Research Article

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Green synthesis of magnesium oxide nanoparticles using endophytic fungal strain to improve the growth, metabolic activities, yield traits, and phenolic compounds content of Nigella sativa L.

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Abstract: Endophytic fungus Penicillium crustosum EP-1 was used to create spherical shape magnesium oxide nanoparticles (MgO-NPs). The MgO-NPs possess a crystalline structure with sizes of 8–35 nm. The weight percentages of Mg and O were 42.44% and 30.13%, respectively, as shown in energy-dispersive X-ray analysis. In addition, analysis involving dynamic light scattering indicated the uniformity of MgO-NPs within the colloidal solution. These NPs displayed a polydispersity index of 0.341 and held a surface charge of −29.7 mV. A field experimental was carried out to assess the outcome of foliar spraying of MgO-NPs at 5, 10, and 20 ppm on the growth, yield, and metabolic activities of Nigella sativa L. Our results indicated that MgO-NPs significantly enhanced various growth parameters, including chlorophyll content (both a and b), total carotenoids, carbohydrate and protein levels in both shoots and seeds, as well as free proline concentration, compared to the control plants at both 55 and 75 days after planting. In addition, all yield traits were markedly increased. Moreover, high-performance liquid chromatography is employed for the identification of phenolic compounds within the seeds. Data indicated that sex phenolic acids, two phenols, and five flavonoids were present with high concentrations due to MgO-NPs treatment as opposed to untreated plants.

Keywords: green synthesis, magnesium oxide nanoparticles, black cumin, growth traits, HPLC, phenolic compounds

1 Introduction

The Nigella sativa L., with common names of the black seed, black cumin, black caraway, or nigella, belongs to the Ranunculaceae family and is characterized as an annual herbaceous flowering plant [1]. Originating in the Mediterranean region, it is now cultivated globally. Renowned for its tiny, black, crescent-shaped seeds, N. sativa has been used for kitchen and medicinal purposes for centuries [2]. The nigella seeds are grown in Egypt due to ideal conditions for planting and watering until the seed pods mature [3]. Widely used as a spice, these seeds impart a distinct flavor profile with earthy, peppery, and slightly bitter notes, found
in various cooking applications worldwide [4]. In addition to cooking uses, *N. sativa* is assumed to have a broad spectrum of health benefits including anti-inflammatory and antioxidant properties, immune system support, treatment of respiratory and digestive diseases, and using their oils topically for various skin diseases [1,2,4]. Because of its nontoxic nature, safety, ease of accessibility, and effectiveness when compared to traditional allopathic drugs, nigella seeds are utilized all over the world [5].

Nanotechnology, a flourishing scientific field and technology, is responsible for synthesizing novel materials at the nanoscale (1–100 nm) [6]. At this scale, the new materials possess unique properties that are significantly different from bulk compounds [7]. Nanotechnology enables researchers to manipulate and synthesis individual atoms and molecules to form new materials, systems, and devices with fascinating functions and properties [8]. The end products from nanotechnology, nanoparticles (NPs), find applications in various fields like pharmaceuticals, medicine, diagnosis, chemistry, catalysts, wastewater treatment, sensors, agriculture, and textile [9–11]. NP synthesis is an important part of nanotechnology and involves three major groups: chemical, physical, and biological. Because of the drawbacks associated with chemical and physical techniques, such as the use of perilous substances, generation of toxic secondary products, harsh fabrication conditions, and high processing expenses, biological methods are preferred [12]. Various biological entities, including bacteria, plants, yeasts, fungi, and actinomycetes, use their metabolites to reduce metal and its oxide and form various NP shapes. The final product is then stabilized and capped to increase its stability and prevent it from aggregating over time [13,14].

Nanotechnology is making significant contributions to agriculture, enabling researchers and farmers to enhance crop production, improve soil health, and reduce environmental impact. NPs can be used in agricultural sectors as nanopesticides, nanofertilizers, soil remediation, plant disease management, and smart farming as nanosensors for monitor plant health, soil conditions, and environmental factors [15,16].

Magnesium (Mg) is an important element for several processes that take place in plants and crops, including biochemical reactions and processes, photooxidation, reactive oxygen species (ROS) synthesis, utilization and partitioning of photo-assimilates, chlorophyll generation, phloem loading, photosynthetic CO_2 fixation, protein synthesis, and photophosphorylation [17,18]. Besides it is safety and synthesized using the green approach, there are several benefits to providing Mg to plants in the form of magnesium oxide NPs (MgO-NPs). The first benefit of MgO-NPs is that they serve as a nano-sized delivery route for magnesium ions, enhancing the efficacy of plants to absorb and utilize the mineral. The increased surface area of the MgO-NPs improves their contact with plant roots and speeds up the uptake of magnesium [17]. In addition, soil magnesium solubility and bioavailability can also be enhanced by using MgO-NPs. Magnesium may be released slowly and may be leached if conventional magnesium fertilizers do not dissolve quickly in the soil. MgO-NPs have a higher surface area; thus, they dissolve faster and make magnesium ions readily available to plants [19]. Also, MgO-NPs can be engineered to release Mg ions slowly, resulting in a continuous supply of Mg over time. This timely release of Mg can assist in keeping plant Mg levels where they need to be, which is good for the plant’s growth and development [11,20]. Therefore, using MgO-NPs as Mg ion supply for plants can increase growth and production while decreasing waste.

The selection of nanomaterials, specifically MgO-NPs that were produced by the endophytic fungal strain *Penicillium crustosum* EP-1, is based on the principles of green synthesis. Due to the fact that it is environmentally benign and sustainable, the usage of biological entities, such as fungi, for the synthesis of NPs is chosen. This is because it eliminates the need for the use of dangerous substances that are connected with chemical and physical procedures. MgO-NPs have several distinct advantages, one of which is that they provide a nano-sized delivery pathway for magnesium ions, which increases the efficiency with which plants absorb magnesium. As a result of the expanded surface area, improved interaction with plant roots is made possible, which speeds up the process of magnesium digestion. In addition, MgO-NPs provide advantages in the form of improved soil solubility, bioavailability, and controlled release of magnesium ions, all of which contribute to the continuous supply of nutrients to plants. Therefore, the novelty of the study lies in its combination of green synthesis, biogenic approach, agricultural application, controlled release features, comprehensive characterization, and a field-based perspective. Also, the detection of phenolic compounds due to various treatments increases the novelty of the current study. These elements collectively contribute to the originality and significance of the research.

Based on the aforementioned knowledge, MgO-NPs were formed by endophytic fungi, *P. crustosum* EP-1, as an eco-friendly, cost-effective, and rapid method for increasing the growth and metabolic traits of *N. sativa* L. The MgO-NPs that were produced underwent characterization using various techniques, including UV-visible (UV-Vis) spectroscopy, FT-IR (Fourier transform infrared spectroscopy), XRD (X-ray diffraction), TEM (transmission electron microscopy), EDX (energy-dispersive X-ray), DLS (dynamic light scattering), and zeta potential analysis. In addition, a field-based experimental study was conducted to assess the impact of foliar application of MgO-NPs on plant growth, metabolic processes, and the detection of phenolic components through high-performance liquid chromatography (HPLC) analysis.
2 Materials and methods

2.1 Microorganism used

*P. crustosum* EP-1 was employed for MgO-NPs biosynthesis. This fungal strain was recognized as an endophytic strain isolated from healthy leaves of *Ephedra pachyclada* collected from Wadi Selebat (N: 28.545493; E: 33.933707), Saint Katherine, South Sinai, Egypt [21]. The fungal strain was identified using morphological and macroscopic examination as well as ITS sequence analysis (molecular identification). The acquired sequence was uploaded with accession number MN954764 in GenBank.

2.2 Biosynthesis of MgO-NPs

2.2.1 Formation of fungal biomass filtrate (FBF)

Two disks of fungus *P. crustosum* EP-1, covered with hyphae, were introduced into malt extract broth media (100 mL), which subsequently incubated for 6 days at 30 ± 2°C while being agitated at 150 rpm. Following, the media containing the fungal inoculum was subjected to centrifugation to harvest the fungal biomass. The collected biomass (10 g) was suspended in distilled water (dH2O, 100 mL) and incubated at 30 ± 2°C for 48 h under continuous agitation at 150 rpm. After that, the mixture was centrifuged at 5,000 rpm for 10 min. The resulting supernatant, identified as the FBF, was collected and used for MgO-NPs synthesis.

2.2.2 Biosynthesis

The collected FBF was used as a biocatalyst for reducing Mg(NO3)2·6H2O (metal precursor) to form a nanostructure as follows: dissolving 76.9 mg of metal precursor in 10 mL dH2O, before being mixed with 90 mL of the collected FBF to obtain 3 mM as a final concentration. The previous mixture was heated at 60°C under mantic stirring (100 rpm) for 1 h, pH = 8 which was adjusted using 1 N NaOH, and finally incubated for overnight at room temperature. Following the incubation period, the cloudy white solid, identified as Mg(OH)2, was gathered, extensively washed with dH2O to remove any contaminants, and then dried in an oven at 100°C for a duration of 2 h [22]. After that, Mg(OH)2 that formed underwent calcination at a high temperature (400°C) for 4 h to form the end product MgO-NPs as shown in Eqs. 1 and 2 [23].

\[
\begin{align*}
\text{Mg(NO}_3\text{)}_2 \times 6\text{H}_2\text{O} & \xrightarrow{\text{Fungal metabolites}} \text{Mg(OH)}_2\cdot\text{(white ppt.)} \\
\text{Mg(OH)}_2 & \xrightarrow{400^\circ\text{C}} 4\text{h} \text{MgO-NPs}
\end{align*}
\]

2.3 Characterization

2.3.1 UV-Vis spectroscopy

Changes in the color from colorless to turbid white were checked by UV-Vis spectroscopy (JENWAY-6305, UK). For this purpose, a cuvette containing 2 mL of the resulting solution was subjected to absorbance analysis at various wavelengths (200–600 nm) to identify the maximum surface plasmon resonance peak (SPR).

2.3.2 Fourier transform infrared (FT-IR)

The functional groups present in both the FBF and the biosynthesized MgO-NPs were identified using a Cary 630 FT-IR instrument from Tokyo, Japan. For the analysis, 200 mg of MgO-NPs were blended with potassium bromide (KBr), compressed into a disc, and subsequently scanned across wavenumbers 400–4,000 cm\(^{-1}\). Likewise, a KBr was mixed well with FBF drops and subjected to examination at the same wavenumbers to identify the functional groups within the filtrate.

2.3.3 X-ray diffraction (XRD)

The XRD was carried out to examine the structural characteristics (whether crystalline or amorphous) of the fungus-mediated-MgO-NPs. The XRD measurements were conducted utilizing an X’ Pert Pro instrument from Philips in Eindhoven, the Netherlands, within a 2θ value range spanning from 10° to 80°. The X-ray source employed was Cu-Kα, with a wavelength (λ) of 1.54 Å, operating at a current of 30 mA and a voltage of 40 kV.

The average size of the crystallite in the fungal-mediated MgO-NPs was calculated using the Debye–Scherrer equation, as shown below:

\[
\text{Average crystallite size} = \frac{0.9\times\lambda}{\beta\cos\theta}
\]

where 0.9 is the constant of Debye–Scherrer, \(\lambda\) represents the wavelength of the X-rays, which is equivalent to 1.54 Å. Meanwhile, \(\beta\) stands for half of the maximum intensity, and \(\theta\) corresponds to Bragg’s angle.
2.3.4 Morphological and elemental composition

The structure (shape and size) of the MgO-NPs were investigated using transmission electron microscopy (TEM) utilizing an instrument manufactured in Japan by JEOL 1010. After dissolving the synthesized compound in ultrapure water, it was sonicated and then dropped onto a carbon-copper TEM grid. Before being moved to the TEM holder for examination, this grid was desiccated in a vacuum overnight to guarantee proper deposition.

Energy-dispersive X-ray (EDX) examination employing a JEOL JSM-6360LA apparatus from Japan’s Thermo-Fisher Scientific was utilized to analyze the synthetic MgO-NPs for both qualitative and quantitative composition. An EDX holder containing a trace amount of the NP solution was covered with gold using a sputter coater.

2.3.5 Dynamic light scattering (DLS) and zeta potential (ζ) analysis

DLS was utilized to analyze the size distribution of the synthesized NPs in the colloidal solution. During the scattering process, the synthesized NPs were ultrasonically dispersed in ultrapure water (Milli-Q H2O) to limit the chance of artifact peak or shadow forming. In addition, the Smoluchowski model was used with a zeta-sizer (Nano-ZS, Malvern, UK) to examine the NPs’ surface charge. The pH of the extremely pure water used in the experiments was around 6.5, and the temperature was kept constant at 25°C. The count rate for the 12 zeta runs was 55.2 kcps.

2.4 Experimental design

The black seeds (N. sativa L.) were sourced from the Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. These uniform nigella seeds were sown in naturally occurring loamy soil within an open-field setting, specifically in a plot measuring 12 m in width and 15 m in length. This plot was located at the Botanical Garden, within the Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.

For the field experiment, the design of completely randomized was employed, comprising four blocks. Each block was subdivided into four individual plots, representing the following treatments: Control, 5 ppm of MgO-NPs, 10 ppm of MgO-NPs, and 20 ppm of MgO-NPs.

The seeds were planted on a single side of the ridge, maintaining a spacing of 15 cm between each hill. Adequate irrigation was provided as needed throughout the growth phase. The growing plants received two rounds of spraying with the treatments mentioned earlier. The initial spray was administered 45 days after planting, followed by the second spray at the 63-day mark. For analysis, five plants from each treatment were randomly selected at two growth stages: when the plants were 55 days old (stage I) and when they reached 75 days of age (stage II) since planting. The culmination of the growth season, which spanned 205 days, marked the point at which the analysis of the harvested seeds from various treatments, as well as the control, was conducted.

2.5 Plant analyses

2.5.1 Growth measurement

The length of the shoot and root, fresh and dry weights of the shoot, and branch number/plant were detected at two harvest stages after 55 and 75 days of planting, whereas the flower number/plant was detected one time at stage II. To determine the dry weight of the shoot, the collected shoots were placed in an oven at 70°C and allowed to dry until a consistent weight was achieved [24].

2.5.2 Photosynthetic pigments

One gram of freshly harvested leaves was powdered and immersed in 100 mL of 80% acetone. After that, Whatman filter paper (no. 1) was used to filter the liquid. The resultant filter was placed in a volumetric flask with a 100 mL capacity, and the volume was brought up to the 100 mL mark using 80% acetone. Optical densities were measured at 470, 649, and 665 nm to find out how potent the extract was. The concentration of chlorophyll a, b, a + b, and carotenoid was detected using the following equations [25].

\[
mg \text{ chl a/g tissue } = 11.63(A_{665}) - 2.39(A_{469}) \\
mg \text{ chl b/g tissue } = 20.11(A_{469}) - 5.18(A_{665}) \\
mg \text{ chl a + b/g tissue } = 6.45(A_{665}) + 17.72(A_{669}) \\
mg \text{ carotenoids/g tissue } = 1,000 \times OD_{470} - 1.82 \text{ Chl a} - 85.02 \text{ Chl b}/198
\]

where the optical density is represented as A and chlorophylls a and b are represented as Chl a and Chl b, respectively.
2.5.3 Carbohydrate and protein contents

UV spectrophotometry (UNICO Vis Model 1200, USA) was used to determine the total soluble carbohydrates using the anthrone-sulfuric acid method described by Unbreit et al. [26]. Total soluble protein concentrations were determined with a UV spectrophotometer (UNICO Vis Model 1200, USA) and the Bio-Rad protein assay according to the methods described by Lowry et al. [27].

2.5.4 Free proline

The contents of free proline were measured using the method of Bates et al. [28]. Ten milliliters of sulfosalicylic acid was used to thaw out about 0.5 g of frozen leaf tissue at 4°C. Using No. 2 Whatman filter paper, this fresh homogenate was filtered. In a test tube, 4 mL of the filtrate was mixed with 4 mL of glacial acetic acid and 4 mL of acid ninhydrin solution. To prepare the acid ninhydrin solution, 1.25 g of ninhydrin was dissolved in 30 mL of glacial acetic acid and 20 mL of 6-M phosphoric acid with mild heating, agitation, and chilling until completely dissolved. After reacting for an hour in a water bath, this mixture was left to cool. After adding 4 mL of toluene to the reaction mixture and rapidly whirling it with a test tube, the chromophore-containing toluene was successfully separated from the aqueous phase. After separating the aqueous phase from the toluene layer, the absorbance of the chromophore in the toluene was measured at 520 nm in a UV spectrophotometer (UNICO Vis Model 1200, USA). Micromoles per gram of fresh weight (mol·g⁻¹ F.Wt) were used to quantify the proline concentration.

2.5.5 Detection of phenolic compounds using HPLC

In a stoppered container, 1 g of powdered dried N. sativa seeds was soaked in 50 mL (85%) of methanol and allowed to remain at room temperature for 24 h. Conventional extraction was carried out in a sonicate for 30 min at 4°C. The extract was filtered and concentrated under vacuum at 40°C by using a rotary evaporator to provide crude extract. Equipment from the Agilent 1260 series was utilized for the HPLC analysis that was achieved. The Eclipse C18 column (4.6 mm × 250 mm internal diameter, 5 nm particle size) was utilized to accomplish the separation. Both water (A) and acetonitrile containing 0.05% trifluoroacetic acid (B), delivered at a flow rate of 0.9 mL·min⁻¹, were included in the mobile phase. The flowrate was measured in mL·min⁻¹. The linear gradient that was designed for the mobile phase went as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (82% A); 15–16 min (82% A); and 16–20 min (82% A). The detection was carried out with the assistance of a multiwavelength detector that was calibrated to 280 nm. A volume of 5 mL was used for the injection of each sample solution. The temperature of the column was kept at a steady 40°C.

2.6 Statistical analysis

Each treatment was conducted three times, and the experiment was set up using a split-plot design. Sigma Plot v14 was not used for statistical analysis of the gathered data. Mean differences between treatments were determined mostly through the use of analysis of variance. Post hoc comparisons were performed using Tukey’s test (truly significant difference) at a P ≤ 0.05 significance level. The content of phenolic compounds in N. sativa seeds data was also inflicted to a hierarchical two-way cluster analysis. The centered approach for normalized data was used in this study.

3 Results and discussion

3.1 Biosynthesis of MgO-NPs

In the present study, MgO-NPs were synthesized by utilizing the cell-free filtrate of the endophytic fungal strain P. crustosum EP-1 as a green method. Green NP synthesis involves the utilizing natural, nontoxic, cost-effectiveness, and environmentally friendly substances as reducing, capping, and stabilizing agents, resulting in reduced environmental impact, enhanced biocompatibility, and promising applications in various sectors [6]. The reducing and capping agents in green methods for NP synthesis are the metabolites secreted by biological entities. In this work, the metal precursor was reduced using metabolites released by the endophytic fungal strain EP-1, which had been isolated from the healthy leaves of the medicinal plant.

The high efficacy of endophytic fungi to synthesize NPs of varied size, shape, and activity is one of the reasons that these organisms have received more interest in recent years. These fungi reside within the plant tissues and work in harmony with their host [29]. Using endophytic fungi in NP manufacturing is advantageous because of the diversity of bioactive metabolites they may create [30].
As they coexist with the plant host, they are subjected to different environmental stressors, leading to the synthesis of different secondary metabolites [31]. These bioactive metabolites are used as reducing and stabilizing agents during NP formation, making the process more efficient and controllable. Moreover, as a result of their unique genetic makeups, the NPs synthesized by endophytic fungi exhibit a wide range of forms, sizes, and surface functionality [32]. This natural variability between metabolites secreted by endophytic microbes opens the way for researchers to tailor NPs with unique properties for specific applications, such as environmental remediation, drug delivery, cosmetics, pharmaceuticals, improvement the agriculture yield, and catalysis [33]. Due to these remarkable properties of endophytic fungi, they offer several advantages over conventional fungi in NP synthesis.

### 3.2 Characterizations

#### 3.2.1 UV-Vis spectroscopy

The turbid white formation due to the mixing of FBF with metal precursor refers to the successful synthesis of MgO-NPs. The emergence of the new color could be due to the efficacy of fungal metabolites to reduce the NO3 to NO2 and use the liberated electron to reduce the Mg2+ to Mg(OH)s (white precipitate), which was collected and subjected to calcination at high temperature to form MgO-NPs [34,35]. The density of the milky-white substance was determined by assessing its absorbance across a spectrum of wavelengths from 200 to 600 nm, to identify the SPR peak. As seen, the maximum SPR for the synthesized MgO-NPs was located at 285 nm (Figure 1a). The SPR values usually influence the shape, size, and distribution of synthesized NPs. Regarding this finding, Jeevanandam and coauthors stated that the MgO-NPs sizes formed by an aqueous extract of three different plant leaves, *Andrographis paniculata*, *Amaranthus tricolor*, and *Amaranthus blitum*, were small and homogenous when the maximum SPR ≤ 300 nm, and it became large and more heterogenous at SPR ≥ 300 nm [36]. Therefore, we predict the small sizes and well-arranged MgO-NPs synthesized in the current study.

#### 3.2.2 FT-IR

FT-IR was used to identify the functional groups found in the FBF and their function in the reduction of metal precursor to generate MgO-NPs, which causes the appearance of new peaks or alters the intensity of the existing peaks (Figure 1b). As shown, the spectra of FBF contain three peaks at wavenumbers of 3,433, 2,078, and 1,633 cm⁻¹ [32]. The peak at 3,433 cm⁻¹ signifies the vibration of N–H and O–H overlapping [37], which changed to 3,395 cm⁻¹ after the synthesis of MgO-NPs with a similar intensity. The sulfonic acid groups and a moiety of carbohydrates that originated from fungal metabolites were represented by broadband at a peak of 2,078 cm⁻¹ [38]. This observation was confirmed by EDX analysis. The peak at 1,633 cm⁻¹ demonstrated the presence of polysaccharides in the FBF, confirming the overlap of CO with N–H [39]. Some new peaks were observed after fabrication of MgO-NPs represented at wavenumbers of 2,495, 1,430, 1,071, 870, and 520 nm. Peak 2,495 cm⁻¹ represented the nitrogen-containing amines (NH₂) and carbamates (CN) groups [40]. Moreover, peak 1,430 cm⁻¹ corresponded to binding CH₃ vibration (methyl group), whereas silicate (Si–O) or silanol (Si–OH) groups were involved in peak at a wavenumber of 1,071 cm⁻¹ [40,41]. Peaks at 870 and 520 cm⁻¹ could be relevant to the characteristic absorbance of the MgO-NPs at this specific wavelength. It reinforces the presence of the Mg–O bonds and the formation of MgO-NPs [22,36].

![Figure 1: (a) UV-Vis spectroscopy, which reveals the SPR peak at 285 nm, and (b) FT-IR analysis for both the FBF and MgO-NPs.](image-url)
3.2.3 XRD

The XRD analysis was employed to examine the crystalline nature and crystallographic structure of the MgO-NPs formed using a cell-free filtrate of P. crustosum EP-1. As shown, there are five prominent peaks at 2θ° of 37.08°, 42.68°, 62.36°, 74.92°, and 78.62°, represented by the crystallographic planes (111), (200), (220), (311), and (222), respectively (Figure 2a). These peaks are associated with distinct MgO-NPs crystallographic planes according to the JCPDS card number 75-0447 [42]. This reveals that crystalline MgO-NPs were successfully fabricated due to the formation of NPs with a well-defined and ordered crystal structure, as evidenced by the presence of intense and sharp peaks. Recently, the crystalline structure of MgO-NPs synthesized by Rhizopus oryzae was confirmed due to the presence of intense peaks at 2θ° of 36.8° (111), 42.7° (200), 62.3° (220), 45.2° (311), and 78.7° (222) [23]. The presence of extra peaks in XRD could be attributed to the capping agent from the fungal extract, which was confirmed by EDX analysis. Moreover, the XRD analysis confirms the presence of oxides in the form of Mg(OH)2 and MgO. According to the study by Lekota et al. [43], the peaks at 2θ of 37.1° (111) and 74.9° (311) correspond to Mg(OH)2, while the diffraction peaks at 2θ of 42.6° (200), 62.3° (220), and 78.6° (222) indicate the presence of cubic MgO-NPs. Based on the XRD, the crystallite size of MgO-NPs was calculated using the Debye–Scherrer equation based on the width of the highest peak at the 2θ value of 42.68° (200). The result showed that the average MgO-NPs crystallite size was 22 nm. In a similar study, the average MgO-NPs crystal size was calculated based on XRD according to a plane (200) to be 17.4 nm [44]. However, the average crystal size of MgO-NPs according to the Debye–Scherrer equation was 40.7 nm and decreased to 36.6 nm after being doped with cerium (Ce) NPs [45].

3.2.4 Morphological structure

The shapes, sizes, and elemental compositions are important parameters that should be detected because they influence NP’s applications. TEM and SEM-EDX are considered the main apparatus used for this purpose. As shown, the cell-free filtrate of P. crustosum has the potential to form MgO-NPs with spherical shape and well arranged without aggregation with sizes in the ranges of 8–35 nm (Figure 2b). The activity of NPs varied based on their sizes and shapes. For instance, Bacillus subtilis treated with MgO-NPs showed varying degrees of inhibition depending on the NP sizes used. The highest percentage of inhibition (96.1%) was found for a size of 35.9 nm, and this proportion gradually reduced to 94.5% for particles of 47.3 nm and 75.7% for microparticles of 2,145.9 nm [46]. Zhang et al. reported that the activity of

![Figure 2: Characterization of MgO-NPs fabricated by fungal strain P. crustosum. (a) The XRD analysis for detecting the crystalline nature, (b) the TEM analysis showing the spherical shape, and (c) the EDX chart showing the elementary mapping of synthesized MgO-NPs.](image-url)
ZnO as an antibacterial agent against Escherichia coli was increased either by increasing the NP concentrations or by decreasing the NP sizes [47]. The authors found that the antibacterial activity is shown by both the NP and micro-particle ZnO suspensions. When comparing the micron-sized ZnO suspension to the nano-sized ZnO suspension, the latter is superior in terms of antibacterial activity against E. coli. Cheon et al. reported that the antibacterial activity of NPs against E. coli, Staphylococcus aureus, and Pseudomonas aeruginosa was shape dependent [48]. The authors reported that the highest antibacterial activity was recorded for spherical NPs followed by disk and triangular shapes.

Successful creation of MgO-NPs is indicated by the existence of peaks of O and Mg in the bending energy in the range of 0.5–1.5 keV [49,50]. Figure 2c shows that Mg and O accounted for roughly 42.44% and 30.13% of the weight and 35.63% and 32.41% of the atomic percentages, respectively, of MgO-NPs. Weight and atomic percentages of Mg and O remained at 18.1% and 28.1%, respectively, when MgO-NPs were synthesized using a cell-free filtrate of fungal strain Aspergillus terreus S1 [51]. There are additional peaks for C, Cl, and Ca at lower weights and atomic percentages, as seen by the EDX diagram. The bioactive metabolites that coat and stabilize MgO particles are probably to blame for these spikes [52,53].

3.2.5 DLS analysis

One essential technique for characterizing NPs is the DLS. It offers important details regarding the size distribution of the colloidal sample. It can show if NPs are polydisperse, or vary in size, or monodisperse, or uniform in size. Also, DLS analysis is a useful tool for detecting the aggregation or agglomeration of NPs, which have direct impacts on stability, reactivity, and effectiveness [54]. Herein, the average particle size of fungal-mediated –MgO NP synthesis was 52.8 nm (Figure 3a). The obtained size indicates that the fungal strain P. crustosum, used in the fabrication process was successful in producing relatively small NP sizes. This smaller size is significant for the integration of NPs in different biomedical and biotechnological applications. This finding is due to the smaller sizes, which tend to have high stability in suspension and decrease the aggregation or settling over time [55]. Also, smaller sizes have higher surface area-to-volume ratios, which can enhance catalytic activity [56].

As depicted in the results, it is clear that the average size of the MgO NPs varies due to various analytical techniques. It is important to note that the size measurements obtained by DLS are superior to those obtained through XRD and TEM investigations. This disparity can be explained by the fact that the experimental settings for TEM and DLS are very different from one another. DLS measurements are carried out in an aqueous solution, whereas TEM analysis is performed on dehydrated particles (solid particles). This difference in measuring environment inevitably leads to variances in the estimation of particle diameter. It is essential to emphasize that DLS offers a hydrodynamic diameter, which indicates the particle size while they are in a hydrated state [57]. In addition, it is important to realize that the diameter obtained from DLS may be affected by the nonuniform distribution of NPs within the colloidal solution, in addition to the presence of protective compounds derived from cell-free extract that encapsulate or capping the NPs. Both of these factors can affect the diameter. During the process of calculating the size, these elements pose the possibility of influence from other sources [54,58].

The DLS analysis is a powerful technique used for the detection of the polydispersity index (PDI), which is a crucial parameter in NP characterization as it provides valuable insights into the NP uniformity within a sample [59]. The efficacy of DLS in determining the PDI lies in its ability to provide reliable information about the size distribution of NPs in suspension.
to measure the fluctuations in light scattering caused by the Brownian motion of particles suspended in a liquid medium. By analyzing these fluctuations, DLS can generate a size distribution profile that reveals whether the NPs in a sample are monodisperse or polydisperse [60]. The PDI is measured on a scale from 0 to 1. A PDI value of 0–0.4 indicates a perfectly monodisperse sample where all particles are the same size. Conversely, a higher PDI value suggests a broader distribution of particle sizes, indicating polydispersity [59]. Herein, the PDI of MgO-NPs fabricated by fungal strain was 0.341, which reveals the homogeneity of synthesized NPs in the solution.

3.2.6 Zeta potential (ζ) analysis

Detecting the ζ analysis for MgO-NPs is important in NP characterization and various applications. The ζ potential, which is a measure of the electric surface charge, provides valuable insights into the stability and behavior of NPs in a colloidal system [61]. In general, dispersion systems with ζ potential values in a range of ±10 mV are considered highly unstable, while those in a range of ±10 to ±20 mV are considered stable. Systems with potential values of ±20 and ±30 mV are classified as moderately stable dispersions, whereas those with potential values greater than ±30 mV are classified as extremely stable dispersions [8]. In the current study, the ζ potential of green synthesized MgO-NPs was −29.7 mV, which indicates the stability of NPs in the colloidal system. The presence of a high absolute ζ potential value, which in the current study is negative, tends to repel each other strongly, hence preventing agglomeration or precipitation over time.

### 3.2.7 Effect of biosynthesized MgO-NPs on the performance of *N. sativa* growth

#### 3.2.7.1 Morphological parameters

Results showed that foliar MgO-NPs application at concentrations of 5, 10, and 20 ppm on N. sativa plant enhanced all growth parameters (i.e., shoot length, root length, weights of fresh shoot, weights of dry shoot, branch number per each plant, and flowers number per each plant) compared to the control. In most cases, the growth criteria were significantly increased with 10 ppm of MgO-NPs during the two stages of the plant growth (Table 1). The obtained results were in agreement with those mentioned by Fatemi and co-authors about the potential of Mg-NPs on the growth performance of *Helianthus annuus* L. [62]. Also, different concentrations (10, 50, 100, and 150 ppm) of MgO-NPs improved the growth and increased the yield of *Brassica juncea* L. plant [11]. The authors reported that the plant growth improvement was remarkable in the increase of leaf area, biochemical constituents, and plant biomass. Moreover, the applications of MgO-NPs are recommended for increasing the growth parameters of many legume crops like black gram, horse gram, lentils, chickpea, and mungbean [63–66].

The enhancement of the plant growth due to MgO-NP treatment could be attributed to different mechanisms including nutrient availability, improved nutrient uptake, enhanced water absorption, antioxidant activity, improved resistance of the plant to various bacterial and fungal pathogens, improved resistance of plant toward various stresses, and phytostimulation. Magnesium (Mg) ions, released from MgO-NPs, are an essential nutrient for plant growth because they are a component of chlorophyll, the pigment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plant growth stages</th>
<th>MgO-NPs treatments</th>
<th>HSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Shoot length (cm)</td>
<td>Stage I</td>
<td>13.7 ± 1.2b</td>
<td>14.3 ± 0.6ab</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>Stage II</td>
<td>20.1 ± 1.1b</td>
<td>20.8 ± 0.8ab</td>
</tr>
<tr>
<td>Shoot fresh weight (g)</td>
<td>Stage I</td>
<td>6.2 ± 0.8b</td>
<td>7.3 ± 0.6ab</td>
</tr>
<tr>
<td>Shoot dry weight (g)</td>
<td>Stage II</td>
<td>11.5 ± 0.5c</td>
<td>13.7 ± 0.5b</td>
</tr>
<tr>
<td>Branch number/plant</td>
<td>Stage I</td>
<td>9.3 ± 0.9c</td>
<td>13.9 ± 0.5b</td>
</tr>
<tr>
<td>Flowers number/plant</td>
<td>Stage II</td>
<td>12.03 ± 1.2c</td>
<td>17.9 ± 0.6b</td>
</tr>
</tbody>
</table>

Data represent means ± SE (n = 5). Different lowercase letters in the same species within a row indicate significant differences (P ≤ 0.05). HSD is honestly a significant difference by post hoc Tukey’s test.
responsible for photosynthesis, and thus can boost photosynthesis and thus plant growth and productivity [67]. Increased nutrient uptake by plant roots is one result of MgO-NPs’ ability to modify the soil’s or growth medium’s physicochemical qualities. They can make nutrients like phosphorus and iron more soluble and available to plants, allowing for easier uptake by the plants [56]. Moreover, the water-holding capacity of soil can be improved or enhanced due to MgO-NP treatment. This finding is beneficial in drought-borne or arid regions, as it allows plants to access water more effectively, reducing water stress and supporting growth even under water-limited conditions [68]. Interestingly, due to antioxidant properties, MgO-NPs can enhance plant growth by scavenging the ROS that formed under different stresses, hence protecting plant cells from oxidative damage and promoting healthier growth [68]. On the other hand, MgO-NPs have antibacterial and antifungal activity, hence promoting the plant growth by protecting plants against phytopathogens that can inhibit or decrease their growth. Some published studies propose that MgO-NPs can act as phytostimulants, nutrient uptake, promoting root growth, and overall plant vigor. This phytostimulatory effect can lead to increased plant growth and biomass [64,69].

### 3.2.8 Biochemical traits

#### 3.2.8.1 Chlorophyll and carotenoid Contents

Based on our findings, spraying of MgO-NPs significantly improved the contents of chlorophyll and carotenoid in *N. sativa* compared to the control plant throughout the two stages of plant growth (Table 2). The highest increases in the chlorophyll a, b, and a + b contents were attained at stage I with 49%, 26%, and 39%, respectively, when the plants were treated with MgO-NPs at a concentration of 20 ppm. In the case of stage II, the maximum increases in the percentages of chlorophyll a and a + b were 71% and 47%, respectively, with MgO-NPs concentration of 5 ppm, while the maximum increase percentages in chlorophyll b was 24% with 10 ppm Mg-NPs as being compared to that of the control. It was found that the highest value of carotenoid content at stage I (0.96 mg·g⁻¹ fresh weight) was achieved at a concentration of 5 ppm MgO-NPs, while at stage II, the highest value (0.79 mg·g⁻¹ fresh weight) was achieved due to spraying with 20 ppm MgO-NPs compared to control (0.49 mg·g⁻¹ fresh weight at stage I and 0.65 mg·g⁻¹ fresh weight at stage II).

In this connection, the application of MgO-NPs might enhance the rate of cell metabolic and chlorophyll destruction and/or increase chlorophyll biosynthesis, resulting in increased chlorophyll accumulation and a faster rate of photosynthesis. This finding is because of the liberation of Mg ions from MgO-NPs, which are considered a component of chlorophyll and hence improve photosynthesis, leading to an increase in the chlorophyll a, b, and a + b contents. The obtained results are matched with the previous study of Singh et al. [70], who reported that the application of the Mg-NPs at concentrations of 125, 250, 500, 750, and 1,000 mg·L⁻¹ showed an increase in the chlorophyll pigments in *Stevia rebaudiana* plant because of the abundance of Mg⁺ ions liberated from Mg-NPs. Moreover, the positive effects of MgO-NPs on chlorophyll and carotenoid contents of plants were recorded by various workers. For instance, Cai et al. [17] investigated the efficacy of MgO-NPs on seedling growth, morphology characters, and physiological activities (chlorophyll and carotenoid) of *Nicotiana tabacum* L. Also, Salcido-Martínez et al. investigated the activity of various Mg-NP concentrations (50, 100, and 200 ppm) on the growth, biochemical, physiological activities, and yield of *Phaseolus vulgaris* L. [71]. The authors reported that the highest biomass and photosynthetic pigments were attained at a concentration of 50 ppm, whereas a concentration of 100 ppm of Mg-NPs was useful for obtaining the highest pod yield and improving the nitrate reductase activity. Moreover, Fatemi et al. reported that the spraying of *H. annuus* with Mg-NPs at a concentration of 0.25 g·L⁻¹ increased the chlorophyll, carotenoid contents, and photosynthetic pigments.

### Table 2: Effects of MgO-NPs on the chlorophyll and carotenoid contents (mg·g⁻¹ fresh weight) of *Nigella sativa* plant

<table>
<thead>
<tr>
<th>MgO-NPs treatments</th>
<th>Chl a (Stage I)</th>
<th>Chl a (Stage II)</th>
<th>Chl b (Stage I)</th>
<th>Chl b (Stage II)</th>
<th>Chl a + b (Stage I)</th>
<th>Chl a + b (Stage II)</th>
<th>Carotenoids (Stage I)</th>
<th>Carotenoids (Stage II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.5 ± 0.1c</td>
<td>1.1 ± 0.1d</td>
<td>2.9 ± 0.2b</td>
<td>0.9 ± 0.1c</td>
<td>6.4 ± 0.3c</td>
<td>1.9 ± 0.1c</td>
<td>0.5 ± 0.1b</td>
<td>0.7 ± 0.04b</td>
</tr>
<tr>
<td>5 ppm</td>
<td>4.0 ± 0.2b</td>
<td>1.8 ± 0.03a</td>
<td>3.2 ± 0.2b</td>
<td>1.04 ± 0.04ab</td>
<td>7.1 ± 0.2b</td>
<td>2.8 ± 0.1a</td>
<td>1.0 ± 0.1a</td>
<td>0.7 ± 0.02b</td>
</tr>
<tr>
<td>10 ppm</td>
<td>5.2 ± 0.1a</td>
<td>1.6 ± 0.03b</td>
<td>3.7 ± 0.1a</td>
<td>1.1 ± 0.04a</td>
<td>8.9 ± 0.2a</td>
<td>2.7 ± 0.1a</td>
<td>0.3 ± 0.1b</td>
<td>0.6 ± 0.1c</td>
</tr>
<tr>
<td>20 ppm</td>
<td>5.3 ± 0.1a</td>
<td>1.2 ± 0.1c</td>
<td>3.7 ± 0.2a</td>
<td>1.0 ± 0.1bc</td>
<td>8.9 ± 0.3a</td>
<td>2.1 ± 0.1b</td>
<td>0.8 ± 0.1a</td>
<td>0.8 ± 0.03a</td>
</tr>
<tr>
<td>HSD</td>
<td>0.14</td>
<td>0.04</td>
<td>0.59</td>
<td>0.07</td>
<td>0.33</td>
<td>0.10</td>
<td>0.12</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data represents means ± SE (n = 5). Different lowercase letters in the same species within a column indicate significant differences (P ≤ 0.05). Chl is represented by a chlorophyll. HSD is honestly a significant difference by post hoc Tukey’s test.
3.2.8.2 Carbohydrate and protein contents

Herein, the application of MgO-NPs through foliar spray significantly enhanced the levels of total soluble carbohydrates and proteins in *N. sativa* across both stages of growth, when compared to the control group (Figure 4). The maximum increases in total soluble carbohydrate contents were about 105% and 64% with 20 ppm MgO-NPs, while the maximum increases in total soluble protein contents were about 106% and 89% with 10 ppm MgO-NPs at stages I and II, respectively, as being compared to that of the controls (Figure 4). The existing results are matched with the studies that investigate the efficacy of spraying *Vigna radiate* and *P. vulgaris* L. on the contents of protein and carbohydrates [65,71]. In the same way, the protein contents in soybeans were significantly increased upon Mg foliar treatment during vegetative growth [72].

The increased level of soluble carbohydrates in the MgO-NPs treated *N. sativa* plant can be due to the vital role of Mg in carbohydrate synthesis and activation of many metabolic enzymes [73]. Interestingly, MgO-NPs may enhance the absorption of essential elements and nutrients, such as nitrogen (N), which is a key component of proteins and a fundamental element in plant metabolism. Enhanced nutrient uptake leads to increased protein synthesis within the plant [74]. Also, the positive impact of Mg ions on the photosynthetic process leads to high production of carbohydrates, as soluble carbohydrates are a product of photosynthesis [75]. The Mg is considered a cofactor for various enzymes responsible for enhancing the metabolic pathways, indirectly affecting proteins and carbohydrates metabolism. The increase in soluble carbohydrates and proteins can be elucidated by the pivotal role of Mg ions in both protein synthesis and the transportation of amino acids [18]. Furthermore, the formation of active ribosome subunits necessitates the presence of Mg to facilitate inter-subunit bridging. Consequently, variations in Mg levels significantly impact protein biosynthesis, consequently altering the concentration of precursor amino acids [18]. Moreover, MgO-NPs could potentially influence the balance of plant hormones, such as cytokinins and auxin, which play vital roles in cell division and differentiation. These hormones can influence protein synthesis and carbohydrate allocation within the plant [76–78]. MgO-NPs have the potential to boost nutrient mineralization and nutrient availability for plants by enhancing positive microbial interactions in the rhizosphere, leading to an increase in the amount of protein and carbohydrates [17].

3.2.8.3 Free proline contents

Figure 5 shows that when MgO-NPs were applied to *N. sativa* plants during either of their two growth phases,
free proline concentrations increased considerably. The maximum increases in the content of free proline were observed regarding the plants treated with 20 ppm MgO-NPs by 67% and 219% at stages I and II, respectively, more than that of the controls. Our results are in agreement with the recent studies about the content of free prolines in yarrow (*Achillea millefolium* L.) and sunflower (*H. annuus* L.) due to treatment with Mg-NPs [62,79].

Furthermore, a study achieved by Howladar et al. [20] showed the impact of foliar application of Mg at concentrations of 0, 0.5, and 1 mM on the growth, yield, and biochemical components of *Pisum sativum* L. The results revealed a significant improvement in the levels of free proline in plants treated with either 0.5 mM or 1 mM Mg through foliar application in comparison to the control. Notably, plants treated with 1 mM Mg exhibited even higher concentrations of free proline than those treated with 0.5 mM Mg. In this aspect, proline has many essential functions: osmoregulation, protection of some enzymes, stabilization of membranes and proteins, the machinery of protein synthesis, regulation of cytosolic acidity, regulation of NAD/NADH ratio, a dip for energy to regulate the redox potential, and scavenging of free radicals (hydroxyl radicals) [80].

### 3.3 Yield characters

The presented results in Table 3 indicate that all yield traits of the *N. sativa* plant were increased as a result of the application of MgO-NPs compared to the control. It was found that the maximum increases in the number of capsules/plants, number of seeds/capsule, seed yield/plant, and weight of 1,000 seeds were achieved with 10 ppm MgO-NPs by 67%, 25%, 52%, and 23%, respectively, more than that of the controls. Our results are also corroborated by Moaveni et al. [81] who studied the influence of MgO-NPs applied topically as a foliar spray at two different doses on sour tea (*Hibiscus sabdariffa* L.) yield characteristics. The authors found that plants given 0.03% MgO-NPs had heavier seeds than the controls. At 0.01% of MgO-NPs, mucilage yield (kg·ha⁻¹) was greatest. Recent research on soybeans and maize also found that MgO-NPs improved yield characteristics [74,82].

Several factors contributed to the increased production of *N. sativa* after foliar treatments of MgO-NPs, including (1) Mg is a crucial element for plants to thrive, and increases their ability to absorb nutrients. It is an essential part of chlorophyll, which is responsible for absorbing light energy during photosynthesis. When applied as a foliar spray, MgO-NPs give plants access to a form of magnesium that is readily absorbed. Magnesium’s greater availability boosts photosynthesis efficiency, which in turn increases biomass production and yield [73]. (2) Mg has a key role in activating enzymes that play important roles in the metabolism of different plants. Mg is a necessary cofactor for enzymes used in glucose metabolism, protein synthesis, and energy transmission. Improved nutrient utilization and plant development are the results of delivering MgO-NPs by foliar spray, which stimulates the production of these enzymes and increases the yield [18]. (3) Mg helps plants absorb light energy and transform it into chemical energy, so more of it can be used in the process of photosynthesis. Increased glucose production and greater yields may derive from the enhanced magnesium availability made possible by MgO-NPs, which can boost photosynthetic efficiency [17]. (4) Mg is involved in the movement of nutrients within the plant. It aids the transport of phosphorus and iron from the plant’s roots to the rest of the plant. Magnesium supplementation has been shown to increase plant health and yield through improving nutrient uptake and transport [75].

### 3.4 Carbohydrate and protein contents in seeds

Data represented in Figure 6 showed that the application of Mg-NPs significantly increased the content of soluble

<table>
<thead>
<tr>
<th>MgO-NPs treatments</th>
<th>No. of capsule/plant</th>
<th>No. of seeds/capsule</th>
<th>Seed yield/plant (g/plant)</th>
<th>Weight of 1,000 seeds (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.0 ± 1.0a</td>
<td>80.7 ± 6.03c</td>
<td>3.3 ± 0.1d</td>
<td>2.1 ± 0.1b</td>
</tr>
<tr>
<td>5 ppm</td>
<td>9.0 ± 1.7a</td>
<td>97.0 ± 2.0ab</td>
<td>4.4 ± 0.2b</td>
<td>2.4 ± 0.1ab</td>
</tr>
<tr>
<td>10 ppm</td>
<td>11.7 ± 2.5a</td>
<td>101.3 ± 3.2a</td>
<td>5.0 ± 0.2a</td>
<td>2.6 ± 0.1a</td>
</tr>
<tr>
<td>20 ppm</td>
<td>7.3 ± 2.1a</td>
<td>87.0 ± 4.4bc</td>
<td>3.8 ± 0.1c</td>
<td>2.2 ± 0.1b</td>
</tr>
<tr>
<td>HSD</td>
<td>2.55</td>
<td>5.56</td>
<td>0.17</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Data represent means ± standard error (n = 5). Different lowercase letters in the same species within a column indicate significant differences (P ≤ 0.05). HSD is honestly a significant difference by post hoc Tukey's test.
carbohydrates and protein in seeds of *N. sativa* compared to the control plant. The maximum increases in the total soluble carbohydrate (52%) and protein (36%) in seeds were obtained at 20 and 10 ppm MgO-NPs, respectively. The existing results are also corroborated by Fatemi et al. [62] on the sunflower plant.

Moreover, many studies show that Mg is an essential macronutrient in the photosynthetic process, as well, an increase in its content increases the yield and quality of crops, in addition to being implicated in carbohydrates and protein synthesis [18]. Recently, Gautam et al. [11] reported that the carbohydrate and protein accumulations in mustard seeds were significantly increased because of MgO-NP application.

### 3.5 Detection of phenolic compounds using HPLC

Herein, eight phenolic acids, three phenolics, and eight flavonoid standards were used for the identification and quantification of phenolic compounds in *N. sativa* before and after MgO-NP treatments. The results showed that sex phenolic acids, two phenols, and five flavonoids were determined in control and treated plants, while methyl gallate, quercetin, and kaempferol were detected only in plants treated with different concentrations of MgO-NPs (Figure 7 and Table 4).

Syringic acid, ferulic acid, and cinnamic acid production were enhanced by the application of 20 ppm MgO-NPs by seven, two, and three folds, respectively compared to control. On the other hand, the production of Chlorogenic acid, Catechol, Daidzein, and Apigenin are negatively affected by treatments with varied MgO-NPs concentrations compared to control.

Interestingly, the antioxidant characteristics of phenolic compounds have been studied extensively, which help protect plants from oxidative stress and damage caused by factors like UV radiation, pathogens, and environmental stressors. In addition to their role in plant defense mechanisms, phenolic compounds also contribute to the color, flavor, and aroma of fruits, vegetables, and beverages such as tea, coffee, and wine.

The possible health benefits of phenolic substances in humans have also attracted interest. Some of these molecules, like the flavonoids and antioxidants found in fruits, vegetables, and seeds, may be beneficial to human health. It is also thought that they may help prevent chronic diseases including heart disease and some forms of cancer. Therefore, the efficacy of MgO-NPs to increase the content of these phenolic compounds in the *N. sativa* plant has a high economic importance.

There appears to be a lack of literature discussing the relationship between MgO-NPs and phenolic chemicals as they pertain to plant biology. Despite the obvious interest in the interactions between MgO-NPs and phenolic compounds, there is no in-depth research or investigations devoted to this topic in the current scientific literature.

Interestingly, data provided in Figure 8 include the content and percentages of various phenolic compounds in different groups (control group and three groups treated with different concentrations of MgO-NPs at 5, 10, and 20 ppm).

The levels of 17 phenolic compounds were measured and found to exhibit variations across the different treatments. This suggests that the application of various MgO-NP concentrations has influenced the production of these phenolic compounds within the plant samples. Some phenolic compounds, such as chlorogenic acid, caffeic acid, and vanillin, consistently displayed increased concentrations in response to MgO-NP treatment. The highest concentration was often observed in the group treated with 10 ppm MgO-NPs. However, the effects of MgO-NPs on...
Figure 7: HPLC for detection of phenolic compounds in *Nigella sativa* seeds at different treatments. (a) Control, (b) plant treated with 5 ppm of MgO-NPs, (c) plant treated with 10 ppm of MgO-NPs, and (d) plant treated with 20 ppm of MgO-NPs.
Table 4: Effects of MgO-NPs on phenolic compounds on *Nigella sativa* seeds based on HPLC analysis

<table>
<thead>
<tr>
<th>Phenolic compounds (µg·g⁻¹)</th>
<th>Control</th>
<th>5 ppm</th>
<th>10 ppm</th>
<th>20 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (mAU*s)</td>
<td>Conc. (µg·g⁻¹)</td>
<td>Area (mAU*s)</td>
<td>Conc. (µg·g⁻¹)</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>132.8</td>
<td>514.28</td>
<td>221.3</td>
<td>830.78</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>193.7</td>
<td>1,189.61</td>
<td>160.2</td>
<td>953.89</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>727.6</td>
<td>2,508.97</td>
<td>928.9</td>
<td>3,105.75</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>8.6</td>
<td>26.14</td>
<td>23.5</td>
<td>69.29</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>2.5</td>
<td>7.55</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>11.6</td>
<td>9.55</td>
<td>17.2</td>
<td>13.76</td>
</tr>
<tr>
<td>Methyl gallate</td>
<td>498.5</td>
<td>3,216.57</td>
<td>403.2</td>
<td>2,522.48</td>
</tr>
<tr>
<td>Vanillin</td>
<td>16.6</td>
<td>32.58</td>
<td>19.3</td>
<td>36.63</td>
</tr>
<tr>
<td>Catechin</td>
<td>170.6</td>
<td>1,894.47</td>
<td>194.3</td>
<td>2,092.17</td>
</tr>
<tr>
<td>Rutin</td>
<td>60.8</td>
<td>316.48</td>
<td>86.5</td>
<td>436.71</td>
</tr>
<tr>
<td>Naringenin</td>
<td>13.03</td>
<td>70.44</td>
<td>15.5</td>
<td>81.05</td>
</tr>
<tr>
<td>Daidzein</td>
<td>38.2</td>
<td>105.83</td>
<td>15.1</td>
<td>40.57</td>
</tr>
<tr>
<td>Quercetin</td>
<td>n.d.</td>
<td>n.d.</td>
<td>10.4</td>
<td>62.26</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>n.d.</td>
<td>0.00</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>23.3</td>
<td>60.91</td>
<td>190.2</td>
<td>482.07</td>
</tr>
</tbody>
</table>

n.d., not detected.

Figure 8: Percentages of phenolic compounds based on HPLC analysis in the presence and absence of MgO-NPs treatments (5, 10, and 20 ppm).
phenolic compounds were diverse. For example, the contents of gallic acid and chlorogenic acid exhibited mixed responses across different MgO-NP treatments, with both increases and decreases observed.

Among the phenolic compounds, gallic acid, chlorogenic acid, caffeic acid, catechol, and catechin had contents exceeding 5%. Caffeic acid displayed the highest content, ranging from 25% to 32%, followed by catechol, which ranged from 23% to 32%, and then catechin, with content ranging from 12% to 19%.

A two-way cluster analysis (Figure 9) revealed that the different treatments could be grouped into two distinct clusters. The first cluster included the control group of untreated plants and those treated with 20 ppm of MgO-NPs, while the second cluster encompassed plants treated with 5 and 10 ppm. This clustering was likely due to the similar phenolic compound content observed between the control and 20 ppm treatment groups. Specifically, gallic acid, chlorogenic acid, caffeic acid, ferulic acid, methyl gallate, catechol, and quercetin exhibited comparable concentrations in these two groups, explaining their grouping together.

4 Conclusions

MgO-NPs were successfully developed from the endophytic fungus *P. crustosum* EP-1 as an environmentally friendly method. These NPs exhibited several noteworthy characteristics, including a spherical, well-arranged structure, a crystalline nature, uniform dispersion in a colloidal solution, and a size range of 8–35 nm. The MgO-NPs were composed of 42.44% magnesium and 30.13% oxygen as shown by EDX analysis and have a negative surface charge with a value of −29.7 mV detected by zeta potential analysis. In a field experiment, the foliar application of MgO-NPs at varying concentrations significantly enhanced the growth parameters, physiological and metabolic activities, yield, and phenolic components of *N. sativa* L. throughout two growth stages. Notably, HPLC analysis revealed an increased presence of six phenolic acids, two phenols, and five flavonoids in seeds subjected to MgO-NPs treatment, with higher concentrations as being compared to that of the control plant. In conclusion, the foliar application of biosynthesized MgO-NPs positively impacted the growth and phenolic content of *N. sativa* plants, offering a likely strategy to improve seed yield and quality.

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Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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