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Effect of Biological Synthesis of Nanoparticles from *Penicillium Chrysogenum* as well as Traditional Salt and Chemical Nanoparticles of Zinc on Canola Plant Oil Productivity and Metabolic

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Abstract

With the rapid advancement of nanotechnologies, it is more likely that biological systems may be exposed to an excessive amount of nanoparticles (NPs). The effect of NPs on plants, on the other hand, has yet to be investigated. The study's objective was to explore the comparative and effect of traditional salt ($ZnSO_4$), chemical and biological zinc oxide nanoparticles (ZnONPs, ZnObNPs) respectively through foliar treatment on photosynthetic traits, soluble sugars, proteins, phenols, oil content, and mineral content. 40, 90 days old seedlings of canola were foliar with distilled water (control), 50 or 100 mg/l of ZnSO4, ZnONPs, and ZnObNPs, respectively. Plants grown with ZnObNPs (100 mg/l) exhibited better growth and photosynthetic characteristics and higher levels of soluble sugars, proteins, phenols, oil content, and mineral content than non-treated plants at 50, 100, and 160 days after sowing (DAS). In conclusion, ZnObNPs can be used to improve canola plants by up-regulating the non-antioxidant defense system as phenol, osmolyte and increased oil content, mineral content.

Keywords: Biological Nano-biosynthesis; chlorophyll; elemental analysis; traditional salt.

1. Introduction

Increased yield for an ever-increasing global population has become a significant issue in terms of food security. Unfortunately, agricultural production has not kept pace with population expansion, especially in Africa, resulting in higher yearly imports and undernourishment [1-3].

The genus Brassica, which belongs to the Cruciferae (Brassicaceae) family, contains many economically important plants farmed in various conditions. Today, oilseed rape (Brassica napus) is Europe's most important source of vegetable oil and the world's second-largest oilseed crop after soybeans [4, 5].

Because of their catalytic and photocatalytic characteristics, NPs have high activity and efficiency in these techniques. However, metal and metal oxide NPs are often synthesized using various physical and chemical techniques [6]. These treatments are costly, take a lot of energy, are poisonous, and are very dangerous. As a result, numerous attempts have been made to create environmentally friendly techniques for synthesizing NPs to overcome the drawbacks of earlier approaches. A suitable alternative approach for producing metal and metal oxide NPs using plant extracts is the biosynthetic process. This green technique is risk-free, easy to use, environmentally beneficial, and cost-effective [7, 8].

The use of supports in the production of NPs has received a lot of attention in recent years. The supports prevent NPs from clumping together and improve their stability and efficiency [9]. The resultant heterogeneous catalysts offer many benefits over homogeneous metal catalysts, including ease of handling, simplicity of preparation, and recyclability [10-12]. As a result, several scientists are working to create a simple and environmentally friendly technique for producing heterogeneous nanocatalysts.

The best material for use as a Zn fertilizer in plants is anticipated to be nanoparticles (NPs) with a small size and high surface area. Nanomaterials are now being used to offer an essential pathway for releasing trace elements gradually and in a regulated manner, and they have found a place and purpose in agriculture [13].

Under normal or stressful conditions, zinc (Zn) serves a variety of critical biochemical and molecular functions in various plant species, including the

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upregulation of Reactive Oxygen Species (ROS) scavenging strategies, the activation of several enzyme systems (about 300 enzymes), and improved nucleic acid biosynthesis [14]. Zn is also involved in the absorption of indole acetic acid, cell growth, and sexual reproduction [15]. Therefore, it has been suggested that Zn supplementation is necessary for protection methods for plants. The application of exogenous zinc on lupin plants substantially enhanced plant growth, photosynthetic pigments, ion concentration [15]. More excellent knowledge of canola physiological responses may aid in programs aimed at increasing oil content. Therefore, this study aimed to evaluate the comparative and effect of traditional salt ZnSO4), chemical, and biological zinc oxide nanoparticles (ZnONPs, ZnObNPs) on the physiological responses (i.e., chlorophyll, soluble sugars, protein, phenol, oil content, and mineral content) of canola.

2.Experimental

Biosynthesis of biological zinc oxide nanoparticles (ZnObNPs):

Preparation of cell-free extract

In a 250 ml flask containing 50 ml of the liquid medium yeast extract medium, a fungal strain (*Penicillium chrysogenum*) was inoculated. First, inoculation was performed using a 0.4 cm diameter disk produced from seven days old culture plates of the investigated strain, which was incubated cultured for four days in a rotary shaker at 200 rpm, 37°C, and an initial pH of 7.0. After incubation, each culture was after an incubation period, and actinomycetes cells were removed from the suspension by filtration through a 0.44 µm PVDF filter; then, they were centrifuged at 10,000 rpm to remove occasional actinomycetes cells and macromolecules [16, 17] [14, 15].

Biosynthesis of ZnObNPs.

The pH was adjusted to 8.5 by mixing a 50 mL aqueous solution of 1 mM zinc nitrate solution with 50 mL fungal supernatants. For 24 hours, the mixture was shaken at 37°C and 200 rpm in the dark in a rotary shaker. Control tests were carried out using an uninoculated medium and zinc nitrate solution to verify the function of actinomyces in nanoparticle formation. The reduction of zinc ions was investigated by collecting approximately 2 ml of the solution at time intervals and using a UV–Vis spectrophotometer to analyze the UV–Vis spectra (JASCO V-560). A color shift was seen in each reaction vessel, resulting in the development of a white suspension. The ZnObNPs suspension was centrifuged for 30 minutes at 12,000 rpm, and the precipitate pellet was dried and weighed [18].

Preparation of Zinc

The required concentration of ZnSO4, ZnONPs, and ZnObNPs was prepared by dissolving 50 or 100 mg $ZnSO_4$, ZnONPs, and ZnObNPs in 1000 mL of distilled water (DW). Four drops of 80 % Tween were used with every prepared solution to maximize dissemination on canola leaves.

Planting techniques, treatments, and sample collection:

Canola seedlings (*Brassica napus*) were planted at the botanical garden of Al-Azhar University's Faculty of Science's botany and microbiology department in Cairo, Egypt. The seeds were planted in plots (10 m length and 4 m width). The area was divided into 21 rows, each 70 cm apart, with hills 20 cm apart. The following were the soil characteristics: 27.79 percent sand; 23.15 percent silt; 49.06 percent clay; pH 7.6; 2.39 mmhos/cm EC; textured clay loam Table 1 explains the chemical characteristics of the soil.

Table 1: shows the chemical characteristics of the soil that was utilized.

The following treatments are represented by twenty-one rows split into seven groups:

T1. Tap water as (control).

T2. 50 mg/l Zinc sulphate as (50 mg/l $ZnSO_4$).

T3. 100 mg/l Zinc sulphate as (100 mg/l $ZnSO_4$).

T4. 50 mg/l Zinc oxide nanoparticles as (50 mg/l ZnONPs).

T5. 100 mg/l Zinc oxide nanoparticles as (100 mg/l ZnONPs).

T6. 50 mg/l biological zinc oxide nanoparticles as (50 mg/l ZnObNPs).

T7. 100 mg/l biological zinc oxide nanoparticles as (100 mg/l ZnObNPs).

The treatments mentioned above were applied twice to canola (Brassica napus) plants (as foliage spraying). The first application was performed when canola plants were 40 DAS old, and the second was performed when canola plants were 90 DAS old. When the plants were 50 DAS, and 100 DAS, plant samples were taken for examination. At the end of the growing season (160 DAS).

Determination of Photosynthetic Pigments

Fresh leaves (0.5 g) were pulverized with acetone (80%), and the homogenate was filtered through Whatman No 1 filter paper. A spectrophotometer was used to measure the absorbance of the filtrate at 470, 652, and 665 nm to determine the levels of chlorophyll-a, chlorophyll-b, and carotenoids [19].

Determination of total soluble sugar, protein, phenol, and oil content

The anthrone reagent was used to determine the soluble sugar content, and absorbance was measured at 625 nm as described by the [20] method. The soluble protein content was estimated following Lowry, et al. [21] using Folin phenol reagent, and the absorbance was recorded at 700 nm using bovine serum albumin as standard. The Folin–Ciocalteu (FC) test was used to estimate the total phenolic compounds, and the spectrophotometer read the absorption at 765 nm as described by Singleton and Rossi [22]. According to Helrich [23], the seed oil content was measured using a soxhlet apparatus and petroleum ether (40-60 ºC).

Estimation of mineral ion contents

Dried powdered tissues were used for the estimation of nitrogen (N), potassium (K), phosphorus (P). N was estimated using micro-Kjeldahl apparatus (Ningbo Medical Instruments Co., Ningbo, China) by Bremner [24], P was determined following Sen Tran, et al. [25], and K was determined using a flame photometer [26].

Statistical methods:

The experimental results were submitted to one-way ANOVA, and Tukey's technique calculated the differences between means. The values are shown as means SE (standard error). Unless otherwise noted, levels of significance were assessed at $p < 0.05$ and the least significant difference (LSD) at a 5% level of probability using SPSS software [27].

3. Results and discussion

Synthesis and characterization of ZnObNPs by the biological method.

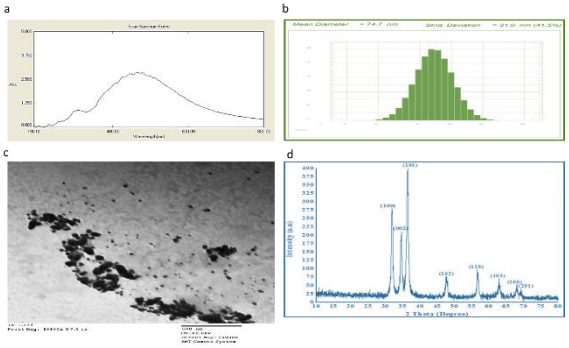
UV-visible spectrophotometer (UV-Vis.), Dynamic light scattering (DLS), Transmission electron microscopy (TEM), and X-Ray Diffraction (XRD) of ZnObNPs synthesized by the biological methods.

The UV–visible spectra of ZnObNPs produced, as shown in Fig.1a, indicate that it has a maximum absorbance (2.739 abs) and a wavelength of 455 nm. DLS was used to monitor the particle size distribution, and the average particle size was calculated using this technique to be 74.7 nm, as shown in Fig. 1b. In contrast, as shown in Fig. 1c, TEM results revealed spherical particles within a nano-scale range of 20.47 nm to 61.21 nm, with an average major diameter of 53.7 nm. Because it reveals the axis, shape, size, and location of the atoms, XRD provides a better picture of the crystal structure of the detected atoms. In Fig. 1d, the XRD pattern for zinc aggregates can be seen, with numerous peaks. For diffractions from the (002), (100), (101), (102), (103), (110), (112), (200), and (201) planes, the pattern may be indexed.

Figure 1 (a-d). a) UV-Visible spectrum; b) DLS image; c) TEM image, and d) XRD image of ZnONPs synthesized by the biological method.

2. Synthesis and characterization of ZnONPs by chemical method

UV-visible spectrophotometer (UV-Vis.), Dynamic light scattering (DLS), Transmission electron microscopy (TEM), and X-Ray Diffraction (XRD) of ZnONPs synthesized by chemical method.



The UV–visible spectra of ZnONPs produced, as shown in Fig. 2a, indicate that it has a maximum absorbance (1.593 abs) and a wavelength of 335 nm. DLS was used to monitor the particle size distribution, and the average particle size was calculated using this technique to be 62.4 nm, as shown in Fig. 2b. In contrast, as shown in Fig. 2c, TEM results revealed spherical particles within a nano-scale range of 19.4 nm to 59.8 nm, with an average major diameter of 48.32 nm. Because it reveals the axis, shape, size, and location of the atoms, XRD provides a better picture of the crystal structure of the detected atoms. In Fig. 2d, the XRD pattern for zinc aggregates can be seen, with numerous peaks. For diffractions from the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) planes, the pattern may be indexed.

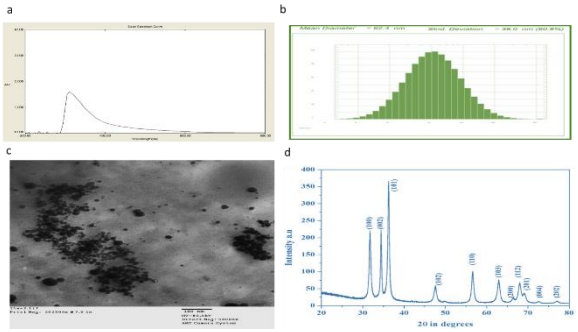


Figure 2 (a-d). a) UV-Visible spectrum; b) DLS image; c) TEM image and d) XRD image of ZnONPs synthesized by chemical method.

Photosynthetic Pigments

The results in Fig. 3 show that as the growth proceeded from 50 to 100 DAS, the photosynthetic parameters increased regardless of the treatment. However, concentrations (100 mg/L) increased further at both stages. The highest values of Chl a, b, carotenoids and total pigments content about 148.7%, 274% and 310.83%, 322.75 and 54.09%, 212.88% and 195.72,

291.76% after treatment with 100 mg/L ZnObNs at 50 and 100 DAS respectively compared to control plants. The impact of Zn on crop output is thought to be due to its effect on CA activity and Rubisco, which increased CO_2 assimilation and photosynthetic capacity, resulting in maximum dry matter production [28, 29]. Furthermore, Zn plays a vital role in sexual reproduction, including floral organ development, gametogenesis, and seed generation. Pollen–stigma contact and pollen tube development are also improved [30]. The positive impact of Zn on yield may be due to (1) an increase in canopy size, which improves light interception and increases photosynthetic rate while reducing senescence processes [31]; (2) a well-developed root system that determines water and ion utilization. In addition, seed quality is favourably influenced by Zn [32]; and (3) the maintenance of higher rates of photosynthesis with a relatively high fluorescence ratio and water use efficiency [33].

Zarasvand [34], found that the phytotoxicity of ZnONPs to maize and rice was determined mainly by the NP itself rather than the Zn^{2+} produced from ZnONP suspensions. Our research also found that the quantity of Zn^{2+} emitted by ZnONPs was minimal. The phytotoxicity of NPs may be assessed using the concentration of photosynthetic pigments, which is often used as an indication in phytotoxicity tests [35-37].

Our findings on the positive effect of ZnONPs or ZnObNPs on photosynthetic pigments agree with Tondey, et al. [38], who discovered that nano-Zn treatment increased total chlorophyll content in maize (*Zea mays*) when compared to control. According to Rai-Kalal and Jajoo [39], chlorophyll levels increased in response to ZnONPs. This implies that zinc oxide nanoparticles can mitigate the harmful effects of bulk zinc on plant health, making them suitable for use as a nano fertilizer. Rajput, et al. [40], compared to control samples, total chlorophylls of barely plant were significantly increased with increasing ZnONP concentrations (300 and 2000 mg/l) in the treated samples.

Gaafar, et al. [41], discovered that foliar spraying Moringa oleifera plants with nano-zinc resulted in higher levels of carotenoids than in normal plant conditions.

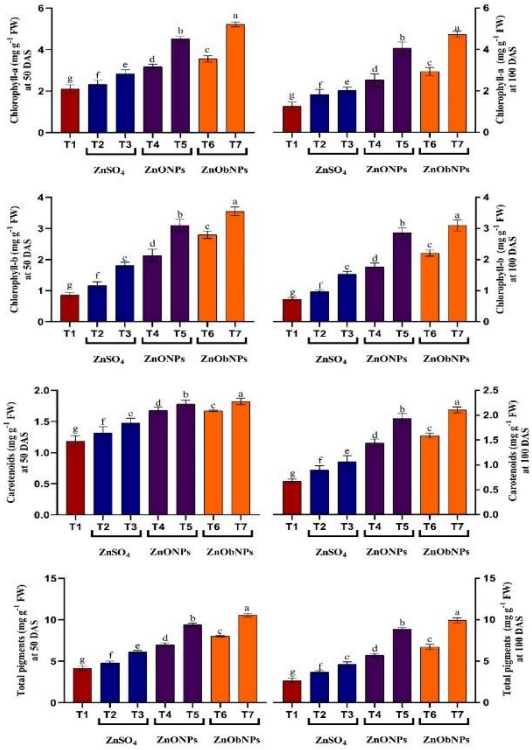


Figure 3 Effect of different concentrations (50, 100 mg/l) of $ZnSO_4$, ZnONPs and ZnObNPs on photosynthetic pigments in the leaves of canola at 50 and 100 DAS respectively. All the data are the mean of five replicates (n = 5) with mean ± SE (standard errors). The various letters (a–g) are substantially different across treatments at the 0.05 level, according to Fishers test.

Total Soluble Sugar:

The current study found (Fig 4) that sugar levels in canola shoots, roots, and produced seeds were substantially enhanced in response to treatments with ZnSo4, ZnONPs, and ZnObNPs.

The beneficial impact of ZnONPs and ZnObNPs on sugar content in leaves and seeds is thought to be due to an increase in starch synthase and CA activity and enhanced Rubisco activity, which promotes seed development [28, 42]. Furthermore, NP has been shown to protect enzyme function by binding the sulphydryl group and preventing disulfide formation, which leads to an increase in protein production and protein content in the seed [40, 43].

Fatollahpour, et al. [44] discovered that foliar spraying of Zn nano-fertilizers at various dosages (30, 60, and 90 mg/l) to pinto bean plants resulted in more significant sugar percentages than those seen in control plant circumstances. According to Zhang, et al. [45], higher glucose content of wheat plants was found at different doses of $ZnSO_4$ and ZnO Nanoparticles. Furthermore, Davarpanah, et al. [46] found that the sugar content of Punica granatum plants rose

substantially in the leaves and seeds of plants treated with zinc as a foliar spray. In addition, foliar ZnNPs treatment increased soluble sugar in the wheat plant, according to Seyed, et al. [47]. Thus, the rise in sugar content in response to zinc nano-fertilizer is most likely due to zinc's role in stimulating the enzymes responsible for photosynthesis, sugar transformation, and biosynthesis [48, 49].

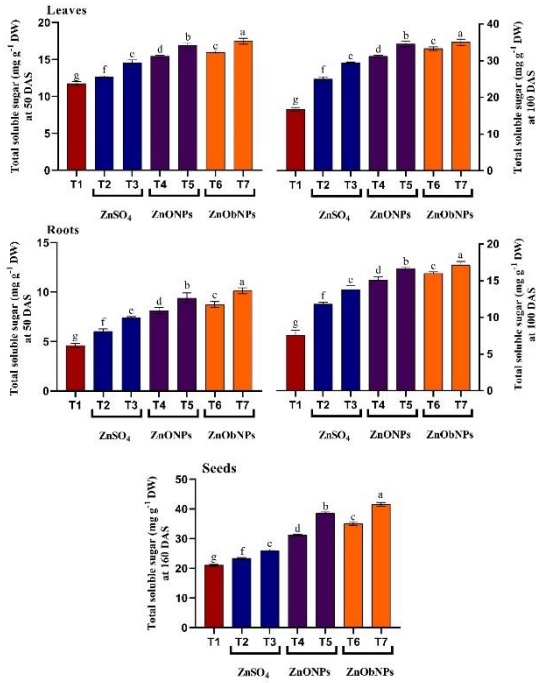


Figure. 4 Effect of different concentrations (50, 100 mg/l) of ZnSO4, ZnONPs and ZnObNPs on total soluble sugar in the leaves, root and seeds of canola at 50, 100 and 160 DAS respectively. All the data are the mean of five replicates (n = 5) with mean ± SE (standard errors). The various letters (a–g) are substantially different across treatments at the 0.05 level, according to Fishers test.

Soluble Proteins

The soluble protein content of canola plants was substantially enhanced by foliar application of ZnObNPs at 50, 100, and 160 DAS, as shown in fig (5). The most significant treatment for shoot, root, and seed protein was 100 mg/l ZnObNPs. These findings are consistent with who Zolfaghari, et al. [50] found that zinc is an essential component of the structure of enzymes involved in amino acid biosynthesis. Because amino acids are the building blocks of protein synthesis, protein content increases when this micronutrient is used. Fatollahpour, et al. [44] found that treatments of nano-zinc at various doses (1.5 g L^{-1}) to pinto bean plants resulted in higher crude protein percentages than those seen under normal plant circumstances. According to Askary, et al. [51], the highest protein content was observed in Catharanthus roseus plants applied with generated nano-fertilizer. Furthermore, Osman, et al. [52] discovered that treatment Zn nanocomposite resulted in substantial rises in protein percent in the seeds of soybean plants.

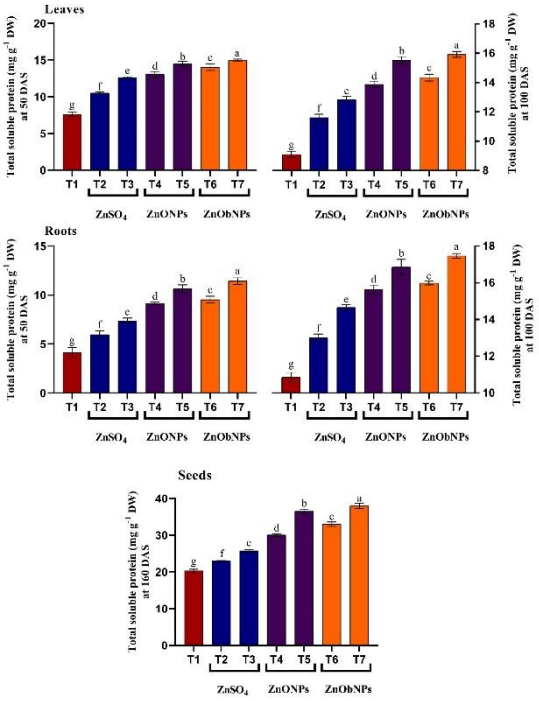


Figure. 5 Effect of different concentrations (50, 100 mg/l) of $ZnSO_4$, ZnONPs and ZnObNPs on total soluble protein in the leaves, root and seeds of canola at 50, 100 and 160 DAS respectively. All the data are the mean of five replicates (n = 5) with mean ± SE (standard errors). The various letters (a–g) are substantially different across treatments at the 0.05 level, according to Fishers test.

Soluble Phenols

Total phenol levels in shoot, roots, and seeds of canola plants were substantially enhanced in response to treatments with $ZnSO_4$, ZnONPs, and ZnObNPs (Fig 6). Phenolic acids are potent antioxidants that protect plants from oxidative damage. In addition, Zn stimulates non-enzymatic antioxidants, which may help with detoxification [53-56]. This was followed by an increase in antioxidant capability and total phenol content. In this regard, many studies have discovered an increase in antioxidant activity due to Zn's effect [44, 53].

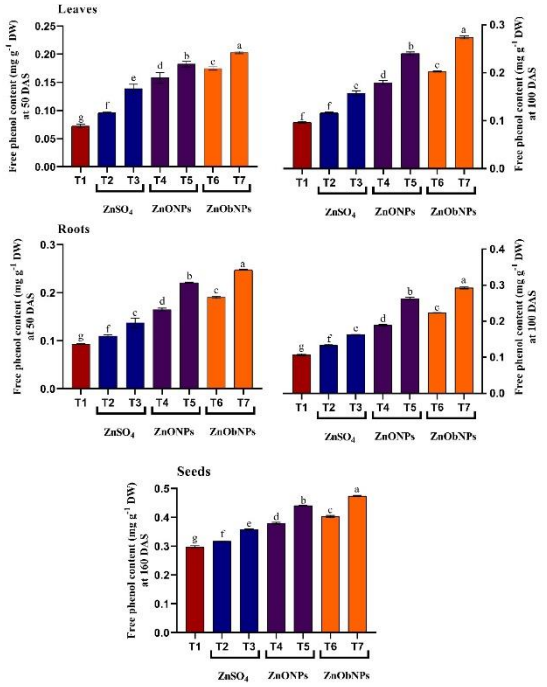


Figure. 6 Effect of different concentrations (50, 100 mg/l) of $ZnSO_4$, ZnONPs and ZnObNPs on total phenol in the leaves, root and seeds of canola at 50, 100 and 160 DAS respectively. All the data are the mean of five replicates (n = 5) with mean ± SE (standard errors). The various letters (a–g) are substantially different across treatments at the 0.05 level, according to Fishers test.

Oil content

Treatment with ZnObNPs enhanced the oil content of the produced canola seeds (Fig 7). It is worth noting that the treatment with ZnObNPs at 100 mg/l resulted in the most significant increase in oil content of produced seeds compared to the other treatments and control. Zinc is required for the production of oil in seeds [50]. Zn treatment has also been shown to decrease fatty acid desaturation in plant tissues and enhance plant growth and biomass [57]. The degree of fatty acid saturation is critical for maintaining membrane fluidity and providing a proper membrane function environment [58, 59]. A typical plant plasma membrane response is an increase in the degree of fatty acid saturation. Furthermore, certain alterations in membrane lipids were thought to be an adaptive response to various environmental stressors to restore optimal physical membrane characteristics. For example, the foliar spray of zinc element on rapeseed cultivars plants substantially enhanced the proportion of seed oil [60].

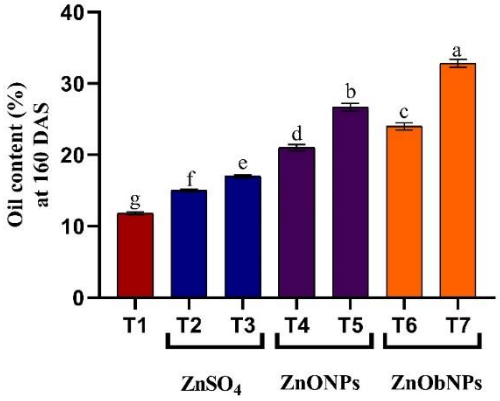


Figure. 7 Effect of different concentrations (50, 100 mg/l) of $ZnSO_4$, ZnONPs and ZnObNPs on total oil content in the seeds of canola at 160 DAS respectively. All the data are the mean of five replicates (n = 5) with mean ± SE (standard errors). The various letters (a–g) are substantially different across treatments at the 0.05 level, according to Fishers test.

Mineral's content

The experiment results in fig (8) indicated that treating canola plants with ZnObNPs increased nitrogen, potassium, and phosphorus content. However, other studies have shown the varying solid influence of Zn on the mineral element concentrations. This result was in agreement with other investigators Rizwan, et al. [61] found that N, P, and K concentration and their uptake of wheat plants were raised by increasing Zn oxide nanoparticles treatment. Also, Sofy, et al. [14] indicated that the treatments of Zn application significantly raised all nutrients in leaves, i.e., N, P, K, as compared with the control treatment in *Pisum sativium* plant. So the zinc supplementation increased crop ion status by encouraging ion transport and accretion in crop organs [61, 62].

4. Conclusion:

Biological synthesis is one of the most cost-effective and naturally safe methods to obtain non-hazardous metallic NPs. Exposure to nanoparticles enhanced the biochemical components of plants, according to a number of researchers, greenhouses, and field experiments. *Penicillium chrysogenum* culture supernatant was used to synthesize NPs in this research, and the effects of the synthesized NPs on *B. napus* were assessed. The findings of this study showed that the use of ZnObNPs had a favourable impact on the biochemical profile. It was found that foliar application of ZnObNPs at 100 mg/l to canola plants increased the biosynthesis of photosynthetic pigments and improved yield by raising the soluble sugar, proteins, phenols, and oil content of canola seeds. That all of the treatments improved the quality and nutritional value of canola seeds

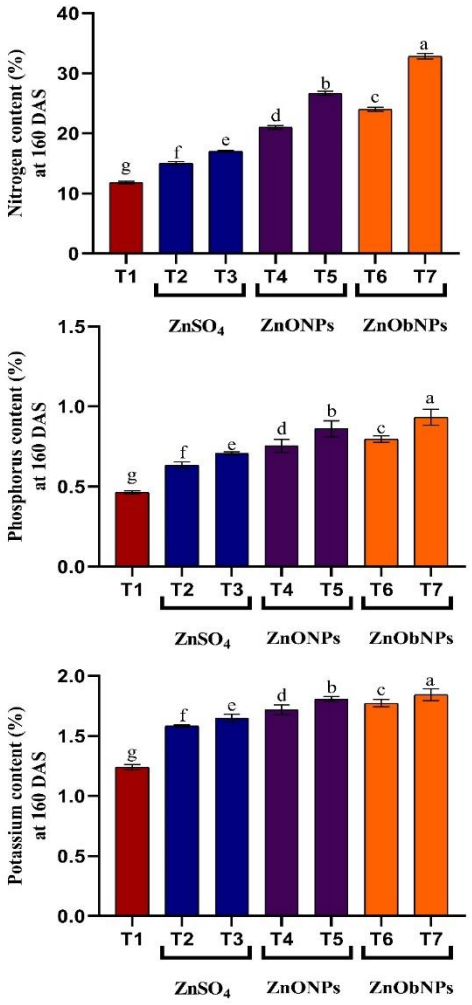


Figure. 8 Effect of different concentrations (50, 100 mg/l) of $ZnSO_4$, ZnONPs and ZnObNPs on mineral content in the seeds of canola at 160 DAS respectively. All the data are the mean of five replicates (n = 5) with mean ± SE (standard errors). The various letters (a–g) are substantially different across treatments at the 0.05 level, according to Fishers test.

Declarations

N/A

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not available.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Conceptualization, HMF; methodology, HMF, and MRS; software, HMF, and MRS; formal analysis, IU, HMF and MRS; investigation, HMF; resources, MRS; writing-original draft preparation, MRS; writing-review and editing, HMF and MRS; supervision.

All authors have read and agreed to the final version of the manuscript.

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