Egyptian Journal of Botany

Zinc Oxide Nanoparticles Induce Changes in the Antioxidant Systems and Macromolecules in the *Solanum nigrum* Callus

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THIS STUDY aimed to explore the effects of zinc oxide nanoparticles (ZnO NPs) on physiological parameters of *Solanum nigrum* calli and the possibility of using it as a remediator for contaminated media. *In vitro* experiments were conducted to understand the mechanism of *S. nigrum* in the remediation of ZnO NPs (0, 50 and 100 mg L^{-1} used). The dry weight of calli subjected to the lowest concentration of ZnO NPs was increased (1.8 fold higher than the control). The activities of lipoxygenase and antioxidant enzymes in the callus were stimulated at the highest level of ZnO NPs. The treatment of ZnO NPs did not change the activity of phenylalanine ammonia-lyase and phenolic compounds while reducing the activity of polyphenol oxidase. The contents of phosphorus and potassium were decreased under ZnO NPs treatments. Amino acids, soluble proteins, soluble carbohydrates, and Zn content were elevated in the ZnO NPs-treated-callus. The infrared spectroscopy analysis proved the differences between most macromolecules. The results indicate that this plant can be used in the remediation of ZnO NPs in the contaminated media.

Keywords: Antioxidant system, Macromolecules, Nanoparticles, Phytoremediation, *Solanum nigrum*, Zinc oxide.

Introduction

Nanoparticles (NPs) from natural and anthropogenic sources can appear in the soil (Morales-Díaz et al., 2017; Kumar et al., 2019). Nowadays, NPs have been widely used in various products and industries, however, their release into the environment threatens living organisms (Zafar et al., 2016).

Entry and toxicity concerning NPs to plants rely on the chemical construction and size that impacts the ligand-receptor interactions (Raliya et al., 2016). ZnO NPs are significantly used in various products as well as in the agriculture and food sector, like pesticides, fungicides, and fertilizers (Ghosh et al., 2016 ; Housseiny & Gomaa, 2019). The toxic impact of NPs has been reported as a result of size, morphology, nature, composition, reactivity, and other NPs capacities (Zaka et al.,

2016). Subsequently, phytotoxicity mechanisms regarding NPs ought to be quite realized earlier than application in the fields. The utmost ordinarily applied nanoparticles in a diversity of usage are ZnO NPs (Ju-Nam & Lead, 2008) that elevated the possibility of direct release to ecosystems, leading to an ecological risk of ZnO NPs. It was reported that ZnO NPs reduced chlorophylls and photosynthetic capacity, whereas it stimulated the transcription of genes linked to the antioxidant capacity in tomato plants (Wang et al., 2018). Furthermore, in *Allium cepa* ZnO NP toxicity induced fragmentation to DNA (Ghosh et al., 2016).

Phytoremediation using the outstanding ability of plants to uptake heavy metals and compounds from environments and metabolize these diverse molecules between their tissues appears promising to decontaminate the polluted-environments (Garbisu & Alkorta, 2003). The

use of vegetation to remove pollution from the surrounding has attracted a lot of attention as a low-expense and intimately to reclaim the contaminated environment (Pilon-Smits, 2005). The efficiency phytoremediation depends on the valid chosen of domestic plant varieties, typically with hyperaccumulators, which may enlarge on the soil of marginal finesse like richness, generation, construction of soils and metals existing (Mkumbo et al., 2012).

Solanum nigrum L. is a widespread and comparatively rapid-growing herb located in many tree regions and polluted environments (Särkinen et al., 2018). Furthermore, it is utilized as a medicinal plant and food in some countries (Rehman et al., 2017). In addition, it has been stated that it has the ability to accumulate heavy metals like Cd, Zn, and Cu in its tissues (Marques et al., 2006; Abdel-Wahab et al., 2019). Nonetheless, there is a lack of data about the efficiency of *S. nigrum* for the phytoremediation regarding ZnO NPs-contaminated surroundings and mechanisms concerned the toleration.

To study the efficacy of *S. nigrum* L. in tolerating ZnO NPs, *in vitro S. nigrum* callus was used. The effect of ZnO NPs on growth, antioxidative responses, accumulation of certain minerals, and Fourier transform infrared spectroscopy (FT-IR) functional groups were assessed. The quantification of Zn accumulation was also performed to affirm if this plant can be used for the phytoremediation of ZnO NPs.

Materials and Methods

Preparation of ZnO NPs solution

ZnO NPs (< 100 nm, 15–25m² g⁻¹ surface area and 99.5% purity; Sigma-Aldrich) were dissolved in distilled H_2O and sonicated (100W, 40 kHz) for 30min.

Plant tissue culture

The culture medium composed of 4.4g/L MS medium (Murashige & Skoog, 1962), 3% sucrose, 1mg/L α-naphthalene acetic acid (NAA), different concentrations of the earlier prepared ZnO NPs (0, 50 and 100mg L⁻¹, which were selected from preliminary experiments) and 0.3% gelrite that was added after adjusting the pH of the medium to 5.7. The culture medium was autoclaved for 15min at 121°C temperature and 105kPa pressure and cooled to room temperature.

Young shoots were collected from wild *S. nigrum* herbs grown in Assuit Governorate (27°11'00"N 31°10'00"E). The leaves were washed under running tap water for regarding 20min, sterilized with 50% commercial bleach containing a few drops of Tween-20 for 8min and then washed for 4-6 times using sterile distilled H_2O. In a 195ml jar, three sterilized (1–1.5cm) leaf segments were placed on 30ml solidified MS media that previously prepared. Twenty jars were used per each treatment. These culture media were transferred to the growth chamber [16/8hrs photoperiod 30μM m⁻² S⁻¹ irradiance, temperature 25±1 °C and 50–60% relative humidity] (Abdel-Wahab et al., 2019). After one-month, some calli were quickly weighed to determine the fresh weight (FW), frozen in liquid nitrogen and stored at -80ºC for physiological parameters analysis. The other calli were dried at 60°C for 48hrs to determine the dry weight (DW) and some minerals.

The activity of enzymes

Frozen calli (0.5g) were ground to a fine powder in liquid N_2 and homogenized in 5ml of 100mM potassium phosphate buffer (pH 7.8) containing 0.1mM ethylenediaminetetraacetic acid (EDTA) and 0.1g polyvinylpyrrolidone (PVP). The homogenate was filtered by centrifuge (18,000rpm at 4 ºC) for 10min and then the supernatant was used for the evaluation of enzymes and some metabolites. All enzyme activities were measured using a Unico UV-2100 spectrophotometer and the data were expressed as the difference in absorbance of wavelengths (DA).

Lipoxygenase (LOX; EC 1.13.11.12) activity was evaluated following the technique of Minguez-Mosquera et al. (1993). The activity was measured at 234nm and expressed as DA_{234} mg protein⁻¹ min⁻¹.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed following the autoxidation of epinephrine as described by Misra & Fridovich (1972). The activity was measured as an increase in absorbance at 480 nm and expressed as DA_{480} mg protein⁻¹ min⁻¹.

The activity of catalase (CAT; 1.11.1.6) was assayed following the consumption of H_2O_2 for 1min as described by the method of Aebi (1984). The activity was calculated as a reduction in absorbance at 240nm and expressed as DA_{240} mg protein⁻¹ min⁻¹.

Peroxidase activity (POD; EC 1.11.1.7) was measured spectrophotometrically following the method of Tatiana et al. (1999). The formation of tetraguaiacol was measured at 470nm and expressed as DA_{470} mg protein^{-1} min^{-1}.

The activity of phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) was assayed following the production of trans-cinnamate as described by Havir & Hanson (1968). The activity was calculated at 290nm and expressed as DA_{290} mg protein^{-1} min^{-1}.

The activity of polyphenol oxidase (PPO; EC 1.14.18.1) was evaluated *via* the formation of purpurogallin namely explained by Kumar & Khan (1982). The activity was measured as an alteration of absorbance at 495 nm and expressed as DA_{495} mg protein^{-1} min^{-1}.

Free and bound phenolics
Free and cell wall-bound phenolics were assessed following the technique of Kofalvi & Nassuth (1995). Phenolics were measured from the gallic acid standard curve and expressed as μg/g FW.

Free amino acids
Free amino acids were assayed through the ninhydrin method using glycine as a standard amino acid according to Moore & Stein (1948) and calculated as mg/g FW.

Soluble proteins
Soluble proteins were measured by Folin reagent according to Lowry et al. (1951). A calibration curve was constructed using bovine serum albumin (BSA) and data were expressed as mg BSA/g FW.

Soluble carbohydrates
Soluble carbohydrates were measured according to the anthrone sulphuric acid method of Fales (1951) and Schlegel (1956) using glucose as a standard curve and expressed as mg/g FW.

Zinc, potassium, and phosphorus
The acid digested sample (1:3:1 ratio of 60% $HClO_4$, concentrated HNO_3 and H_2SO_4) was analyzed for K using flame photometer Carl Zeiss; for Zn using atomic absorption spectrophotometry (Buck model 210 Vgp, USA) (Radi et al., 2018), while P was determined using the technique of Woods & Mellon (1941).

Fourier transform infrared (FT-IR)
The oven-dried sample (~ 100μg) was prepared to identical thickness pellets using potassium bromide (KBr). Infrared transmittance data were measured using Nicolet IS 10 FT-IR wave numbers ranged from 4000-400cm^{-1}. The sample was analyzed with plain KBr pellet as a blank and the data were compared with a reference to identify the functional groups in the sample.

Statistical analysis
Data were analyzed using one-way variance analysis (ANOVA) with Tukey's as post-hoc analysis. The relationship between the different parameters of the tested plant was analyzed using Pearson's correlation. Asterisks indicate a significant correlation (* and ** at 5 and 1%, respectively). All values are means of four independent replicates and analyzed using SPSS version 22.

Results

Effects of ZnO NPs on biomass
Different ZnO NPs concentrations caused variable effects on callus growth (Fig. 1). The treatment of *S. nigrum* calli with 50mg L^{-1} showed a significant increase in DW, and the increase was about 80%, over the control. On the other hand, the 100mg L^{-1} ZnO NPs treatment reduced callus tissues DW by 30%, relative to the control.
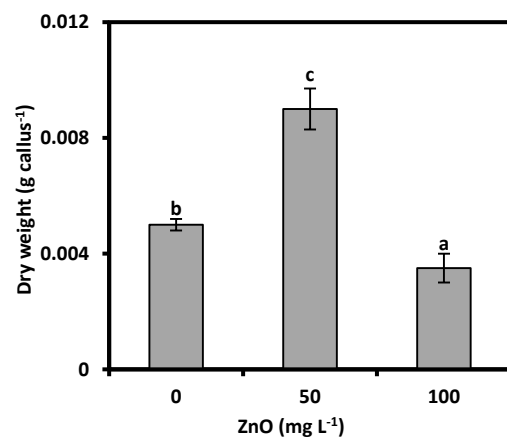

Fig. 1. The dry weight of *S. nigrum* callus grown under different concentrations of ZnO NPs (0, 50, and 100mg L^{-1}) [The data are means± SD (n= 4) [Different letters indicate statistically significant differences according to the Tukey's HSD test (P≤ 0.05)].

Effects of ZnO NPs on the specific activity of enzymes

Figure 2A showed LOX activity in callus tissues of *S. nigrum* that differs with the various concentrations of ZnO NPs. Non-significant variations in the activity of LOX were observed in tissues due to the application of 50mg L^{-1}, compared with the control. However, LOX activity in calli was considerably increased by applying 100mg L^{-1} ZnO that showed an 82.37% increase over the control. It is important to note that LOX activity was non-significant correlated with the DW (-0.539), whereas it was strongly correlated with Zn content in callus tissues (0.966**).

The activity of SOD exhibited a similar response of LOX due to exposure to ZnO NPs (Fig. 2B). Initially, the non-significant effect on SOD activity was observed by applying 50mg L^{-1} ZnO NPs, however increasing concentration to 100mg L^{-1} increased the activity by 66.39%, as compared with the control. Further, the result indicated that there was a substantial positive correlation between the activity of SOD and Zn concentration (0.917**) in *S. nigrum* callus tissues.
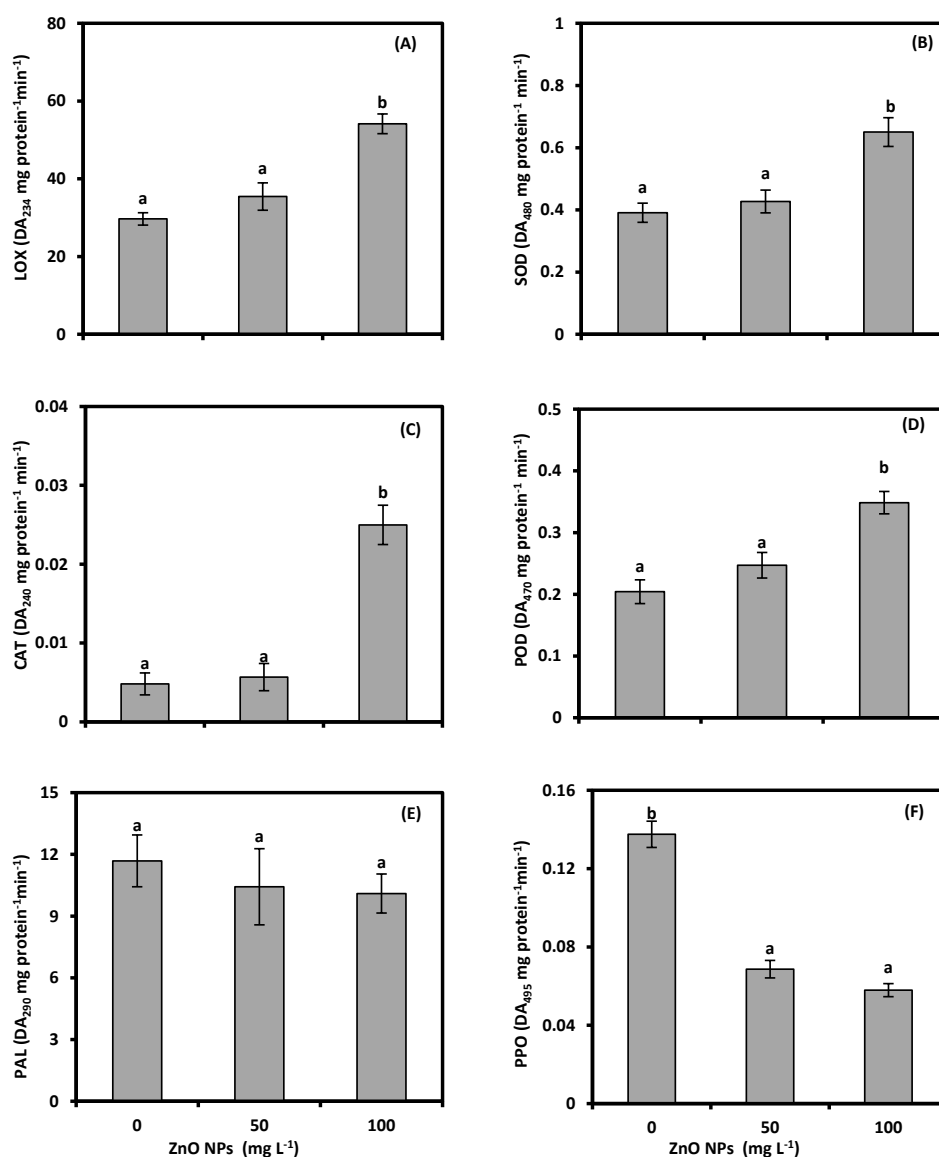

Fig. 2. Lipoxygenase (LOX; A), superoxide dismutase (SOD; B), catalase (CAT; C), peroxidase (POD; D), phenylalanine ammonia-lyase (PAL; E), and polyphenol oxidase (PPO; F) activities of *S. nigrum* callus grown under different concentrations of ZnO NPs (0, 50, and 100mg L^{-1}) [The data are means± SD (n= 4). Different letters indicate statistically significant differences according to the Tukey's HSD test (P≤ 0.05)].

The concentration of 50mg L⁻¹ ZnO NPs insignificantly increased the activity of CAT, whereas a higher increase in CAT activity was recorded (419.39% over the control) at 100mg L⁻¹ (Fig. 2C). The activity of the CAT was considerably correlated (0.929**) with the Zn content of the callus.

Similar to the previous antioxidant enzymes, no considerable alteration in POD activity in *S. nigrum* calli was determined with the existence of 50mg L⁻¹ ZnO NPs within the medium, while the activity raised with the rising of NPs in the media to 100 mg L⁻¹ to achieve 70.63% over the control (Fig. 2D). Furthermore, the results revealed that the activity of POD was significantly correlated with the Zn content of callus tissues (0.955**).

Effects of ZnO NPs on free and bound phenolics and associated enzymes

Results concerning the different concentrations of ZnO NPs on PAL activity in *S. nigrum* calli were given in Fig. 2E. No significant changes in

PAL activity were observed when the callus was exposed to ZnO NPs. The data revealed that PAL activity was only correlated with bound phenolics (-0.786*).

Concerning the effects of ZnO NPs on PPO activity, Fig. 2F showed that ZnO NPs treatments reduced the activity of PPO by 50.07 and 57.88%, after calli exposure to 50 and 100mg L⁻¹, respectively, as compared with controls. Moreover, the results indicated a significant negative correlation between PPO activity and the content of Zn in calli (-0.824**).

The content of free and bound phenolics in calli of *S. nigrum* was non-significantly affected by exposure to the different concentrations of ZnO NPs, as compared with the control (Fig. 3A). Treatment with ZnO NPs showed a strong correlation between free phenolics and DW (0.758*), while bound phenolics represented strong negative correlations with PAL and PPO activities (-0.786* and -0.668*, respectively).
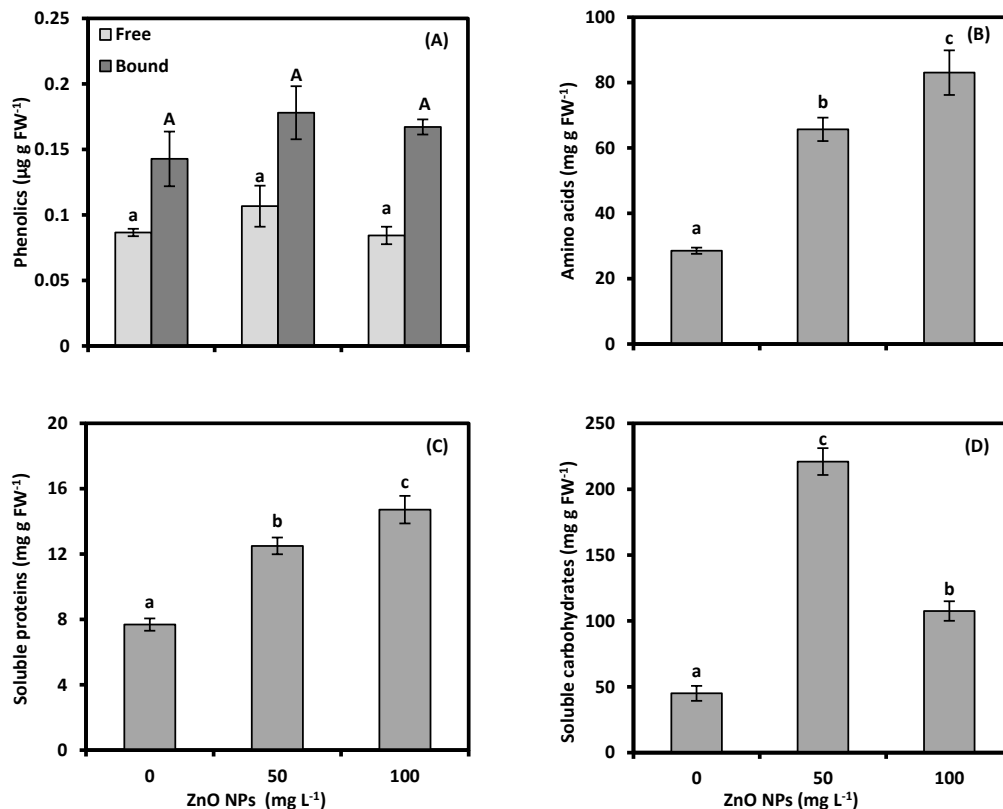

Fig. 3. **Free and bound phenolic compounds (A), amino acids (B), soluble proteins (C) and soluble carbohydrates (D) of *S. nigrum* callus grown under different concentrations of ZnO NPs (0, 50, and 100mg L⁻¹) [The data are means ± SD (n= 4). Different letters (small for free and capital for bound phenolic compounds) indicate statistically significant differences between different treatments according to the Tukey's HSD test (P≤ 0.05)].**

Effects of ZnO NPs on amino acids, soluble proteins, and soluble carbohydrates

As shown in Fig. 3B, the amino acid content was increased by 130.05 and 190.81% with the exposure to 50 and 100mg L⁻¹ ZnO NPs, respectively, relative to the control. The results also showed that amino acids were significantly correlated with zinc content in callus tissue (0.897**).

Similarly, ZnO NPs enhanced the accumulation of soluble proteins within *S. nigrum* callus tissues (Fig. 3C). Its accumulation was found to be 62.60 and 91.46%, after exposure to 50 and 100mg L⁻¹ ZnO NPs, respectively, compared with controls. Furthermore, the content of soluble proteins was significantly correlated with Zn concentration of the callus (0.912**).

The soluble carbohydrates content of *S. nigrum* tissues was considerably increased when plants were exposed to the different concentrations of ZnO NPs (Fig. 3D). Compared to the control, the recorded highest increase was 390.69% at 50mg L⁻¹ ZnO NPs. The accumulation of soluble carbohydrates was notably correlated with DW (0.799**) and negatively with K content (-0.720*).

Effects of ZnO NPs on some essential minerals elements

ZnO NPs treatments differently affected some of the essential nutrients present in callus tissues as shown in the current study (Fig. 4A-C). The content of P in callus tissues was reduced by 39.06% and 53.87% under 50 and 100mg L⁻¹ ZnO NPs, respectively, relative to the control. The results further revealed that the P content strongly negative correlated with the content of Zn^{+2} (-0.876**).

Regarding the content of K^+ in callus tissues, a decrease of 48.72% and 53.36% was observed after the application of 50 and 100mg L⁻¹ ZnO NPs, respectively, compared with controls. Moreover, data revealed that the contents of K^+ and Zn^{+2} have a strong negative correlation with each other (-0.786*).

As seen in Figure 4C, the accumulation of Zn^{+2} was concentration-dependent. The results manifested an increase in Zn^{+2} content in *S. nigrum* calli by increasing the concentration of ZnO NPs within MS media reaching up to 2.2 and 4.8 fold, under 50 and 100mg L⁻¹ treatments, respectively, compared to the control.
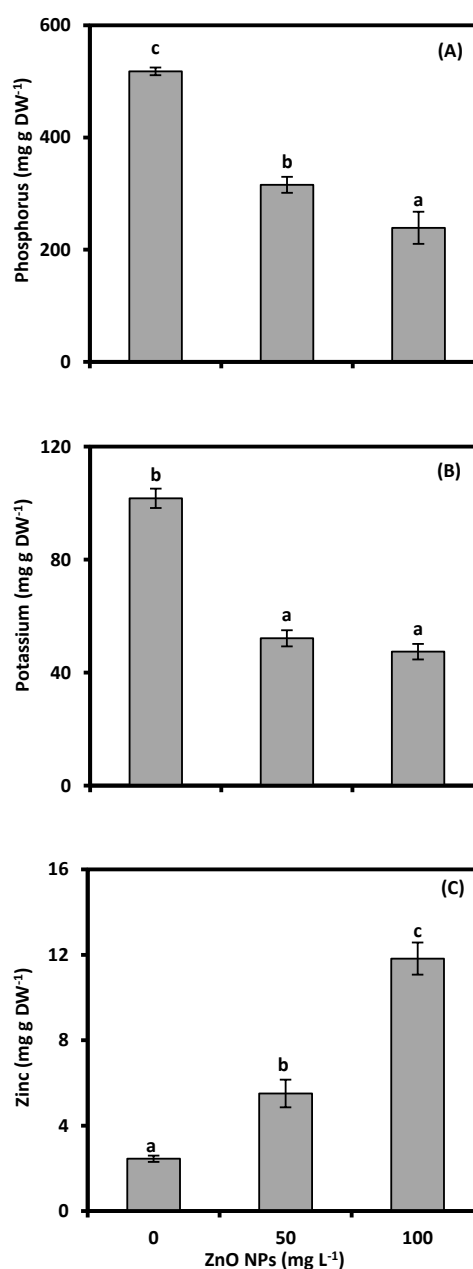

Fig. 4. Phosphorus (A), potassium (B) and zinc (C) contents of *S. nigrum* callus grown under different concentrations of ZnO NPs (0, 50, and 100mg L⁻¹) [The data are means ± SD (n= 4). Different letters indicate statistically significant differences according to the Tukey's HSD test (P≤ 0.05)].

Effects of ZnO NPs on macromolecules

To study the structural changes in macromolecules of *S. nigrum* callus tissues exposed to different concentrations of ZnO NPs, the FT-IR was applied (Fig. 5A-I). The broadband 3342.96cm⁻¹ (control) was shifted by +30.65 and

+38.38cm⁻¹ when the callus was treated with 50 and 100mg L⁻¹ ZnO NPs, respectively (Fig. 5A). The data in Fig. 5B, C, and E revealed that more or less no extended modifications were observed within the bands at 2929.53, 1653.24, and 1384.4cm⁻¹ with the application of various ZnO NPs levels. The transmittance area of the band at 1540.66 cm⁻¹ (control) was disappeared under the treatment of 50mg L⁻¹ ZnO NPs, whereas it was reduced by applying 100mg L⁻¹ ZnO NPs (-24.63cm⁻¹ less than the transmittance area of control) (Fig. 5D). Compared to the control, 50mg L⁻¹ ZnO NPs increased the transmission of the band at 1242.69cm⁻¹ by +15.6cm⁻¹, while the highest concentration (100mg L⁻¹) slightly reduced the transmission by 0.81cm⁻¹ (Fig. 5F).

Treatment with 50mg L⁻¹ ZnO NPs shifted the band at 1055.13cm⁻¹ (control) by -15.3cm⁻¹, whereas the highest concentration (100mg L⁻¹) increased the transmission area by +5.3cm⁻¹, relative to the control (Fig. 5G). The weak sharp band at 824.84cm⁻¹ (control) disappeared by the application of 50 mg L⁻¹ ZnO NPs, while shifted (+7.61cm⁻¹) by applying the highest concentration (100mg L⁻¹) (Fig. 5H). Furthermore, the week sharp band at 778.66cm⁻¹ (control) disappeared by applying ZnO NPs (Fig. 5I). Finally, the shift in the weak sharp band at 610.32cm⁻¹ was observed under ZnO NPs treatments (+7.19 and +9.02cm⁻¹, over the control at 50 and 100mg L⁻¹, respectively) (Fig. 5I).

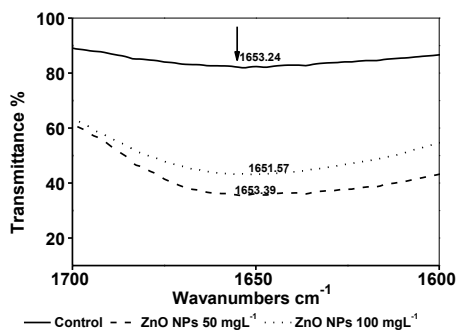**Carbohydrates, proteins, alcohols and phenolic compounds**
(A)
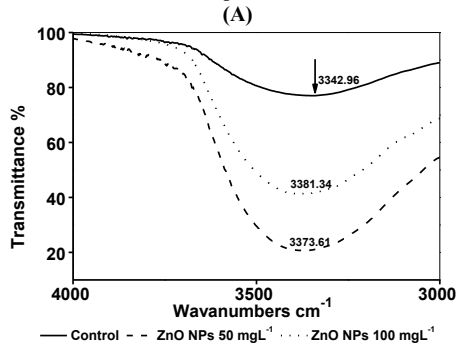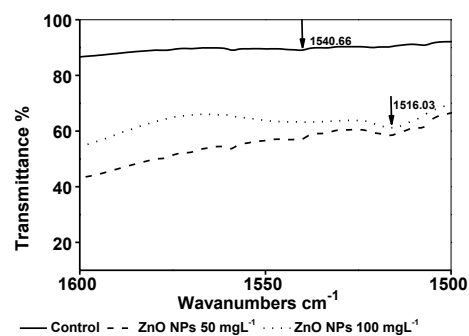
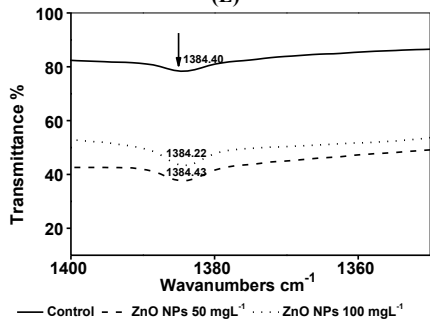**Lipids**
(B)
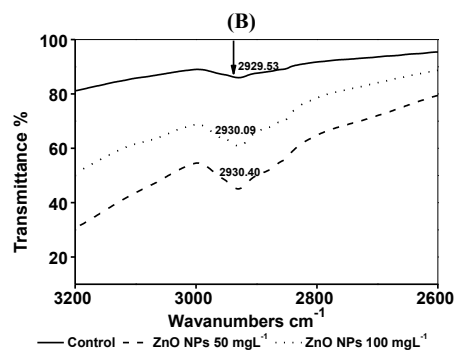
Amide I
(C)

Amide II
(D)

Amide III
(E)

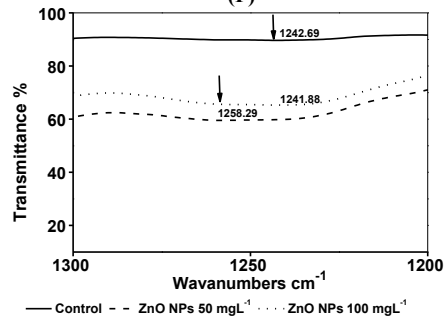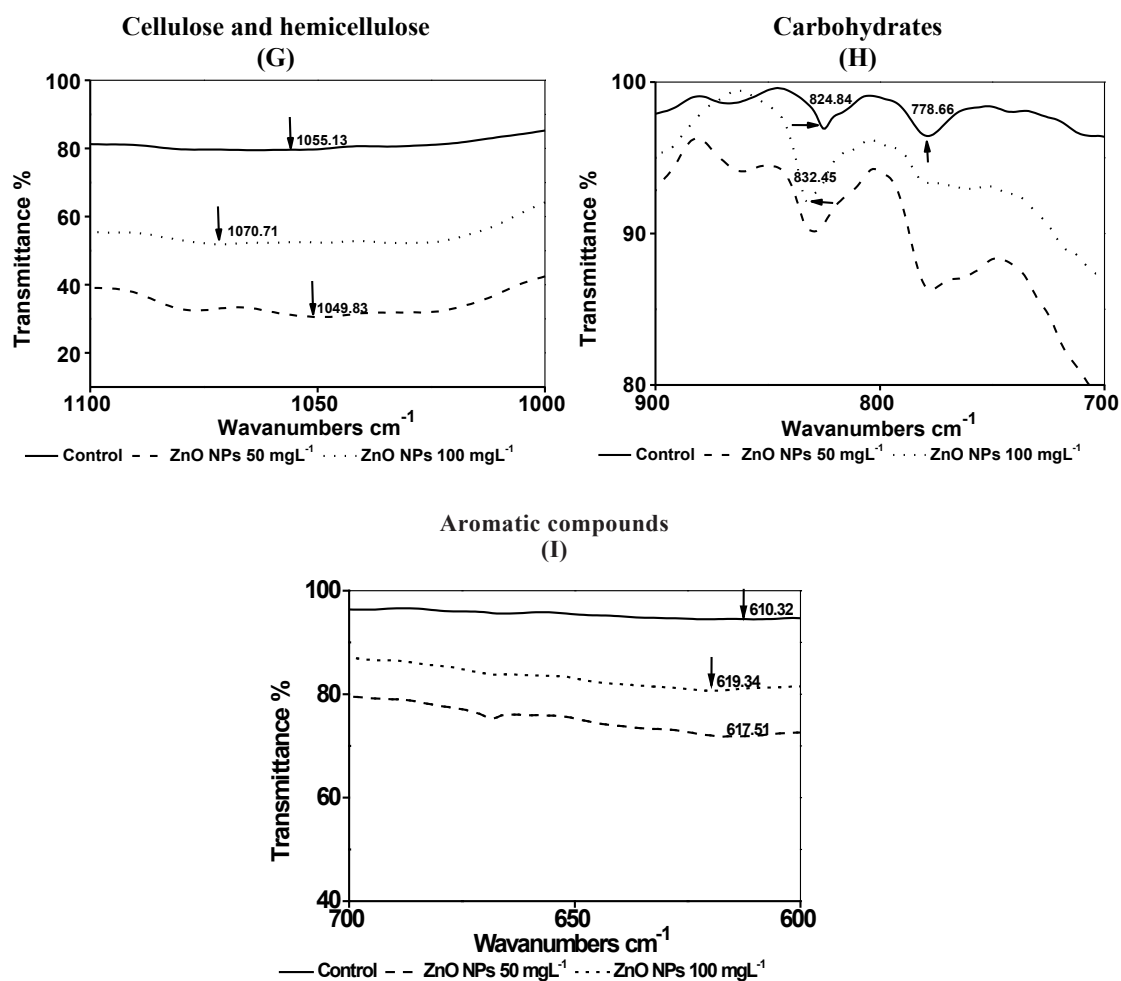**Cellulose and hemicellulose**
(F)

Fig. 5. FT-IR spectra of *S. nigrum* callus grown under different concentrations of ZnO NPs (0, 50, and 100mg L⁻¹). Spectral region associated with carbohydrates, proteins, alcohols, phenolic compounds (A), lipids (B), proteins (amide I) (C), amide II (D), amide III (E), cellulose and hemicellulose (F, G), carbohydrates (H), and aromatic compounds (I).

Interestingly, treatments with ZnO NPs increased the intensity of all FT-IR bands over the control. Compared to the control, the highest intensity of all bands was recorded in the treatment of 50mg L⁻¹ ZnO NPs.

Discussion

Effects of ZnO NPs on biomass
Under these experimental conditions, the lowest concentration of ZnO NPs significantly stimulated the callus growth, while the DW at the highest concentration of ZnO NPs was reduced. The stimulation of growth may suggest the ability of this callus to tolerate the toxicity of low concentrations of ZnO NPs. The negative weak correlation (-0.437) between DW and Zn concentration may confirm this suggestion. Similarly, Zafar et al. (2016) reported that the

rise in *Brassica nigra* shoot length in response to ZnO NPs could be the nutrition action of particles or dissociated ions only at a non-lethal level. In contrast, growth suppression is a common phenomenon in the case of Zn toxicity (Collins & Zinc, 1981). The reduction in calli growth may additionally result from changes in the ultrastructure of the cells (Radi et al., 2018) or the arrest of cellular division (Ghosh et al., 2016).

Effects of ZnO NPs on the activity of the enzymes
The data presented here clarified an insignificant increase in LOX activity at 50mg L⁻¹ ZnO NPs, while the highest concentration of ZnO NPs showed a significant increase, relative to the control. In line with these findings, Tripathi et al. (2006) reported that the activity of LOX was increased by Zn, which stimulated lipid peroxidation in membranes.

The response of the antioxidant enzymes activities in the *S. nigrum* callus was based on the applied ZnO NPs concentrations. Feigl et al. (2015) reported that the ROS synthesis by Zn treatment changed the antioxidant capacity in *Brassica juncea*. In-plant cells, the SOD constitutes the prime line of defence versus ROS (Alscher et al., 2002). In the current study, the lowest level of ZnO NPs failed to exert a significant stimulation on the activity of SOD, while the highest level resulted in a significant one. These results suggested that ZnO NPs at 50mg L^{-1} would possibly haven't toxic impacts on *S. nigrum* callus tissues. The strong positive correlations of SOD with the tested antioxidant enzymes may justify the strength of *S. nigrum* calli to resist ZnO NPs toxicity. The result is in line with recent findings of Wang et al. (2018) who reported that there was an increase in SOD activity with increased ZnO NPs concentrations in the nutrient medium of *Solanum lycopersicum* plants.

In the current study, the highest level of ZnO NPs caused significant stimulation of CAT activity, while the lower level failed to exert significant stimulation. This result may reveal that the highest concentration of Zn stimulated the active oxygen species, so stimulated SOD activity that produced excessive H_2O_2 which increased CAT activity (Wang et al., 2018).

In this study, the presence of 50 mg L^{-1} ZnO NPs within the culture medium considerably failed to affect on POD activity of *S. nigrum* calli, therefore, no oxidative stress may be exerted in calli. Nonetheless, the higher POD activity observed under 100mg L^{-1} ZnO NPs might suggest the role of this enzyme as a defence against ZnO NPs-caused oxidative damage. Also, POD activity exhibited a significant increasing trend when *Allium cepa* root cells were exposed to ZnO NPs (Ghosh et al., 2016).

Effects of ZnO NPs on free and bound phenolics and associated enzymes

In this study, ZnO NPs treatments did not alter PAL activity and phenolic contents (free and bound) in *S. nigrum* callus, whereas PPO activity was reduced. The data suggest that one probable reason for the plant resistance to ZnO NPs stress may be related to the unchanged PAL activity and phenolic compounds as well as the reduced PPO activity. Also, Zafar et al. (2016) and

Mohsenzadeh & Moosavian (2017) found that exposure to ZnO NPs did not cause differences in total phenolic compounds in *Brassica nigra* shoot and rosemary seedling, compared to controls. The negative correlations between PPO and POD activities further confirm earlier reports of Sofo et al. (2005) who suggested that reduced PPO activity, which caused abiotic stress, is associated with improved antioxidant capacity.

Effects of ZnO NPs on amino acids, soluble proteins, and carbohydrates

Amino acids help to trap and chelate the metal ion, thus giving plants the ability to tolerate heavy metals (Singh et al., 2016). The obtained increase in free amino acids in callus tissues under the influence of ZnO NPs may indicate that these metabolites are involved in the high ability of *S. nigrum* to accumulate this metal. In this context, Kozhevnikova et al. (2014) reported that histidine was involved in Zn transport within the plant.

The noticed increase in protein concentration in plants exposed to Cu and Zn NPs by Olkhovych et al. (2016) was confirmed with the present results that showed an increase in the soluble protein content in the *S. nigrum* callus under different concentrations of ZnO NPs. Furthermore, Priyanka & Venkatachalam (2016) suggested that improvements in the soluble protein content may protect cells from oxidative stress caused by ZnO NPs.

Soluble carbohydrates accumulate through many abiotic stress conditions along with oxidative stresses (Couée et al., 2006), suggesting a relationship between carbohydrates and ROS accumulation caused by intra-plant stresses. Recently, Gautam et al. (2019) reported that sucrose acts as a signal molecule against ROS besides that it is an energy source. The significant increase in the accumulation of soluble carbohydrates in the current experiment can be associated with its role as stress management and osmolytes needed for growth. High sucrose level in plant tissues has been reported under abiotic stress that indicates direct/indirect sugar involvement in stress management (Tognetti et al., 1990; Sami et al., 2016). The strong positive correlation between carbohydrates and the DW could confirm previous reports of Mukhopadyay et al. (2012) who concluded that soluble carbohydrates may be providing the energy and osmolytes needed for growth.

Effects of ZnO NPs on some essential minerals elements

Potassium has a protective role against abiotic/biotic stresses, as K reduces the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which reduces the generation of ROS in plants (Hasanuzzaman et al., 2018). In this work, the different concentrations of ZnO NPs significantly reduced the K content in the *S. nigrum* callus. This result was confirmed by the resultant significant negative correlations between concentrations of K, LOX, and Zn. In this regard, Tewari et al. (2004) reported that the accumulation of Zn leads to a deficit of ions and that is why it affects plant growth. In line with the obtained results, Zhao et al. (2012) recorded that Zn uptake is associated with considerable reductions in the content of macronutrients, such as S, K, and Ca, referring that Zn disturbs ionic homeostasis that harms the plant by inducing oxidative damages.

Several studies disclose the negative correlation between the accumulation of inorganic P and Zn in the different plant species (Wang et al., 2009; Bouain et al., 2014). In agreement with these studies, the present finding revealed that the content of P in *S. nigrum* callus tissues gradually decreased with increasing ZnO NPs concentrations in the nutrient media. Strong negative correlations between the content of P, the activities of LOX, SOD, CAT, POD, and Zn content may confirm that Zn considerably disturbs the P homeostasis, which increases the toxicity of Zn in plants.

Zinc is a necessary micronutrient, and shows a significant role in plant metabolism, while excess Zn may also have negative effects on the plant (Cakmak, 2000). In this research, the accumulated Zn in the *S. nigrum* callus by the increase in levels of ZnO NPs was insignificantly correlated with the DW of callus, while it was significantly correlated with LOX activity that could confirm the tolerance of *S. nigrum* to the toxicity of ZnO NPs. An increase in Zn accumulation was also reported during the stress of ZnO NPs on the pomegranate callus (Radi et al., 2018).

Effects of ZnO NPs on macromolecules

The FT-IR analysis regarding *S. nigrum* callus tissues allowed us to get more details about the construction adaptations of macromolecules under the effect of ZnO NPs. The initial peak at 3342.96cm^{-1} (control) is stretched N-H and O-H groups associated with proteins, carbohydrates,

alcohols, and phenolic compounds (Türker-Kaya & Huck, 2017). The obtained data showed that exposure of the callus to ZnO NPs increased the transmittance area and intensity, and this increase could indicate that additional O-H groups were formed to chelate Zn. It was earlier reported that the low concentration (10mg L^{-1}) of ZnO NPs led to a positive shift and high intensity of this band in pomegranate calli, whereas the high concentration (150mg L^{-1}) caused the opposite effect (Radi et al., 2018).

Fatty acids show characteristic transmittance peak at 2950-2845cm^{-1} (Gupta et al., 2015). In this investigation, the different concentrations of ZnO NPs did not induce noticeable shifts at the peak of the fatty acids, whereas the intensity of that peak was increased. This change in the intensity of peaks might reveal that ZnO NPs changed the composition of fatty acids. In line with this result, Radi et al. (2018) showed that ZnO NPs did not induce a noticeable shift at this peak.

The peak at 1653.24cm^{-1} (control) is ascribed to C=O stretching ordinarily conjugated according to a -NH deformation mode, and might also ascribe to the amide I peak (α-helix structure) (Hlihor et al., 2013). In this study, the slight shift within the amide I peak under the highest level of ZnO NPs may indicate some changes in the structure of calli proteins. In this context, Surewicz et al. (1993) stated that the limit in the peak area may detect modifications between protein content and structure, possibly due to the toxicity of metals. On the other hand, the resultant increase in the peak intensity under ZnO treatments agree with the previous report of Nahar & Tajmir-Riahi (1996) who concluded that the rise in the intensity of amide I peak, is considered as a consequence of a direct heavy metal (Cd) – protein binding by the peptide carbonyl group. The peak around 1540.66 cm^{-1} is assigned as amide II, which collaborated in the motion combining both -NH bending and -CN stretching vibration of the group C(=O)-NH in its transform (Pan et al., 2007). Under ZnO NPs treatments, this peak disappeared at the lowest level, while at the highest level it was reduced. These changes might be attributed to the change in amide II under ZnO NPs stress. The increased intensity of the 1384.40cm^{-1} peak, which is related to the amide III (Fawzy, 2016), under ZnO NPs treatments could be linked to Zn-binding proteins.

The peak at 1242.69cm^{-1} is pointed to

stretching vibration C-O that identifies the existence of cellulose and hemicellulose (Rico et al., 2015). ZnO NPs at 50mg L^{-1} raised the transmittance of that peak, whereas 100mg L^{-1} slightly reduced the transmittance. The shift and height of this peak intensity can be indicated in the change and participation of C-O polysaccharides in the accumulation of Zn on the cell wall (Radi et al., 2018).

The decrease in the peak area 1055.13cm^{-1} could suggest a decrease in the synthesis of cellulose, hemicellulose and carbohydrates that are participated in cell growth (Rico et al., 2015). Furthermore, the increase in the peak intensity under ZnO NPs treatments may be caused by the participation of C=O of polysaccharides within the accumulation of Zn on the cell wall (Radi et al., 2018). The peaks at 824.84 and 778.66cm^{-1}, which were found in control and 100mg L^{-1} ZnO, respectively, are normally attributed to carbohydrates (Schrader, 1995). These results might indicate the synthesis of extra carbohydrates under an excessive level of ZnO NPs (Fawzy, 2016). The increase in the transmittance area at 610.32cm^{-1} that is indicated to aromatic compounds (Ramamurthy & Kannan, 2007), might suggest chelation of ZnO NPs.

Interestingly, ZnO NPs treatments increased the intensity of all FT-IR peaks over the control. Compared to the control, the highest intensity for all peaks was recorded in the treatment of 50mg L^{-1} ZnO NPs. This result may reveal that the high peak intensity plays an important role in the chelation of excess Zn. Fawzy (2016) stated that the increase in peak intensity may indicate the interaction of amino, carboxyl, hydroxyl, thiol and phosphate groups with heavy metals.

Conclusion

The results of this study revealed the stimulation of growth by the lowest concentration (50mg L^{-1}) of ZnO NPs. The tolerance of *S. nigrum* calli to ZnO NPs can be attributed to the antioxidant systems, free amino acids, soluble proteins, and soluble carbohydrates. Furthermore, FT-IR analysis indicated the role of macromolecules in the ZnO NPs chelating process. The current results showed that the *S. nigrum* callus may be able to resist the toxicity of ZnO NPs, this means that it can be used in the remediation of ZnO NPs from contaminated media.

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أكسيد الزنك النانو يستحث التغيرات في أنظمة مضادات الأكسدة والجزيئات الكبيرة في كالوس نبات عنب الديب

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تهدف هذه الدراسة إلى استكشاف آثار الجسيمات النانوية لأكسيد الزنك (ZnO NPs) على العمليات الفسيولوجية المختلفة لكالوس نبات عنب الديب وإمكانية استخدامها كعلاج للوسائط الملوثة. اجريت تجارب معملية لفهم آلية عمل كالوس عنب الديب في علاج سمية الجسيمات النانوية لأكسيد الزنك (0، 50، و100 مجم/لتر). وأوضحت النتائج زيادة نمو الكالوس والمتمثل في زيادة الوزن الجاف للكالوس المعرضة لأدنى تركيز من اكسيد الزنك النانو ليصل الي 1.8 ضعف الكالوس غير المعامل، ومع زيادة التركيز ثبط نمو الكالوس. أيضا حفز التركيز العالي من جسيمات اكسيد الزنك النانوية أنشطة إنزيمات الليبوجينيز والانزيمات المضادة للأكسدة في الكالوس. في حين معاملة اكسيد الزنك النانو لم تغير من نشاط انزيم فينيل ألانين الأمونيا والمركبات الفينولية، بينما خفضت من نشاط انزيم أوكسيديز بوليفينول. انخفضت محتويات الفوسفورووالبوتاسيوم تحت معاملات اكسيد الزنك النانو. ومع ذلك، فإن محتوي كل من الأحماض الأمينية، والبروتينات الذائبة، والكربوهيدرات الذائبة، والزنك ارتفع في الكالوس المعامل بأكسيد الزنك النانو. أثبت التحليل الطيفي للأشعة تحت الحمراء الاختلافات بين معظم الجزيئات ذات الوزن الجزئي الكبير. ويشير هذا البحث إلى امكانية استخدام هذا النبات لعلاج الاوساط الملوثة بجسيمات النانوية لأكسيد الزنك.