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Effect of Green Synthesized Zinc Oxide Nanoparticles on the Physiological Parameters and Gum Production of the Saravan Landrace of Guar (*Cyamopsis tetragonoloba* L.)

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Abstract

Introduction: The plant species *Cyamopsis tetragonoloba* L. (Fabaceae) is a source of guar gum that has a variety of applications in the pharmaceutical industry because of its therapeutic properties. Other parts of the plant are also used as food and fodder. This study was conducted to evaluate some physiological changes and gum production of guar in response to foliar spraying with synthesized zinc oxide nanoparticles (ZnO-NPs).

Methods: ZnO-NPs were synthesized using guar gum by the co-precipitation method. The properties of the ZnO-NPs were investigated using various analytical techniques, including ultraviolet-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), transmission electron microscope (TEM), energy dispersive X-ray (EDX), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The nanoparticles were sprayed on the leaves of the Saravan landrace of guar to study their effects on the physiological properties of the plant. The experiments were performed in a completely randomized design with three replications.

Results: Treatment of plants with ZnO-NPs resulted in an improvement of some physiological properties. After exposure to 500 mg/L of ZnO-NPs, the contents of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, gum, and soluble protein were increased by 47.88%, 78.43%, 54.37T, 29.41%, 55.08%, and 52.12%, respectively. Additionally, the amount of Zn and insoluble sugars showed the highest increase, by 3.96 and 4.31 times, respectively. In contrast, soluble sugars content as well as the activity of catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) reduced 0.69-, 2.23-, 1.60-, and 3.91-fold, respectively.

Conclusion: ZnO-NPs improved the physiological properties of guar at different concentrations, with the best effects obtained at the maximum concentration of 500 mg/L. The results of this study suggest that the ZnO-NPs synthesized by the biocompatible process are appropriate candidates to prepare guar gum to be used in pharmaceutical and therapeutic-based approaches.

Keywords: Ascorbate peroxidase, Chlorophyll, *Cyamopsis tetragonoloba*, Guar gum, Nanoparticles, Zinc oxide

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Introduction

Cyamopsis tetragonoloba L., commonly known as cluster bean or guar, is the source of guar gum, one of the major naturally occurring plant polysaccharides widely used in various industries.[1] It has a broad application as gelling/viscosity agent in the food, textile, cosmetic, and pharmaceutical industries.[2] It also has therapeutic properties such as antidiabetic, anti-proliferative, anti-inflammatory, and antimicrobial effects as well as applications in the control of bowel movements, colon cancer, hypolipidemic, hypoglycemic, and heart disease.[2,3] Guar gum has been reported to be helpful in the treatment of SARS-COV-2 infections.[4] Various parts of guar plant, including the seeds and green pods, are used as food and fodder.[5]

Gums in general are mainly polysaccharides found in various parts of different plant species and are potent candidates for green synthesis and stabilization of nanoparticles such as

zinc oxide nanoparticles (ZnO-NPs).[6] Although ZnO-NPs can be synthesized by many different techniques; biological methods are relatively fast, inexpensive, and environmentally friendly.[7] Plant materials containing reducing agents such as polysaccharides, phenolics, flavonoids, and terpenoids are more suitable for the synthesis of nanoparticles.[8]

Zinc (Zn) is an essential element for many structural and functional proteins in living cells.[9] It plays a vital role not only as a cofactor for many enzymes, but also in the protection and resistance of the structural integrity of cell membrane.[10] The effect of Zn on plants depends on the application form, i.e., ionic, bulk, or nanoscale; the nanoscale form is used in this study.[11] Half of the world's agricultural soils are severely zinc deficient, and zinc deficiency poses a risk to human health. Nearly one-third of the world's population consumes Zn improperly. To solve this problem, adding zinc to agricultural products can help protect human health. ZnO-NPs can be applied either to the soil or directly to the leaf surface.[12] The foliar spray used here is a successful and efficient low-cost nutrition method that increases yield even in deficient soils.[13] Direct application of ZnO-NPs to the soil may reduce the mobility of nanoparticles by binding to soil colloids.[14] In the present work, green synthesis of ZnO-NPs was carried out using guar gum to study its effect at different concentrations on physiological parameters of a landrace of guar from Saravan.

Material and Methods

Preparation of Guar Gum

Seeds of a local guar landrace, namely Saravan, were provided by the Agricultural Research Center, Iranshahr, Sistan and Baluchestan province, Iran. The taxonomic validation of this landrace was previously performed.[15] Seeds were treated with warm water and rubbed manually to separate endosperm from the husk. The endosperm was air-dried and pulverized in a grinder to obtain a pale-yellow powder.

Synthesis of ZnO-NPs by Co-precipitation Procedure

The green synthesis of ZnO-NPs was carried out by the co-precipitation method with a few modifications to the procedure.[16] 0.3 g guar gum was added to 40 mL of deionized water and mixed for 90 minutes at 60°C. Meanwhile, 4.5 g of Zn $(NO_3)_2.4H_2O$ was added to 15 mL of deionized water and stirred for 10 minutes. The zinc nitrate solvent was mixed with the guar gum solution for 2 hours at 80°C. Then, 10 mL NaOH (2 M) was added drop-wise to the mixture until pH 12 was reached, a reflux tube was used to avoid evaporation. After 8 hours, the attained white solution was centrifuged for 15 minutes at 3000 rpm. The supernatant was discarded and the precipitate was eluted three times with 96% ethanol. It was transferred to the oven at 100°C for 12 hours before it was calcined at 600°C for 1 hour to form ZnO-NPs.

Characterization of ZnO-NPs

After dispersing the synthesized ZnO-NPs in ethanol using a sonicator, their absorbance was measured at 300-600 nm by an ultraviolet-visible (UV-Vis) spectrophotometer (NACH DR 5000). The morphology, and size of nanoparticles was examined after gold coating procedure with a field emission scanning electron microscope (FE-SEM, TESCAN MIRA3 XMU) at a voltage of 15 kV. In addition, the size and shape of the nanoparticles were further analyzed using a transmission electron microscope (TEM, Zeiss-EM10C-100kV). Energy dispersive X-ray system (EDX) attached to SEM was used for chemical study of ZnO-NPs. Fourier transform infrared spectroscopy (FTIR, Bruker Tensor II) was recorded in the area of 400-4000 cm^{-1}. X-Ray diffraction (XRD, D8 Advance Bruker AXS) was performed at a voltage of 40 kV, a current of 30 mA with Cu-Kα radiation (λ = 0.15406 nm).

Culture of Plants

The seeds were sown in a clay loamy soil containing 30% vermicompost in the green-house of Sistan and Baluchestan University, Zahedan, Iran. During the treatment period, the greenhouse temperature was between 25 ± 2°C and the humidity was 30-35%. Drip irrigation was done twice a week.

Exposure to ZnO-NPs

Different concentrations (0, 25, 50, 100, 200, and 500 mg/L) of the synthesized ZnO-NPs were provided. In order to prevent NP aggregation, the suspensions were sonicated for 45 minutes. Foliar spray was performed twice, 20 and 27 days after planting. Experiments were performed in a completely randomized experimental design with three replicates.

Assessing the Concentration of Zn

The harvest was carried out 34 days after sowing at the vegetative stage to evaluate physiological traits. Zinc content in leaf tissues was measured using a method previously reported by Niesiobedzka.[17] 0.5 g dried leaves of guar digested with 5 mL mixture of perchloric acid and nitric acid 1:3 on a heater. Fully digested samples were suspended in 2.5 mL of 10% hydrochloric acid in a final volume of 10 mL and assayed by means of an atomic absorption spectrophotometer (Analytik Jena - novAA 400 P).

Photosynthetic Pigments Assay

Measurements of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in leaves performed using the method explained in Ref.[18] 0.01 g of fresh leaves was homogenized in a mortar containing 10 mL of ethanol

95%. The absorption of the solutions was then measured by a JENWAY 6305 spectrophotometer to evaluate the quantity of chlorophyll a, chlorophyll b, and carotenoids at wavelengths of 645, 663 and 470, respectively. The amounts of the pigments were computed using the following formulas:

$C_a = 13.36A_{663} - 5.19A_{645}$, $C_b = 27.43A_{645} - 8.12A_{663}$, $C_{a+b} = 5.24A_{663} + 22.24A_{645}$, $C_{x+c} = 1000A_{470} - 2.13C_a - 97.64C_b/209$

where (*C*a), (*C*b), (*C*a+b), and (*C*x+c), represent chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid, respectively and "A" represents the absorbance of the samples.

Measurement of Soluble and Insoluble Sugars

Measurement of the sugars was accomplished on the basis of the phenol-sulfuric acid method.[19] 5 mL of ethanol 70% was added to 50 mg of dry material and incubated at 4°C for one week. The extracts were centrifuged at 5000 g for 30 minutes. Supernatants were utilized to measure soluble sugars and residual sediments to quantify insoluble sugars. 500 µL of supernatant was mixed with 1 mL of phenol 5% and 5 mL of H_2SO_4 96%, stirred rapidly and was shaken after 30 minutes. The sediment was boiled in 10 mL distilled water for 15 minutes, filtered, and mixed with phenol and H_2SO_4. The absorption of each solution was measured at 485 nm. The sugar content was expressed using a glucose standard curve and reported in mg per gram dry weight.

Estimation of Polysaccharides in Gum

0.1 g of guar gum was shaken in 5 mL ethanol and heated for 15 minutes to remove monosaccharides and oligosaccharides.[20] This process was repeated after centrifugation and removal of supernatant. The acquired precipitate was mixed with 10 mL of acidified distilled water with HCl (pH = 3.5) and heated at 96°C for 10 minutes. The phenol-sulfuric acid method was applied to assess the amount of gum polysaccharide content in the solution.[19]

Protein Content and Antioxidant Enzyme Assay

0.1 g of samples was homogenized in ice-cold phosphate-buffered solution (50 mM, pH = 7). The mixture was centrifuged at 5000 g for 20 minutes at 4°C. The protein concentration in the supernatant (leaf extract) was determined using bovine serum albumin as a standard according to Bradford's study. [21]

Catalase (CAT) activity was determined based on the protocol suggested in Chance and Maehly's study.[22]

The CAT activity was calculated using the decomposition of H_2O_2 by reducing the absorption at 240 nm (extinction coefficient, $\varepsilon = 40 mM^{-1} cm^{-1}$). The sampling mixture (1.5 mL) included 975 µL of 50 mM phosphate buffer (pH 7.0), 500 µL of leaf extract and 500 µL of 200 mM H_2O_2. CAT activity was expressed as µmol H_2O_2 decomposed min^{-1} per mg of protein. Peroxidase (POD) activity was assayed using the oxidation of guaiacol and increasing the absorption at 470 nm (extinction coefficient, $\varepsilon = 25.5 mM^{-1} cm^{-1}$). The extraction mixture (1 mL) contained 350 µL of 10 mM phosphate buffer (pH 7.0), 300 µL of guaiacol (4 mM), 50 µL of leaf extract and 300 µL of 5 mM H_2O_2 for 1 min [22]. POD activity was expressed as the enzyme quantity required for the oxidation of 1 µM of guaiacol to tetragaiacol min^{-1} mg^{-1} protein.

To evaluate ascorbate peroxidase (APX), the oxidation of ascorbic acid was assessed by decreasing the absorbance at 290 nm (extinction coefficient, $\varepsilon = 2.8 mM^{-1} cm^{-1}$) based on the protocol suggested in Ref. [23] The sampling mixture (1 mL) consisted of 300 µL of 50 mM phosphate buffer (pH 7.0), 200 µL of 2 mM EDTA, 200 µL of 5 mM ascorbic acid, 200 µL of 50 mM bovine serum albumin, 50 µL of leaf extract, and 50 µL of 1 mM H_2O_2. APX enzyme-specific activity was stated as µmol ascorbic acid oxidized min^{-1} mg^{-1} protein.

Statistical Analysis

All data were submitted to statistical analysis using SPSS software and the results were presented as the mean of three replicates ± SE (standard error). The significance of differences between treatments was evaluated by one-way ANOVA ($P < 0.05$) followed by Duncan's multiple range test.

Results

Characteristics of ZnO-NPs

UV-Vis Spectrum

The UV-Vis absorption spectrum of the green synthesized ZnO-NPs in water is shown in Figure 1. The maximum absorption appeared at 375 nm, which can be ascribed to the intrinsic band-gap absorption of ZnO owing to the electron transfer from the valence to the conduction band.[24]

Morphologic Properties of ZnO-NPs and EDX Analysis

A hexagonal morphology for the synthesized ZnO-NPs (Figure 2A) was observed in FE-SEM and confirmed by TEM analysis (Figure 2b). According to the histogram of particle size distribution of ZnO obtained from FE-SEM data, the average diameter of nanoparticles was ~40 nm (Figure 2C). EDX spectra of ZnO-NPs revealed correct elemental composition and stoichiometry (Figure 2D). The obtained peaks clearly indicated presence of oxygen and Zn elements.

FTIR analysis

FTIR spectrum showed the peak of Zn-O around 409-544 cm^{-1} (Figure 3). The peak for OH stretching was observed at 3373.35 cm^{-1}.[25]

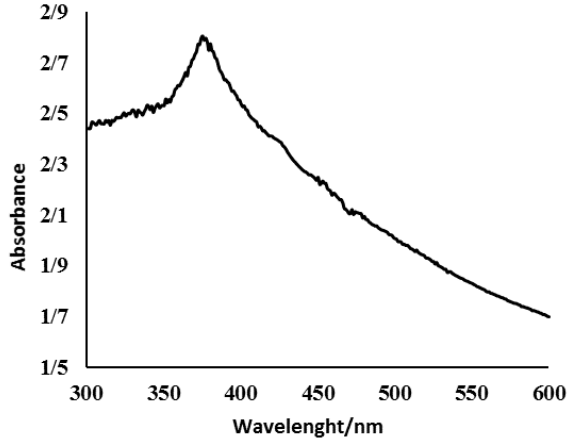

Figure 1. UV-Visible Spectrum of Synthesized ZnO-NPs.

XRD Analysis

XRD analysis of the ZnO-NPs revealed an average size of 40 nm. For the nanoparticles, estimated according to Scherrer's formula. ($D = k\lambda/\beta\cos\theta$) (Figure 4), where D is the average size of ZnO-NPs, K is the Scherrer constant around 0.9, λ is the X- rays' s wavelength of 1.54 Å, β is the peak width at half maximum (FWHM), and θ is the Bragg diffraction angle. The diffraction peaks at 2θ of 31.77°, 34.40°, 36.26°, 47.48°, 56.59°, 62.85°, 66.37°, 67.93°, 69.04°, 72.53° and 76.87°, were referred to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202), respectively. The diffraction peaks of the pattern correspond to the characteristic hexagonal phase (wurtzite structure) of ZnO-NPs. The sharp diffraction peaks show that the ZnO-NPs have a crystalline structure.[26]

Effect of ZnO-NPs on Guar Plant

Effect of ZnO-NPs on Zn Concentration in the Leaf

According to the data of Table 1, an increase in the concentration of ZnO-NPs is accompanied by an increase in the Zn content in guar leaves. The highest Zn concentration was observed in the plants treated with 500 mg L^{-1} ZnO-NPs (114.16 µg g^{-1} FW), and the lowest amount was in the control group (28.81 µg g^{-1} FW).

Influence of ZnO-NPs on Chlorophyll and Carotenoid Content

Increasing the concentration of ZnO-NPs increased the level of chlorophyll a, chlorophyll b, Total Chlorophyll,

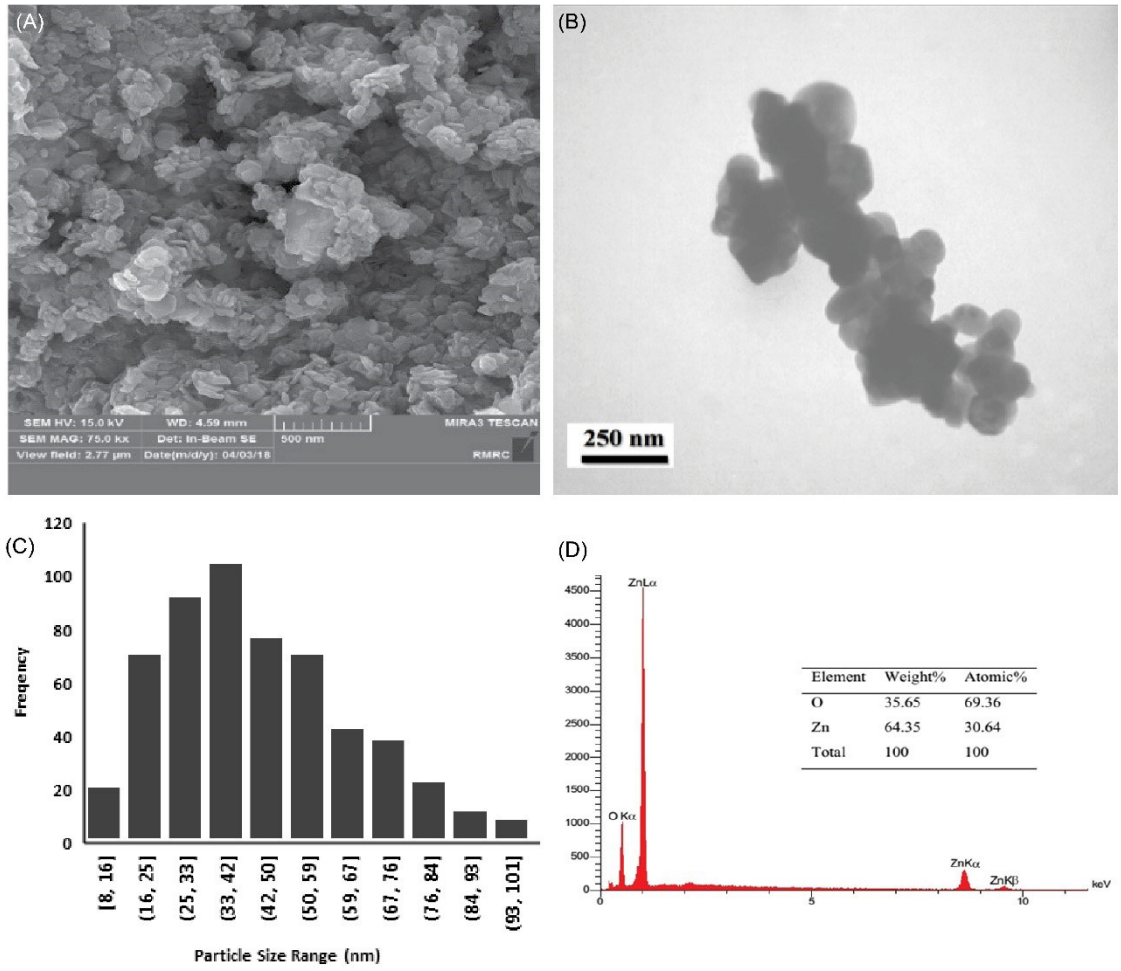

Figure 2. **(a)** FE-SEM micrographic image of the synthesized ZnO-NPs, **(b)** TEM micrographic image of ZnO-NPs, **(c)** Particle size distribution of ZnO-NPs, **(d)** EDX spectroscopy of the ZnO-NPs, inset: atomic composition of the elements in percent.

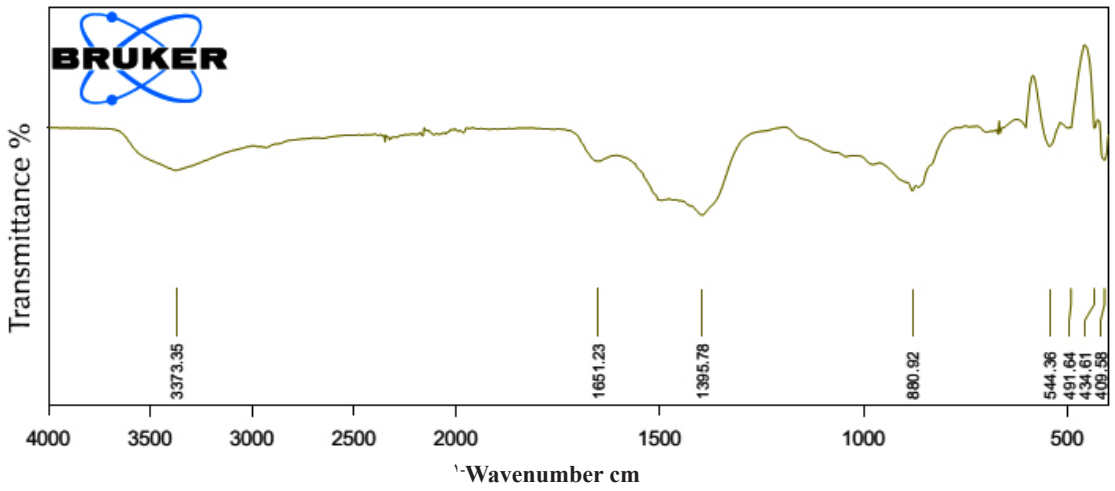

Figure 3. FTIR spectrum of ZnO-NPs.

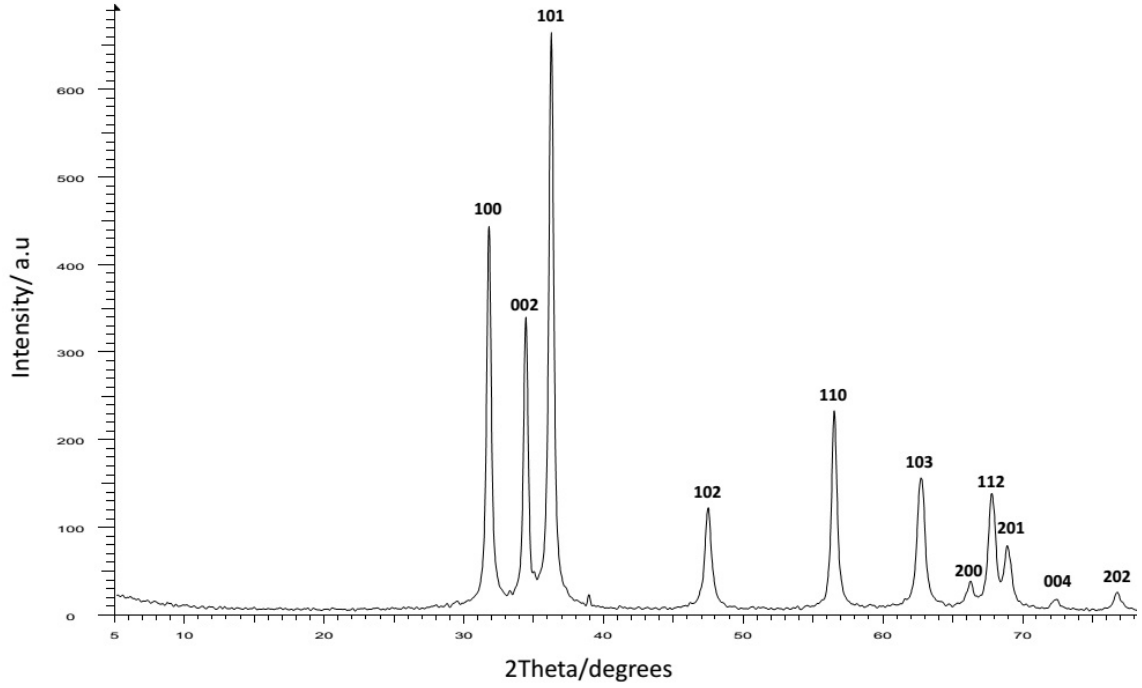

Figure 4. XRD spectrum of ZnO-NPs.

and carotenoids by 47.88%, 78.43%, 54.37%, and 29.41%, respectively, in leaves of guar compared to control. The highest pigment values were obtained with 500 mg L^{-1} ZnO-NPs (Table 1).

Sugar Content

The results obtained for soluble and insoluble sugars are shown in Table 2. The data showed that the soluble sugar content slightly decreased with increasing concentration of ZnO-NPs and reached its lowest value at the highest concentration of ZnO-NPs (500 mg L^{-1}) after a decrease of 30.57%. In contrast, the increase in the concentration of the nanoparticles was accompanied by an increase by 4.31 folds in insoluble sugar content at the maximum concentration of ZnO-NPs.

Gum

According to Table 2, the production of gum was improved by increasing the concentration of nanoparticles. The highest amount of gum was obtained at a concentration of 500 mg L^{-1} of ZnO-NPs, 1.55 times higher than the control sample.

Protein Content

The content of soluble proteins increased after treatment with ZnO-NPs (Table 2). The maximum concentration of ZnO-NPs (500 mg L^{-1}) increased the soluble protein content by 52.12% compared to the control.

Antioxidant Enzymes (CAT, POX, APX)

The activity of the studied antioxidant enzymes

Table 1. Effect of ZnO-NPs Treatments on Zn Concentration and Photosynthetic Pigments of Saravan landrace of Guar

Concentration of ZnO NPs (mg L^{-1})	Zn Concentration in Leaves (µg g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
0	28.81 ± 1.70 d	2.13 ± 0.05 c	0.51 ± 0.05 c	2.63 ± 0.10 c	0.68 ± 0.04 cd
25	33.26 ± 7.53 d	2.20 ± 0.17 bc	0.49 ± 0.04 c	2.69 ± 0.20 c	0.64 ± 0.03 d
50	57.28 ± 2.35 c	2.34 ± 0.14 bc	0.56 ± 0.02 c	2.89 ± 0.16 bc	0.73 ± 0.02 c
100	68.02 ± 5.85 c	2.47 ± 0.20 bc	0.68 ± 0.02 b	3.15 ± 0.22 bc	0.73 ± 0.02 c
200	84.99 ± 0.63 b	2.67 ± 0.16 b	0.72 ± 0.06 b	3.39 ± 0.22 a	0.80 ± 0.01 b
500	114.16 ± 0.41 a	3.15 ± 0.09 a	0.91 ± 0.04 a	4.06 ± 0.11 a	0.88 ± 0.02 a

Data represent means ± SE of replications. Similar lower letters indicate that there is no significant difference at $P < 0.05$.

Table 2. Effect of ZnO-NPs Treatments on Soluble Sugars, Insoluble Sugars, Gum, Soluble Protein, and Antioxidant Enzymes on Saravan landrace of Guar

Concentration of ZnO-NPs (mg L-1)	Soluble Sugar (mg g-1 DW)	Insoluble Sugar (mg g-1 DW)	Gum (mg g-1 DW)	Soluble Protein (mg g-1 FW)	CAT (Umg-1 Protein)	POX (Umg-1 Protein)	APX (Umg-1 Protein)
0	1.57 ± 0.29 a	0.132 ± 0.008 f	4.23 ± 0.08 c	1.88 ± 0.14 b	1.03 ± 0.03 a	7.37 ± 0.20 a	7.17 ± 0.55 a
25	1.44 ± 0.02 ab	0.230 ± 0.024 e	4.35 ± 0.17 c	2.28 ± 0.23 ab	0.75 ± 0.02 b	7.11 ± 0.87 a	6.19 ± 0.18 ab
50	1.29 ± 0.01 ab	0.329 ± 0.007 d	5.04 ± 0.29 b	2.63 ± 0.07 a	0.74 ± 0.02 b	5.10 ± 0.54 b	5.46 ± 0.33 b
100	1.17 ± 0.05 ab	0.451 ± 0.023 c	5.27 ± 0.21 b	2.73 ± 0.28 a	0.68 ± 0.03 bc	4.50 ± 0.41 b	1.32 ± 0.48 c
200	1.19 ± 0.06 ab	0.523 ± 0.005 b	6.19 ± 0.18 a	2.86 ± 0.19 a	0.60 ± 0.06 c	4.54 ± 0.42 b	1.49 ± 0.14 c
500	1.09 ± 0.01 b	0.570 ± 0.011 a	6.56 ± 0.16 a	2.86 ± 0.07 a	0.46 ± 0.01 d	4.58 ± 0.41 b	1.83 ± 0.48 c

Data represent means ± SE of replications. Similar lower letters indicate that there is no significant difference at $P < 0.05$.

significantly decreased in the treated plants compared to the control group (Table 2). After exposure to 500 mg L^{-1} concentration of ZnO-NPs, the activity of CAT, POX, and APX was reduced 2.23-, 1.60-, and 3.91-fold, respectively, compared with the control group.

Discussion

In this study, guar gum was used for the first time in the co-precipitation process for the synthesis of ZnO-NPs. The plant materials such as gums, can be used as reducing, stabilizing and biocompatible agents which makes them suitable for the synthesis of nanoparticles. Thus, in the current work the guar gum was used for the green synthesis of ZnO-NPs.[27] Various studies have used nanoparticles of different sizes as fertilizers or pesticides. In foliar spraying, the size of nanoparticles is inversely related to their penetration.[28] Nanoparticles with a size of 200 to 300 nm,[29] even up to an average of 500 nm have been shown to penetrate the leaves of various plants.[30] Therefore, we used nanoparticles with a size of 40 nm to ensure their successful penetration into leaves.

Because leaves are the main organs for photosynthesis and carbohydrate production, the main part of examinations were focused on their features. The seeds were also assayed as an important part of plant in gum production.

It is the first study of its kind to report on the effects of ZnO-NPs on the landrace Saravan. According to the obtained results, ZnO-NPs have a positive influence on the Zn concentration of the leaf and also on the yield of the guar plant. This observation reveals the possible absorption of zinc by the tissue of the guar leaves. Similar results have been reported in some previous studies. For example, in *Vigna radiata*, application of zinc in the root environment increased the zinc content of the plant.[31]

Exposure to ZnO-NPs resulted in an increase in photosynthetic pigment contents. In a previous study on wheat, ZnO-NPs and Fe-NPs significantly increased photosynthetic pigment content (chlorophyll a, chlorophyll b, carotenoids), and biomass.[32] Zinc increases chlorophyll content because it not only acts as a structural component, but also participates in the chemical catalysis of enzymes. In addition, zinc is a cofactor for the progress of pigment biosynthesis.[31] Overall, exposure of guar to ZnO-NPs has a positive effect on photosynthetic pigment content.

The content of soluble sugar in guar leaves decreased with increasing ZnO-NPs concentration and reached its highest value at the maximum ZnO-NPs concentration (500 mg/L), while the content of insoluble sugar tended to enhance with increasing ZnO-NPs concentration. In fair agreement with our results, exposure of winter wheat to supporting nanomaterials boosted grain yield and protein content and reduced the amount of soluble sugar.[33] Similarly, in willow plant, treatment with Zn significantly increased the amount of carbohydrates.[34] It is known that several Zn-dependent enzymes in leaves are responsible for the metabolism of carbohydrate.[35] Zn is also necessary for several photosynthetic reactions.[36] One of the functions of Zn in promoting sugars may be related to its role in maintaining cell membrane stability, which is due to NADPH-dependent oxygen production.[37] In our study, increasing the concentration of ZnO-NPs elevated the amount of insoluble carbohydrates in the leaves. The

simultaneous decrease in the amounts of soluble sugars confirmed their consumption in metabolic pathways to produce different classes of polysaccharides (insoluble sugars).

The content of guar gum improved with the increase of the ZnO-NPs concentration. There are few studies on the effects of zinc on the gums of various plants. In a previous study on the guar plant, increasing Zn in soil from 0 to 10 kg ha^{-1} increased gum content from 25.52% to 31.37%.[38] In another similar study, application of ZnO-NPs at a concentration of 10 mg L^{-1} to guar leaf resulted in a 7.5% increase in gum content.[39] Zinc affects the polysaccharide content of seeds by improving photosynthetic metabolism, which increases the ability of seeds to produce sugars. In addition, zinc can increase nutrient uptake by elongating the roots, which directly increases the accumulation of seed carbohydrates.

The level of soluble proteins increased after ZnO-NPs treatments. Zinc is not only involved in the regulation of gene expression, but is also a cofactor for RNA polymerase and protein synthesis. Thus, it is conceivable that its concentration affects protein biosynthesis. Alternatively, the reason for the protein increase may be related to the role of zinc in gene expression, activity of enzymes and factors required for protein synthesis. A similar pattern was observed in previous studies. In *Cicer arietinum*, a significant increase in protein content was indicated after exposure to 100 mg L-1 ZnO-NPs.[39] Consistent with these findings, the amount of protein synthesis in bean was drastically reduced under zinc deficiency. Furthermore, structural damage to ribosomes and RNA molecules was observed under zinc deficiency, confirming the crucial effect of zinc on protein biosynthesis.[40]

The activity of the antioxidant enzymes, significantly reduced in the treated plants. The results were consistent with previous findings showing a reduction in the activity of antioxidant enzymes after zinc treatment, possibly due to reducing stress conditions.[40] Similarly, the activity of antioxidant enzymes in tangerine was decreased at the optimal concentration of zinc.[41] It should be noted that plants use antioxidant enzymes to overcome the damage caused by reactive oxygen species (ROS). Therefore, one may conclude that the concentrations of ZnO-NPs used did not induce ROS production in the treated plants. In this study, the effects of ZnO-NPs up to 500 mg L^{-1} were investigated. The physiological behavior of guar at the higher concentrations is unclear. Assessing the higher concentrations and study of their physiological impacts may be regarded as the goals of further researches.

Conclusion

In this study, ZnO-NPs with an average size of 40 nm were green-synthesized using guar gum. Determination of their characteristics using UV-Vis, SEM, TEM, EDX, FTIR, and XRD showed that they are suitable candidates for physiological applications. Subsequently, the effects of the synthesized ZnO-NPs on Saravan landrace of guar were studied. The ZnO-NPs were sprayed on the leaves of guar at concentrations of 0, 25, 50, 100, 200 and 500 mg L^{-1}. Application of ZnO-NPs increased Zn concentration and the content of photosynthetic pigments of guar plant in a concentration dependent pattern. Accordingly, after exposure to 500 mg L^{-1} of ZnO-NPs, the gum production was increased by 55.08%. Concurrently, the amounts of soluble proteins and insoluble sugars increased and the amount of soluble sugars dropped. The activity of antioxidant enzymes gradually decreased showing that the applied concentrations of ZnO-NPs did not induce a significant oxidative stress. The results suggest that ZnO-NPs synthesized with the bio-friendly procedure are suitable candidates for improving physiological properties, and guar gum for use in pharmaceutical, and therapeutic approaches.

Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Competing Interest

The authors declare that there are no competing interests.

Funding/Support

Not applicable.

Authors' Contributions

SB: Conduction of the experiments, data analysis, preparing the original draft of manuscript. JV: Conceptualization. MV: Consultation. AM: Research supervision, preparing the manuscript.

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