoparticles with antimicrobial (Pageni et al. 2018), antioxidant and anticancer properties (Zhang et al. 2016) are already reported. These nanotechnologies may provide unique resources for evaluating and developing effective, new and nontoxic drug formulations. While the methods of chemical and physical processes can produce well-defined and pure nanoparticles, they tend to be more expensive, toxic to nature and energy consuming (Madhumitha et al. 2016). The plant-mediated NPs are less likely to cause ecological damage and can be a potential replacement for synthetic insecticides.

Syzygium cumini (*S. cumini*), generally called as Jamun or Naval Pazham, is a traditional medicinal plant used for the effective treatment of diarrhea, diabetes mellitus, inflammation, ulcers and also possesses antineoplastic, chemopreventive and radioprotective properties. Anthocyanins, ellagic acid, glucoside, isoquercetin, kaempferol and myricetin containing compounds are predominantly present in the plant. The dark violet-colored fruit is rich in sugar, mineral salts and vitamins and has an astringent taste (Ayyanar and Subash-Babu 2012; Swami et al. 2012). The presence of alkaloid, jambosine and glycoside jambolin or antimellin in the seeds stops the diastatic conversion of starch to sugar. *S. cumini* is also reported to contain flavonoids and high phenolics with significant antioxidant activity (Branco et al. 2016; Balyan and Sarkar 2016) and is rich in protein and calcium.

Nanoparticle synthesis using *S. cumini* such as silver, ferric, gold and copper (Kumar and Yadav 2012; Banerjee and Kannan 2011; Venkateswarlu et al. 2014; Rana et al. 2016) has been reported, but none have focused on zinc oxide nanoparticles.

Zinc oxide has found its use in gas sensors, magnetism, optics, catalysts, high adsorption capability, treatment of wastewater and antimicrobial activities (Madhumitha et al. 2016; Ravichandran et al. 2014; Zhang et al. 2013; Yang et al. 2013). Our research group has already reported some work on ZnO (Surendra et al. 2016; Fowsiya et al. 2016).

In the current study, we aimed at exploring the larvicidal activity of ZnO NPs synthesized using the seed extract of *S. cumini* to control *A. aegypti* as there are nil reports on the same.

Materials and methods

The *S. cumini* fruits were collected from Vellore local market (June 8, 2016, Vellore 12.92°N79.13°E). Seeds were taken, dried and powdered to obtain the extract. Zinc acetate was procured from AVRA chemicals, Hyderabad. Distilled water was used during the preparation.

S. cumini seed extract preparation

About 5 g of grounded seed powder of *S. cumin*i was added to 100 ml of double distilled water and kept at 60 °C in water bath for half an hour. Buchner funnel was used to filer the solution. Finally to get the crude extract, the excess water in the filtered solution was evaporated by keeping it in the hot air oven.

ZnO NPs synthesis

Twenty milligrams of *S. cumini* aqueous seed extract was dissolved in 80 ml of 1 mM zinc acetate. The reaction mixture was kept in water bath at 60^0 C. Samples were taken every 5 min for 45 min. Once the NPs formation was confirmed, the resultant solution was centrifuged and placed in furnace at 450 °C for 3 h. The powdered sample thus resulted was characterized using multiple techniques for NPs confirmation.

Larvicidal activity

The collected larvae of *A. aegypti* from Palar River, Vellore, were confirmed by Dr. K. Elumalai, Entomologist, Government Arts College, Nandanam, Chennai. Reusable paper cup was used to place the larvae at room temperature for 2 weeks. Soon after the second week, the larvicidal activity of the ZnO NPs was performed for concentrations 15, 30, 60 and 120 ppm. The experiment was performed in replicates of five. After 24 h of exposure to ZnO NPs, the mortality percentage of larvae along with LC_{50} and LC_{90} values was calculated using ANOVA LSD Tukey's test (Roopan et al. 2012; Velayutham et al. 2013).

Ovicidal activity of ZnO NPs

The *A. aegypti* eggs were collected to test against ZnO NPs to determine the ovicidal rate at different concentrations such as 15, 30, 60 and 120 ppm by the antifeedant method. Control used here was Neemazal. After the ZnO exposure, the ovicidal rate was recorded at various intervals of 24, 24, 48, 72 and 96 h with replications of five (Su and Mulla 1998; Elango et al. 2016).

Characterization

The absorption spectra of the samples were taken from a range of 200–800 nm using a UV–Vis spectrophotometer (double beam spectrophotometer 2202). Distilled water was kept as the blank. The powdered form of zinc oxide nanoparticle was subjected to X-ray diffraction (Advanced Powder XRD, model D8 Bruker, Germany), Fourier-transform infrared (FTIR) spectroscopy for identifying the functional groups, scanning electron microscopy (SEM) to study the morphological structure and transmission electron microscopy (TEM) to identify the size and shape of the particle. In addition, larvicidal and ovicidal activities were also carried out using the synthesized nanoparticles.

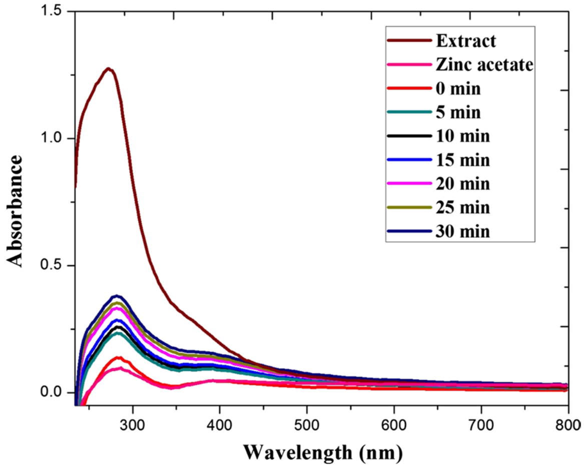


Fig. 1 UV–Vis absorption spectra of ZnO NPs

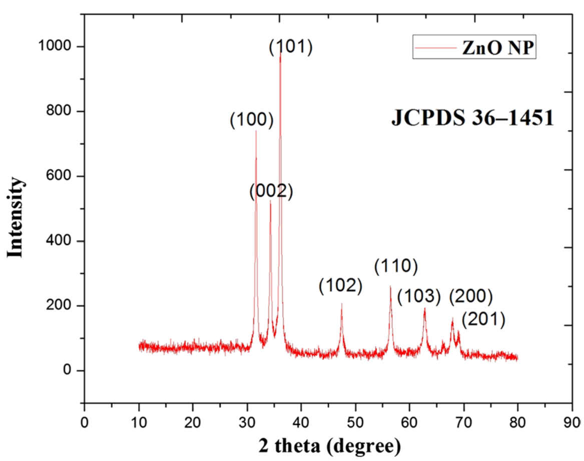


Fig. 2 XRD pattern

Results and discussion

UV–visible spectroscopy

ZnO NPs formation was examined via UV–Vis spectrophotometer at every 5-min interval. Wavelength range was fixed from 200 to 800 nm. Spectra showed a surface plasmon resonance at around 282 nm as shown in Fig. 1. Size, shape and dielectric constant of the reaction media affect the absorption, i.e., the surface plasmon resonance (Fowsiya et al. 2016).

XRD of ZnO

The crystalline phase and phase purity were analyzed using XRD analysis as shown in Fig. 2. The synthesized ZnO NPs were indexed as hexagonal phase, and it was well matched with the standard JCPDS card no (36–1451). The characteristic reflection planes were (100), (002), (101), (102), (110), (103), (112), (200) and (201) in the 2θ regions of 31.67°, 34.29°, 36.26°, 47.80°, 56.67°, 62.93°, 67.86° and 69.17°. Moreover, there was no other peak present in the XRD pattern; hence, the synthesized ZnO nanoparticles were pure. The peaks were very sharp, and the broad peak area was observed which clearly indicates that the high-crystalline nature.

FTIR

Functional group analysis of the *S. cumini* extract and ZnO NPs was done by subjecting the samples to FTIR. The spectrum (Fig. 3) revealed that the broad band at 3300 cm^{-1} seen in the extract corresponding to the phenolic group was reduced in ZnO NPs. Absorption bands around 416.62 cm^{-1} confirm the ZnO stretching. The intensity of the 1400–1600 peak (–COOH group) was reduced which indicates elimination of organic compound from ZnO NPs (Koupaei et al. 2016) as depicted in Fig. 3. Therefore, it can be concluded that the phenolic group and other organic compounds' presence in the extract act as a capping and stabilizing agent in the formation of zinc oxide nanoparticles.

SEM

The surface morphology and the average particle size were observed from SEM analysis as shown in Fig. 4. The ZnO nanoparticles were little agglomerated and accumulated with one another. Moreover, the sponge-like dusts were spread over with the average particle size of 300 nm. Further, the morphological analysis confirmed the amorphous nature. The sponge-like dust may be the soft carbon particles which may be from the plant extract.

TEM

The *S. cumini*-mediated synthesized ZnO NPs were analyzed with TEM to find out the particle size. Results (Fig. 5a, b) confirm the shape to be of spherical form. By utilizing the method of histogram analysis with the aid of image J software, we calculated the average size as 50–60 nm as shown in Fig. 5c. Furthermore, the selected area electron diffraction (SAED) pattern confirmed that the hkl values were in agreement with that of the XRD results (Fig. 5d). Higher surface energy and aqueous media synthesis of NPs can be the reason for agglomeration of particles (Fowsiya et al. 2016).

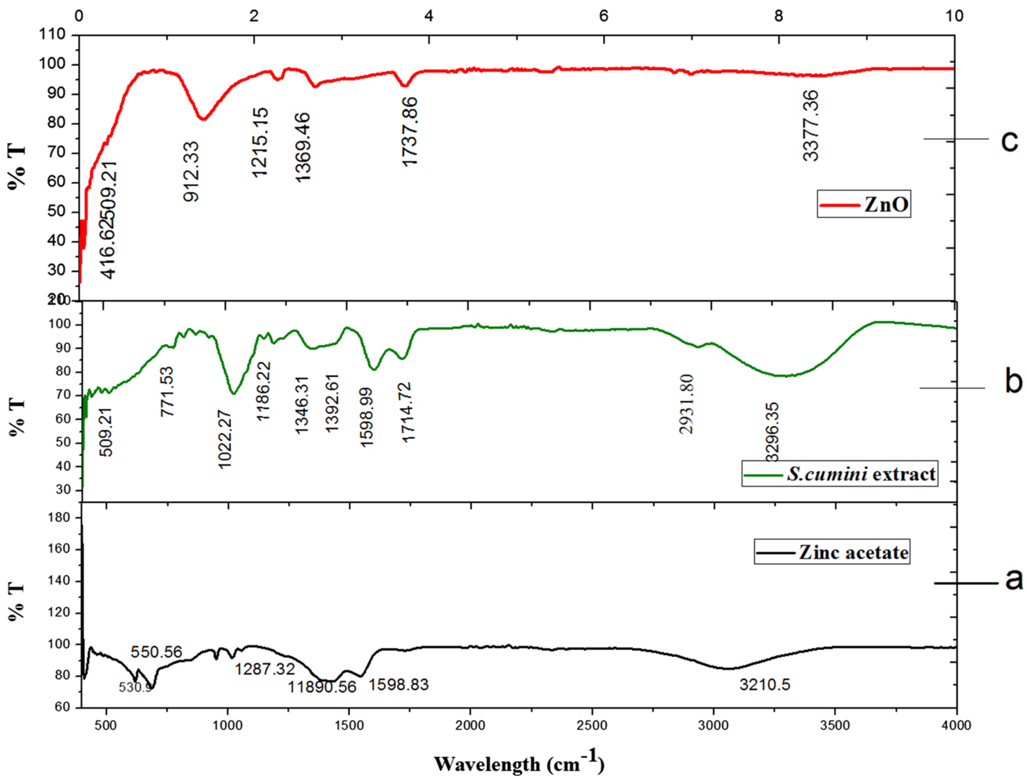


Fig. 3 FTIR spectrum: **a** zinc acetate, **b** *S. cumini* extract and **c** ZnO NPs

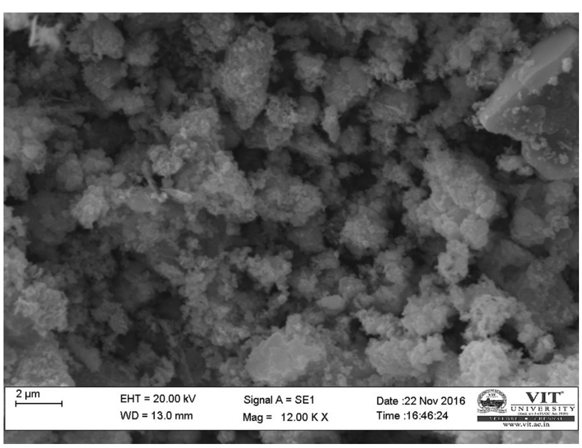


Fig. 4 SEM image of ZnO NPs

Larvicidal activity

Larvicidal activity of the prepared ZnO NPs was tested for various concentrations of 15, 30, 60 and 120 ppm against the dengue vector *A. aegypti*. Mortality rate of 21.4 ± 2.3, 35.2 ± 3.6, 59.6 ± 5.2 and 88.6 ± 1.2 was exhibited by 15 pm, 30 ppm, 60 ppm and 120 ppm, respectively. Therefore, it was concluded that at maximum concentration, highest mortality was achieved, i.e., 120 ppm followed by 60 ppm. Values were found to be statistically significant at $p < 0.05\%$ (LSD, Tukey's test). The LC_{50} value was 51.94 ppm with upper confidence limit (UCL) of 59.35 ppm and lower confidence limit (LCL) of 44.68 ppm. LC_{90} was found to be 119.99 ppm with LCL and UCL of 106.54 and 139.36 ppm, respectively. Chi-square value observed was 1.269 as presented in Table 1. The results were compared with studies. *S. cumini* plant-mediated synthesized ZnO NPs had high larvicidal activity. *Sargassum wightii*-mediated prepared ZnO NPs have LC_{50} value (49.22 ppm) compared to our result (Ishwarya et al. 2018a, b). *Ulva lactuca*-fabricated ZnO NPs were screened for larvicidal activity against *A. aegypti*, which showed an IC_{50} value of 22.38 ppm (Ishwarya et al. 2018a, b).

Ovicidal activity

ZnO NPs were tested for their ovicidal activity against *A. aegypti* eggs for various concentrations, and from the results, it is inferred that 82% mortality rate was achieved at higher concentration of ZnO NPs which is listed out in Table 2. Our results relate to the literature (Veni et al. 2017), i.e., *Terminalia chebula extracts* against A. aegypti. The ovicidal activity of ZnO NPs was reported and may be affected by diverse factors, predominantly egg age and contact period.

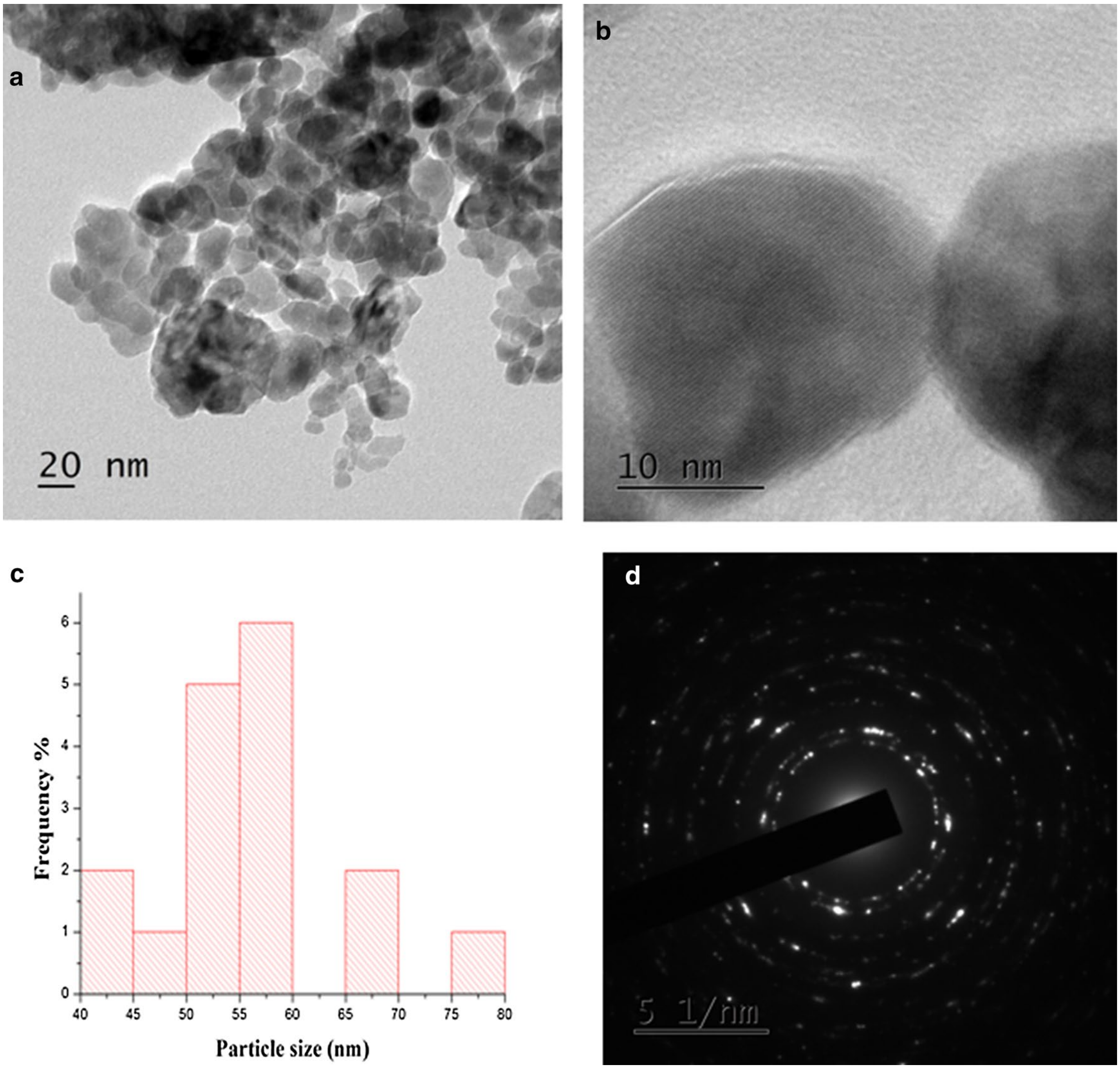


Fig. 5 **a**, **b** TEM images of ZnO NPs, **c** particle size histogram and **d** SAED pattern

Table 1 Results of larvicidal activity of ZnO NPs tested against the *Aedes aegypti*

Concentration (ppm)	Mortality (%)	LC_{50} (ppm)	95% Confidence limits (ppm)		LC_{90} (ppm)	95% Confidence limits (ppm)		χ^2 value
			LCL	UCL		LCL	UCL	
ZnO								
Control	1.6 ± 0.8	51.94	44.68	59.35	119.99	106.54	139.36	1.269
15	21.4 ± 2.3							
30	35.2 ± 3.6							
60	59.6 ± 5.2							
120	88.6 ± 1.2							

Conclusion

Phytochemical synthesis of ZnO nanoparticles was achieved utilizing *S. cumini* seed extract, and it is one of the easiest and less expensive nanoparticle fabrication methods. The agglomerated spherical shaped nanoparticles were having an average size of 55–60 nm. XRD patterns of the ZnO NPs were with no impurities and matched with JCPDS 36–1451. In addition, larvicidal and ovicidal

Table 2 Results of ovicidal activity against *A. aegypti*

Concentrations tested (ppm)	Ovicidal activity Sample 1
15	14.6 ± 1.6
30	32.2 ± 6.3
60	62.0 ± 2.9
120	81.6 ± 1.4
Neemazal	100 ± 0.00

activity against *A. aegypti* which resulted in statistically significant $p < 0.05$ revealed maximum mortality rate of 80% at maximum concentrations.

Acknowledgments We thank SAS, Vellore Institute of Technology, Vellore, for providing XRD, FTIR, SEM facilities and STIC, Cochin, for TEM facility.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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