



Preparation of Nanofertilizer from Biogenic Zn-Nanoparticles Synthesized by *Azotobacter* spp. and its Impact on Growth Promotion of *Coriandrum sativum*

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ABSTRACT

Two isolates of *Azotobacter* spp. were isolated from the agricultural soil habitat of Jejuri village, Pune, Maharashtra, and identified using morphological characterization and biochemical tests. Zinc nanoparticles (Zn NPS) were synthesized from *Azotobacter* spp. and characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and FESEM. XRD analysis showed the nanocrystalline form of zinc oxide NPs, and the Scherrer equation determined a mean crystallite size of approximately 42 nm. The FESEM spectra revealed the spherical shape and agglomeration of the biosynthesized nanoparticles. Various functional groups were involved in the capping and stabilization of the zinc oxide NPs, as confirmed by FTIR analysis. A pot experiment was conducted to study the effect of nanofertilizer prepared from zinc nanoparticles on parameters such as plant height, shoot length, root length, and dry leaf weight of *Coriandrum sativum* (coriander plant). In addition, parameters such as photosynthetic pigments, hormones, sugars, polyphenols, and proteins were tested. Mean values of measurements recorded on the 9th, 10th, 11th, and 12th days, along with standard deviation (SD) and percentage gain relative to the control measurements, were computed and reported. On the 12th day, the increase in height of the plant, length of the shoot, length of the root, and dry weight of the leaves in percentage were 9.24, 9.84, 10.86, and 11.71, respectively. On the 9th, 10th, and 11th days, the growth pattern was the same. To strengthen these results, advanced statistical analysis, such as analysis of variance (ANOVA), was performed and found that the growth in height of the plant, length of the shoot, length of the root, and weight of dry leaves significantly increased with respect to increasing days as well as increasing concentrations of nano fertilizer. Furthermore, to test significant differences in the mean chlorophyll content of both types of chlorophyll 'a' and chlorophyll 'b', a t-test for equality of population means was applied, and the results are reported. The results revealed that the nanofertilizer enhanced plant growth and health.

INTRODUCTION

Nanofertilizers are a novel approach to enhancing plant growth and productivity by utilizing nanoparticles and beneficial microorganisms. *Azotobacter* spp. are known for their nitrogen-fixing abilities, which help in converting atmospheric nitrogen into a form that plants can readily use. By incorporating *Azotobacter* spp. into nanofertilizers, researchers aim to improve nutrient availability to plants, leading to enhanced growth and yield. Nanofertilizer have emerged as a promising approach to enhance nutrient efficiency and plant growth in agriculture. Zinc is an essential micronutrient for plant growth, influencing various physiological processes, enzyme activation, and ion homeostasis (Yang et al. 2020, Alsafran et al. 2022).

Azotobacter can convert atmospheric nitrogen to ammonia, which is useful to plants (Prajapati et al. 2008). Zinc nanoparticles can be supplied for foliar use or

can be located in the roots of plants, and both application techniques efficiently transport Zn because of their size and surface area ratios (Czyzowska & Barbasz 2022). Nanofertilizers can improve crop yield by mitigating environmental contamination, ecological stress and plant illnesses. However, the unsystematic use of nanofertilizers may be challenging (Khatri & Bhateria 2023). Nano-biofertilizers have emerged as a promising approach to enhance nutrient efficiency and plant growth in agriculture. Incorporating *Azotobacter* spp. into nano-biofertilizers could potentially enhance their efficacy in delivering nutrients to plants. Nanofertilizers are a new generation of fertilizers that employ advanced nanotechnology to provide an effective and sustainable method for fertilizing crops (Yadav et al. 2023). Nanofertilizers, especially when new technologies such as nano-encapsulation and controlled release of nutrients are applied, can increase plant nutrient uptake, improve fertilizer efficacy, enhance soil fertility, and reduce environmental effects (Ardali et al. 2024). This study will evaluate the influence of these nano-biofertilizers on coriander growth parameters.

MATERIAL AND METHODS

Collection of Soil Sample

Soil samples were collected from the habitat of Jejuri village, Pune, Maharashtra, India. The soil was collected in a polythene bag, transported to the laboratory, and stored at 4°C for further isolation and identification of *Azotobacter* spp.

Isolation of *Azotobacter* spp.

1 g soil sample was added to 100 mL Ashby's broth and incubated at 37°C for 5-6 days in a shaker incubator. After incubation, a loopful of the enriched broth was aseptically streaked on sterile Ashby's agar petri plates and incubated at 37 °C for 4-5 days in an incubator.

Identification of *Azotobacter* spp.

Azotobacter spp. were identified by morphological characterization, Gram staining, and biochemical tests. The plates were observed to identify colony characteristics, such as color, size, shape, elevation, and opacity. Biochemical tests were performed according to "Bergey's Manual of Determinative Bacteriology" (Bergey et al. 1994).

Synthesis of Zinc Oxide Nanoparticles

The isolated microorganism was inoculated into sterile nutrient medium and incubated for 48 h at 35°C on an incubatory shaker. The broth was centrifuged at 10,000 rpm

for 5 min. To 25 mL of supernatant, 0.5 M zinc acetate was added and incubated at 35 °C for 24 h under shaking. The creamy white precipitate was collected, washed twice with deionized water, and oven-dried (Metalab) at 150 °C for 24 h (Belely et al. 2021).

Characterization of Zinc Nanoparticles

UV-Vis spectroscopy: The color change was measured using a Spectrophotometer at a wavelength of 200-600 nm to obtain the extreme peak of (ZnNPs)

FTIR spectrometry: The role of microbial metabolites in CFF in the decrease, capping, and stabilization of ZnO-NPs was studied using FT-IR (Shimadzu, Japan). Approximately 0.2 g of synthesized ZnO-NPs was mixed with potassium bromide and exposed to high pressure to form a disk, which was scanned at 500–4000 cm^{-1} wavelength.

X-ray diffraction (XRD): The synthesized nanoparticles were analyzed using a Rigaku Miniflex (600G model) to determine their phase, purity, and crystallite size using Cu Ka radiation with an angle of 2θ from 10° to 80°. The crystallite size of the material was calculated using the Debye–Scherrer equation:

$$D = \frac{0.9\lambda}{\beta \cos\theta}$$

Where D is the crystal size, λ is the wavelength of the X-ray, θ is the Bragg angle in radians, and β is the full width at half maximum of the peak in radians.

FESEM analysis: FESEM analysis (FEI Nova Nano SEM 450) of the biosynthesized ZnONPs was conducted at Savitribai Phule Pune University (India) to determine the morphology and structure of the nanoparticles. To obtain FESEM images, the synthesized particles were mixed with acetone and allowed to dry on a glass slide to obtain a thin layer for analysis.

Preparation of Nanofertilizer

The synthesized ZnNPs and talcum powder were weighed in a desired ratio of 1:10. Zinc (Zn) nanoparticles were mixed with talcum powder in deionized water. A magnetic stirrer was used to ensure the uniform dispersion of ZnNPs and talcum powder in water for 30 min to achieve a homogeneous suspension. The suspension was dried, and the powder was collected and stored in an airtight container to prevent agglomeration and moisture absorption. The concentration of ZnNPs required in parts per million (ppm) was then determined. For different concentrations (20 ppm, 40 ppm, 60 ppm, 80 ppm), the amount of nanofertilizer and water was adjusted accordingly. The prepared nanofertilizer solution

was applied to plants via foliar spray. The solution was transferred to a spray bottle, and the plants were sprayed evenly to ensure thorough coverage of the leaves.

Pot Experiment

The pot experiment was carried out, of which four were considered sample pots and one was a control. Each pot contained 800 gm of soil and was treated with increasing concentrations of nanofertilizer (20, 40, 60, and 80 ppm). The current investigation considered the improvement of plant growth with different concentrations of nanofertilizer. In addition, it studies the plant's physiological responses and determines how Zn uptake affects the roots and shoots of the plant. The plants were then grown for 20 days.

Growth Parameters of *Coriandrum sativum*

The growth parameters, viz., shoot and root lengths, were calculated on the 9th, 10th, 11th, and 12th days, whereas dry and wet biomasses were studied after the entire treatment. The plants were cut into shoots and roots for the measurement of shoot length and root length, respectively, using a scale (Mane et al. 2010).

Estimation of Chlorophyll 'a' and 'b' by the Acetone Method

The estimation method for chlorophyll in leaves given by Arnon (1949) was used. A 0.5 g sample of coriander leaves from the crop was collected. Plant material was homogenized in a mortar with a pestle, and 20 mL of 80% acetone was added at 0–4 °C in the dark for extraction. A pinch of magnesium carbonate was added for chlorophyll stabilization. (Magnesium is at the center of the chlorophyll molecule). The aliquot was filtered using Whatman No.1 filter paper by suction using a Buchner funnel. The filtrate was washed with 80% acetone, and the filtrate was then collected. Then, 80% acetone was added to obtain a final volume of 100 mL of the filtrate. The optical density of the filtrate was read at 663 and 645 nm for chlorophyll 'a' and 'b,' respectively, on a UV-VIS double beam spectrophotometer (Shimadzu, Japan), using 80% acetone as a blank.

The chlorophyll yield was calculated using the following formula:

$$\text{Final Chlorophyll 'a'/'b' / Total (mg. } 100^{-1}\text{g)} = \frac{X/Y/Z \times \text{Volume of extract} \times 100}{1000 \times \text{weight of plant material}}$$

Estimation of Protein by Bradford's Method

In clean test tubes, 1 mL of the sample was prepared, and

5 mL of Bradford's reagent was added. The test tubes were then placed in the dark for 5 min. The absorbance was measured at 595 nm using UV-vis spectrometry (Bradford 1976, Sadasivam & Manickam 1996).

Detection of Hormone (IAA) by Salkowski Reagent

To 1 mL of the test sample, 4 mL, Salkowski's reagent was added. The samples were then incubated in the dark for 30 min. Readings were recorded at 530 nm using UV-Vis spectrometry (Emami et al. 2019).

Estimation of Polyphenol by the Folin-Denis (1915) Method

Extraction of polyphenol: 0.5 grams of dried sample was added to 70% methanol. The sample was kept in a shaker incubator for 15 min and centrifuged at 3000 rpm for 5 min. The pellet was collected, and the polyphenol was re-extracted using 5mL of 70% methanol. The sample was centrifuged at 3000 rpm for 5 min. The pellet was then collected.

Quantitative estimation of total phenolic content: The concentration of the dried leaf extract (0.2-1 mg. mL⁻¹) in methanol was properly mixed. A 0.5 mL sample was added to each test tube, and 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent was added. Then, 2 mL of 75% Na₂CO₃ solution was added. The tubes were covered and allowed to rest for 30 min until a blue color was obtained. Readings were recorded at a wavelength of 700 nm using a spectrophotometer.

Estimation of total carbohydrate by the phenol sulphuric acid method: Preparation of the sample: 100 mg of dried powder of coriander leaves was taken in a boiling tube containing 100 mL of distilled water. The sample was hydrolyzed by exposing it to a boiling water bath with 5 mL of 2.5 nm hydrochloric acid for 3 h and then cooled at room temperature. The solution is then neutralized with Na₂CO₃ until effervescence ceases. The volume was adjusted to 50 mL. The sample was then centrifuged, and the supernatant was used as the sample.

Estimation of total carbohydrate: To 1 mL of the sample, 1 mL of phenol solution was added. Then, 5 mL of 96% sulfuric acid was added to each tube. The tubes were then kept for 10 min in an ice bath and thoroughly mixed. After 10 min, the contents of the tube were mixed and placed in a water bath at 25-30°C for 20 min. The color was read at 490 nm. (Sadasivam & Manickam 1996).

RESULTS AND DISCUSSION

Isolation and Identification of Isolates

Two bacterial isolates were isolated and characterized based on their morphological characteristics. Ashby's agar medium

was used for the isolation of these bacterial isolates. Both colonies were circular, opaque, and smooth in morphological appearance. Gram staining was performed for these isolates, and both isolates showed Gram-negative rods. Two promising isolates were biochemically characterized using eight biochemical tests (sugar fermentation, nitrate reduction, urease, citrate, catalase, oxidase, and starch hydrolysis). Both isolates were positive for glucose, mannitol, urease, citrate, and catalase and negative for nitrate reduction, oxidase, and starch hydrolysis tests. Morphological and biochemical characteristics of isolates, and with reference to Bergey's manual, isolate AS1 and AS2 were identified as *Azotobacter* spp.

Preparation of Zn Nanofertilizer (ZnNF)

In the present study, two isolates of *Azotobacter* spp. were screened for the synthesis of zinc nanoparticles for the preparation of nanofertilizers. Of these two isolates, AS1 showed the maximum zinc nanoparticle production; therefore, isolate AS1 was used for the preparation of Zn nanofertilizer using talc powder.

Characterization of Synthesized ZnO-NPs

UV-Vis spectrometry: UV-visible spectrum of zinc nanoparticles was observed at 200–800 nm wavelength to confirm the presence and evaluate the characteristics of the zinc nanoparticles based on their optical properties. The absorption spectrum of 0.5M ZnNPs was observed at 360 nm. The presence of this peak at 360 nm indicates that the synthesized nanoparticles were zinc nanoparticles. The intensity of the absorption peak at 360 nm was notably high, which correlated with the high concentration of zinc nanoparticles in the solution (Fig. 1). A higher peak

suggests a greater number of nanoparticles that absorb light at specific wavelengths. The peaks were observed to be sharp and narrow. This indicates a relatively uniform nanoparticle size distribution. The control spectrum did not show any significant absorption near 360 nm, confirming that the peak in the test sample was attributable to the Zn nanoparticles. Biosynthesis involves the utilization of several active components secreted by various biological organisms (plants, microorganisms, etc.) to reduce and stabilize new substances at the nanoscale (Salem et al. 2021).

Fourier Transform Infrared (FTIR) Spectroscopy: FTIR analysis was conducted in the range of 4000 cm^{-1} to 500 cm^{-1} to determine the nature of the probable covering agent of nanoparticles responsible for the effective stabilization of Zn nanoparticles by *Azotobacter* spp. A peak was found at $1581.62912\text{ cm}^{-1}$ for 0.5 M ZnNPs, which is indicative of C=O stretching vibrations, suggesting the presence of carbonyl groups. 3325.23 cm^{-1} indicates the presence of -OH hydrogen bands (Hamza et al. 2020), which could be the capping agent or stabilizers used in the synthesis process. The absence of the zinc stretching peak in the control spectrum confirms that the observed zinc peaks in the zinc nanoparticles were due to the presence of zinc nanoparticles (Fig. 2).

X-ray diffraction (XRD) pattern

X-ray diffraction of dried ZnO NPs was studied using a Rigaku Miniflex 600G to characterize the crystallinity of the nanoparticles. The synthesized ZnO nanoparticles were crystalline with a crystallite size of 42 nm, calculated using the Debye–Scherrer equation. (Fig. 3). The samples showed prominent diffraction peaks at $2\theta = 31.77^\circ$, 34.42° , and 36.25° , corresponding to the lattice planes of (100), (002), and (101), respectively, whereas some small peaks were

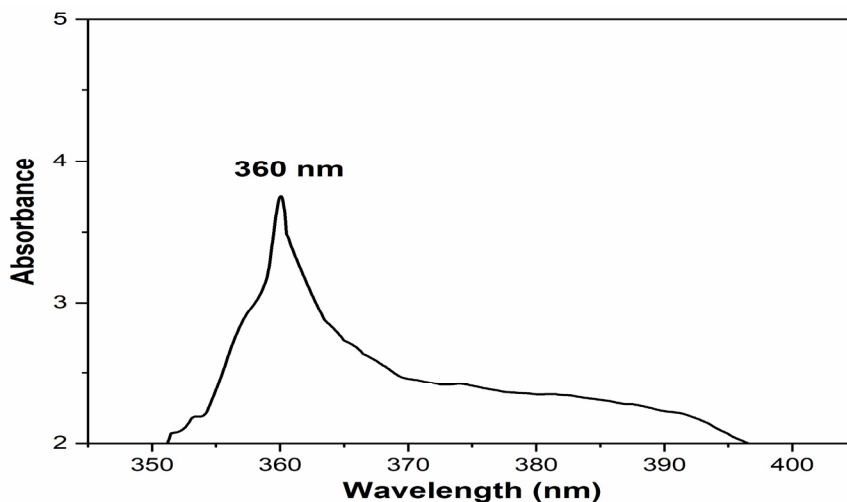


Fig. 1: UV-Vis of 0.5 mM Zinc Nanoparticles.

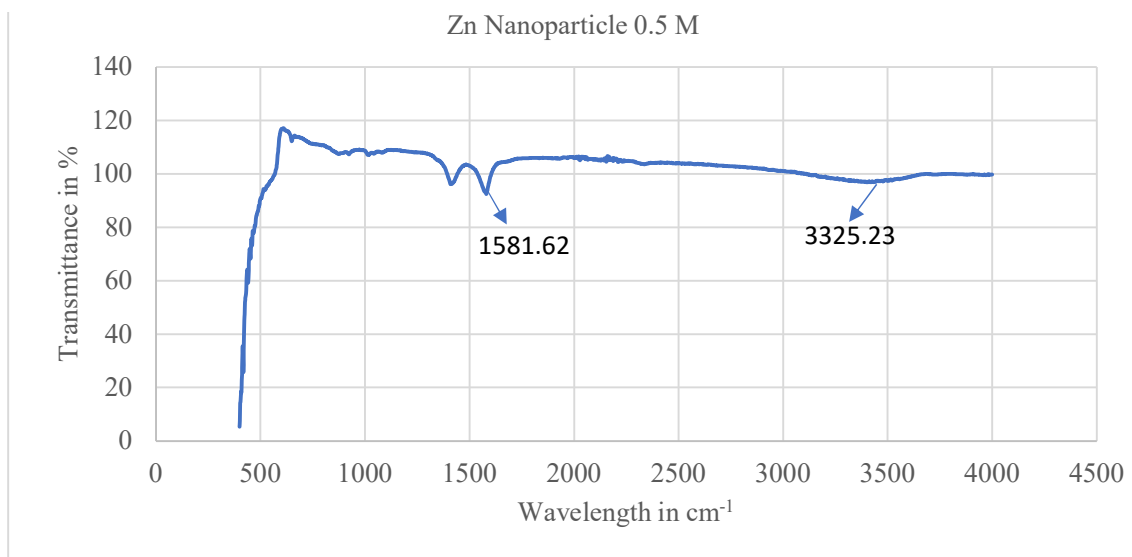


Fig. 2: FTIR of Zn nanoparticles.

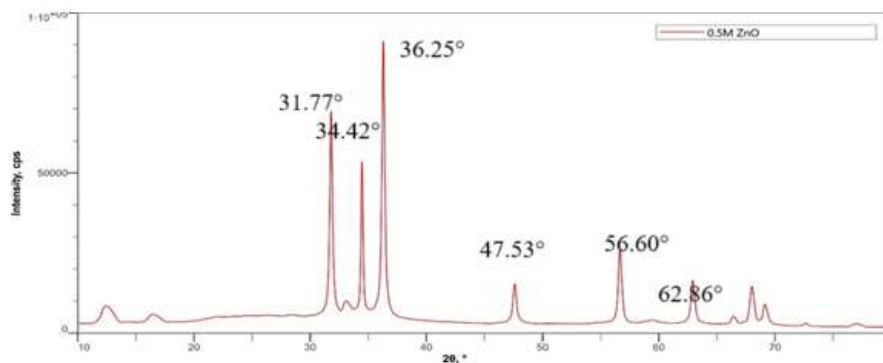


Fig. 3: X-ray Diffraction (XRD) Pattern.

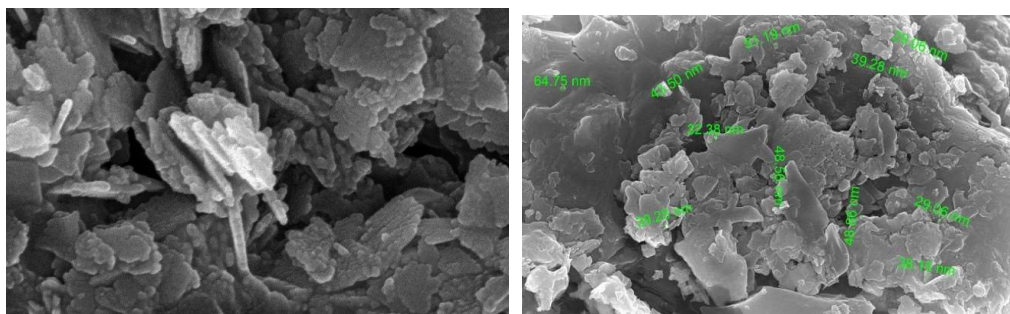


Fig. 4: FESEM spectra of ZnO.

observed at $2\theta = 47.53^\circ$, 56.60° , 62.86° , 66.38° , 67.96° , and 69.10° , corresponding to the lattice planes of (102), (110), (103), (200), (112), and (201), respectively (JCPDS: 36-1451) (Madhumitha et al. 2019). These observations confirmed that the ZnO NPs had a hexagonal wurtzite structure.

FE-SEM spectra: The surface morphology and particle size were determined using FE-SEM. The particle dimensions and morphology of the prepared ZnO nanoparticles were examined using field-emission scanning electron microscopy (FESEM). From the image shown in Fig. 4, it is clear that the particles are in the nano form (Minhas et al. 2023). All the nanoparticles are



Plate 1: Pot experiment.

spherical or agglomerated, and the average particle diameter is 42 nm, which corresponds to the XRD result.

Pot Experiment

Out of five planted *Coriandrum sativum* plants, one was used as a control with zero ppm nanofertilizer, and the other four were given the fertilizer at different ppm (Plate 1). Tests were conducted to evaluate the chlorophyll, protein, polyphenol, and carbohydrate contents using leaves as samples from each of the five plants. The plant sample with 80 ppm nanofertilizer showed maximum values of all contents, and there was maximum growth in the roots and shoots of the plant.

Effect of Nanofertilizer on Plant Growth of *Coriandrum sativum*

In the present study, a pot test was conducted to investigate the efficacy of nanofertilizers on parameters such as plant height, shoot length, root length, and dry leaf weight of *Coriandrum sativum* (coriander plant). The observations were recorded on the 9th, 10th, 11th, and 12th days. On each of these four days, measurements on five sample species were recorded, and the mean values are presented in Tables 1-4 along with standard deviation (SD) and percentage gain relative to control measurements. From Tables 1-4, it is clear from the results that Zn nanofertilizer had a positive effect on the growth of plant height, shoot length, root length, and weight of dry leaves on each of the four days considered. As shown in Table 1, the height of the plant was 7.36 cm on the 12th day in the control group, whereas it increased by 8.04 cm in the culture treated with 80 ppm ZnNF on the same day, resulting in a 9.24% increase in plant height. From Table 2, the length of the shoot was 3.86 cm on the 12th day in the control, whereas it was observed to be increased by 4.24 cm in the culture treated with 80 ppm ZnNF on the same day, which resulted in a 9.84% increase in plant shoot length. From Table 3, the length of the root was 3.50 cm on the 12th day in the control, whereas it was observed to be increased by 3.88 cm in culture treated with 80 ppm of

ZnNF on the same day, which resulted in a 10.86% increase in root length of the plant. From Table 4, the dry weight of leaves was 0.070 gm on the 12th day in the control group, whereas it was observed to be increased by 0.078 gm in the culture treated with 80 ppm of ZnNF on the same day, which resulted in an 11.71% increase in the dry weight of leaves.

The values in Tables 1-4 are the mean of five measurements along with \bar{x} SD. The values in the third row are percentage variations relative to control values.

Further, analysis of variance (ANOVA) (Snedecor and Cochran 1989) is carried out to study the significance of growth in height of plant, height of shoot, height of root, and weight of dry leaves of coriander leaves with respect to days 9th, 10th, 11th, 12th and for various concentrations of nano fertilizer (control, 20, 40, 60, 80) at 5% level of significance (l.o.s) denoted by α , i.e., $\alpha=0.05$. The following results were obtained:

- i) With respect to days, the p-values corresponding to growth in height of plant, height of shoot, height of root,

Table 1: Effect of nanofertilizer on the height of the plant of *Coriandrum sativum*.

Zinc Nanofertilizer (ppm)	Plant height in cm			
	9 th day	10 th day	11 th day	12 th day
Control	4.02 (\bar{x} 1.90) 100	5.76 (\bar{x} 0.96) 100	6.5 (\bar{x} 0.98) 100	7.36 (\bar{x} 1.14) 100
20	4.12 (\bar{x} 1.82) 102.49	5.88 (\bar{x} 0.92) 102.08	6.64 (\bar{x} 1.00) 102.15	7.52 (\bar{x} 1.083) 102.17
40	4.20 (\bar{x} 1.67) 104.48	6.00 (\bar{x} 0.94) 104.17	6.80 (\bar{x} 0.94) 104.62	7.66 (\bar{x} 1.06) 104.08
60	4.332 (\bar{x} 1.85) 107.46	6.12 (\bar{x} 0.97) 106.25	6.94 (\bar{x} 0.92) 106.77	7.84 (\bar{x} 0.97) 106.52
80	4.38 (\bar{x} 1.83) 108.96	6.18 (\bar{x} 1.01) 107.29	7.02 (\bar{x} 0.98) 108.00	8.04 (\bar{x} 1.03) 109.24

Table 2: Effect of nanofertilizer on the length of the shoot of *Coriandrum sativum*.

Nanofertilizer (ppm)	Shoot length in cm			
	9 th day	10 th day	11 th day	12 th day
Control	2.84 (∓ 0.72) 100	3.04 (∓ 0.69) 100	3.56 (∓ 0.66) 100	3.86 (∓ 0.70) 100
20	2.92 (∓ 0.13) 102.82	3.14 (∓ 0.35) 103.29	3.68 (∓ 0.24) 103.37	3.98 (∓ 0.26) 103.11
40	3.00 (∓ 0.10) 105.63	3.20 (∓ 0.16) 105.26	3.80 (∓ 0.32) 106.74	4.06 (∓ 0.15) 105.18
60	3.04 (∓ 0.19) 107.04	3.24 (∓ 0.21) 106.57	3.82 (∓ 0.32) 107.30	4.14 (∓ 0.15) 107.25
80	3.1 (∓ 0.12) 109.15	3.32 (∓ 0.13) 109.21	3.94 (∓ 0.34) 110.67	4.24 (∓ 0.21) 109.84

Table 3: Effect of nanofertilizer on the length of the root of *Coriandrum sativum*.

Nanofertilizer (ppm)	Root length in cm			
	9 th day	10 th day	11 th day	12 th day
Control	2.38 (∓ 0.15) 100	2.76 (∓ 0.27) 100	2.94 (∓ 0.34) 100	3.50 (∓ 0.45) 100
20	2.46 (∓ 0.09) 103.36	2.86 (∓ 0.39) 103.62	3.04 (∓ 0.25) 103.40	3.62 (∓ 0.28) 103.43
40	2.52 (∓ 0.15) 105.88	2.92 (∓ 0.42) 105.80	3.10 (∓ 0.24) 105.44	3.70 (∓ 0.25) 105.71
60	2.56 (∓ 0.15) 107.56	2.96 (∓ 0.34) 107.25	3.18 (∓ 0.22) 108.16	3.84 (∓ 0.25) 109.71
80	2.62 (∓ 0.13) 110.08	3.06 (∓ 0.24) 110.87	3.28 (∓ 0.22) 111.56	3.88 (∓ 0.15) 110.86

and weight of dry leaves are 6.1511×10^{-18} , 3.1174×10^{-16} , 2.5956×10^{-15} , and 5.56544×10^{-17} , respectively, which are less than $\alpha = 0.05$. Hence, various growths and weights of dry leaves are significantly increasing at the 5% level of significance with respect to days.

- ii) With respect to the concentration of nano fertilizer, the p-values corresponding to growth in height of plant, height of shoot, height of root, and weight of dry leaves are 1.9420×10^{-07} , 7.0655×10^{-09} , 6.8599×10^{-08} , and 7.74266×10^{-06} , respectively, which are very less to $\alpha = 0.05$. Hence, various growths and weights of dry leaves were significantly increased at the 5% level of significance with respect to various concentrations of nano-fertilizer.

Table 4: Effect of nanofertilizer on the weight of dry leaves of *Coriandrum sativum*.

Nanofertilizer (ppm)	Weight of dry leaves in gm			
	9 th day	10 th day	11 th day	12 th day
Control	0.032 (∓ 0.0084) 100	0.038 (∓ 0.0130) 100	0.050 (∓ 0.0158) 100	0.070 (∓ 0.0200) 100
20	0.033 (∓ 0.0079) 103.75	0.039 (∓ 0.0098) 103.16	0.052 (∓ 0.0130) 104.80	0.074 (∓ 0.0142) 105.14
40	0.0338 (∓ 0.0081) 105.62	0.040 (∓ 0.0089) 105.26	0.053 (∓ 0.0076) 105.60	0.074 (∓ 0.0069) 105.43
60	0.035 (∓ 0.0066) 108.75	0.041 (∓ 0.0056) 108.42	0.054 (∓ 0.0083) 108.00	0.076 (∓ 0.0052) 109.14
80	0.035 (∓ 0.0038) 110.00	0.043 (∓ 0.0041) 112.63	0.057 (∓ 0.0059) 111.20	0.078 (∓ 0.0023) 111.71

Microsoft Excel (2019 version) was used to perform the statistical analysis.

Estimation of Chlorophyll

Readings were taken at wavelengths of 663 nm and 645 nm for chlorophyll 'a' and 'b' on a UV-visible spectrophotometer, respectively, and calculated using the formula reported in Fig. 5. The maximum concentration required to increase the photosynthetic content in *Coriandrum sativum* is 80 ppm.

From Fig. 5, it is observed that the yield of chlorophyll content in mg per 100 g ($\text{mg} \cdot 100 \text{ g}^{-1}$) of coriander leaves increases as the concentration of nano fertilizer increases for both Chlorophyll 'a', Chlorophyll 'b', and total Chlorophyll. In addition, it was observed that the yield of chlorophyll 'a' was greater than that of 'b' for all concentration levels except the control. Further statistical analyses were performed to strengthen the above conclusions. First, to check the normality of both data sets on chlorophyll 'a' and chlorophyll 'b', we used a Z-test because the sample sizes were small, i.e., five for both data sets. The Z-score for the data on chlorophyll 'a' was -0.0497, which is very close to 0, and that for chlorophyll 'b' was 1.008, which is moderately close to 0. Hence, for a small sample, we assumed that the data were from a normal population. A t-test for equality of population means (Snedecor & Cochran 1989) is applied to test the difference in chlorophyll content ($\text{mg} \cdot 100 \text{ g}^{-1}$) in both the types chlorophyll 'a' and chlorophyll 'b' in the coriander leaves for various concentrations of nanofertilizer (control, 20, 40, 60, 80) at 5% level of significance (i.o.s.) denoted by α , i.e. $\alpha = 0.05$. The probability of rejection in terms of the p-value is $0.041332 < 0.05$. Hence, there is considerable support to conclude that the chlorophyll content ($\text{mg} \cdot 100 \text{ g}^{-1}$) in both

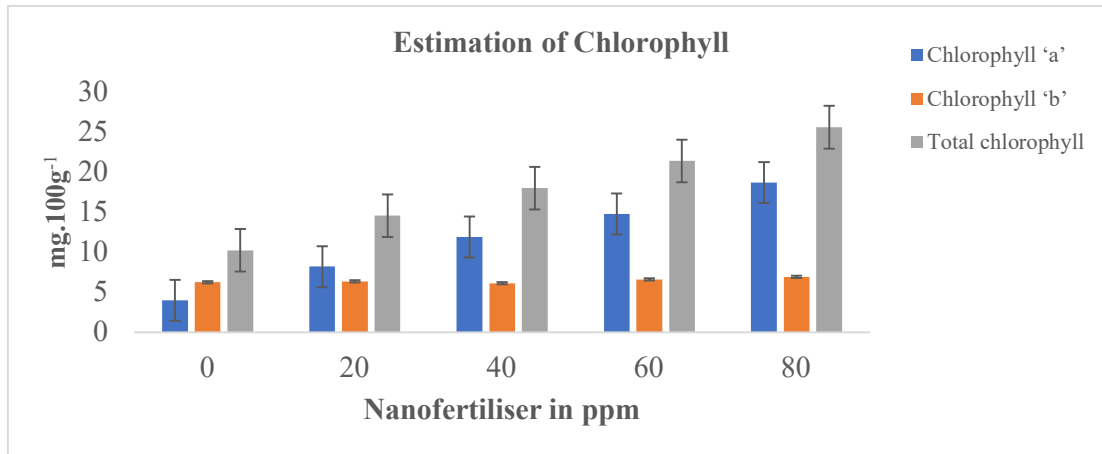


Fig. 5: Estimation of chlorophyll content in coriander leaves.

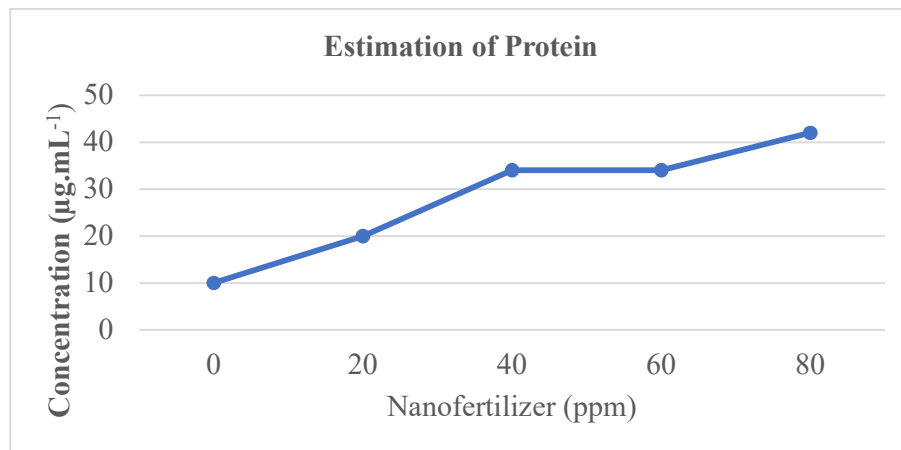


Fig. 6: Estimation of Protein.

Chlorophyll 'a' and Chlorophyll 'b' of the coriander leaves for various concentrations of nanofertilizer is significantly different. We used Microsoft Excel (2019 version) for the data analysis.

In these experiments, it was observed that at 80 ppm of a 0.5 M concentration of zinc nanoparticles, there was a significant improvement in various plant growth parameters. This included plant height, shoot length, root length, leaf area, dry weight, chlorophyll content, protein content, IAA content, carbohydrate content, polyphenol content, and total carbohydrate content (Figs. 5 to 9). Based on these findings, we determined that 80 ppm is the optimal concentration for the application of zinc nano-fertilizer. This concentration demonstrated the most substantial positive effects on plant growth and development, suggesting its potential as an effective nanofertilizer for enhancing crop performance.

CONCLUSION

In this study, zinc nano fertilizer was successfully synthesized using the direct precipitation method with *Azotobacter spp.* Zinc acetate and NaOH were used as precipitating agents in aqueous solutions. The UV-Vis spectrum exhibited a peak between 350 and 365 nm, which is within the range of Zn nanoparticles. FTIR spectra exhibit the existence of the CO triple bond stretching. The functional groups of phyto-ingredients, which are agents for capping and stabilizing, were identified by FTIR analysis, which recognized the formation of zinc nanoparticles. XRD analysis confirmed the formation of Nanocrystalline ZnO NPs. FESEM images clearly confirmed that the nanoparticles were in spherical or agglomerated form, and the average particle diameter was 42 nm, which corresponds to the XRD result. The foliar application of 80ppm biosynthesized zinc nano NPs improved the constituents of *Coriandrum sativum*.

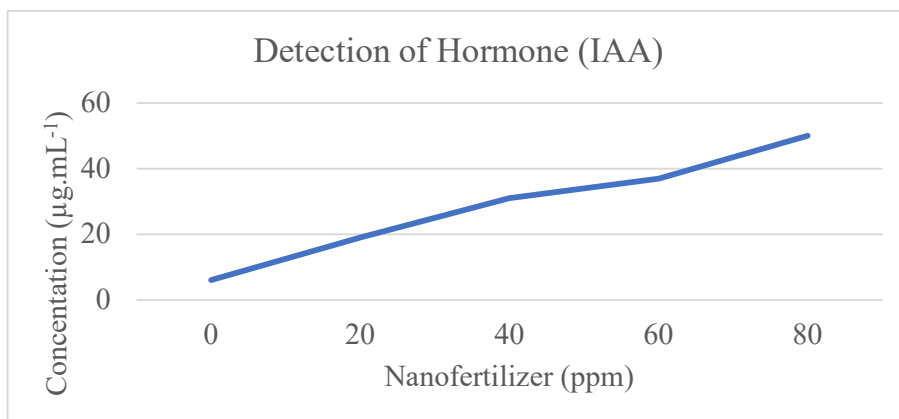


Fig. 7: Detection of hormone (IAA).

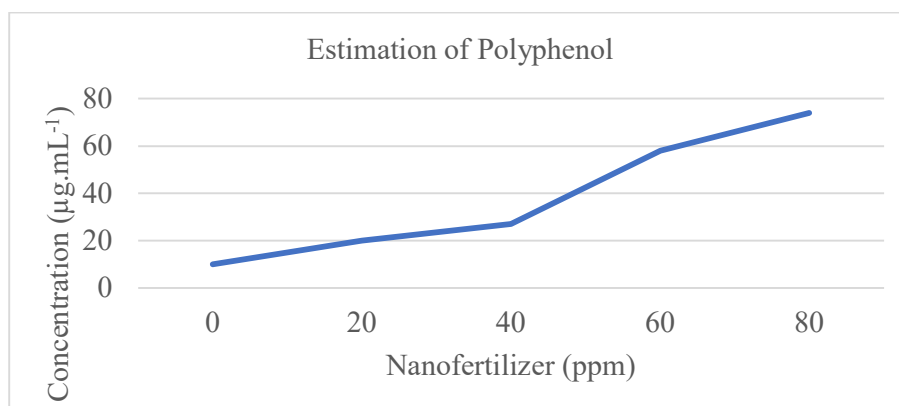


Fig. 8: Estimation of Polyphenols.

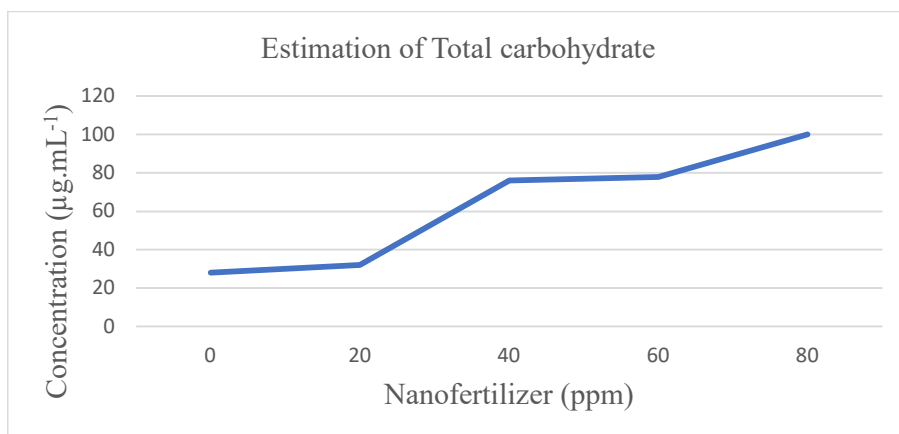


Fig. 9: Estimation of Total carbohydrate.

Mean values of the measurements of the height of the plant, length of shoots, length of roots, and weight of dry leaves of coriander leaves on the 9th, 10th, 11th, and 12th day, along with \pm SD, and percentage gain relative to the control

were computed and reported. On the 12th day, the increase in height of the plant, length of the shoot, length of the root, and weight of dry leaves in percentage were 9.24, 9.84, 10.86, and 11.71, respectively. On the 9th, 10th, and 11th days, the

growth patterns were the same. Furthermore, ANOVA was performed and found that the growth in length of the plant, length of the shoot, height of the root, and weight of dry leaves were significantly increased with respect to increasing days as well as increasing concentrations of nano-fertilizer. After carrying out a t-test for equality of chlorophyll 'a' and chlorophyll 'b' contained in coriander leaves for various concentrations of nanofertilizer, it was found that they were significantly different. This nano-fertilizer also increases the concentration of chlorophyll, protein, polyphenols, carbohydrates, and hormones, which are required for plant growth improvement. The agronomic attributes, such as plant height, dry biomass, and root height, were significantly increased due to the foliar application of zinc nano fertilizer. This study provides the simplest method for the synthesis of zinc nano-fertilizer via biological synthesis and sustainable agriculture. In the future, such experiments can be conducted using this nanofertilizer on other crops or using different nanofertilizers to *enhance the health and productivity of Coriandrum*.

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