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Characterisation and application of biosynthesis of zinc-oxide nanoparticles from Spinach leaf extract

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Abstract

The nanoparticles present a range of characterization challenges that affect the detailed and appropriate characterization of nanoparticles. Thus understanding the problems faced during characterization of nanoparticles and selecting a suitable characterization technique are of utmost importance. Specifically, nanoparticle characterization is performed to assess the surface area and porosity, pore size, solubility, particle size distribution, aggregation, hydrated surface analysis, zeta potential, adsorption potential and shape, size of the interactive surface, crystallinity, fractal dimensions, orientation, intercalation and dispersion of nanoparticles and nanotubes in nano-composite materials. Several techniques can be used to determine nanoparticle parameters, including UV- visible spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM), powder X-ray diffraction and Atomic Force Microscope (AFM) and basically they have been specifically used in agriculture and medicine as antibacterial, antifungal and antioxidants. Antibacterial activity of synthesized ZnO nanoparticles was carried out for both gram positive and gram negative bacteria and its effect against *Helicoverpaarmigeralarvae.*

Keywords: antimicrobial activity, biosynthesis, characterisation, spinach leaf, zinc-oxide nanoparticles

Introduction

Nanotechnology is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scale (Tarafdar *et al*,. 2012) [1]. This technology is rapidly growing to produce nanoproducts and nanoparticles that have novel and size related physico-chemical properties which differ significantly from particles at large scale (Kalpana and Devi Rajeswari, 2018) [2]. The unique properties of nanoparticles, especially in the size range of 1-100 nm result in high surface to volume ratio. As the particle size decreases, not only the ratio of surface area to volume increases but also the physical, chemical and biological properties of the particles differ compared to their bulk counterparts (Jeevanandam *et al*,. 2017) [3]. It is a rapidly growing field with its wide application in science and technology for manufacturing of new materials at Nano scale level (Patra and Baek, 2014) [4]. It includes the synthesis, characterization, exploration and utilization of nanostructured materials.

The term "nanotechnology" was used for the first time by Richard Feynman in 1959, in that time it has considered as Modern nanotechnology (Patra and Baek, 2014) [4]. There are two general strategies for the synthesis of nanomaterials: the top-down approach, wherein a larger structure is broken down into smaller pieces using chemical, physical, and biological energy; and the bottom-up approach, in which material is synthesized from the atomic level using various chemical, physical, or biological reactions to make a large nanostructure. (Das et al., 2017) [5].

Zinc-oxide (ZnO) nanopowders are available as powders and dispersions. These nanoparticles exhibit antibacterial, anti-corrosive, antifungal and UV filtering properties. Some of the synonyms of zinc-oxide nanoparticles are oxydatum, since oxicum, permanent white, ketozinc and oxozinc. Few features of ZnO nanoparticles are given below, Large surface to volume ratio, High UV absorption, Anti-bacterial, Anti-fungal, Anti-corrosive and UV filtering properties, Antioxidant activity. Reported that the toxicity of ZnO nanoparticles to gram-negative (Escherichia coli), gram-positive (*Pseudomonas aeruginosa*) bacterial systems, and primary human immune cells. Zinc-oxide nanoparticles are presently under intensive study for applications in the field of optical devices, sensors, catalysis, biotechnology, DNA labeling, drug delivery, medical, chemical and biological sensors and as a catalyst(Fan Z. and Lu J.G,2005)[6].

Materials and Methods

Biosynthesis of ZnO nanoparticles using Spinach leaf extract Preparation of Spinach leaf extract

The fresh Spinach leaves (Fig 1a) were collected from the University of Agricultural Sciences, Raichur. First, they were thoroughly washed with the tap water to remove debris and other contamination and finally washed with the distilled water. The cleaned Spinach leaves were finely cut into small pieces (Fig 1b) and dried under solar tunnel dryer. The dried leaves were ground using a pulveriser to make into affine powder (Fig 1c) and passed through a 150mesh sieve (105µm). 10grams of dried powder was added to 100 ml of ethanol and kept for 24 hour in a 250 ml Beaker and filtered through what man filter paper. The filtrate was stored at 4 ºC for further experiments (Fig 1d).

Biosynthesis of ZnO nanoparticles using spinach leaf extract

The Spinach leaves Extract (50 ml) was boiled at 70 °C using magnetic stirrer. 1 mM Zinc nitrate solution was prepared using distilled water (Fig 1e). Then, different concentrations (2.5, 5, 7.5 and 10 ml) of filtered plant

extract was added to the 100 ml of silver nitrate solution. Added mixture was kept for incubation at different temperature 10, 30 and 50 °C or until colour changed. A change in the colour from dark green to pale yellow indicates the formation of zinc-oxide nanoparticles (Amrita *et al*., 2015) [7]. Obtained ZnO nanoparticles solution was kept for further analysis under refrigerated condition (Fig 1f).

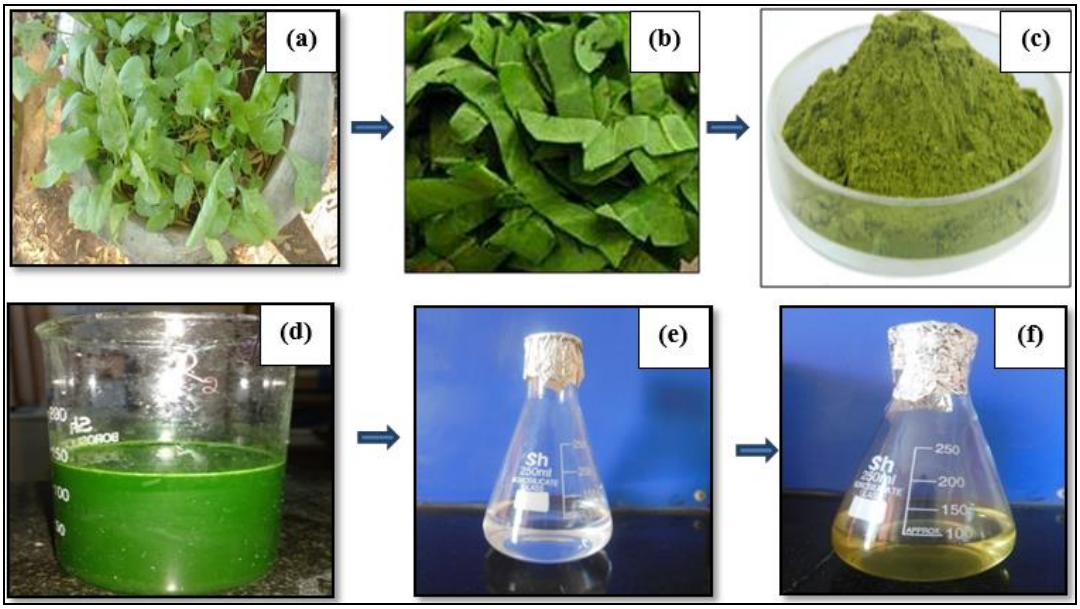

Fig 1: Process for biosynthesis of ZnO nanoparticles using Spinach leaf extract (a and b) Fresh spinach leaves (c) Spinach leaf powderd (d) Spinach leaves extract solution (e) Zinc Nitrate hexa hydrate Solution (f) Biosynthesized ZnO nanoparticles

Characterisation of biosynthesized ZnO nanoparticles

Characterizations of the Bio-synthesized ZnO Nanoparticles were done by using Varies analytical techniques such as UV-Vis spectroscopy (UV-1800; Kyoto, Schimadzu, Japan), Particle analyser (ZETA Sizer, Nano ZS, Malvern, England), Scanning Electron Microscope (SEM) (EVO-18; Carl Zeiss; Germany).X-Ray Diffraction (XRD) (Ultima-4, RIGACO, Japan) and Atomic Force Microscope (AFM) (Trial SPM, Version 6.4.3, Trieste, Italy).

Antibacterial activity of biosynthesised ZnO nanoparticles

Antibacterial activity of biosynthesized ZnO nanoparticles was determined by well diffusion method. Test bacteria such *as Escherichia coli* and *Pseudomonas aeruginosa* were procured from the Microbial Type Culture Collection Centre (MTCC), Chandigarh. Procured cultures were maintained in appropriate media for further use. The inoculums prepared from the stock culture were maintained on nutrient agar slant at 4 ºC and sub cultured on nutrient broth using a sterilized wire loop and maintained for further study.

Muller Hinton agar was used as a media to cultivate the bacteria. The sterilised media was poured in the petri discs and kept for 30 min for solidification. After 30 min, the incubated culture (2drops) was spread on media with the help of spreader. Then, holes were made on solidified media containing bacterial culture and ZnO nanoparticles (2 ml) at different concentration (50, 100 and 150 µl/ml) were poured into the wells. The cultured plates were incubated at 37 ºC for 24 h. After 24 h of incubation, the zone of inhibition was determined. Media without nanoparticles was used as control (Poovizhi and Krishnaveni, 2015b) [8] (Kaviya *et al*., 2011) [9]. Diameter of the zone of inhibition was recorded in mm using the following formula.

$$\text{Zone of inhibition (mm)} = \text{Diameter of zone (mm)} - \text{Diameter of well (mm)}$$

Effect of bio-synthesized ZnO nanoparticles on Spodoptera in castor leaves

A laboratory strain of Spodoptera was obtained from Department of Zoology, University college of Science, Tumkur University, Tumkur. They were reared on castor bean leaves under constant conditions at 25 ± 2°C and 65±5 % RH. The 2nd larval instars were used in all laboratory experiments. The dipping technique was applied to examine the effect of silver nanoparticles on toxicological and some biological aspects of the 2nd instar larvae of Spodoptera. 1, 3 and 5mM Zinc Nitrate concentration was prepared by dilution the tested compound with distilled water. Castor bean leaves were dipped for 15 second in each concentration (50, 100 and 150 µl/ml), then left to dry at room temperature and were offered to ten 2nd instar larvae, three replicates were carried out for each concentration, larvae were allowed to feed on the treated leaves for 48 h and then removed, the fresh untreated leaves were provided to the larvae until pupation. Other three replicates were dipped in distilled water for the same period as check. Mortalities were recorded daily (Jameel et al., 2020) [10].

Results and Discussion

The biosynthesis of zinc-oxide nanoparticles were carried out using spinach leaves extract and Zinc nitrate hexahydrate solution as a precursor. The reaction of nanoparticle synthesis started after introducing at different concentration of Zinc nitrate hexahydrate solution. A change in the colour from dark green to pale yellow indicated the formation of zinc-oxide nanoparticles. The colour change was due to excitation of surface plasmon vibration (Amrita *et al*., 2015) [7]. The size of the biosynthesized zinc-oxide nanoparticles were reduced by centrifuging (5000 rpm for 15 min) and ultrasonication (5 min for 25 ºC). Among the different concentration of plant extract and different incubation temperature, 5ml and 10 °C

has given the best size and stable ZnO nanoparticles. The convertion of nanoparticles may be due to biomolecules and bioreducing agents from the plants such asenzymes, proteins, flavonoids, terpenoids and cofactors present in the plant parts (Ravindran *et al.*, 2016) [11].

UV-Vis spectroscopy

The reduction of biosynthesized zinc-oxide nanoparticles were characterized by UV-Visible spectrophotometer. UV-Visible spectroscopy is an important technique to monitor the formation of metal nanoparticle in aqueous solution. The biosynthesis of zinc-oxide nanoparticles from the reaction mixture was confirmed by evaluating its optical properties. This analysis showed an absorbance peak at 329 nm, which was specific for ZnO nanoparticles as shown in Fig. 2. The result is in agreement with the findings of (Senthilkumar and sivakumar, 2014) [12] with 325 nm and (Singh *et al.* (2011) [13] with 368 nm.

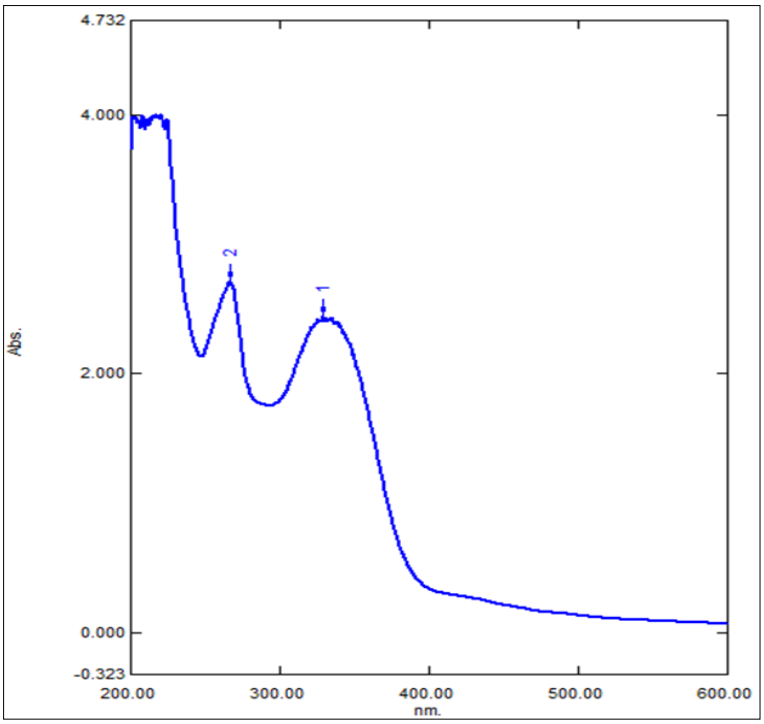

Fig 2: UV-Visible spectrum analysis of biosynthesized zinc-oxide nanoparticles from Spinach leaf extract

Particle size analysis

The results of zetasizer revealed that average particles diameter of biosynthesized zinc-oxide nanoparticles was 23.05 nm as shown in Fig. 2. The variation in particle size was probably due to change in climatic conditions during biosynthesis. This similar result is obtained as 20.3 nm. (Supraj a*et al.* (2015) [14].

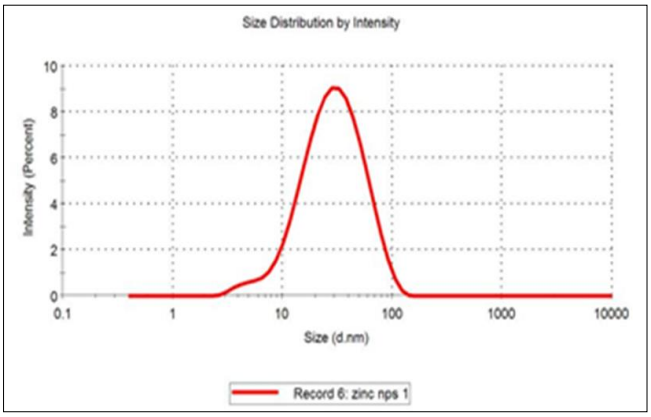

Fig 3: Particle size analysis of ZnO nanoparticles using Zetasizer

Scanning electron microscope (SEM)

SEM technique was Explain the shape of ZnO nanoparticles.

The SEM images of the ZnO Nanoparticles are shown in (Fig.4.) The morphological study of the SEM image demonstrated that, the biosynthesised ZnO nanoparticles were in spherical shape with 1µm and it can also observe that some of the particles are agglomerated. This may be due to the availability of different quantity and nature of capping agents present in the leaf extract (Al-Bedairy and Habeeb Alshams, 2018) [15].

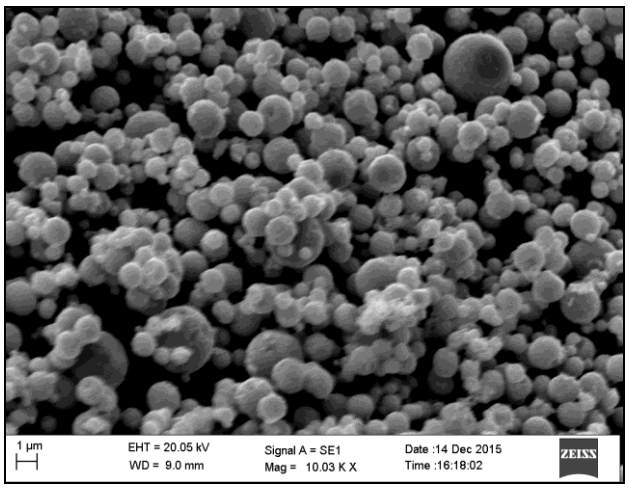

Fig 4: Morphology analysis of ZnO nanoparticles using Scanning Electron Microscope

X-ray diffraction (XRD)

XRD analysis showed distinct diffraction peaks (Fig. 5) and the Braggs reflections were observed in the XRD pattern at 2θ= 31.880°, 34.506°, 36.330°, 47.69°, 56.678°, 63.00°, 68.09°,and 69.23°. These Braggs reflections clearly indicated the presence of (100), (002), (101), (102),(110), (112),(112) and (201) sets of lattice planes and All diffraction peaks of sample correspond to the characteristic hexagonal wurtzite structure of zinc-oxide nanoparticles. Similar X-Ray diffraction patterns are obtained by earlier studies also for green synthesis of zinc-oxide nanoparticles by (Senthilkumar and sivakumar, 2014) [12].

The average particle size (D) of synthesized nanoparticles was 22 nm which was calculated using the well-known Debye-Scherrer equation. X-Ray Diffraction result obtained with synthesized ZnO nanoparticles confirms its presence and reveals about structure and size information as follows, Synthesized ZnO NPs have characteristic hexagonal wurtzite structure. The average size of crystallite was 22nm.

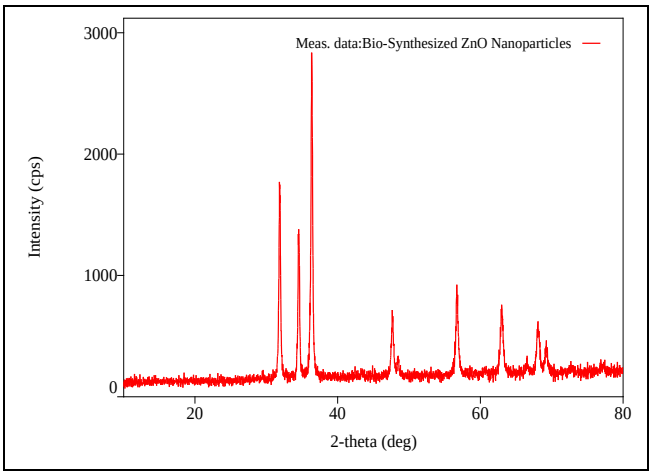

Fig 5: Crystallinity analysis of ZnO nanoparticles using X-Ray Diffraction

Atomic force microscope (AFM)

AFM analysis is explained as mechanical properties of ZnO nanoparticles. The topographical study of ZnO nanoparticles is explained in the surface roughness is 34.731nm, due to rough surface area of biosynthesized ZnO nanoparticles. The thin lines indicate on the 2D image (Fig. 6a) showed the presence of ZnO nanaperticles and 3D image (Fig.6b) depicted the average height and width of the nanoparticles. This study revealed that (Dinesha *et al*., 2021) [16]. Topography and profile image obtained by AFM for CZnO nano-adsorbent was indicated by the height (Y-axis) and width (X-axis) of the particles. Surface roughness value of 6.75 nm was obtained due to the rough surface area of the CZnO nano-adsorbent.

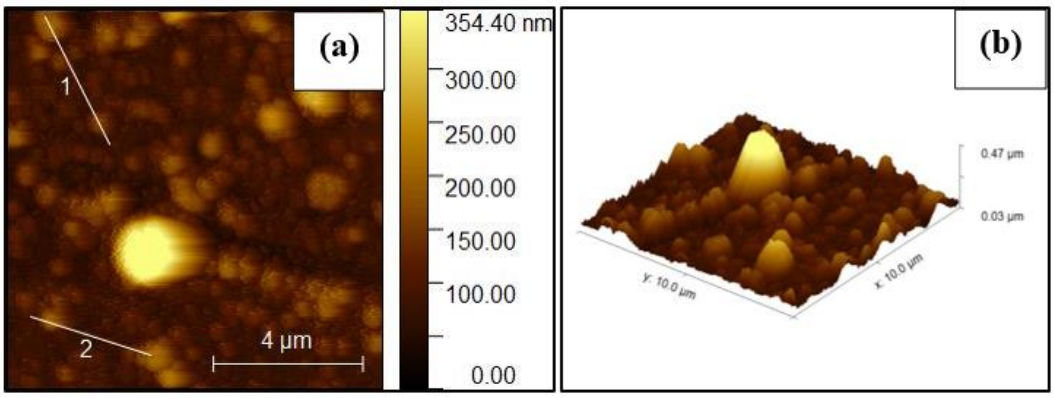

Fig 6: Mechanical properties of ZnO nanoparticles a) 2D image and b) 3D image

Antibacterial activity of biosynthesised silver nanoparticles

The mechanism of bactericidal effect of ZnO nanoparticles against bacteria is well known. ZnO Nanoparticle may attach to the surface of the cell membrane and disturbs its power function such as permeability and respiration. The results of this study clearly demonstrated that the ZnO Nanoparticle inhibits the growth and multiplication of the tested bacteria such as *Escherichiacoli* and *Pseudomonas aeruginosa.* The zone of inhibition was found for both the bacteria at all the concentration of ZnO Nanoparticle (Table 1). The maximum zone of inhibition (at 150 µl/ml of ZnO Nanoparticle) was foundto be 21.66 and 21.00 mm for *Escherichia coli*and *Pseudomonas aeruginosa*, respectively. The similar Antimicrobial activity Results obtained by (Jayappa, *et al* 2020) [17]. Whereas in the case of control, no zone of inhibition was observed. The effective antimicrobial activity is due to a spherical shape and small size of ZnO Nanoparticle. The zone of inhibition of *Escherichia coli* and *Pseudomonas aeruginosa* at different concentrations of ZnO Nanoparticleis shown in (Fig 7).

Table 1: Antibacterial activity of ZnOnanoparticleson*E.coli and P.aeruginosa*

Sl. No	ZnO Nanoparticles Concentration (µl/ml)	Zone of Inhibition (mm)	
		Escherichia coli	*Psedomonues aeruginosa*
1	50	16.33	17.00
2	100	17.66	18.00
3	150	21.66	21.00

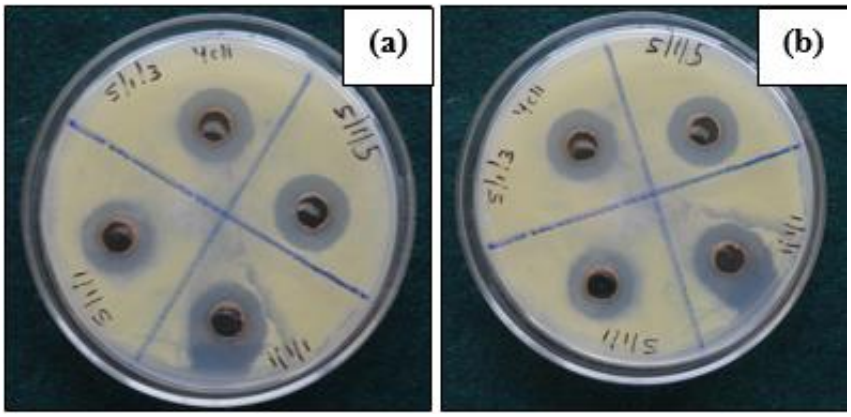

Fig 7: Zone of Inhibition of a) *Escherichia coli* and b) *Pseudomonas aeruginosa*

Effect of ZnO nanoparticles on Spodoptera in castor leaves

The obtained results in Tables summarized the toxicity and latent effect of ZnO Nanoparticles at different concentrations against the 2nd larval instar of Spodoptera. Data clearly showed that the larval mortality percentage showed positive correlation with the concentrations of the tested ZnO Nanoparticles and time after exposure. The mortalities were increased as concentrations and time after treatment increased. Mortality rate was low during the first three days. The highest cumulative larval mortalities were observed with the maximum conc. level (150 µl/ml). The pupation per cent and pupal weight resulted from the treated 2nd instars larvae with the different concentrations of ZnO Nanoparticlesgave highly reduction compared to untreated larvae. Among all, 150 µl/ml showed better effect in terms of mortality (76.67), pupation % (23.33), pupal weight (0.273 ± 0.14) against Spodoptera on 12th day. This study revealed that by (Jameel *et al*., 2020) [10]. The (Table2). Pertaining the data regarding the effect of ZnO Nanoparticles against Spodoptera in castor leaves.

Table 2: Influences of ZnO Nanoparticles on some biological aspects of Spodoptera (2nd larval instar)

Conc. µl/ml	% Cumulative larval mortality at indicated days						6th Larval weight (g) ± SE	Pupation %	Pupal weight (g) ±SE
	2	3	5	7	9	12			
T_0 (0)	0.00	0.00	0.00	0.00	3.33	6.67	0.39±0.04	93.33	0.38±0.04
T_1(50)	3.30	13.33	16.67	36.70	43.33	53.33	0.29±0.00	46.67	0.28±0.29
T_2 (100)	6.67	16.67	20.00	40.00	46.60	73.33	0.26±0.01	26.67	0.27±0.12
T_3 (150)	13.33	20.00	23.33	46.70	53.33	76.67	0.27±0.01	23.33	0.27±0.14

Conclusion

The biological synthesis provides a simple and efficient route for the synthesis of ZnO nanoparticles using Spinach leaf extract. The biomolecules present in the plant extract probably, terpenoids, flavonoids and saponins were responsible for the zinc-oxide nanoparticles takes place by the reduction of zinc ions by changing the quantity of plant extract and incubation time at room temperature the size of nanoparticles was varied. At optimum condition (Plant extract 5 ml and 10 °C incubation time) the average diameter of ZnO nanoparticles was 23.05 nm. This study also indicated that ZnO nanoparticles can be used as effective antibacterial materials against various microorganisms which can endanger human beings and it's also recorded highest mortality against insects.

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