

# Green Synthesis of Zinc Oxide Nanoparticles using Leaf Extract of *Causonis trifolia* (L.) and its Applications on Germination and Growth Enhancement of Mustard Seeds

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The present work reported the seed germination and plant growth activity studies on mustard seeds using bio-inspired green synthesized zinc oxide nanoparticles (ZnO NPs). The ZnO NPs were synthesized *via* green method utilizing *Causonis trifolia* (L.) plant leaf extract as a capping as well as reducing agent. The synthesized zinc oxide nanoparticles (ZnO NPs) have been characterized by using powder XRD (X-ray diffraction), FTIR (Fourier transforms infrared spectroscopy), emission SEM (scanning electron microscopy) and UV-visible spectroscopy. Based on various characterizations, it was proved that the nanoparticles were hexagonal Wurtzite form with more or less spherical in shape having an average particle size of 58 nm that contains 78% of metallic zinc. Seed germination and plant growth activity studies of ZnO NPs have been performed on mustard seeds through the pot method. The result revealed a high seed germination rate (91.7%) of mustard seeds when comparing observed in control pots (62.1%). Further dose-dependent studies also performed under the same condition. The measurements of each plant shoot length and root length have performed after six days. The mustard plants root grown in pots with ZnO NPs at 1000 mg/Kg (6.9 cm) have grown 627% higher and plant in pots with ZnO NPs at 1000 mg/Kg (9.8 cm) has grown 316% at 100 mg/Kg (6.7 cm) has grown 216% higher than the control pot (3.1 cm). The results indicate that the synthesized ZnO NPs could potentially be used as an enrichment fertilizer to improve agricultural output.

Keywords: Green synthesis, Zinc oxide nanoparticles, Nano-fertilizers, Causonis trifolia (L.), Mustard seeds, Seed germination.

## **INTRODUCTION**

Nanotechnology has demonstrated promising approach for supporting the sustainable agriculture, especially crop production and protection, with a particular emphasis on nanofertilizers, nano-biosensors, nano-pesticides, along with nanoenabled remediation techniques for polluted soils [1]. Increasing chemical fertilizers' use contaminates the environment (by volatilization and leaching) and increases production costs [2].

Nanoparticles improve the agricultural inputs efficacy by facilitating the targeted delivery of nutrients on-site, hence reducing the consumption of agricultural inputs. The involvement of nanomaterials in plant protection also ensures an increase in crop yield [3]. There are different techniques available to perform syntheses by physical and chemical methods, but biological methods have played an important role in synthesizing nanomaterials [4,5]. The advantages of plant-derived

nanoparticle synthesis compared to conventional synthesis methods include rapid synthesis, cost-effectiveness, enhanced stability and multiple sizes and shapes. Among all plants, the pharmacologically important plants have proven the best sources for nanoparticle synthesis because of various phytochemicals [6]. Due to the exceptional physical properties and the better antibacterial, anti-tumor and drug-carrying properties, metal nanoparticles are considered the most effective for biomedical applications [7,8].

Element zinc is considered as an essential micronutrients for living organisms and plays significant role on the action of several enzymes. It acts as regulator of phytohormones and involves synthesis of chlorophyll and also essential during the metabolism of carbohydrates in plants [9]. Zinc oxide nanoparticles (ZnO NPs) show great potential for its use as nanofertilizers due to its unique and desirable properties in agriculture [10]. The available forms of zinc in soil are water-soluble

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complex and exchangeable, which was readily available to the plant. The deficiency of zinc in plants reduces the metabolism of nitrogen and slows down the process of photosynthesis that results reduces the flowering, development of fruit and ultimately reduces the crop production [11]. ZnO acts as source of zinc in agriculture fertilizer and can used to coat seeds, immerse roots and inject to trees to alleviate the deficiency of zinc. ZnO NPs are significantly soluble in water and also plants are able to absorb and accumulate them in their biomass [12,13].

The weak herbaceous climber *Cayratia trifolia* L., belongs to *Vitaceae* family and was native to India, Australia and Asia [14]. The whole plant was reported to be having tannins, terpenoids, flavonoids and steroids [15]. The leaves of the plant contain stilbenes such as ampelopsin, reveratrol, piceid and viniferin [16]. Various extracts of the plant was reported to be having vast pharmacological activities such as antibacterial, antiviral, hypoglycemic, antiprotozoal, diuretic and anticancer activity [17].

No reports is available in literature for the synthesis of nanoparticles using *Cayratia trifolia* L., the present study was intended to synthesize the ZnO NPs using aqueous leaf extract. Further, the synthesized zinc oxide nanoparticles were studied for its applicability for the germination of seeds of mustard seeds (*Brassica nigra* L.) using pot experiment.

# EXPERIMENTAL

Fresh leaves of *Cayratia trifolia* L. was collected from the coastal region of Machilipatnam, Krishna district, India. The analytical grade zinc sulphate, sodium hydroxide, nitric acid and other chemicals utilized in the study were procured from Fisher-Scientific, Mumbai, India.

Powder XRD (X-ray diffraction) studies were performed by utilizing Shimadzu 7000 powder X-ray diffractometer having ( $\lambda = 1.541$  Å) CuK $\alpha$  radiation. The functional group analysis of synthesized ZnO nanoparticles was carried using Shimadzu IR Prestige (Shimadzu Corporation, Japan) instrument. SEM analysis was performed utilizing a ZEISS GEMINI, SUPRA 55 scanning electron microscope instrument. Techcomp-UV2301, Hitachi 2.2 spectrophotometer was used to measure the UV-Visible absorption wavelength of the synthesized nanoparticles.

**Preparation of plant extract:** The leaves of *Cayratia trifolia* were cleaned with water absorbent and cut in to small pieces and allowed to dry. Approximately 10 g of plant powder was dissolved in 100 mL of distilled water and then boiled at 80 °C for 1 h. The extract has been gathered in a separate conical flask using the conventional filtration process. The extract was utilized for the synthesis of ZnO NPs.

**Synthesis of ZnO nanoparticles:** About 15 mL of leaf extract of *Cayratia trifolia* was taken into100 mL of conical flask and 2.195 g of zinc sulphate was added makeup to 50 mL using distilled water. The reaction mixture has been stirred utilizing magnetic stirrer and stirring continued to 6 h. During the reaction period, a change in the colour of the solution has been noted. After 6 h, 2 M of NaOH has been mixed with the solution as well as placed in an incubator during the night at 60 °C. After 15 min of incubation, the solution was centrifuged

at 14000 rpm for 15 min and the precipitate was rinsed three times with rubbing alcohol and distilled water. The precipitate has been dried at 400 °C in an oven before being ground into a fine powder.

**Characterization:** Synthesized ZnO NPs were studied for their physical and structural properties using various techniques. Surface chemistry and organic functional groups were studied by FTIR in the range of 4000-400 cm<sup>-1</sup> and conformation of ZnO NPs synthesis and absorption activity was studied by using UV-visible spectrophotometer. The crystalline ZnO NPs structure was characterized by the X-ray diffraction profiles at an acquisition rate of 2°/min at 40 kV and 30 mA. The surface morphology of ZnO NPs was studied with the scanning electron microscopic studied.

Pot experiment for seed germination activity: The fertile red soil was collected from the agricultural field and transported to the research location for analysis. A local market in Guntur provided the mustard seeds (Brassica nigra) which were used in this experiment. The pot method was used to study the growth enhancement activity of ZnO NPs [18]. The soil was air-dried as well as passed through a sieve with 1 cm opening, after which phosphorus (P), nitrogen (N) and potassium (K), contents of the soil was estimated by analyzing the soil samples. Plastic pots with a height of 15 cm and a diameter of 45 cm, as well as a pot pad, was filled with soil (1.25 kg). Every pot was homogenized with an aqueous solution of ZnO NPs at the dosages of 10 mg, 100 mg and 1000 mg/Kg (to observe the optimum dosage), as well as conventional zinc sulphate at the dosages of 100 mg and 1000 mg/Kg (as zinc source for comparison), for germination trials with three duplicates of each treatment. The nanoparticles adjusted soils was stabilized for 24 h prior to usage; 100 seeds was picked and seedlings developed four leaves after being transplanted. For the pot experiment research, water was poured to the pot to a level around 60% of the maximum field capacity, the pot was collected after 10 days.

A number of parameters were determined from the observed data, including the germination rate:

# Germination rate (%) = $\frac{\text{Number of germinated seeds}}{\text{Total seed number for testing}} \times 100$

shoot and root length, along with number of leaves per plant. The zinc metal content in the roots, stem and leaves was determined using atomic absorption spectroscopy (Shimadzu, Japan) as per reported procedure [19]. The total chlorophyll (both A and B) content of plants grown in pot experiment was evaluated as per the procedure reported by Shinde *et al.* [20]. The chlorophyll A and B concentrations in the samples were determined using the method reported by Arnon [21].

#### **RESULTS AND DISCUSSION**

The one-step green synthesis of zinc oxide nanoparticles (ZnO NPs) utilizing an aqueous leaf extract of *Cayratia trifolia* was accomplished at room temperature. The reaction mixture of containing zinc sulphate and aqueous plant leaf extract turn in dark brown colour during incubation. The change in colour of reaction mixture was considered as the preliminary conform-

ation for the formation of nanoparticles. A change in colour was monitored using UV-visible spectrophotometer at the scan range of 700-200 nm. The spectra clearly show the wavelength maxima at 349 nm, which confirms the formation of ZnO NPs (Fig. 1). The colour change in ZnO NPs is caused by the coherent excitation of all of the 'free' electrons produced by the phenolic compounds in the plant leaf extract, which results in the colour change. The wavelength maxima was in correlation with the findings reported in literature proves that the particles formed in the study were ZnO NPs [22,23].



Fig. 1. UV-visible spectra of ZnO NPs synthesized using aqueous leaf extract of *Cayratia trifolia* 

**FT-IR studies:** The bands in the IR spectrum indicate the stretching modes that correspond to the functional groups responsible for the observed reduction of zinc ions into ZnO (Fig. 2). The peaks identified at 3396 cm<sup>-1</sup> and 2865 cm<sup>-1</sup> correspond to O-H stretching and intramolecular hydrogen bonded alcohol, respectively. A peak at 1735 cm<sup>-1</sup> confirms the presence of C-H bending vibrations in aromatic compounds and bond at 1353 cm<sup>-1</sup> represents the presence of phenolic functional group. Broad peak at 3472 cm<sup>-1</sup> indicates N-H stretching in primary amine and 2171 cm<sup>-1</sup> confirms C=C stretch in alkynes. A peak corresponds to the Zn–O functional group was identified at low wavenumber in the FT-IR spectrum, which agreed with literature [24,25].



Fig. 2. FT-IR spectra of ZnO NPs synthesized using aqueous leaf extract of *Cayratia trifolia* 

**SEM studies:** From the SEM analysis, the average size of synthesized ZnO nanoparticles was found to be approximately 58 nm. The shape of the synthesized ZnO nanoparticles was found mostly circular in shape and few of them are irregular as observed from the SEM image. The energy dispersive X-ray (EDX) profile of the ZnO nanoparticles reveals the presence of carbon (C), oxygen (O) and potassium (K). The % composition of zinc was observed to be 78%, which confirmed that the synthesized nanoparticles contain high quantity of zinc metal. The SEM-EDX spectra of the nanoparticles synthesized using aqueous leaf extract of *C. trifolia* is presented in Fig. 3.



Fig. 3. SEM-EDX spectra of ZnO NPs synthesized using aqueous leaf extract of *Cayratia trifolia* 

**XRD studies:** The XRD spectra shows 2θ characteristic peaks corresponding to planes of the crystal lattice structure were identified at 31.6° (100), 34.2° (002), 36.1° (101), 47.5° (102), 56.5° (110), 62.9° (103), 66.1° (200), 67.8° (112), 68.7° (201), 71.7° (004) and 76.6° (202). The diffraction peaks identified for the synthesized nanoparticles as shown in Fig. 4 were in good correlation with the hexagonal Wurtzite form of the particles with standard JCPDS card No. 89-0510. The diffraction peaks were observed to be narrow and robust peaks confirms that the synthesized nanoparticles were in uniform size. The size of the nanoparticles was calculated by adopting Debye-Scherrer's equation and results confirms that the nanoparticles were in the average particle size of 58 nm.



Fig. 4. XRD spectra of ZnO NPs synthesized using aqueous leaf extract of *Cayratia trifolia* 

**Germination studies:** Many cellular activities including the production of chlorophyll and certain carbohydrates as well as the conversion of starch to sugars require zinc as a critical component. Zinc stimulates the activity of enzymes, which are responsible for production of certain proteins in plants [26]. The availability of zinc in plant tissue helps the plant's ability to survive cold temperatures. Zinc is a necessary component in the formation of auxins, which help in the control of growth and the elongation of stems. The uptake of nano-size zinc may high from the soil due to its size and bioavailability. Due to the slower absorption of traditional fertilizers by plants, the synthesis of nanoparticles fertilizers has become more essential in recent years. This is because conventional fertilizers are needed in huge amounts due to plants' slow absorption rate [27]. The effect of synthesized ZnO NPs to boost plant growth of mustard seeds was studied. Except for the nanoparticle treatment, all of the plants have been cultivated in the same size pots and with the same type of soil to ensure that all of their growth parameters were the same.

The mustard seeds were potted in the soil and studied for seed germination along with plant growth. The role of various ZnO NPs concentrations on germination rate, growth of root and shoot and number of leaves in a plant was measured. The ZnO NPs was studied at10 mg/Kg, 100 mg/Kg and 1000 mg/ Kg concentration on prepared soil. The zinc sulphate (ZnSO<sub>4</sub>) at 100 mg/Kg and 1000 mg/Kg were studied to control zinc in soil. A high germination rate (91.7%) of mustard seeds was found in the pots consists of soil with1000 mg/Kg, whereas less germination rate was observed in control pots (62.1%).

The results revealed that the dose-dependent germination rate was found in the mustard seeds. After 6 days, the shoot and root lengths of each potted plant have been measured to assess the effectiveness and concentration-dependent effects of the produced ZnO NPs on plant growth augmentation. It was necessary to perform the growth experiments three times in order to anticipate statistically credible growth patterns. All mustard plants showed healthy growth with drop of high ZnO NPs concentration than control plant.

The mustard plant root grown in pots with ZnO NPs at 1000 mg/Kg (6.9 cm) has grown 627% higher than the control pot (1.1 cm). Growth heights of the root were reported based on the final heights after 6 days. The plants in pots with ZnO NPs at 100 mg/Kg (4.8 cm) have grown 436% higher than the control pot (1.1 cm). Similar results were achieved with pots consist of zinc sulphate *i.e.* growth of plants root were grown 572 % at 1000 mg/Kg (6.3 cm) and 381% at 100 mg/Kg (4.3 cm) than the control pots. The images of different stages of the pot experiment for germination was presented in Fig. 5.

It has been reported that zinc in the form of nanoparticles significantly affects shoot growth. Mustard plant shoot length increased by 316% at 1000 mg/Kg (9.8 cm) and by 216% at 100 mg/Kg (6.7 cm) when compared to the control pot (3.1 cm). Whereas pots containing zinc sulphate increased plant shoot growth by 261% at 1000 mg/Kg (8.1 cm) and 187% at 100 mg/Kg (5.8 cm) compared to control pots. The images of roots and shoot parts that have grown after six experiment days are presented in Fig. 6.

A total number of leaves formed in a plant was counted and found similar results in the pot containing both ZnO NPs and zinc sulphate. The average number of leaves in the plant was 4.5 in pots consist of ZnO NPs at 1000 mg/Kg and 4.1 in 1000 mg/Kg of zinc sulphate. The average number of leaves in the plant was 3.2 in pots consisting of ZnO NPs at 1000 mg/Kg and 3.0 in 1000 mg/Kg of zinc sulphate. However, pots enriched with ZnO NPs significantly boosted the growth of mustards hoots and leaves.

The most significant result from the growth experiments was that mustard plants grown in soil supplemented with ZnO NPs and zinc sulphate at 1000 mg/Kg and 100 mg/Kg experienced greater root and shoot development, as well as increased leaf production. The highest effect on growth with ZnO NPs was observed in the shoot growth of the plants. The results showed that nanoparticles have great promise to serve as a leading Zn enriching fertilizer for the goal of increasing agricultural output. The results of the Zn estimation indicated a significant difference in zinc content between the various dose conditions. Zinc accumulation in roots was found high in 173.8 mg/Kg in soil with 1000 mg/Kg of ZnO NPs. On the other hand, 123.9 mg/Kg, 55.3 mg/Kg of Zn was found in soil with 1000 mg/Kg of ZnO NPs, respectively.

**Chlorophyll contents:** The chlorophyll content of the leaves was measured and results confirm that the plants treated with ZnO NPs show high quantity of chlorophyll than control as well as zinc sulphate treated plants. The uptake and accumulation of zinc metal on the plant body was observed to be very high for the plants treated with synthesized ZnO NPs. This study confirms the significant dose-dependent relationship between zinc content and nanoparticles application. The results observed during the pot experiment of growth promotion activity study are presented in Table-1. Based on the archived results, it can be confirmed that nano treatment significantly enhances the seed germination as well as the growth of mustard seeds.

### Conclusion

In this work, an environmentally benign route for synthesizing ZnO NPs and characterized by PXRD, SEM, FT-IR and UV-visible spectroscopy were carried out. The spectral results confirmed the formation of spherical shape ZnO NPs with an average particle size of 58 nm. The synthesized nanoparticles were hexagonal Wurtzite form with 78% of the metallic zinc content on it. The synthesized ZnO NPs were evaluated for seed germination and growth enhancement on mustard seeds. The growth of the shoot and root from the mustard seed with ZnO NPs significantly increase by 436-627%. Although the development pattern differs across roots and shoots, the soil treated with high ZnO NPs concentration exhibited the fastest and most significant growth compared to the other control soil pots. The nanoparticles treated plants exhibited more leaf germination than the untreated soils. The present study also reported the uptake of zinc content in mustard plants root and found a significant increase in the soil treated with ZnO NPs. Plant growth and seed germination enhancement activities of the synthesized ZnO NPs confirms that ZnO NPs was successful in promoting the growth of seeds and plants. The key benefit of the current study is that the nanoparticles as fertilizer was administered in a little amount and able to enhance significantly the production rate as well as life span of plants. Therefore, the Vol. 35, No. 4 (2023)



Fig. 5. Images of seed germination activity of mustard seeds



Fig. 6. Images of growth promotion activity of shoot and roots of mustard plants

TABLE-1 RESULTS OBSERVED DURING SEED GERMINATION AND GROWTH PROMOTION STUDY OF SYNTHESIZED ZnO NPs ON MUSTARD SEEDS									
Sample studied	Germination	Root length	ength Shoot length Total number		Chlorophyll content (mg/g)		Zn metal		
	rate (%)	(cm)	(cm)	of leaves	А	В	content (mg/g)		
Control	62.1	1.1	3.1	2.5	19.1	11.6	48		
ZnO NPs at 10 mg/Kg	63.9	1.3	3.3	2.5	19.6	11.8	51		
ZnO NPs at 100 mg/Kg	89.1	4.8	6.7	3.2	20.8	14.7	58		
ZnO NPs at 1000 mg/Kg	91.7	6.9	9.8	4.5	23.5	16.6	61		
Zinc sulphate at 100 mg/Kg	61.2	4.2	5.9	3.0	18.5	11.7	56		
Zinc sulphate at 1000 mg/Kg	63.8	6.3	8.1	4.1	17.6	10.4	41		

green synthesized ZnO NPs is highly beneficial to fertilizer in improving bioenergy crop production.

## **CONFLICT OF INTEREST**

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

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