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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 by 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 crystalline size of ~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, which were confirmed by FTIR analysis. A pot experiment was formulated to study the effect of nanofertilizer prepared from zinc nanoparticles on parameters such as the height of the plant, length of shoot, length of root, and dry weight of leaves of *Coriandrum sativum* (coriander plant). Also, parameters like Photosynthetic pigments, hormones, sugars, polyphenols, and proteins were tested. Mean values of measurements recorded on the 9^{th}, 10^{th}, 11^{th}, and 12^{th} day along with $\mp$ standard deviation ($\mp$SD), and percentage gain relative to control measurements are computed and reported. It was found that on 12^{th} day increase in height of plant, length of shoot, length of root, and dry weight of leaves in percentage are 9.24, 9.84, 10.86, and 11.71 respectively. On 9^{th}, 10^{th}, 11^{th} days the growth pattern is same. To strengthen these results advanced statistical analysis, say analysis of variance (ANOVA), is carried out and found that growth in height of plant, height of shoot, height of root, and weight of dry leaves are significantly increasing with respect to increasing in days as well as increasing concentrations of nano fertilizer. Further, to test significant differences in mean chlorophyll contained in both the types of chlorophyll 'a' and chlorophyll 'b' a t-test for equality of population means is applied and results are reported. The results revealed that the nanofertilizer is responsible for enhancing plant growth and health.

1. INTRODUCTION

Nanofertilizer are a novel approach to enhance 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 the nanofertilizer, 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 processes like physiological, enzyme activation, and ion homeostasis (Yang et al. 2020 and 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 both as foliar use or located in the roots of plants, and both techniques of application efficiently transport the Zn because of their size and surface area ratio (Czyzowska and Barbasz 2022). Nanofertilizers can improve crop yield by mitigating environmental contamination, ecological stress, and plant illnesses. However, the unsystematic usage of nanofertilizer may be challenging for their use (Khatri and 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 of fertilizing crops (Yadav et al. 2023). Nanofertilizers, especially when applying new technologies in fertilizers such as nano-

encapsulation and controlled release of nutrients, can increase plant nutrient uptake, improve fertilizer efficacy, enhance soil fertility, and reduce environmental effects (Ardali et al. 2024). The study will evaluate the influence of these nano-biofertilizers on coriander growth parameters.

2. MATERIAL AND METHODS

2.1. Collection of soil sample

The soil sample was collected from the habitat of Jejuri village, Pune, Maharashtra, India. The soil was carried in a polythene bag, which was transported to the laboratory and stored at 4^0C, for further isolation and identification of *Azotobacter* spp.

2.2. Isolation of *Azotobacter* spp.

1g soil sample was added in 100 ml Ashby's broth and incubated at 37^0 C for 5-6 days in a shaker incubator and after incubation, a loopful of enriched broth was streaked on sterile Ashby's agar petri plates aseptically and incubated at 37^0C for 4-5 days in incubator.

2.3. Identification of A*zotobacter* spp.

The identification of *Azotobacter* spp. was done by morphological characterization, gram's staining, and biochemical tests. The plates were observed to identify colony characters such as the colour of the colony, size, shape, elevation, opacity, etc. Biochemical tests were prepared referring to "Bergey's Manual of Determinative Bacteriology" (Bergey et al. 1994).

2.4. Synthesis of zinc oxide nanoparticles

The isolated microorganism was inoculated in sterile nutrient media and incubated for 48 h at 35^0 C on an incubatory shaker. The broth was centrifuged at 10,000 rpm for 5 min. In 25 mL of supernatant add 0.5 M concentration of zinc acetate and incubate at 35 ◦C for 24 h under a shaking state. The creamy white precipitate was collected, washed twice with deionized water, and kept for oven drying (Metalab) at 150 ◦C for 24 h (Belely et al. 2021).

2.5. Characterization of zinc nanoparticles

2.5.1. 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)

2.5.2. FTIR Spectrometry

The role of microbial metabolites in CFF in the decrease, capping, and stabilizing of ZnO-NPs was studied by FT-IR (Shimadzu, Japan). About 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 to 4000 cm^{-1}wavelength.

2.5.3. X-ray Diffraction (XRD)

The synthesized nanoparticles were analyzed using Rigaku Miniflex (600G model) to determine their phase, purity, and crystallite size utilizing Cu Kα radiation with an angle of 2θ

from 10 to 80°. The crystallite size of the material was calculated from the Debye Scherrer's 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 Braggs angle in radians and β is the full width at half maximum of the peak in radians.

2.5.4 FESEM :

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

2.6. Preparation of nanofertilizer

The synthesized ZnNPs and talcum powder were weighed in the desired ratio of 1:10. The zinc nanoparticles were mixed with talcum powder in deionized water. A magnetic stirrer was used to ensure uniform dispersion of the ZnNPs and talcum powder in water for 30 minutes to achieve homogeneous suspension. The suspension was dried and 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 determined. For different concentrations (20ppm, 40ppm, 60ppm, 80ppm), 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 plants were sprayed evenly ensuring thorough coverage of the leaves.

2.7. Pot experiment

The pot experiment was carried out of which four were considered as sample pots and one as control. Each pot containing 800 grams of soil and placed in increasing order of nanofertilizer concentration considered viz 20ppm, 40ppm, 60ppm, 80ppm. The current investigation considers the improvement of plant growth with different concentrations of nanofertilizer. Also, it studies the plant's physiological responses and determines how Zn take up affects the roots and shoots of the plant. The plants were grown for 20 days. Dry and wet-weight biomass of the roots and shoots were measured on digital weighing balance (Contech).

2.8. Growth parameters of *Coriandrum sativum*

The growth parameters viz. shoot and root length were calculated on the , 9^{th},10^{th} , 11^{th} , and 12^{th} day whereas dry and wet biomass was studied after the whole treatment. The plants were cut into shoots and roots for the measurement of shoot length and root length respectively with the help of scale (Mane et al. 2010).

2.9. Estimation of chlorophyll 'a' and 'b' by Acetone method

The estimation method of chlorophylls in leaves given by Arnon (1949) is used. A sample of 0.5 g coriander leaves from the crop is taken. Homogenise plant material in a mortar with a pestle and with the addition of 20 ml 80% acetone at 0 to 4ºC in the dark. Add a pinch of magnesium carbonate for chlorophyll stabilization. (Magnesium is at the centre of the chlorophyll molecule). Filter the aliquot using Whatman No.1 filter paper by suction using Buchner's funnel. Wash the filtrate with 80% acetone and collect the filtrate. Add 80% acetone to get 100ml final volume of filtrate. Read the optical density of the filtrate 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 yield of chlorophylls is calculated by using the following formula:

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

2.10. Estimation of protein by Bradford's method

In clean test tubes, 1 ml of sample was prepared, and 5 ml of Bradford's reagent was added. The test tubes were placed in the dark for 5 minutes. Readings were recorded at 595 nm under UV-vis spectrometry (Marion, M., Bradford 1976, Sadasivam & Manickam 1996).

2.11. Detection of hormone (IAA) by Salkowski reagent

To 1 ml of sample test sample 4 ml of Salkowski's reagent was added. The sample was incubated in the dark for 30 minutes. Readings were recorded at 530nm under U.V-Vis spectrometry (Emami et al. 2019).

2.12. Estimation of polyphenol by Folin – Danis (1915) method

2.12.1. Extraction of polyphenol:

0.5 grams of dried sample was added to 70% methanol. The sample was kept in a shaker incubator for 15 minutes and then centrifuged at 3000 rpm for 5 minutes. The pellet was collected and re-extracted the polyphenol with 5ml of 70% methanol. Centrifuge the sample at 3000 rpm for 5 minutes. The pellet was recollected.

2.12.2. Quantitative estimation of total phenolic content:

The concentration of dried leaf extract (0.2 mg – 1mg/ml) in methanol was properly mixed. A sample of 0.5 ml was taken in each test tube then 2.5 ml of 10-fold diluted Folin-ciocalteu reagent. 2ml of 75% Na_2CO_3 was added. The tubes were covered and rested for 30 minutes until the blue color was obtained. Readings were recorded at wavelength 700nm on a spectrophotometer.

Estimation of total carbohydrate by phenol sulphuric acid method

2.12.1. Preparation of sample:

100mg of dried powder of coriander leaves was taken in a boiling tube containing 100ml of distilled water. The sample was hydrolyzed by exposing it to a boiling water bath with 5 ml of 2.5N hydrochloric acid for 3 hrs then cooled at room temperature. It is then neutralized with

Na_2CO_3 till effervescence ceases. Volume was made up to 50ml. The sample was then centrifuged and the supernatant was treated as the sample.

2.12.2. Estimation of total carbohydrate:

To 1 ml of sample add 1 ml of phenol solution. Add 5 ml of 96% sulphuric acid to each tube. Then tubes were kept at 10 minutes in an ice bath and thoroughly mixed. After 10 minutes, the contents in the tube were mixed and placed in a water bath at 25 – 30 for 20 minutes. The color was read at 490 nm. (Sadasivam & Manickam 1996).

3. RESULTS AND DISCUSSION

3.1. Isolation and Identification of isolates:

Two bacterial isolates were isolated and characterized by their morphological characteristics. For the isolation of these bacterial isolates, Ashby's agar medium media was used. 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 test. Morphological, and biochemical characteristics of isolates and with reference to Bergey's manual isolate AS1 and AS2 were identified as *Azotobacter* spp.

3.2. Preparation of Zn nanofertilizer:

In the present study for the preparation of nanofertilizer, two isolates of *Azotobacter* spp. were screened out for synthesis of zinc nanoparticles. Out of these two isolates, AS1 showed maximum zinc nanoparticles producer so isolate AS1 is used for the preparation of Zn nanofertilizer using talc powder.

3.3. Characterization of Synthesized ZnO-NPs

3.3.1. UV-Vis Spectrometry

UV-visible spectrum of zinc nanoparticles was observed at 200 nm to 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 exhibited at 360 nm. The presence of this peak at 360 indicated that the synthesized nanoparticles are indeed zinc nanoparticles. The intensity of the absorption peak at 360 nm was notably high which correlates with a high concentration of zinc nanoparticles in the solution (Fig. 1). A higher peak suggests a greater number of nanoparticles absorbing light at those specific wavelengths. The peaks were observed to be sharp and narrow. This indicates a relatively uniform size distribution of the nanoparticles. The control spectrum did not show any significant absorption near 360 nm, confirming that the peak in the test sample is attributable to the zinc nanoparticles. Biosynthesis involves the utilization of several active components secreted by various biological lives (plants, microorganisms etc.) to reduce and stabilize new substances at the nanoscale (Salem et al. 2021).

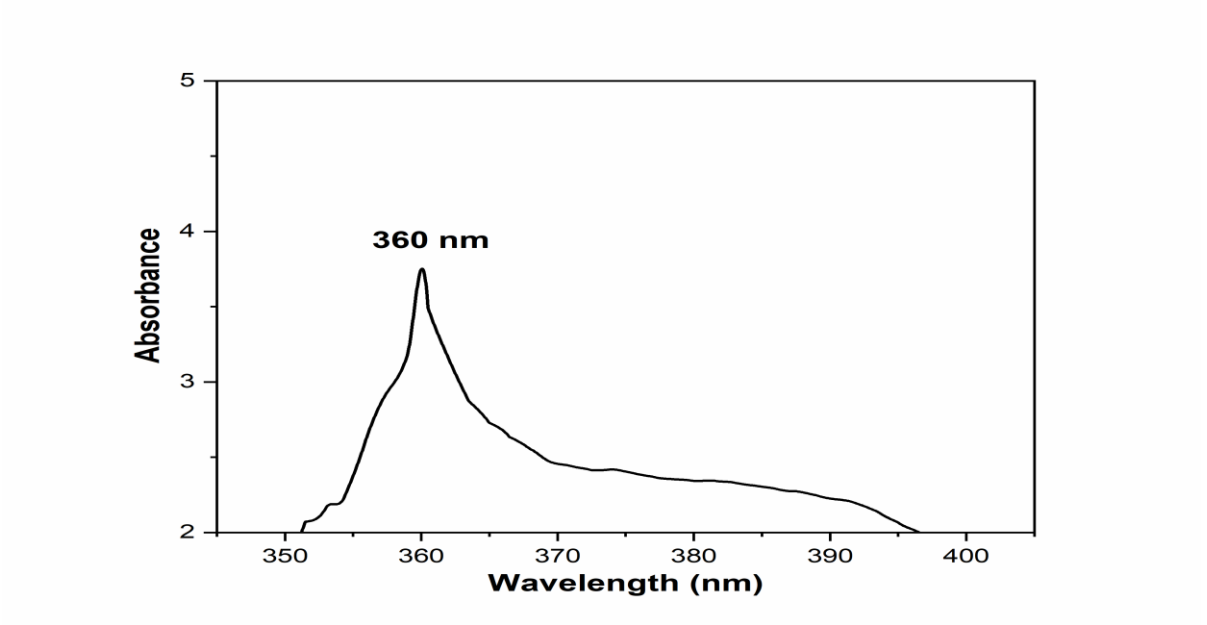

Fig. 1: UV-Vis of 0.5 mM Zinc Nanoparticles

3.3.2. Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR study was conducted in the range from 4000 cm^{-1} to 500 cm^{-1} of wavelength to find out the nature of the probable covering agent of nanoparticles that is responsible for the effective stabilization of zinc nanoparticles by *Azotobacter* spp. Peak is found at 1581.62912cm^{-1} for 0.5 M ZnNPs was indicative of C=O stretching vibrations, suggesting the presence of carbonyl groups. 3325.23cm^{-1} indicated presence of -OH hydrogen bands (Hamza et a. 2020), This 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).

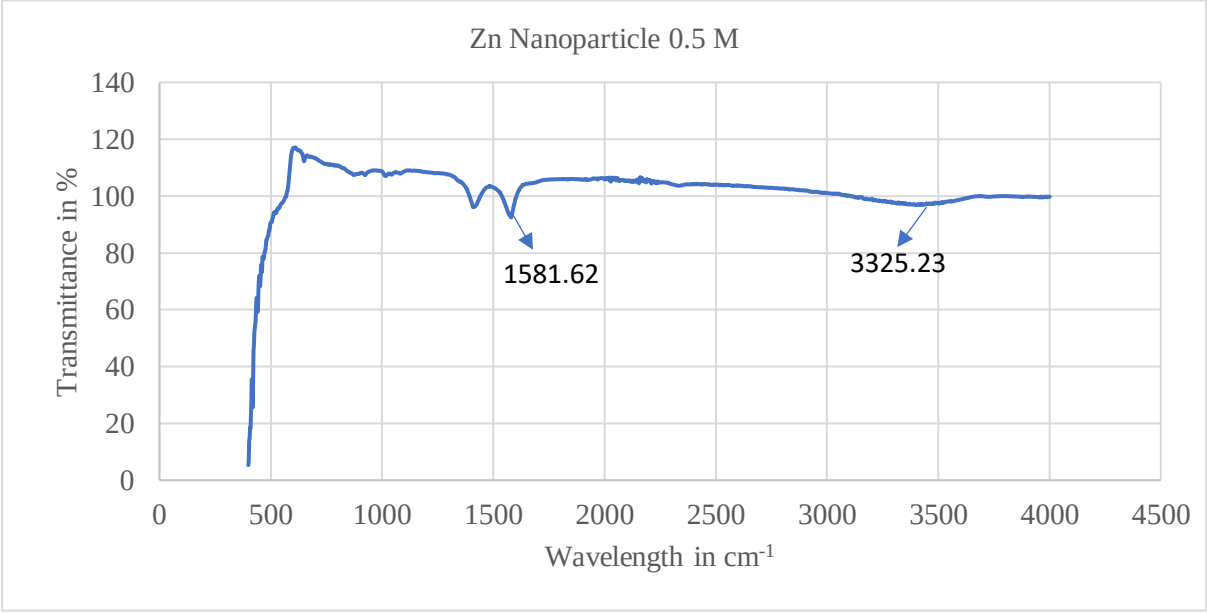

Fig. 2: FTIR Zinc Nanoparticles.

3.3.3. X-ray Diffraction (XRD) Pattern

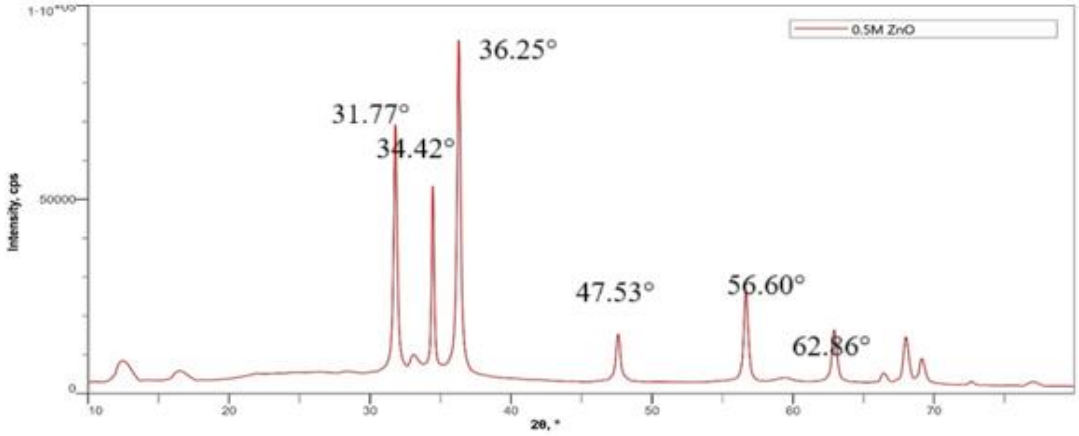

Fig. 3: X-ray Diffraction (XRD) Pattern

X-ray diffraction of dried ZnO NPs was studied using a Rigaku Miniflex 600G to characterize the crystallinity of the nanoparticles. It was observed that the synthesized ZnO nanoparticles were crystalline with a crystallite size of 42nm, calculated by using Debye–Scherrer equation. (Fig. 3). The samples showed prominent diffraction peaks at 2θ 31.77°, 34.42°, and 36.25°, corresponding to lattice planes of (100), (002), and (101), respectively, whereas some small peaks observed at 2θ 47.53°, 56.60°, 62.86°, 66.38°, 67.96°, and 69.10°, corresponding to 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 showed a hexagonal wurtzite structure.

3.3.4. FE-SEM Spectra

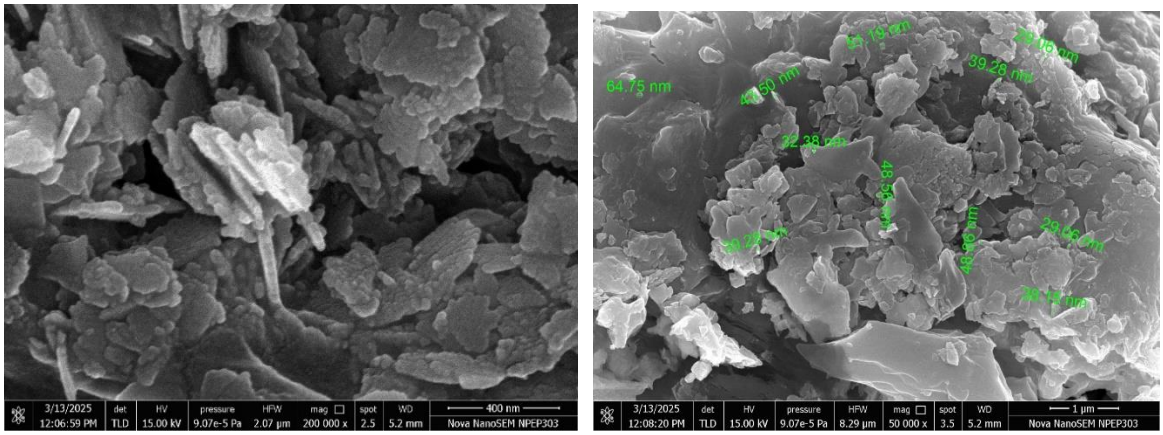

Fig. 4: FESEM spectra of ZnO

Surface morphology and particle size is determined by using FESEM. The particle dimensions and morphology of the prepared ZnO nanoparticles was examined by Field emission scanning electron microscope (FESEM). From the image given in Fig. 4, it is clearly found that the particle is in nano form (Minhas et al. 2023). All the nanoparticles are in spherical shape or

agglomerated form and the average particle diameter is 42 nm which corresponds to the XRD result.

3.4. Pot experiment

Out of five planted *Coriandrum sativum* plants, one was used as a control with zero ppm nanofertilizer, other four were given the fertilizer in different ppm (Plate 1). Tests were conducted to evaluate the chlorophyll, protein, polyphenols, and carbohydrate content using leaves as a sample of each of the five plants out of them the plant sample which has 80 ppm nanofertilizer shows maximum values of all contents also there was maximum growth in root and shoot of the plant.

Plate 1: Pot experiment.

3.5. Effect of nanofertilizer on plant growth of Coriandrum sativum:

In the present, a pot test was formulated to investigate the efficacy of nanofertilizer on parameters such as the height of the plant, length of shoot, length of root, and dry weight of leaves of *Coriandrum sativum* (coriander plant). The observations are recorded on 9th, 10th, 11th and 12th day. On each of these four days, measurements on 5 sample species are recorded and mean values are presented in Tables 1-4 along with $\mp$ standard deviation ($\mp$SD), and percentage gain relative to control measurements. From Tables 1-4, it is clear from the results that Zinc nanofertilizer had a positive effect on the growth of height of the plant, length of shoot, length of roots, and dry weight of leaves on each four days considered. From Table 1, the height of the plant was 7.36 cm. on the 12^{th} day at control while it was observed to be increased by 8.04 cm. in the culture treated with 80 ppm of Zinc nanofertilizer on the same day which resulted in 9.24% increase in height of the plant. From Table 2, the length of the shoot was 3.86 cm. on the 12^{th} day at control while it was observed to be increased by 4.24 cm. in the culture treated with 80 ppm of Zinc nanofertilizer on the same day which resulted in 9.84% increase in shoot length of plant. From Table 3, the length of the root was 3.50 cm. on the 12^{th} day at control while it was observed to be increased by 3.88 cm. in culture treated with 80 ppm of Zinc nanofertilizer 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 12^{th} day at control while it was observed to be increased by 0.078 gm. in the culture treated with 80 ppm of Zinc nanofertilizer on the same day which resulted in 11.71% increase in dry weight of leaves of plant.

Table. 1: Effect of nanofertilizer on height of 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 (∓1.90) 100	5.76 (∓0.96) 100	6.5 (∓0.98) 100	7.36 (∓1.14) 100
20	4.12 (∓1.82) 102.49	5.88 (∓0.92) 102.08	6.64 (∓1.00) 102.15	7.52 (∓1.083) 102.17
40	4.20 (∓1.67) 104.48	6.00 (∓0.94) 104.17	6.80 (∓0.94) 104.62	7.66 (∓1.06) 104.08
60	4.332 (∓1.85) 107.46	6.12 (∓0.97) 106.25	6.94 (∓0.92) 106.77	7.84 (∓0.97) 106.52
80	4.38 (∓1.83) 108.96	6.18 (∓1.01) 107.29	7.02 (∓0.98) 108.00	8.04 (∓1.03) 109.24

The values are the mean of five measurements along with ∓SD. The values in the third row are percentage variations relative to control values.

Table. 2: Effect of nanofertilizer on height of shoot of Coriandrum sativum.

Nanofertilizer (ppm)	**Shoot height 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

The values are the mean of five measurements along with ∓SD. The values in the third row are percentage variations relative to control values.

Table. 3: Effect of nanofertilizer on height of root of Coriandrum sativum.

Nanofertilizer (ppm)	**Root height in cm**			
	9th day	10th day	11th day	12th 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

The values are the mean of five measurements along with ∓SD. The values in the third row are percentage variations relative to control values.

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

Nanofertilizer (ppm)	**Weight of dry leaves in gm**			
	9th day	10th day	11th day	12th 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)	0.043 (∓0.0041)	0.057 (∓0.0059)	0.078 (∓0.0023)

	110.00	112.63	111.20	111.71

The values are the mean of five measurements along with∓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 significance in growth in height of plant, height of shoot, height of root, and weight of dry leaves of coriander leaves with respect to days $(9^{th}, 10^{th}, 11^{th}, 12^{th})$ 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. α=0.05. The following results are found:

i) With respect to days the p-values corresponding to growth in height of plant, height of shoot, height of root, 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 weight of dry leaves are significantly increasing at 5% level of significance with respect to days.

ii) With respect to 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 than $\alpha = 0.05$. Hence, various growths and weight of dry leaves are significantly increasing at 5% level of significance with respect to various concentrations of nano fertilizer.

To perform this statistical analysis, we used office Excel (2019 version).

3.5.1. Estimation of chlorophyll:

Readings were taken at wavelength 663nm and 645nm for chlorophyll 'a' and 'b' on U.V Visible spectrophotometer, respectively, and calculated by the formula reported in Fig. 4. Maximum concentration required to increase photosynthetic content in *Coriandrum sativum* is 80 ppm.

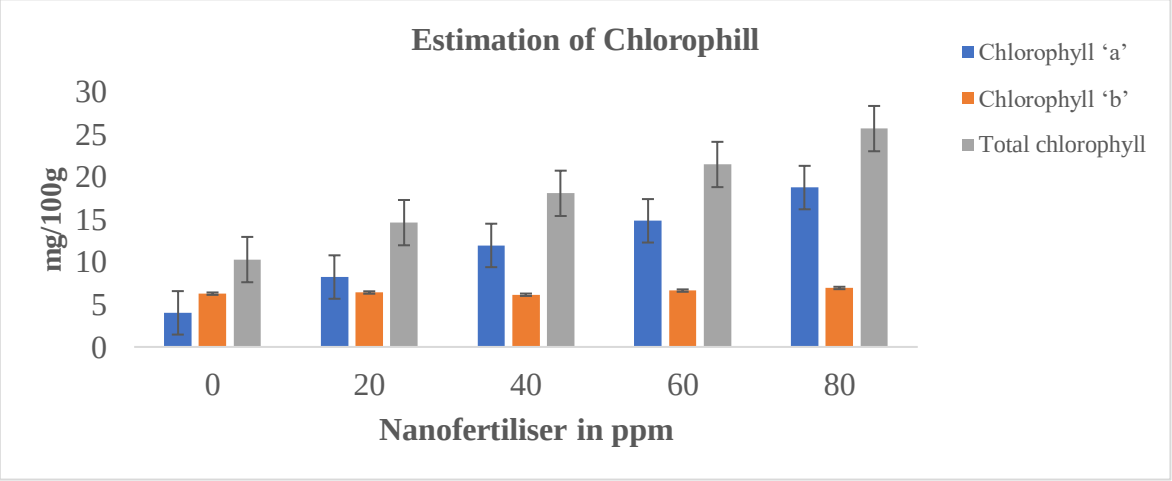

Fig. 5: Estimation of chlorophyll content in coriander leaves.

From Fig. 4, it is observed that the yield of chlorophyll content in mg per 100 g (mg/100g) of the coriander leaves goes increasing as the concentration of nano fertilizer increases for both Chlorophyll 'a', Chlorophyll 'b', and total Chlorophyll. Also, it is observed that the yield of Chlorophyll 'a' is more than that of 'b' for all concentration levels except control. Further statistical analysis is carried out to strengthen the above-concluding remarks. First, to check normality of both data sets on chlorophyll 'a' and chlorophyll 'b', we used Z-test since the sample sizes are small *i.e.* 5 for both data sets. The Z-score for the data on chlorophyll 'a' is -0.0497 which is very close to 0 and chlorophyll 'b' is - 1.008. which is moderately near to 0. Hence, for small sample, we assume the data is from normal population. A t-test for equality of population means (Snedecor and Cochran 1989) is applied to test the difference in chlorophyll content (mg/100g) 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 (l. o. s.) denoted by α, *i.e.*$\alpha = 0.05$. The probability of rejection in terms of p-value is $0.041332 < 0.05$. Hence, there is considerable support to conclude that the chlorophyll content (mg/100g) in both the types of chlorophyll 'a' and chlorophyll 'b' of the coriander leaves for various concentrations of nanofertilizer is significantly different. We used office Excel (2019 version) for this data analysis.

3.5.2. Estimation of Protein:

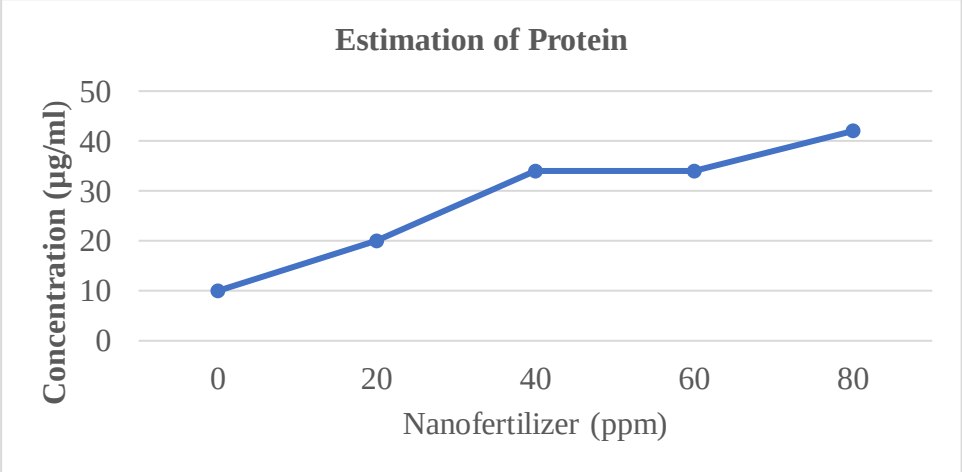

Fig. 6: Estimation of Protein.

3.5.3. Detection of hormone (IAA):

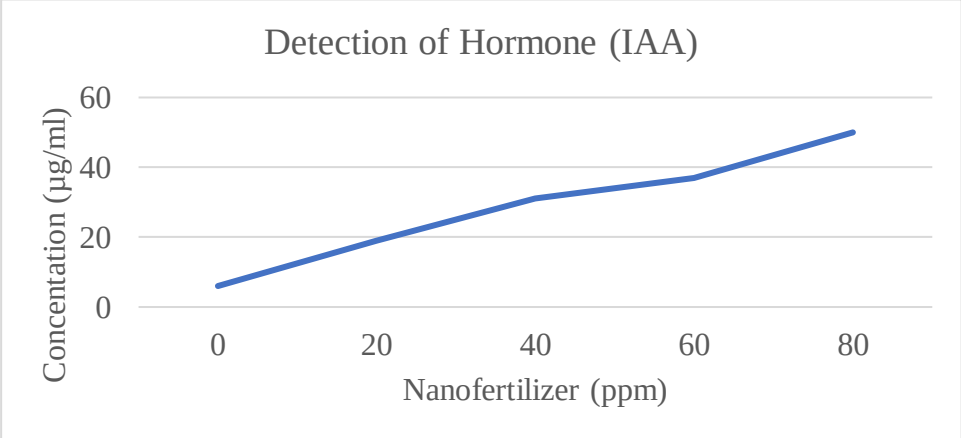

Fig. 7: Detection of hormone (IAA)

3.5.4. Estimation of Polyphenols:

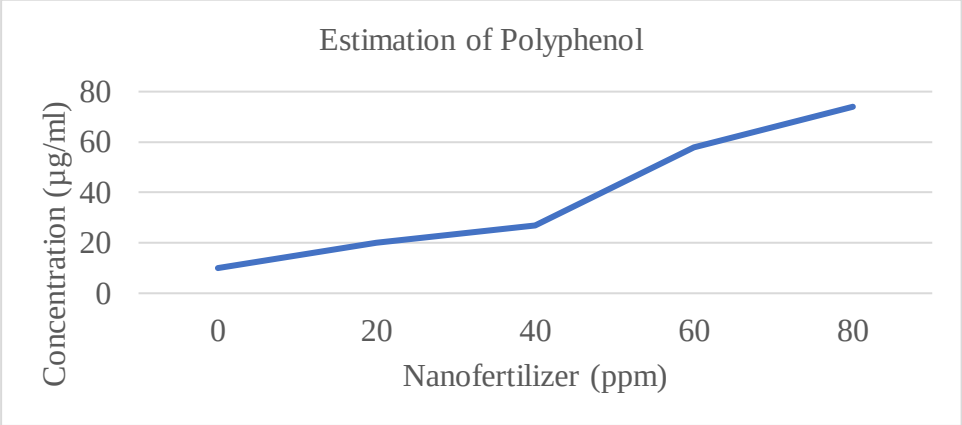

Fig. 8: Estimation of Polyphenols

3.5.5. Estimation of total carbohydrate

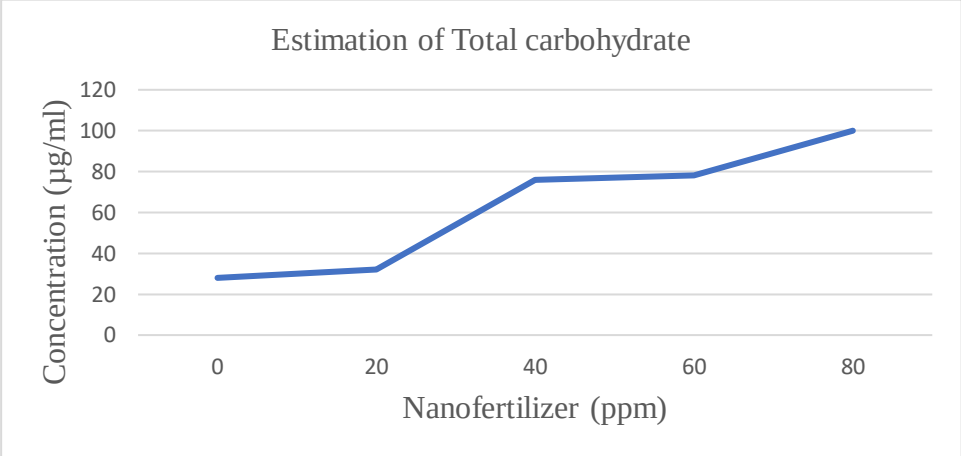

Fig. 9: Estimation of Total carbohydrate

Zinc nanoparticles were synthesized using *Azotobacter* spp. The biosynthesis approach was chosen due to its eco-friendly nature and potential for producing nanoparticles with unique properties. The synthesized Zn NPs were characterized by UV-Vis spectroscopy, FTIR, to confirm their formation. The UV-Vis spectrum of the synthesized ZnNPs displayed a characteristic surface plasmon resonance (SPR) peak at approximately 360 nm, indicating the formation of ZnNPs. This is consistent with previous reports where ZnNPs exhibit an SPR peak in the range of 350-370 nm. The appearance of this peak confirms the presence of nanoscale zinc particles. FTIR study was done to identify functional groups involved in the capping and stabilization of ZnNPs. The spectrum revealed peaks at 1396 cm^{-1}, 1396 cm^{-1}, and 1400 cm^{-1}, C=O stretching, respectively. These functional groups likely originate from biomolecules produced by *Azotobacter* spp., suggesting their role in the reduction and stabilization of ZnNPs. XRD confirmed the crystalline nature with a size of 42nm

In these experiments, it is 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 (Fig. 5 – 8). Based on these findings, we determined that 80 ppm is an 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 nano fertilizer for enhancing crop performance.

4. CONCLUSION

In this study, nano fertilizer of zinc was successfully synthesized by direct precipitation method using *Azotobacter spp.* Zinc acetate and NaOH as precipitating agents in aqueous solution. UV-Vis spectrum provided a peak between 350nm to 365nm that exists within the range of zinc nanoparticles. FTIR exhibits the existence of CO triple bond stretching. Functional group of Phyto ingredients which are agents for capping and stabilizing identified by FTIR analysis that recognized the formation of zinc nanoparticles. XRD analysis confirmed the formation of Nanocrystalline ZnO NPs. FESEM images clearly confirmed the nanoparticles were in spherical or agglomerated form and the average particle diameter is 42 nm which corresponds to the XRD result. Foliar application of 80ppm biosynthesized zinc nano fertilizer improved the constituent of *Coriandrum sativum*. Mean values of measurements of the height of the plant, length of shoots, length of roots, and dry weight of leaves of coriander leaves on the 9^{th} , 10^{th} , 11^{th} , and 12^{th} day along with $\mp$SD, and percentage gain relative to control are computed and reported. It was found that on 12^{th} day increase in height of plant, length of shoot, length of root, and dry weight of leaves in percentage are 9.24, 9.84, 10.86, and 11.71 respectively. On 9^{th}, 10^{th}, 11^{th} days the growth pattern is same. Further, ANOVA is carried out and found that growth in height of plant, height of shoot, height of root, and weight of dry leaves are significantly increasing with respect to increasing in days as well as increasing concentrations of nano fertilizer. After carrying out t-test for equality of chlorophyll ‘a’ and chlorophyll ‘b’ contained in coriander leaves for various concentrations of nanofertilizer is significantly different. This nano fertilizer is also responsible for increasing the concentration of chlorophyll content, 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 gave the simplest way for the synthesis of zinc nano fertilizer via biological synthesis and sustainable agriculture. In future, one can conduct such experiments using this nanofertilizer on other crops or different nanofertilizers to *Coriandrum* enhance their health and productivity.

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