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ORIGINAL ARTICLE

Biosynthesis of zinc oxide nanoparticles using leaf extract of *Calotropis gigantea*: characterization and its evaluation on tree seedling growth in nursery stage

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Abstract Green synthesis of zinc oxide nanoparticles was carried out using *Calotropis* leaf extract with zinc acetate salt in the presence of 2 M NaOH. The combination of 200 mM zinc acetate salt and 15 ml of leaf extract was ideal for the synthesis of less than 20 nm size of highly monodisperse crystalline nanoparticles. Synthesized nanoparticles were characterized through UV–Vis spectroscopy, dynamic light scattering (DLS), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), EDX (energy dispersive X-ray), and AFM (atomic force microscopy). Effects of biogenic zinc oxide (ZnO) nanoparticles on growth and development of tree seedlings in nursery stage were studied in open-air trenches. The UV–Vis absorption maxima showed peak near 350 nm, which is characteristic of ZnO nanoparticles. DLS data showed that single peak is at 11 nm (100%) and Polydispersity Index is 0.245. XRD analysis showed that these are highly crystalline ZnO nanoparticles having an average size of 10 nm. FTIR spectra were recorded to identify the biomolecules involved in the synthesis process, which showed absorption bands at 4307, 3390, 2825, 871, 439, and 420 cm^{-1}. SEM images showed that the particles were spherical in nature. The presence of zinc and oxygen was confirmed by EDX and the atomic % of zinc and oxygen were 33.31 and 68.69, respectively. 2D and 3D images of ZnO nanoparticles were obtained by AFM studies, which indicated that these are monodisperse having size ranges between 1.5 and 8.5 nm. Significant enhancement of growth was observed in Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*), and Milkwood-pine (*Alstonia scholaris*) seedlings in foliar spraying ZnO nanoparticles to nursery stage of tree seedlings. Out of the three treated saplings, *Alstonia scholaris* showed maximum height development.

Keywords *Calotropis* leaf · ZnO nanoparticle · DLS · XRD · FTIR · SEM with EDS · AFM · Tree seedling growth

Introduction

In the recent years, the use of metal nanoparticles gained greater interest due to their diverse applications in the field of medicine, biology, physics, chemistry, and material sciences (Kumar et al. 2014). Nanotechnology can be defined as manipulation of matter through certain chemical and/or physical processes to create materials with specific properties which can be used in particular applications. Nanoparticles can be defined as particles that have at least one dimension less than 100 nm in size (Thakkar et al. 2014). Unlike bulk materials, they have unique optical, thermal, electrical, chemical, and physical properties. Hence, they find a variety of applications in the areas of medicine, chemistry, environment, energy, agriculture, information and communication, heavy industries, and consumer goods (Panigrahi et al. 2004). In recent years, nanotechnology has emerged as a state-of-the-art and cutting-edge technology with multifarious applications in a wide array of fields. It is a very broad area comprising nanomaterials, nanotools, and nanodevices. Amongst nanomaterials, majority of the research has mainly focused on nanoparticles as they can be easily prepared and manipulated.

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Physical and chemical methods are conventionally used for the synthesis of nanoparticles; however, due to several limitations of these methods, such as use of toxic compounds, requirement of very high temperature and pressure, high expense, and time consumption, research focus has recently shifted towards the development of clean and eco-friendly synthesis protocols (Herlekar et al. 2014). It has been demonstrated that many biological systems including higher plants and algae, diatoms, bacteria, yeasts, fungi, and human cells can transform inorganic metal ions into metal nanoparticles via the reductive capacities of the proteins and metabolites present in these organisms. But synthesis of nanoparticles using microbes is difficult because it involves elaborate process of maintaining cell cultures, intracellular synthesis, and multiple purification steps. Hence, it is significant to note that the nanoparticle production using plant displays important advantages over other biological systems as plants are easily available and the procedure of biogenic synthesis is cost effective and less tedious as compared to biosynthesis using fungal source, which requires very long, tedious, and aseptic culture. The active biological compound present in plant parts like enzyme itself acts as a reducing and capping agent, thereby reducing the overall cost of synthesis process (Jain et al. 2009). In addition to enzymes, other biomolecules such as flavanoids, terpenoids, glycosides, alkaloids, inositols, resins, saponins, terpenes, volatile oil, tannins, steroids, and quinine also act as reducing and capping agents. In biogenic synthesis method, small nanoparticles can be produced during large-scale production (Kajbafna et al. 2009), and external experimental conditions like high energy and high pressure are not required causing significant energy saving (Kajbafna et al. 2012). Zinc oxide is an inorganic compound with the molecular formula ZnO. It appears as white powder and is nearly insoluble in water. The powder ZnO is widely used as an additive in numerous materials and products including ceramics, glass, cement, rubber, paints, plastics, lubricants, adhesives, ointments, sealants, pigments, food, batteries, ferrites, and fire retardants. In the earth crust ZnO is present as zincite mineral but mostly ZnO used for commercial purposes is produced synthetically. Nowadays, the unique properties of nanomaterials have motivated the researchers to develop many simpler and inexpensive techniques to produce nanostructures of technologically important materials. Several metal oxide nanoparticles are produced with possible future applications. Among them, ZnO is considered to be one of the best exploited at nanodimensions. The wide band gap and large excitonic binding energy have made ZnO important both for scientific and industrial applications (Wang et al. 2004). ZnO nanoparticles have fascinated research interest and a lot of work has been done using various plant sources. Leaf extract of *Calotropis gigantea* using $ZnNO_3$ salt produced nanoparticles of size 30–35 nm (Vidya et al. 2013). ZnO nanoparticle of size 100–200 nm were produced by zinc acetate salt with *Acalypha indica* leaf extract (Gnanasangeetha and Thambwani 2013). *Parthenium hysterophorus* leaf extract with $ZnNO_3$ salt produced ZnO nanoparticles of 16–108 nm size (Sindhura et al. 2014). ZnO nanoparticles of size ranging from 30 to 35 nm were produced using *Hibiscus rosasinensis* with $ZnNO_3$ salt (Devi and Gayathri 2014). Zinc acetate salt with leaf extract of *Azadirachta indica* produced ZnO nanoparticle of size 25 nm (Oudhia et al. 2015). Peel extract of *Punica granatum* with $ZnNO_3$ salt produced ZnO nanoparticle (Mishra and Sharma 2015). *Murraya koengii* leaf extract with $ZnNO_3$ salt produced ZnO nanoparticle of size 50 nm (Divyapriya et al. 2014). Leaf extract of *Camellia sinensis* with zinc acetate salt produced ZnO nanoparticle of size 16 nm (Senthilkumar and Sivakumar 2014). Extensive research is going on for commercializing ZnO nanoparticles throughout the world due to their unique properties. *Olea europa* leaf extract with $ZnSO_4$ salt produced ZnO nanoparticles of 20 nm diameter (Awwad et al. 2014). ZnO nanoparticles of size less than 50 nm were synthesized using dried sap of roots and shoots of *Astragalus gummifer* (Darrudi et al. 2013). Biosynthesis of ZnO nanoparticle of size ranging from 50 to 200 nm was done using *Citrus aurantifolia* with zinc acetate salt (Samat and Nor 2013).

Nanofertilizer technology is very innovative but scantly reported literature is available in scientific journals. Substituting nanofertilizer for traditional methods of fertilizer application is a way to release nutrients into the plants in a gradually and controlled manner, thus preventing autrification and pollution of water resources (Naderi and Abedi 2012). There has been a wide use of chemical products such as fertilizers and minerals which are used to increase the yield of various products in the field at the same time. These chemicals result in various side effects in the plants and soil, and harm the environment in different ways. Nano-ZnO particle (20 nm), nano-FeO (100 nm), and nano-CuO (40 nm) at ppm level showed increased shoot and root length in Mung seedling by foliar spray (Dhoke et al. 2013). Foliar spray method is more practical for an agronomic standpoint as plants can absorb essential elements through their leaves more efficiently compared to root feeding. Biologically synthesized ZnO nanoparticles are quickly transported through the plant and included in the metabolic processes. Mungbean seed germination in lowest concentration (20 mg) of ZnO suspension solution showed good shoot and root growth results (Jayarambabu et al. 2014). Silver nanoparticles synthesized from banana (*Musa balbisiana*), neem (*Azadirachta indica*), and black tulsi (*Ocimum tenuiflorum*) showed significant increase in the root and shoot length of germinated seedlings of Mung bean (*Vigna radiata*) and Chickpea (*Cicer arietinum*) (Banerjee et al. 2014). Nanofertilizer technology is very innovative as some reports and patents strongly suggest that there is a vast scope of formulation of nano-fertilizers. Central Arid Zone Research Institute, Jodhpur, developed the infrastructure and standardized biosynthesis methods for synthesis and characterization of large number of nanonutrients and their effective use in crop improvement. Significant increase in yield was observed in Pearl Millet (*Pennisetum americanum*) due to foliar application of zinc oxide nanoparticles as fertilizer (Tarafdar et al. 2014). Currently, research is underway to develop nanocomposite to supply all the essential nutrients in suitable proportion through smart delivery system. Preliminary results suggest that balanced fertilization may be achieved through nanotechnology (Tarafdar et al. 2012). Nanofertilizer nutrients can be encapsulated by nanomaterial cooled with a thin protective film or delivered as emulsions or nanoparticles. Nanomaterials could even be used to control the release of the fertilizer such that the nutrients are only taken up by the plants and not lost to unintended targets like soil, water, or microbes. Recent reports indicated that biosynthesized silver nanoparticles using seed exudates of *Spinosa arvensis* showed antifungal (*Neofusicoccum parvum*) activities (Khatami et al. 2015) and may be responsible for increased seed germination.

The plant *Calotropis gigantean* belonging to the family Asclepiadaceae, also called as Akra, Shwet akra, and Madara, is distributed throughout India, especially in dry vast land. The different parts of the plant are used in Indian traditional medicine for the treatment of painful muscular spasm, dysentery, fever, rheumatism, asthma, and as an expectorant and purgative. Bioactive compounds such as 15β-hydroxycardenolides (1,2) and a 16α-hydroxycalactinic acid methyl ester (3) along with eleven known compounds including 16α-hydroxycalotropagenin, coroglaucigenin, 16α-hydroxycalotropin, calactinic acid, calotoxin, desglucouzarin, 12β-hydroxycoroglaucigenin, frugoside, calotropagenin, dienoic acid, and mevalonolactone are found in the leaves of this plant (Singh et al. 2011). *Calotropis* yields a durable fiber that is useful for ropes, carpets, fishing nets, and sewing thread. Floss, obtained from seeds, is used for stuffing purposes. Extracts of different plant parts such as root, stem, and leaf affect germination and seedling vigor of many agricultural crops. It is also used as green manure and improves soil nutrients and improves moisture binding capacity of soil. The plant is tolerant of dry and salty conditions and can easily be established in over-cultivated areas to help improve the soil conditions and reinvigorate the land (Seeka and Sutthivaiyakit 2010). Moreover, this plant is widely distributed predominantly in Thar Desert area of Rajasthan. Hence, such medicinal and commercial uses of *Calotropis* plants have increased our interest and lead us to work on this plant species. Biosynthesis of nanoparticles is an exciting recent addition to the large repertoire of nanoparticle synthesis methods and now nanoparticles have entered a commercial exploration period. Gold, silver, copper, and zinc have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in areas such as photolysis, diodes, piezoelectric devices, fluorescent tubes, laser, sensor, photography, biological labeling, photonics, and surface-enhanced Raman scattering detection. Till date, use of ZnO nanoparticles is mainly restricted in agriculture applications for enhanced productivity in crop plants. But there is not a single report showing the use of ZnO nanoparticles in nursery stage for enhanced growth and development of tree seedlings. Defence Laboratory Jodhpur is working on arboriculture camouflage for military applications in the desert area of Rajasthan. In the desert area of Rajasthan, the growth of trees is very slow due to adverse climatic conditions (high temperature up to 50 °C, low humidity, less rainfall, and saline soil). To get enhanced growth and development, large canopy in short-time foliar spraying of ZnO nanoparticles may be desirous to overcome the climatic problems and enhanced growth and development of seedlings at nursery stage.

In this paper, we reported the biosynthesis of stable colloidal zinc oxide nanoparticles using leaf extract of *C. gigantea*. This plant is an important medicinal plant and predominantly distributed in Thar Desert area of Rajasthan. Details of biosynthesis, characterization, and its effects on enhanced growth and development of tree seedling in nursery stage are described.

Materials and methods

Reagents

Zinc acetate dihydrate (Lot # MKBQ7110v) was obtained from Sigma-Aldrich and NaOH from E-Merck, India.

Collection of plant material

Fresh leaves of *C. gigantea* were collected from Defence Laboratory, Jodhpur campus (26.2717°N, 73.0378°E). Collected leaves were thoroughly washed under tap water and then were washed with Milli-Q water and chopped with knife. The leaves were kept in oven for drying at 60 °C for 3 days, and dried leaves were powdered using home mixer blender and stored in an air-tight container at room temperature till further use.

Preparation of leaf extract

Glass goods and plastic wares were thoroughly cleaned in chromic acid and washed repeatedly in tap water followed by final wash in Milli-Q water before drying in hot air oven. Glass goods, plastic wares, and Milli-Q water required for biosynthesis and characterization of nanoparticles were autoclaved before use. 5 g of dried leaf powder was transferred to a 250-ml beaker containing 100 ml Milli-Q water and heated for 15 min at 60 °C. The solution was kept for cooling at room temperature and then filtered using Whatman filter paper no. 1. The filtrate was collected in amber bottle and was stored at 4 °C for further experiment.

Biosynthesis of ZnO NPs

15 ml of leaf extract of *Calotropis* was added to 2.195 g of zinc acetate dihydrate dissolved in 35 ml of distilled water (overall concentration 200 mM solution). The reaction mixture was kept on magnetic stirrer for 6 h. After 6 h, 2 M NaOH (4 g of NaOH pellet in 50 ml of Milli-Q water) was added to the solution and it was placed in incubator at 60 °C with magnetic stirring for overnight. White mixture was centrifuged at 14,000 rpm for 15 min. Precipitate was subjected to washing with alcohol and distilled water three times each. Precipitate was dried in an incubator at 40–50 °C and fine powder was prepared with the help of ceramic pestle and mortar. Fine powder was used for characterization with AFM, SEM, FTIR, XRD, EDS, UV–Vis, and DLS.

Characterization of biosynthesized ZnO NPs

pH analysis

Zinc acetate aqueous solution (200 mM) showed pH 6.58. pH of aqueous *Calotropis* leaf extract was 6.23. The changed pH of reaction mixtures was recorded using digital pH meter (Eutech Cyberscan pH 300) during the synthesis of zinc oxide nanoparticles.

UV–Vis spectroscopy

The reduction of zinc ion was monitored by measuring optical density through UV–Vis spectroscopy of the reaction medium after diluting small aliquots of reaction mixture ten times diluted with Milli-Q water and transferred to cuvette, and analysis was done using UV–Vis spectrophotometer (Ocean Optics, USA).

Dynamic light scattering (DLS) analysis and zeta potential measurement

Particle size distribution and average size of zinc oxide nanoparticles were obtained through particle size analyzer. Liquid sample before centrifugation was diluted ten times using Milli-Q water and transferred to cuvette, and analysis was done using DLS (Malvern Zetasizer, Nano Z500 UK). The sample holder temperature was maintained at 25 °C. The measurements depend on the size of the particle core, surface structure, particle concentration, and the type of the ion in the mixture. The zeta potential of the synthesized nanoparticles was determined in water as dispersant.

X-ray diffraction

The formation and quality of compounds were investigated by X-ray diffraction technique. For this purpose, synthesized zinc oxide NPs were centrifuged (1400 rpm; 8 °C) for 15 min, pellet was washed three times with ethanol and finally with sterile Milli-Q water for three cycles. The purified ZnO NP precipitate was dried in oven at 60 °C and powdered with ceramic mortar–pestle. Powdered sample was analyzed using X-ray diffractometer (X'Pert PRO-PAN Analytical, Europe). The scanning was done in the region of 2θ from 20° to 80°.

FTIR analysis

FTIR was used to identify the possible functional groups involved in the reduction of zinc ion and capping of reduced zinc oxide nanoparticles. FTIR spectrum was recorded using Shimadzu, Japan, infrared (IR) double-beam spectrophotometer. FTIR analysis of dried ZnO nanoparticles (NPs) was carried out through potassium bromide (KBr) pellet method in 1:30 ratios (NPs:KBr) and spectrum was recorded in transmittance mode at a resolution of 4 cm^{-1}. The peaks (stretching) obtained were plotted as transmittance in *Y*-axis and wave number (cm^{-1}) in *X*-axis. The spectrum was recorded in the wave number range 500–4500 cm^{-1} and analyzed subtracting the spectrum of pure KBr.

SEM analysis

Scanning electron microscopy (SEM) analysis was carried out using Carl Zeiss Japan, model machine. Thin film of nanoparticle powder sample was prepared on carbon-coated tape by adhering small amount of dried fine powder of sample on the grid, excess sample was removed with the help of blotting paper. The film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min. The SEM analysis was used to determine the surface structure of biogenically synthesized ZnO NPs.

Energy-dispersive X-ray spectroscopy

Sample used for SEM was used as it is and the same instrument was used. EDX analysis was carried out to determine the chemical purity, elemental composition, and stoichiometry of the synthesized zinc oxide nanoparticles.

Atomic force microscopy

Freshly cleaved mica sheet was adhered on substrate using double-sided tape. Diluted sample prepared using ultrasonicator was placed upon the substrate using drop technique with autoclaved microtip. This sample was placed and dried in incubator at 37 °C for 1 h. Analysis was done using Z-03 scanner in AFM (NT-MDT Solver Pro, Russia).

Evaluation of ZnO NP effects on tree seedling growth

One-year-old saplings developed in Defence Laboratory, Jodhpur, nursery were used for the treatment of zinc oxide (ZnO) nanoparticles (NPs). Six trenches were prepared in a specific distance within the iron net house in open air. Hundred saplings of each species (Neem—*Azadirachta indica*, Milkwood-pine—*Alstonia scholaris*, and Tilpapra/Karanj—*Pongamia pinnata*) were placed in control and treated sets (fifty saplings in each trench). Initial heights were recorded with measuring scale. Spraying of biogenic ZnO NPs at 30 mg per liter synthesized from *Calotropis* leaf extract was carried out through aerosol foliar spraying. The optimal concentration (30 mg/l) for seedling treatment was determined in tree seed germination studies (data not included here) before the nanoparticle foliar spray treatment. Watering of the trenches was carried out in specific interval in a general practice (twice a week) of nursery development. After 3 months of treatment, the height of each sapling was measured and spraying of ZnO NPs at 30 mg per liter was done once again and after 6 months the final height of each sapling was recorded for statistical data analysis.

Statistical data analysis

Analytical determination was carried out from the average mean data of 50 plants in a set (control and treated). All experimental data were expressed as mean ± standard error.

Results and discussion

Color change

Reduction of zinc is confirmed by color change of the reaction mixture from pale yellow to white (Fig. 1; Table 1).

Variation in pH during biosynthesis of ZnO NPs

UV–vis spectroscopy

Optical properties of ZnO nanoparticles were characterized using UV–Vis spectrophotometer. The UV–Vis absorption curve of ZnO nanoparticles is shown in Fig. 2. Zinc oxide formation was confirmed as the absorption peak (lambda max) was found near 350 nm. This result correlates with the already reported results, in which absorption peak was found at 360 nm (Jayarambabu et al. 2014) (Table 2).

Dynamic light scattering analysis

The size of synthesized zinc oxide nanoparticles when analyzed by DLS shows the mean size of synthesized ZnO nanoparticles as 11 nm (100%) in a single peak (Fig. 3) and Polydispersity Index (PDI) is 0.245.

Zeta potential

Figure 4 shows the measured zeta potential value of biosynthesized zinc oxide nanoparticles in the colloidal solution. The nanoparticles possess a negative zeta potential value of −20.7 mV, which indicated that even after the storage of 3 months at room temperature these are highly stable due to electrostatic repulsive force. The high negative value confirms the repulsion among the particles and the negative indicates that nanoparticles are stable. Measurement of zeta potential depends on the movement of nanoparticles under influence of an applied electric field. This movement depends upon surface charge and the local environment of the particle.

X-ray diffraction

The dried powdered sample was used to perform XRD for confirming the size of ZnO nanoparticles. The graph (Fig. 5) showed main peaks corresponding to 2θ values of 32.25°, 34.88°, 36.70°, 47.97°, 56.99°, 63.23°, 66.79°, 68.33°, 69.43°, and 77.39°. This result is almost similar to already reported results in *O. europa* leaf extracts in which 2θ values were obtained at 31.84°, 34.50°, 36.32°, 47.59°, 56.63°, 66.89°, 67.98°, 69.09°, and 76.98° (Awwad et al. 2014). The size of nanoparticles was in the range of 8–12 nm and the average size of nanoparticles determined by XRD was 10 nm (using Debye–Scherrer equation).

FTIR analysis

To determine the functional groups responsible for the synthesis of ZnO NPs in *C. gigantea* leaf extract, FTIR analysis was performed. The FTIR spectrum of *C. gigantea*

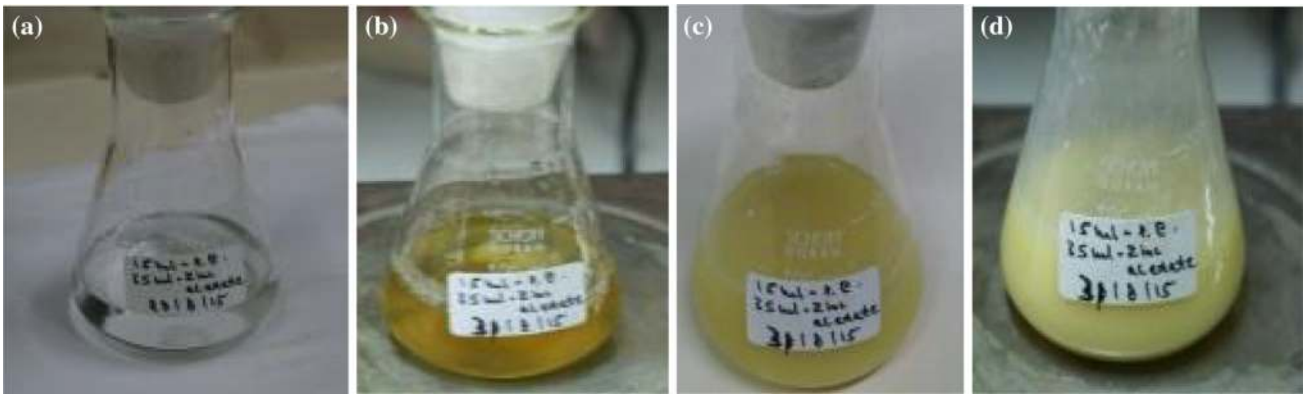


Fig. 1 **a** 200 mM zinc acetate solution (transparent), **b** 35 ml of zinc acetate solution + 15 ml of leaf extract (*yellowish white*), **c** after 6 h (*pale yellow color*), **d** after 24 h (*white color* intensity increased)

Table 1 Change in color of solution during formation of zinc oxide nanoparticles using *Calotropis* leaf extract

Solution	Before reduction	After reduction	Color intensity	Time
Calotropis leaf extract	Light brown			
200 mM zinc acetate	Transparent	Pale yellow	+	Immediate
		Yellowish white	++	After 6 h
		White	+++	After 24 h

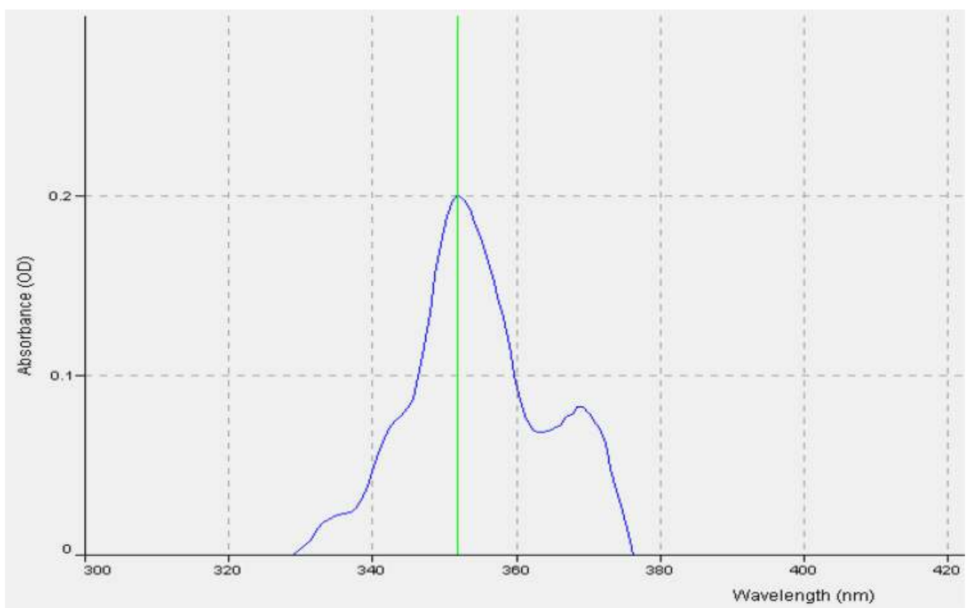


Fig. 2 UV–vis absorption spectra of zinc acetate solution, plant extracts and synthesized ZnO nanoparticles after 24 h

leaf extract is shown in Fig. 6, which shows absorption bands at 4307, 3390, 2825, 871, 439, and 420 cm^{-1} which are characteristic of –OH stretching vibration and –CH stretching vibration. The band at 871 cm^{-1} is due to asymmetrical and symmetrical stretching of zinc carboxylates resulting in the involvement of carboxylic groups in protein of *Calotropis* leaf extract. This result coincides with the already reported result of biosynthesis of ZnO nanoparticles using *O. europa* leaf extract (Awwad et al. 2014). Studies confirmed the presence of phytochemicals (eicosatrienoic acid methyl ester, hexatriacontaine, trimethyl undecatriene, and trifluoroacetic acid), volatile essential oil (phytol), and flavanoids (varinging, quercitrin, hesperitin, and kaempferol). It also contains acalyphamide, 2-methylanthraquinone, tri-*o*-methyl ellagic acid, sitosterol, glucoside, stigmasterol, quinine, tannins, resins, and

Table 2 Change in pH of solution during formation of zinc oxide nanoparticles using *Calotropis* leaf extract

Solution	Before reduction (pH)	After reduction (pH)
Calotropis leaf extract	6.23	–
200 mM zinc acetate	6.58	–
15 ml of leaf extract + 35 ml of 200 mM zinc acetate	6.14	11.40 (after 6 h)
	11.12 (immediately after adding NaOH)	12.50 (after 24 h)

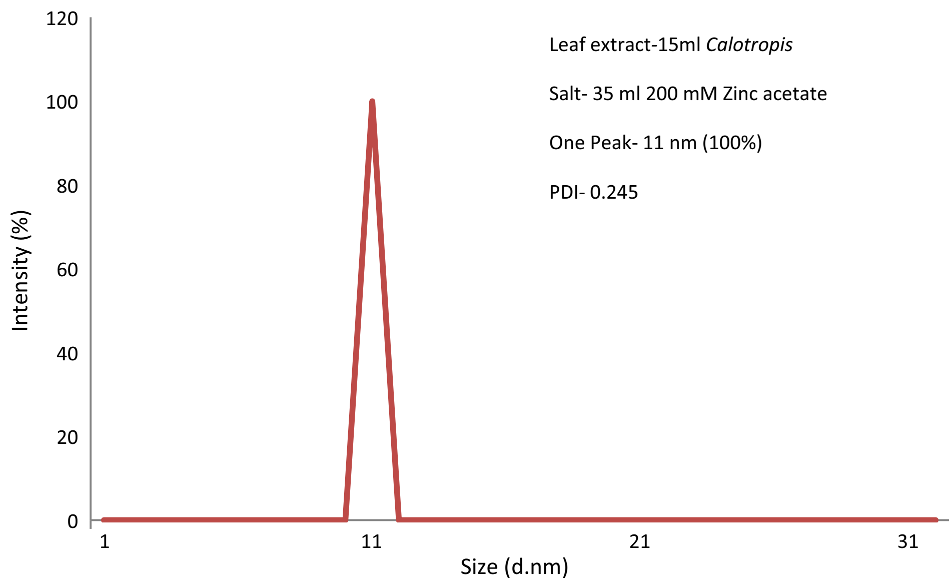


Fig. 3 Size distribution and mean particle size of synthesized zinc oxide nanoparticles

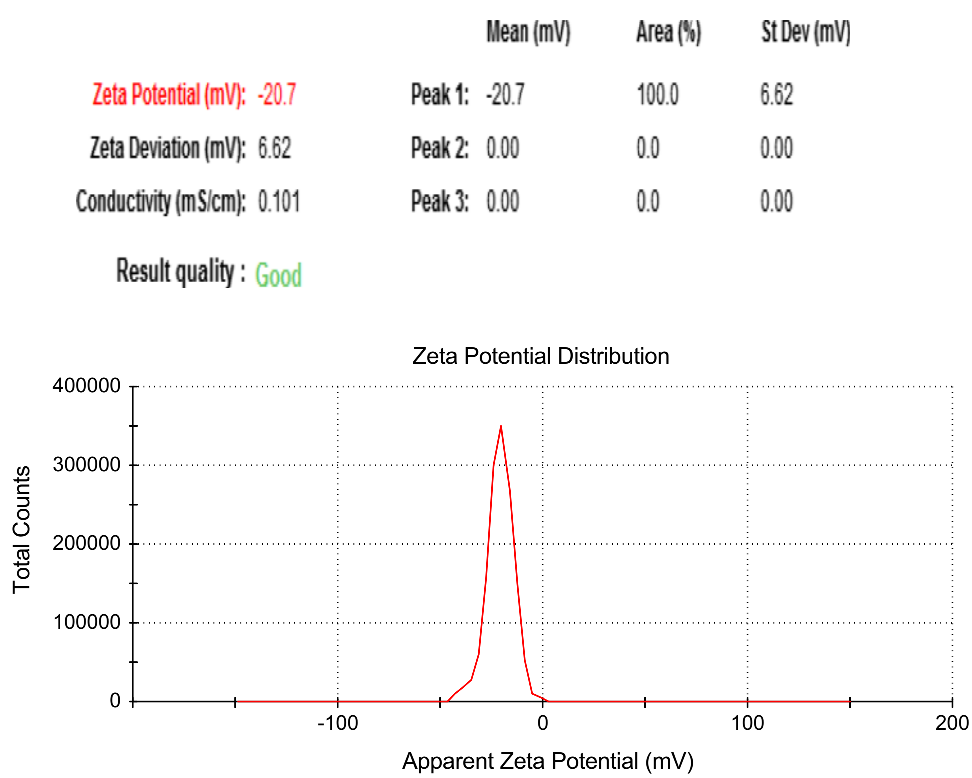


Fig. 4 Zeta potential measurement

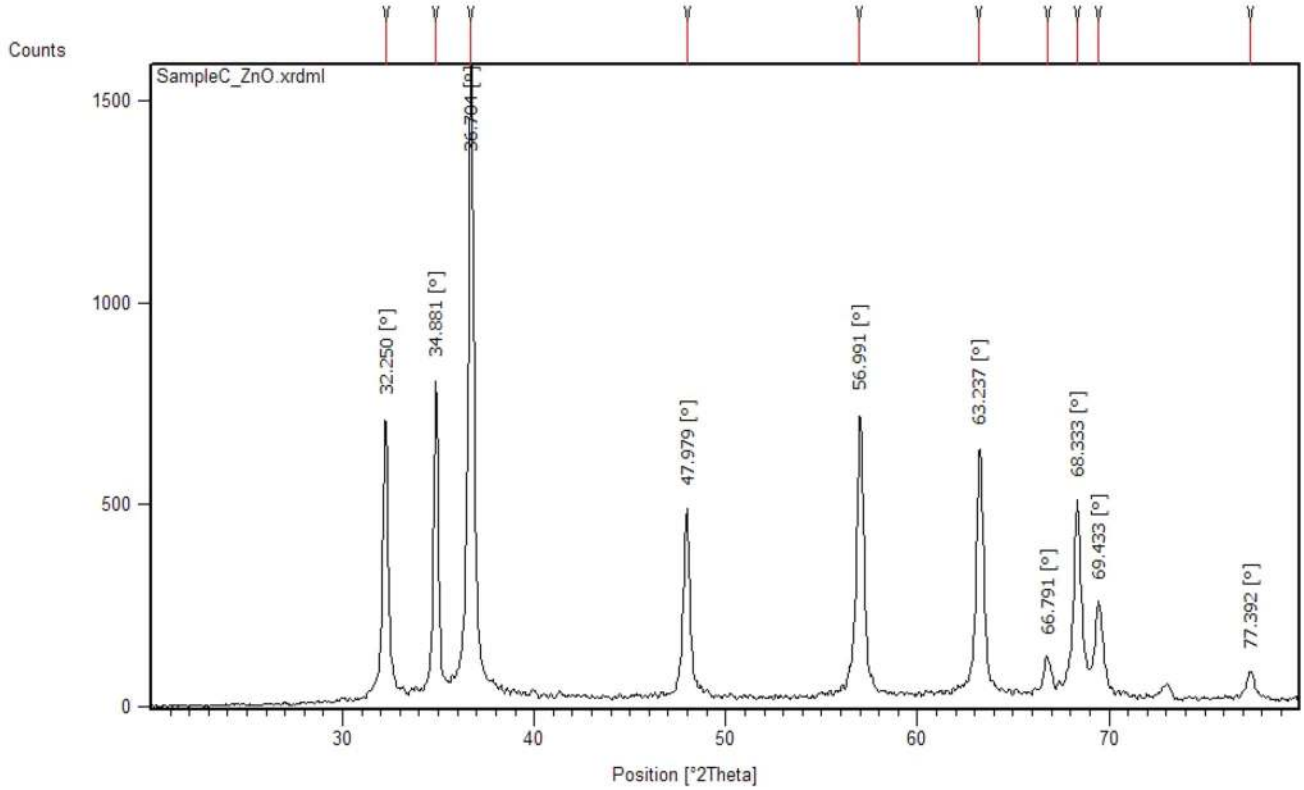


Fig. 5 XRD graph of biosynthesized zinc oxide nanoparticles

essential oils. Results inveterate that –OH stretching around 3390 cm^{-1} and –CH stretching around 2825 cm^{-1} are responsible for strong capping on the ZnO nanoparticles. This result matches with the already reported result of biosynthesis of ZnO nanoparticles using *Acalypha indica* leaf extract (Gnanasangeetha and Thambwani 2013).

SEM analysis

SEM was employed to analyze the structure of nanoparticles that were formed. SEM image has shown individual ZnO nanoparticles as well as a number of aggregates. SEM image in Fig. 7 shows that these are spherical-shaped nanoparticles. This SEM result coincides with results already reported, which shows formation of spherical-shaped nanoparticles and aggregated molecules in *Calotropis* leaf extract (Vidya et al. 2013).

Energy-dispersive X-ray spectroscopy

EDX analysis was carried out to determine the elemental composition and stereochemistry of the synthesized zinc oxide nanoparticles. In Fig. 8, zinc and oxygen signals detected that the synthesized nanoparticles are in pure state of chemical nature. The single peak of Zn and O is found between 0 and 2, and two peaks of Zn were found in between 8 and 10. These results correlate with the already reported results in which similar peaks have been observed in ZnO NP synthesis using *Acalypha indica* leaf extract (Gnanasangeetha and Thambwani 2013).

Further analysis was done to find weight % and atomic % of zinc and oxygen elements present in the biogenically synthesized sample using *Calotropis* leaf extract (Table 3).

Atomic force microscopy

To validate the surface morphology of biogenic ZnO NPs, drop-coated two- and three-dimensional AFM images were taken in noncontact mode. Result showed variability in morphological features of biosynthesized zinc oxide nanoparticles (Fig. 9a, b). The sizes range from 1.5 to 8.5 nm and the particles are more or less homogeneous in size range and monodisperse nature.

Evaluation of ZnO NP effects on tree seedling growth

For studying the effects of ZnO NPs on growth and development of three important tree seedlings (*Azadirachta indica*, *Alstonia scholaris*, and *P. pinnata*) in nursery stage, they were treated with ZnO NPs at a final concentration of

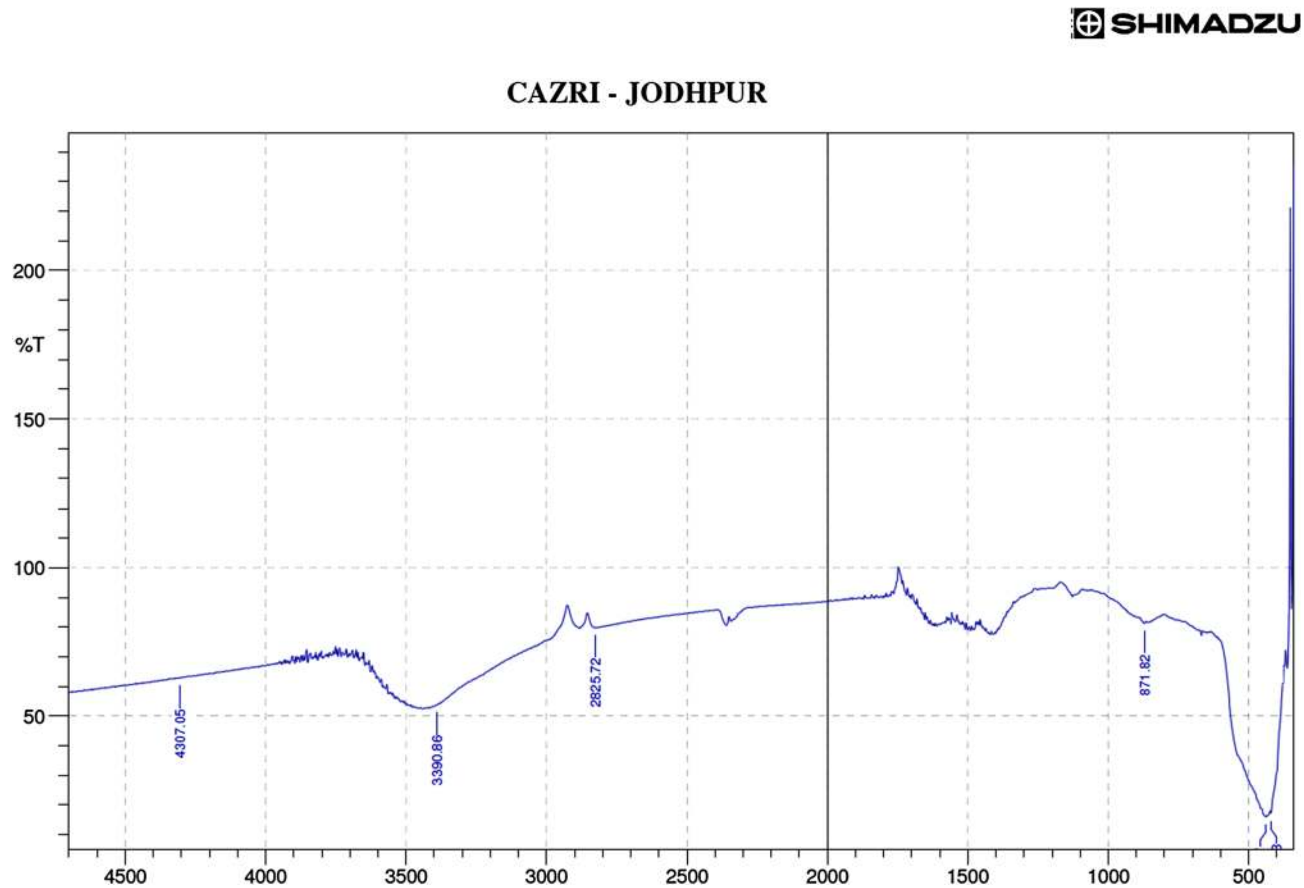
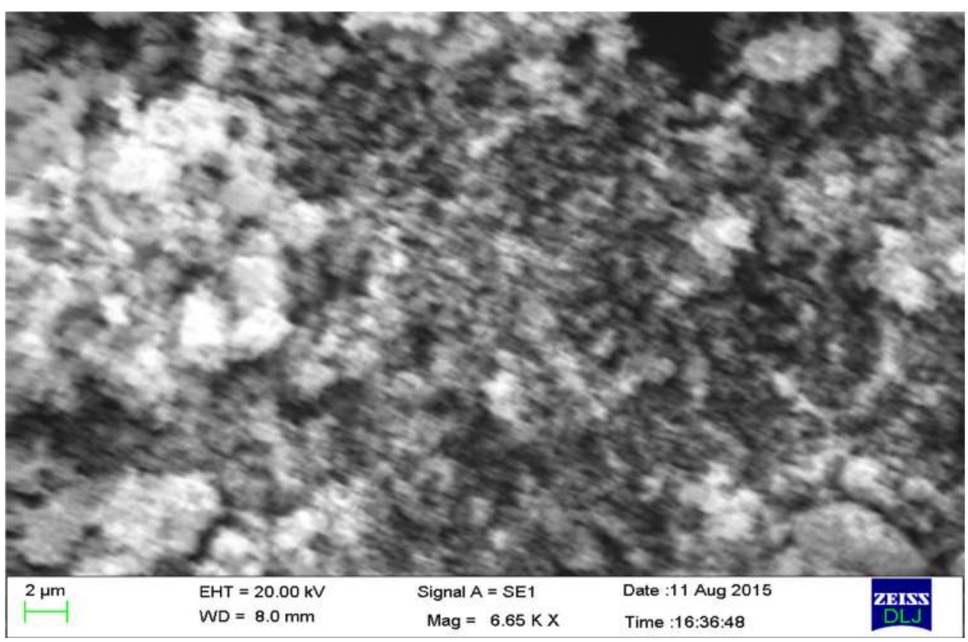


Fig. 6 FT-IR spectrum of the biosynthesized ZnO nanoparticles

Fig. 7 SEM image of zinc oxide nanoparticles



30 mg/l for each treatment. After treatment, seedling growth in terms of plant height was measured. Increased height in all three plant species could be observed compared to control sets (Fig. 10). Significantly enhanced growth and development was evident in *Alstonia scholaris* compared to other two species (Fig. 11).

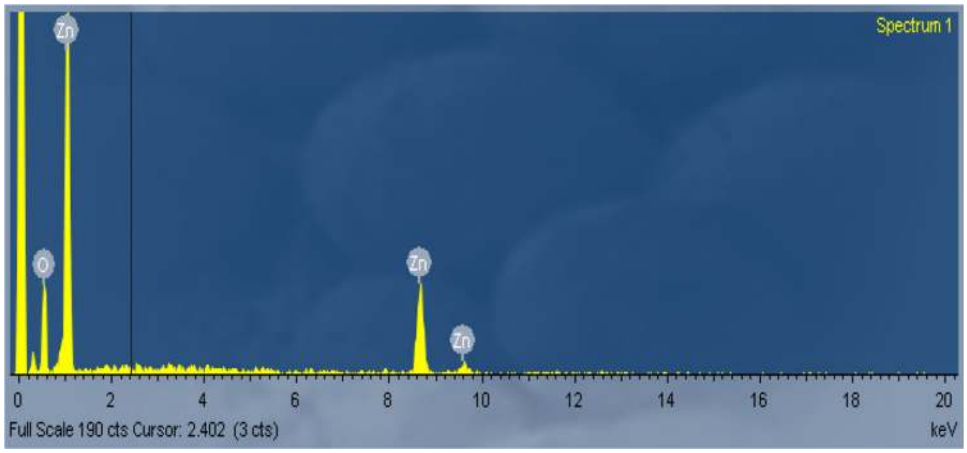


Fig. 8 EDS spectrum of synthesized zinc oxide nanoparticles

Table 3 EDX analysis showing weight % and atomic % of zinc and oxygen elements present in the sample

Element	Weight %	Atomic %
Zinc	59.85	31.31
Oxygen	40.15	68.69

Conclusions

Green-synthesized nanoparticles have a potential role in the form of nanofertilizers in the current scenario, this work may help in future development of nanonutrients for plant growth and development. Recently, we have reported the potential use of endemic hot desert plant Rohida's leaf and flower (*Tecomella undulata*) extract for the synthesis of silver nanoparticles (Chaudhuri et al. 2016; Chaudhuri and Malodia 2017). In Thar Desert area of Rajasthan *Calotropis* plants are available throughout the year and they can easily survive in high temperature without any irrigation. So, Thar Desert bioresources can effectively be used for the biosynthesis of nanoparticles and their use as nanonutrients. The colloidal solution of zinc oxide nanoparticles is used as fertilizer for fast growth and development in tree seedlings. This type of nanofertilizer is a plant nutrient which is more than a conventionally used fertilizer because it not only supplies nutrients for the plant but also revives the soil to stay in organic state without any harmful factors of chemical fertilizers. One of the advantages of nanofertilizers is that they can be in very small amounts compared to chemical fertilizers. Nanopowders can be successfully used as fertilizers and pesticides as well for increased agricultural crop productivity. In the field of crop plant growth and yield, the role of various types of nanomaterials gradually increased. Nanoparticles are found to be effective in seed germination in lettuce and cucumber (Shah and Belozerova 2009; Barrena et al. 2009), and enhanced growth and yield in wheat (Razzaq et al. 2016). Enhanced seedling growth and diosgenin content (Jasim

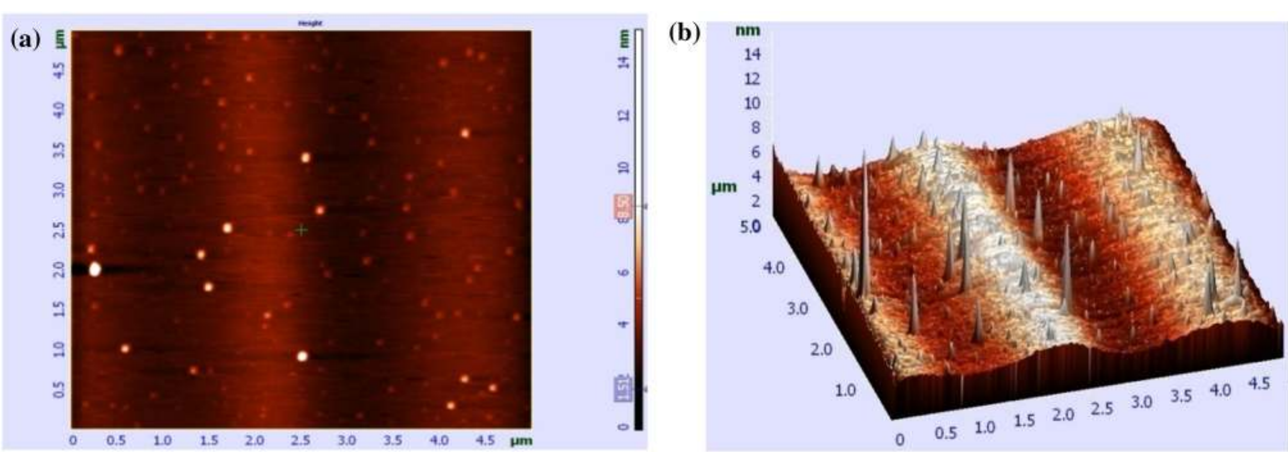


Fig. 9 **a** AFM 2D image. **b** AFM 3D image

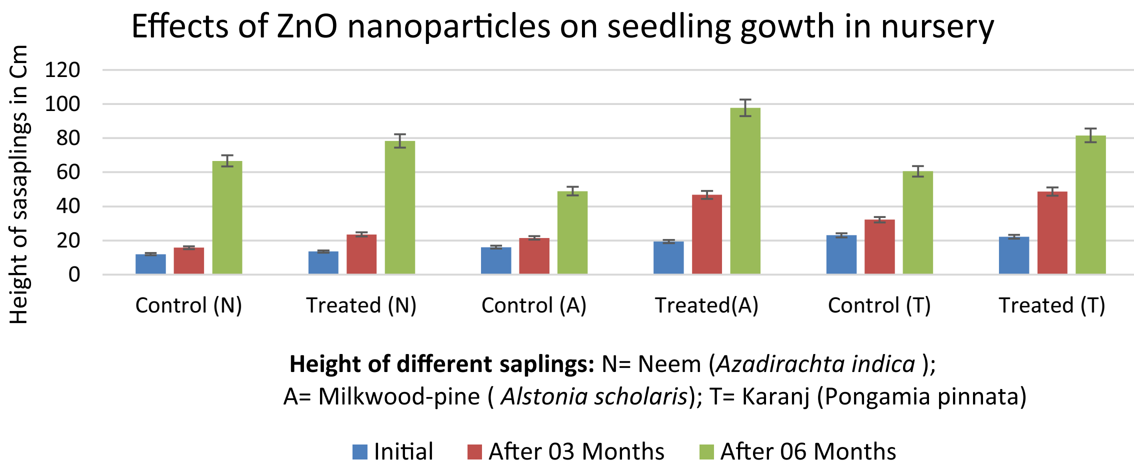


Fig. 10 Effects of ZnO nanoparticles (30 mg/l) on growth and development of nursery saplings. Data represent mean of 50 replicates and *vertical bars* represent ± standard error

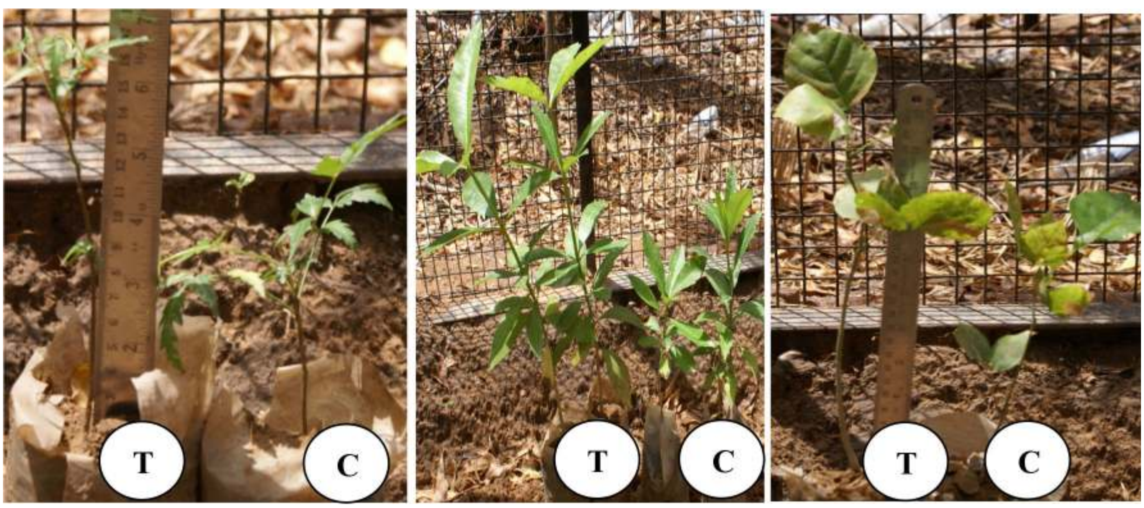


Fig. 11 Comparison between treated (T) and control (C) saplings of *Azadirachta*, *Alstonia,* and *Pongamia* after 3 months of ZnO nanoparticles foliar spraying (treatment)

et al. 2016) were also reported in silver nanoparticle-treated seeds of fenugreek (*Trigonella foenum-graecum* L.). In military camouflage applications, fast growth and large canopy trees are ideal for natural concealment. In the desert area of Rajasthan, plant growth is very much slow due to harsh climatic conditions. The formulation of nanonutrients using nanoparticles and their foliar spraying in nursery seedlings may enhance the growth and development, and stress tolerance to biotic and abiotic factors of identified saplings in nursery stage, which could be used in military camouflage package (arboriculture camouflage).

References

Awwad AM, Albiss B, Ahmad AL (2014) Green synthesis, characterization and optical properties of zinc oxide nanosheets using *Olea europa* leaf extract. Adv Mater Lett 5:520–524

Banerjee P, Satpathy M, Mukhopadhayay A, Das P (2014) Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresourc Bioprocess 1:1–10

Barrena R, Casals E, Colon J, Font X, Sanchez A, Puntes V (2009) Evaluation of the ecotoxicity of model nanoparticles. Chemosphere 75:850–857

Chaudhuri SK, Malodia L (2017) Phytosynthesis and characterization of silver nanoparticles synthesized from flower extract of Roheda (*Tecomella undulata* G. Don). Defence Life Sci J 2:65–73

Chaudhuri SK, Chandela S, Malodia L (2016) Plant mediated green synthesis of silver naoparicles using *Tecomella undulata* leaf extract and their characterization. Nano Biomed Eng 8:1–8

Darrudi M, Oskuee RK, Kargar H (2013) Sol-gel synthesis, characterization and neurotoxicity effect of zinc oxide nanoparticles using gum tragacanth. Ceram Int 40:4827–4831

Devi RS, Gayathri R (2014) Green synthesis of zinc oxide nanoparticles by using *Hibiscus rosa-sinensis*. Int J Curr Eng Technol 4:2444–2446

Dhoke SK, Mahajan P, Kamble R, Khanna A (2013) Effects of nanoparticles suspension of mung seedlings by foliar spray method. Nanotechnol Dev 3:1–5

Divyapriya S, Sowmia C, Sasikala S (2014) Synthesis of zinc oxide nanoparticles and microbial activity of *Murraya koeniggi*. World J Pharm Pharm Sci 12:1635–1645

Gnanasangeetha D, Thambwani DS (2013) Biogenic production of zinc oxide nanoparticles using *Acalypha indica*. J Chem Biol Phys Sci 1:238–246

Herlekar H, Barne S, Kumar R (2014) Plant mediated green synthesis of iron nanoparticles. J Nanoparticles. doi:10.1155/2014/140614

Jain J, Arora S, Rajkumar JM, Khandelwal S, Pahnihar KM (2009) Silver nanoparticles in therapeutics: development of an antimicrobial gel formation for topical use. Mol Pharm 5:1388–1401

Jasim B, Roshmi T, Mathew J, Radhakrishnan EK (2016) Plant growth and diosgenin enhancement effect of silver nanoparticles in Fenugreek (*Trigonella foenum-graecum* L.). Saudi Pharm J. doi:10.1016/j.jsps.2016.09.012

Jayarambabu N, Sivakumari B, Prabhu YT (2014) Germination and growth characteristics of mungbean seeds affected by synthesized zinc oxide nanoparticles. Int J Curr Eng Technol 5:3411–3416

Kajbafna A, Shayegh MR, Mazhammi M (2009) Nanostructure sword like ZnO wires: rapid synthesis and characterization through a microwave assisted route. J Alloy Compd 1:293–297

Kajbafna A, Ghorham H, Parnikar A, Sambrey JP, Sadrhezhaad SK (2012) Effects of morphology on photocatalytic performance of ZnO nanostructures synthesized by rapid microwave irradiation methods. Superlattices Microstruct 4:512–522

Khatami M, Pourseyedi S, Khatami M, Hamidi M, Zaeifi M, Soltani L (2015) Synthesis of silver nanoparticles using seed extract of *Spinosa arvensis* as a novel bioresource, and evaluation of their antifungal activity. Bioresourc Bioprocess 2:1–7

Kumar PPNV, Pammi SVN, Kollu P et al (2014) Green synthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their antimicrobial activity. Ind Crops Prod 52:562–566

Mishra V, Sharma R (2015) Green synthesis of zinc oxide nanoparticles using fresh peels extract of *Punica granatum* and its antimicrobial activities. Int J Pharma Res Health Sci 3:694–699

Naderi MR, Abedi A (2012) Application of nanotechnology in agriculture and refinement of environment pollutants. J Nanotechnol 1:18–26

Oudhia A, Kulkarni P, Sharma S (2015) Green synthesis of ZnO nanotubes for bioapplications. Int J Curr Eng Technol 1:280–281

Panigrahi S, Kendu S, Ghosh SK, Nath S, Pal T (2004) General methods of synthesis of metal nanoparticles. J Nanopart Res 4:411–414

Razzaq A, Ammara R, Jhanzab HM, Mahmood T, Hafeez A, Hussain S (2016) A novel nanomaterial to enhance growth and yield of wheat. J Nanosci Technol 2(1):55–58

Samat NA, Nor RM (2013) Sol-gel synthesis of zinc oxide nanaoparticles using *Citrus aurantafoli*a extracts. Ceram Int 39:545–548

Seeka C, Sutthivaiyakit S (2010) Cytotoxic cardenolides from the leaves of *Calotropis gigantea*. Chem Pharm Bull 5:725–728

Senthilkumar SR, Sivakumar T (2014) Green tea *Camellia sinensis* mediated synthesis of zinc oxide nanoparticles and studies on their antimicrobial activities. Int J Pharm Pharm Sci 6:461–465

Shah V, Belozerova I (2009) Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. Water Air Soil Pollut 197:143–148

Sindhura KS, Prasad TN, Selvam P, Hussain OM (2014) Synthesis, characterization and evaluation of effect of phytogenic zinc nanopaticles on soil exoenzymes. Appl Nanosci 4:819–827

Singh RP, Shukla P, Singh PK (2011) Biological approach of ZnO nanoparticle formation and characterization. Adv Mater Lett 2:313–317

Tarafdar JC, Xiang Y, Wang WN, Dong Q, Biswas P (2012) Standardization of size, shape and concentration of nanoparticle for plant application. Appl Biol Res 14:138–144

Tarafdar JC, Raliya R, Mahawar H, Rathore I (2014) Development of zinc nanofertilizers to enhance crop production in pearl millet (*Pennicetum americanum*). Agric Resourc 3:219–226

Thakkar KS, Mhatre SS, Parikh RY (2014) Biological synthesis of metabolic nanoparticles. Nanomedicine 2:257–262

Vidya C, Hiremath S, Chandraprabha MN, Venugopal I, Jain A, Bansal K (2013) Green synthesis of ZnO nanoparticle by *Calotropis gigantea*. Int J Curr Eng Technol 4:118–120

Wang X, Ding Y, Summers CJ, Wang ZL (2004) Large scale synthesis of 6 nm wide ZnO nanobelts. J Phys Chem 26:8773–8777