Journal of Medical Bacteriology

Biosynthesis of Zinc Nanoparticles of *Capparis Spinosa* Plant Extract and the it's Investigation on Morhpophysiological Properties of the *Moringa Olifera* Plant

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ARTICLE INFO

Article type:
Research Article

Article history:
Received: 26 Jul 2023
Revised: 12 Aug 2023
Accepted: 17 Oct 2023
Published: 21 Nov 2023

Keywords:
Antioxidant Activity, Ascorbate Peroxidase, Capparis Spinosa, Guaiacol Peroxidase, Moringa Olifera.

ABSTRACT

Background: The article aim was biosynthesis of zinc nanoparticles by the fruit and stem of *Capparis Spinosa* plant extract and the investigation of growth factors and some physiological properties of the *M. Oleifera* plant.

Methods: Four levels of zinc nanoparticles (0, 125, 250, and 500) ppm were considered as treatments. Water extract of the Capparis Spinosa plant were obtained using 10 grams of stem and fruit were separately dried. Some properties like below information were measured: Ultraviolet-visible spectroscopic analysis, Infrared spectroscopy, Measurement of chlorophyll a and b and carotenoids, total phenol, antioxidant activity, total protein and antioxidant enzymes. The data of this research were factorially conducted in the form of completely randomized blocks with three replications.

Results: The effect of zinc nanoparticles on growth parameters shows that the length and weight of the shoot and root are significant at the five percent probability level. Also, the length and dry weight of shoots and roots are significant at the five percent probability level. The effect of zinc nanoparticles on the concentration of chlorophyll a, chlorophyll b and carbohydrates is significant at the five percent probability level. The main effect of zinc nanoparticles on the amount of total phenol, flavonoid, and DPPH of the *M. Oleifera* medicinal plant was significant. The effect of stem and fruit extract of zinc nanoparticles on the amount of ascorbate peroxidase, catalase, and guaiacol oxidase enzymes.

Conclusion: The results showed that nano made from fruit and stem significantly increases root length, protein content and total phenol content, and activity of catalase and ascorbate peroxidase enzymes.

- ***Please cite this paper as:*** Khajeh H, Fazeli-Nasab B, Mirzaei AR, Farzanfar H. Biosynthesis of Zinc Nanoparticles of *Capparis Spinosa* Plant Extract and the it's Investigation on Morhpophysiological Properties of the *Moringa Olifera* Plant. *J Med Bacteriol.* 2023; **11** (5, 6): pp.17-29.

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Introduction

M. Oleifera is the large amount of oil in its seeds and its similarities to Tamarix, which is known as *M. Oleifera* in the regions where it grows (1). This plant, which is found over a significant portion of the country's southeast in the provinces of Hormozgan, Sistan and Baluchistan, is one of few that can be cultivated in both tropical and desert environments. *M. Oleifera* is a desert plant that thrives in dry climates and has significant dietary, therapeutic, environmental, industrial, and economic significance. Numerous research have been conducted on several species of this genus, including M. olifera species, to determine their nutritional content, protein content derived from vegetative parts, and various therapeutic benefits. The findings of these research suggest that they have a significant amount of potential for usage in the food and pharmaceutical sectors. Due to this, several uses for various *M. Oleifera* species have been presented (2, 3).

Due to the oil-rich seeds of this plant, the native residents of its surroundings have long utilised it. They have even gathered the seeds and transported them to the Arab nations south of the Persian Gulf. Harvesting seeds from this type of tree causes its branches to break because they are so delicate and easily separate from the tree. As a result, the mature bases of this species, which were previously found in plains and lands close to villages, have frequently sought refuge in heights and difficult-to-reach places. This species is likewise at danger of going extinct in its other habitats. Therefore, one of the study priority for this species is to fully understand this species from many biological perspectives and to have access to various ways of optimal seedling production, especially seedlings with high genetic potential (1, 4).

Many researchers have been interested recently in the production of nanoparticles by plants and microorganisms as a biocompatible and environmentally friendly technique. Physical and chemical approaches can be replaced by biological ones that use plant extracts to create metal nanoparticles (5, 6), due to the fact that they are crucial in the alteration of harmful compounds through the regeneration of metal ions (7)

A broad variety of topics are covered by the discipline of applied knowledge and technology known as nanotechnology. The confinement of particles or objects smaller than one micrometre, typically between nine and one hundred nanometers, is its principal subject. Nanotechnology is the study and use of novel properties of substances and systems that exhibit novel physical effects in these dimensions and are primarily affected by the predominance of quantum over classical qualities. The fourth industrial revolution's nanotechnology is a massive phenomenon that has permeated all scientific fields and is one of the new technologies that is evolving as quickly as possible. Nanomaterials are often defined as substances with at least one dimension smaller than 100 nanometers (8, 9). Zinc insufficiency is the element with the most impact on crop yield among those with low intake (10, 11). Zinc plays an essential role in the synthesis of proteins, RNA, and DNA (11). Although plants only need a little quantity of zinc (5–100 mg/kg), if there isn't enough of it available, plants will experience physiological stress due to the dysfunction of multiple enzyme systems and other zinc-related metabolic processes. Zinc is necessary to keep the root cell membranes intact (12, 13). Zinc is also quite good in making water use more efficient. Applying zinc to the soil reduces the effects of water stress and strengthens plant roots (14). Under water stress, spraying zinc sulphate solution increased the proportion of seed oil protein and the quantity of seeds in the cob. The effects of zinc on pollination, fertilisation, and fruit production are significant, together with those of boron and nitrogen (15). All photosynthetic tissues include this element in a portion of the anhydrase enzyme, which is necessary for the manufacture of chlorophyll. Additionally, tryptophan synthesis,

which is a precursor to the production of auxin, depends on zinc (16).

Divalent zinc from the soil solution is mostly taken by the plant. varying phases of plant development require varying amounts of zinc absorption. As a result, absorption was at its highest during the start of expansion and gradually decreased thereafter. Plants can also take zinc in the form of Zn (OH) + in soils with an alkaline response. Plants can absorb zinc through two active and passive processes. Zinc ions are electrostatically absorbed into the cell walls of plant root cells during passive absorption. The higher layers of the soil profile, which contain the most organic matter (i.e., surface soil), are where the majority of the zinc that plants may absorb. Because of its surface stabilisation, zinc loses some of its solubility, which reduces how useful it is to plants (17, 18). High soil pH causes zinc shortage in calcareous and alkaline soils. With each unit rise in soil pH, the solubility of zinc drops by 100 times, controlling the activity of the zinc ion and its solubility in the soil (19).

It was shown that the zinc element was much more absorbed by the sprouts at a concentration of 500 mg/liter, and that at higher concentrations, the buildup of nanoparticles reduced absorption. Additionally, the length of the soybean root shrank as the nanoparticle concentration rose (20). It has reported that the application of zinc nano oxide up to 2 mg/liter increases and higher amounts decrease root length (21). It has related that the Treatment of peanut seeds with zinc nanoparticles with a concentration of 1000 mg/liter resulted in a significant increase in germination, shoot length, root length, and vigor index compared to other concentrations of the same substance and varying concentrations of other zinc-containing substances such as zinc sulfate (22, 23). It has reported that the treatment of peanut seeds with zinc nanoparticles with a concentration of 1000 mg/liter has caused a significant increase in germination, shoot length, root length, and strength index compared to other concentrations of the same substance and variable concentrations of other zinc-containing substances such as zinc sulfate chelate (23, 24). The article aim was the effect of different concentrations on the biosynthesis of zinc nanoparticles by the fruit and stem of Capparis Spinosa plant extract and the investigation of growth factors and some physiological properties of the *M. Oleifera* plant.

Materials and Methods

The University of Zabol's Biotechnology Research Institute served as the site of this study. The seeds needed for this experiment were found in Saravan City's outlying districts. After being washed numerous times in regular water and 5% sodium hypochlorite for one minute, the seeds were sterilised. After that, distilled water was used to rinse it three times. It was utilised for germination in plastic pots that were 15 cm tall by 10 cm wide and filled with 1.5 kg of coco peat perlite soil when it was 1 mm in the Petri dish. Four levels of zinc nanoparticles (0, 125, 250, and 500) ppm were considered as treatments, which included 12 pots, and 3 pots for each treatment. The day and night temperature of the growth chamber was 28±2 and 20±2 degrees Celsius, the photoperiod was 14 hours of light and 10 hours of darkness, and the relative humidity was 60-70%. After 15 days, when the samples had 4-5 leaves, the leaves of the plants treated with nanoparticles were sprayed with zinc nanoparticles in the mentioned concentrations.

Making the extract

Ten grammes of stem and fruit from the Capparis Spinosa plant were dried separately, crushed, and extracted with water using the maceration process. Briefly, 100 cc of distilled water was added as a solvent after the first 5 grammes of plant extract powder were weighed. For 48 hours, the plant powder was in contact with the solvent on a magnetic stirrer. After the allotted time, it was filtered, concentrated using a rotary device (R100,

Jal Tehiz, Iran), and then an extract with a concentration of 10 mg/ml was created for future research.

Fabrication of biological zinc nanoparticles

For the biogenesis of high-purity zinc oxide nanoparticles, 20 ml of Louis plant leaf extract was mixed with 200 ml of zinc nitrate solution (1.5 mmol), and the mixture was then treated with 10 ml of sodium hydroxide (1 M). The obtained mixture was incubated on a stirrer (MS300HS, Jaltajhiz, Iran) in the dark at 60 degrees Celsius. After 24 hours, white color development was noticed, indicating the emergence of zinc oxide nanoparticles. The resultant solution was centrifuged (Sigma, model 2.16, USA), rinsed with distilled water, then with ethanol, and dried in order to further purify it. In order to verify the structure of artificial zinc oxide nanoparticles, the following techniques were used:

Ultraviolet-visible spectroscopic analysis

Using a UV-Vis spectrometer (Agilent, Cary 300 Spectrophtometer, USA) between 200 and 700 nm, the ultraviolet-visible spectroscopic analysis of zinc nanoparticles was assessed after 120 minutes of the additional reaction time of the extract to zinc nitrate and the colour change of the reaction.

Infrared spectroscopy (Fourier-transform infrared spectroscopy)

Different functional groups on molecular compounds may be recognised using infrared spectroscopy, which allows us to infer the compounds' potential structures. Infrared spectroscopy was carried out using the PerkinElmer (Japan) Spectrum Two equipment.

Measurement of growth parameters

In order to determine the fresh weight of the root and aerial portion for each treatment, the height of the aerial part and the length of the root were measured individually after harvesting the plant samples and separating the aerial part of the plant from the subterranean section. The root and shoot samples were then put in a paper envelope and heated to 80 degrees Celsius in an MTY-O108F oven for 72 hours to finish drying. It was taken out of the oven after 72 hours, and the dry weight was calculated. The samples' dry and wet weights were then precisely quantified to within 1% using a digital scale.

Measurement of chlorophyll a and b and carotenoids

In order to count the pigments, 80% acetone was used. First, 80% acetone was made for this use. Then, 0.5 g of each sample was thoroughly pulverised in a Chinese mortar with 4 ml of acetone, and the resulting powder was read by a spectrophotometer at wavelengths of 470 for carotenoid, 663 for chlorophyll a, and 645 for chlorophyll b.

Measurement of total phenol

We initially collected 0.5 grammes of the plant's fresh aerial tissue to calculate the total phenol content. Then, 5 ml of acidic methanol (5.99% methanol with 1% hydrochloric acid at a ratio of 99:1) was rubbed into the surface. The extracts were put inside the falcon and left there for 24 hours in the dark with a 4 degree Celsius temperature. The falcons were removed from the refrigerator after 24 hours and centrifuged at 4000 rpm for 10 minutes. One millilitre of ether was added to the supernatant solution after centrifugation, and following the creation of the supernatant phase, chlorophylls were transferred to the supernatant phase. At 280 nm, the total phenol absorbance was measured (25).

Measurement of antioxidant activity

Inhibition of DPPH free radicals was assessed using the Barros et al. (2007; Barros et al.) technique. The principle behind this technique is to convert the purple solution of 2 and 2-diphenyl-1-picryl-hydroxyl in methanol to the yellow solution of diphenyl-picrylhydrazine. Two milligrammes of DPPH were dissolved in 50 millilitres of methanol to make the DPPH solution, which was then combined with 250 microliters of the extract. The absorbance at 517 nm wavelength was measured with a spectrophotometer after these samples had been incubated for 30 minutes at room temperature and complete darkness. Using the formula below, the amount of free radical inhibition was computed (26):

(Ac-As)/Ac×100 = percentage of free radical inhibition

AC: absorbance for the control sample

AS: Absorption rate of plant sample

Measurement of total protein and antioxidant enzymes

Potassium phosphate buffer has to be made initially in order to extract protein and other enzymes. Two one-molar solutions of KH2PO4 and K2HPO4 were created for this purpose, along with a one-molar phosphate buffer that was created at a ratio of 39 to 61. When 50 mM buffer was needed for protein extraction, the previously prepared buffer was diluted, and 50 mM buffer with a pH of 7 was created.

Determination of total protein concentration in the Bradford method

After mixing 50 microliters of each sample's extracted extract with 750 microliters of Bradford solution and vortexing the mixture, the absorbance was measured at a wavelength of 595 nm to determine how much protein was present in each sample. Bovine protein BSA was used to create the standard curve, and the stock concentration was 1/32 micrograms/ml (27).

Preparation of enzyme extract

To do this, 0.5 grammes of each plant sample were thoroughly pulverised with liquid nitrogen in a Chinese mortar before being placed to falcons, who had already received 3 mm of 50 mM phosphate buffer. They were then centrifuged for 30 minutes at 13000 rpm and 4 degrees Celsius. The sampler then scraped off the top layer and transported it to 2 ml microtubes.

Measurement of catalase enzyme activity

The catalase enzyme activity was assessed using the Nicholls and Schonbaum technique. First, a blank was prepared in the spectrophotometer cuvette by adding 250 microliters of 100 mM phosphate buffer, 250 microliters of 70 mM H2O2, and 500 microliters of distilled water. The ingredients mentioned above were then combined with 50 microliters of the extract from each sample, and the mixture was read for 60 seconds at a wavelength of 240 nanometers (28).

Measurement of ascorbate peroxidase enzyme activity

The ascorbate peroxidase reaction produces dehydroascorbate, which was detected in 60 seconds at a wavelength of 290 nm, and the quantity of enzyme activity was quantified (29). We initially dissolved 0.5 mM ascorbate in 100 mM phosphate buffer for this purpose. Add 50 microliters of each extract to the aforementioned solution, then combine 850 microliters of 0.5m ascorbate with 150 microliters of H2O2 (used as a blank), and measure the enzyme activity in 60 seconds at a wavelength of 290 nanometers.

Measurement of guaiacol peroxidase enzyme activity

The peroxidase enzyme, also known as guaiacol peroxidase in certain sources, converts hydrogen peroxide to water by utilising the phenolic molecule guaiacol 16 as an electron donor. The phenolic molecule guaiacol donates its electron to peroxide-hydrogen during this reaction, which is catalysed by the enzyme glutathione peroxidase, and reduces it to water. This catalytic process produces tetraguaiacol, whose maximum absorption occurs at a wavelength of 470 nm, from the equivalent guaiacol. A mixture of 3000 microliters of 50 mM phosphate buffer with pH=10, 7 microliters of 3% hydrogen peroxide, 3 microliters of 200 mM guaiacol, and 50 microliters of enzyme extract was used to test the activity of this enzyme. The absorbance was then measured for 60 seconds at 10-second intervals at a wavelength of 470 nm (30).

Statistical software

The data of this research were factorially conducted in the form of completely randomized blocks with three replications and were analyzed using Statistix ver1 software.

Result

The effect of zinc nanoparticles on growth parameters of the M. Oleifera medicinal plant

The length and weight of the shoot and root are significant at the five percent probability level, according to the findings of the analysis of variance and Table 1 on the influence of zinc nanoparticles on growth parameters. Additionally, at a five percent likelihood level, the length and dry weight of shoots and roots are important.

Photosynthetic pigments

The simple effect of zinc nanoparticle treatment on the quantity of chlorophyll a and b and carbohydrates in *M. Oleifera* plant leaves yielded results that indicate that the effect of zinc nanoparticle treatment on the concentration of chlorophyll a, chlorophyll b, and carbohydrates is significant at the 5% probability level.

Effect of nano zinc particles on phytochemical enzymes

The major impact of zinc nanoparticles on the protein content in the stem and fruit of the medicinal plant *M. Oleifera* is considerable at different doses, as shown in Table 2.

According to the study's findings, the major influence of zinc nanoparticles on the quantity of total phenol, flavonoid, and DPPH of the medicinal plant *M. Oleifera* was significant at the 5% probability level (Table 2).

According to Table 2, the major impact of zinc nanoparticles on the quantities of the enzymes ascorbate peroxidase, catalase, and guaiacol oxidase in the stem and fruit of the *M. Oleifera* medicinal plant is substantial.

Infrared spectroscopy

Each molecule has its own set of bands. The fingerprint zone is defined as 400-1300 cm-1 of wave number. These bands are solely used to contrast the spectra of different compounds. The majority of the bands fall between 1300 and 4000 cm-1 wave numbers. To identify functional groups in unidentified compounds, these bands are employed. The zinc infrared spectroscopy diagram is shown in Figure 1. The graph of infrared spectroscopy for zinc nanoparticles is shown in Figure 2. The spectroscopic diagram of zinc and zinc nanoparticles is shown in Figure 3.

Discussion

The promotion of safe and ecologically acceptable ways for the manufacture of nanoparticles without the use of any harmful or chemical substances is one of the most crucial elements of nanotechnology. Nanoparticle synthesis may be done using a variety of techniques. The utilisation of nanoparticles is complicated by the chemical processes needed to create them, which result in hazardous byproducts and environmental degradation. The major objectives of this study are to manufacture zinc nanoparticles biologically for easier exploration and to provide favourable circumstances for the best possible creation of zinc nanoparticles. Because there are less waste products, non-consumable components, and hazardous residues in the environment when zinc nanoparticles are

Table 1. Statistical analysis of fresh weight and dry weight of *M. Oleifera* roots.

	p-value in nanobiology on fruit	**p-value in nanobiology on stem**
Fresh weight of leaves	0.016	0.001
Dry weight of leaves	0.000	**
Fresh weight of roots	0.000	0.461
Dry weight of roots	0.016	**
Green leaves	0.465	0.001
Percentage of green leaves	0.000	0.054

** Denominator of F-test is zero or undefined.

Table 2. Statistical analysis of growth parameters of *M. Oleifera* plants.

	p-value in nanobiology on fruit	**p-value in nanobiology on stem**
Chlorophyll a	0.000	0.000
Chlorophyll b	0.000	0.000
Carbohydrate	0.000	0.000
Content Phenol Total	0.000	0.000
Flavonoid	0.000	0.000
DPPH	0.000	0.000
Enzyme Ascorbate Peroxidase	0.000	0.000
Enzyme Catalase	0.001	0.084
Enzyme Catalase	0.000	0.007
Protein	0.000	0.000
Enzyme Guaiacol Oxidase	0.014	0.002
Enzyme Guaiacol Oxidase	0.003	0.000

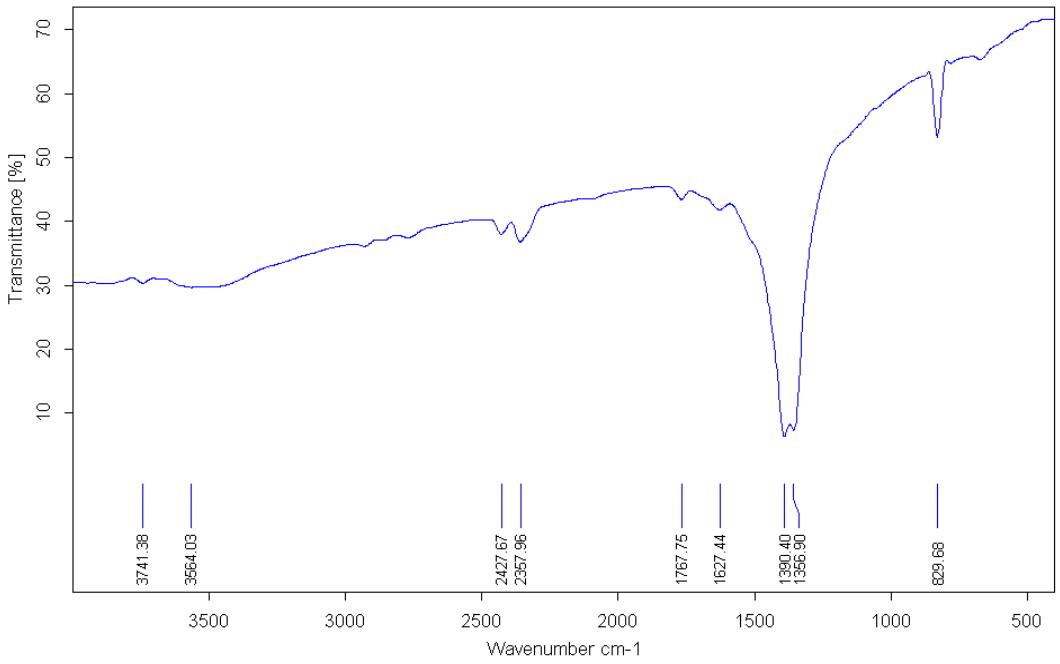

Figure 1. Infrared spectroscopy diagram for Zn.

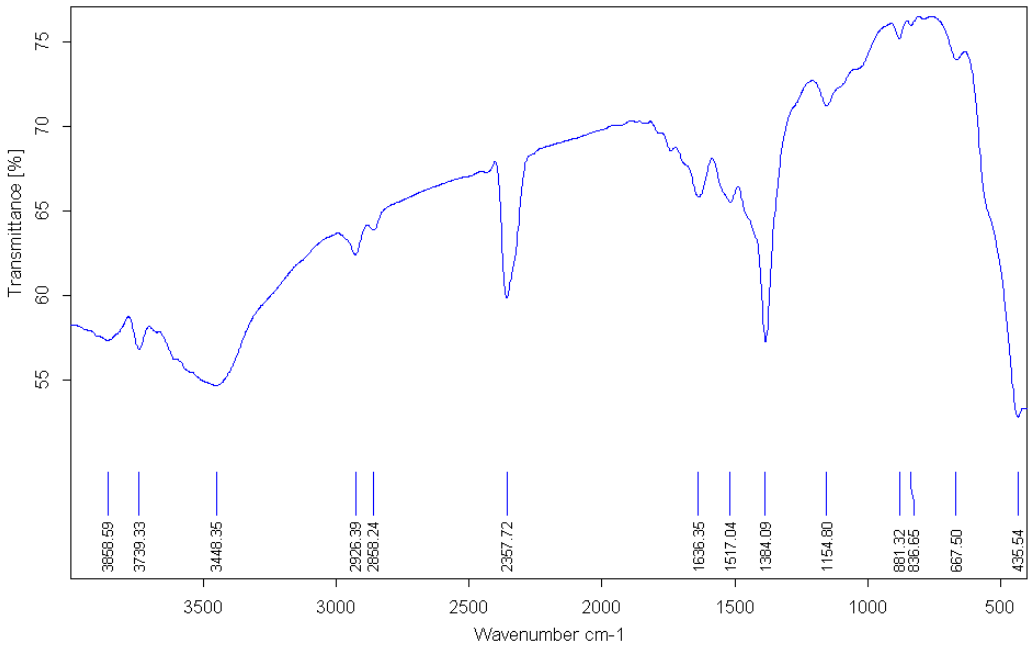

Figure 2. Infrared spectroscopy diagram for zinc nanoparticles.

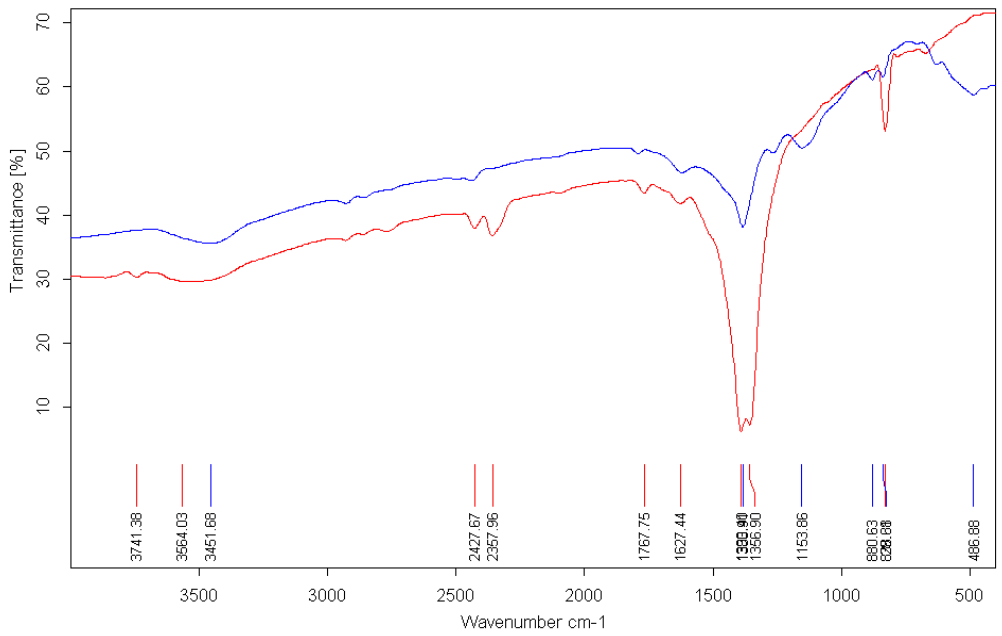

Figure 3. Spectroscopy chart of Zn and Zn nanoparticles.

created biologically as opposed to chemically, they are more useful. This work can serve as the foundation for in-depth investigations on the use of zinc nanoparticles as a biological approach because of the numerous uses and useful properties of nanoparticles in nanobiotechnology (31, 32).

The findings of the current study demonstrated that the growth characteristics of the *M. Oleifera* plant are affected differently depending on the concentration of zinc nanoparticles. These metrics have therefore risen at low concentrations, although the fresh and dry weight of the shoot and root remains similar with earlier studies. That is, the aforementioned characteristics are increased by low concentrations and decreased by large amounts. The more novel and unique the features and activities of the particles are, the smaller their size (33, 34).

The expansion of internal node length has been linked to an increase in auxin, which has been linked to the rise in plant growth since zinc is involved in the manufacture of auxin. It was discovered that the weight of the wheat plant grows as the amount of zinc increases (34, 35). It has been established that adding zinc to beans increased the quantity of soluble carbohydrates in the plant, which in turn raised the amount of dry matter. This rise demonstrates the crucial function that zinc plays in enhancing photosynthetic activity and raising the yield of plant products (34, 36).

The element zinc has many structural and functional roles in many metabolic processes of plants, but its overabundance as a heavy metal in soils for plants, the number 18 is regarded as a growth-limiting signal. Thus, the high level of nano oxide is regarded as stress. Applying zinc raises the leaf area index, which is acceptable given that zinc is necessary for the plant to produce food and for the leaves to synthesize chlorophyll (10, 37).

According to research, the amount of zinc element in the plant also increased as zinc nano oxide concentration rose. The concentration and absorption of zinc were shown to be positively and significantly correlated with the amount of zinc ingested (10, 34).

Other studies have demonstrated that adding zinc oxide nanoparticles to wheat plants causes their tissues to have more total zinc than control plants do (10, 38). Researchers found in a different study that the zinc content in corn's roots, stems, and leaves increases as zinc treatment increases. As a result, aerial sections have more of it than roots do (39, 40). Additionally, zinc indirectly affects the quantity of chlorophyll in plants since it is a necessary component of the chlorophyll molecule in the metabolism of nitrogen (41, 42). Different zinc element concentrations were found in pumpkin plants, and it was found that as the zinc element concentration increased, the amount of chlorophyll dropped. On the other hand, large levels of this element in the root environment hinder the absorption of iron elements and cause the reaction of iron shortage, which will impair the production of chlorophyll (37, 43).

In varying doses, saltiness increases the activity of the enzymes ascorbate peroxidase and catalase. The activity of these enzymes is also increased by foliar spraying with zinc nanoparticles (44, 45).

Conclusion

The results showed that nano made from fruit and stem significantly increases root length, fresh and dry weight in aerial parts of stem and root, protein content and total phenol content, and activity of catalase and ascorbate peroxidase enzymes at the five percent level.

Acknowledgements

The authors thank of the Agricultural Biotechnology Institute and the University of Zabol from it,s supports.

Funding Information

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics approval and consent to participate

No human or animals were used in the present research. The authors declare not used any patients in this research.

Conflict of interest

All authors declare no conflict of interest.

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