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Activating antioxidant enzymes, hyoscyamine and scopolamine biosynthesis of *Hyoscyamus niger* L. plants with nano-sized titanium dioxide and bulk application

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ABSTRACT

Application of nanotechnology is now widely distributed overall the life, especially in agricultural systems. This study intended to indicate the impacts of nano-sized titanium dioxide particles (NT) and bulk (BT) on antioxidant enzymes activities including superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT), and variations of two major tropane alkaloids such as hyoscyamine (HYO) and scopolamine (SCO) in *Hyoscyamus niger* L. Plants were treated with different concentrations of NT and BT (0, 20, 40 and 80 mg l^{-1}). Alkaloids extracted were identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. Results showed that SOD activity increased with increasing titanium dioxide concentration in both nano-particles and bulk treated plants. However, the highest and the lowest POX activity were observed in plants exposed to NT at 40 mg l^{-1} and control, respectively. Generally, all tested enzymes activities were higher in NT treated plants that those of BT except CAT activity at 80 mg l^{-1}. The highest alkaloids content values, HYO: 0.286 g kg^{-1} and SCO: 0.126 g kg^{-1}, were achieved in plants treated with NT at 80 and 20 mg l^{-1}, respectively. The maximum and minimum plant biomass and subsequently total alkaloids yield were obtained in plants exposed to NT at 40 mg l^{-1} and controls, respectively. Our results suggest that NT in appropriate level (40 mg l^{-1}) may act as an elicitor for biochemical responses and tropane alkaloids biosynthesis in *H. niger* plants.

Key words: black henbane, tropane alkaloids, antioxidant enzymes, nano-anatase TiO_2

IZVLEČEK

AKTIVIRANJE AKTIVNOSTI ANTIOKSIDACIJSKIH ENCIMOV, BIOSINTEZE HIOSCIAMINA IN SKOPOLAMINA PRI ČRNEM ZOBNIKU (*Hyoscyamus niger* L.) Z NANO IN CELOKUPNIMI DELCI TITANOVEGA DIOKSIDA

Uporaba nanotehnologije je v svetu danes zelo razširjena v znanostih o življenju, še posebej v kmetijstvu. V raziskavi je bil preučevan vpliv nano delcev (NT) in celukopnih delcev (BT) titanovega dioksida na antioksidacijsko aktivnost encimov kot so superoksid dismutaza (SOD), peroksidaza (POX) in katalaza (CAT) in vpliv tega obravnavanja na variabilnost vsebnosti dveh glavnih tropanskih alkaloidov, hiosciamina (HYO) in skopolamina (SCO) v črnem zobniku (*Hyoscyamus niger* L.). Rastline so bile tretirane z naslednjimi koncentracijami NT in BT delcev: 0, 20, 40 and 80 mg l^{-1}. Ekstrahirani alkaloidi so bili analizirani in določeni s plinsko kromatografijo (GC) in plinsko kromatografijo povezano z masno spektroskopijo (GC-MS). Rezultati so pokazali, da se je aktivnost SOD povečala pri tretmajih z NT in BT delci z naraščanjem njihove koncentracije. Aktivnost POX pa je bila največja pri rastlinah izpostavljenih NT delcem pri 40 mg l^{-1} in najmanjša pri kontroli. Nasplošno so bile aktivnosti vseh testiranih encimov večje pri rastlinah tretiranih z NT delci kot pri tretmaju z BT delci, razen aktivnosti CAT pri tretmaju z 80 mg l^{-1}. Največji vsebnosti alkaloidov, HYO: 0.286 g kg^{-1} in SCO: 0.126 g kg^{-1}, sta bili doseženi pri rastlinah tretiranih z NT delci pri koncentracijah 80 in 20 mg l^{-1}. Največja biomasa in največji pridelek alkaloidov sta bila dosežena pri rastlinah tretiranih z NT pri 40 mg l^{-1} in najmanjša pri kontroli. Rezultati kažejo, da NT delci v primernih koncentracijah (40 mg l^{-1}) delujejo kot elicitorji za biokemične odzive in biosintezo tropanskih alkaloidov pri črnem zobniku.

Ključne besede: črni zobnik, tropanski aklaloidi, antioksidacijski encimi, nano-delci TiO_2

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1 INTRODUCTION

Alkaloids are a diverse group of low-molecular-weight, nitrogen-containing compounds found in about 20% of plant species. Solanaceous plants are regarded as rich sources of alkaloids, namely the pharmaceutical by interesting tropane derivatives. Tropane alkaloids, especially hyoscyamine (HYO) and scopolamine (SCO) are widely used in medicine for their mydriatic, antispasmodic, anticholinergic, analgesic and sedative properties (Zehra *et al.,* 1998). SCO, which is the 6,7-epoxide of HYO, is the most valued of the two tropane alkaloids (due to fewer side effects on nervous system), its worldwide demand being 10 times higher than that for HYO and its racemic form, atropine (Hashimoto *et al.,* 1993). The synthetic production of these alkaloids is more expensive than their extraction from plant materials and they are, therefore, currently industrially extracted from various solanaceous plants belonging to the genera *Atropa*, *Duboisia*, *Datura, Scopolia* and *Hyoscyamus*.

Black henbane (*Hyoscyamus niger* L.) has a very long history of use as a medicinal plant. A cosmopolitan, strong-scented annual or biennial herb, which all it's parts (root, leaf, and seed) contain tropane alkaloids such as HYO and SCO (Cuneyt *et al.,* 2004). These metabolites are synthesized in roots and then transported to the aerial parts of the plant (Oksman, 1987).

It has been exhibited that signal molecules are very potential elicitors for induction of plant secondary metabolites. Recent years, the applications of signal components as elicitors have evolved an effective strategy for the production of target secondary metabolites in plant cell cultures. However, it is still uncommon for commercial application. It therefore, application of elicitors in vivo is an easy and direct channel to promote the yield of plant secondary metabolites at the whole plant scale. Nanonmaterials could act as signal compounds to make metabolic and physiological responses but the underlying mechanisms are not fully understood (Hatami and Ghorbanpour, 2014).

The development of nanotechnology on physiology and biochemistry has expanded the application area of nanomaterials in different fields due to their unique characteristics (Scrinis and Lyons, 2007). Also, this technology could open up new approaches in plant sciences and in agricultural researches. In recent years, many scientists have studied the effects of nanomaterials on seed germination and plant growth with the aim to promote its use for agricultural productions. Most of these studies are focused on the potential toxicity of nanoparticles on higher plants and positive, negative or inconsequential effects were presented. Most recently it was revealed that the use of appropriate concentration of nano-TiO_2 increased the seed germination parameters and early growth of some medicinal and aromatic plants (Hatami and Ghorbanpour 2014). According to Lu *et al.,* (2002) treatment of soybean (*Glycine max*) plants with a mixture of nano Sio_2 and Tio_2 increase nitrate reductase activity, stimulate its antioxidant system, and accelerate germination and growth. It is reported that silver nanoparticle treatment of *Brassica juncea* seedlings induced the activities of specific antioxidant enzymes (Priyadarshini *et al.,* 2012). However, the mechanism of these nanoparticles has not been completely established yet. Also, in the field of medicinal and aromatic plants, the use of nanomaterials is relatively new and needs more researches. However, some studies have reported negative effects of TiO_2 nanoparticles (NT) on higher plants that varied between plant tissues, growth stages, plant species, applied concentrations, and specific properties of nanoparticles (Castiglione *et al.,* 2011). Thus, the exploration of their extensive application in agriculture and plant science is still in debate (Kurepa *et al.,* 2010). Therefore, the present study was carried out to elucidate the potential effects of nanosized TiO_2 (NT) and bulk (BT) application on antioxidant enzymes including SOD, POX and CAT activity and elicitation of two main tropane alkaloids such as HYO and SCO on *Hyoscyamus niger* L. plants.

2 MATERIALS AND METHODS

2.1 Transmission electron microscopy (TEM) image of nano-dioxide titanium

Nano-sized TiO_2 were provided from the Iranian Nanomaterials Pioneers Company, NANOSANY (Mashhad, IRAN). The size of the TiO_2 nanoparticles was estimated to be 10-15 nm in diameter. A transmission electron microscopy (TEM) image of the TiO_2 particles is also provided (Fig. 1). The crystal properties of TiO_2 nanoparticles were examined by X-ray diffraction (XRD), which showed that used TiO_2 nanoparticles were all present in the anatase form (Fig. 2).

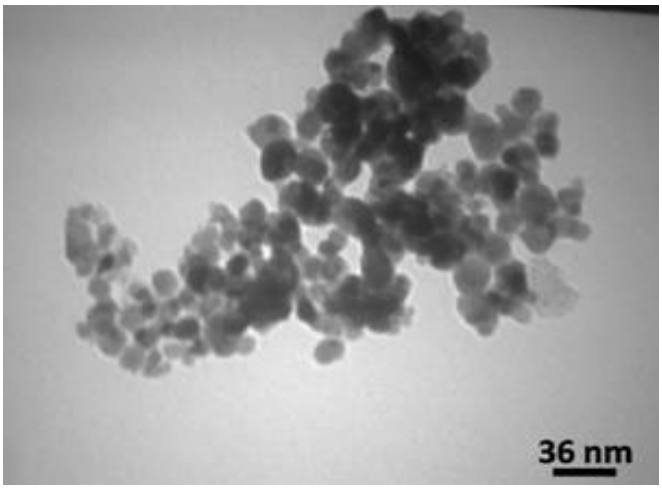

Figure 1: Transmission electron microscopy (TEM) image of TiO_2 nanoparticles. Distribution of particles size was estimated to be 10-15 nm, scale bar = 36 nm

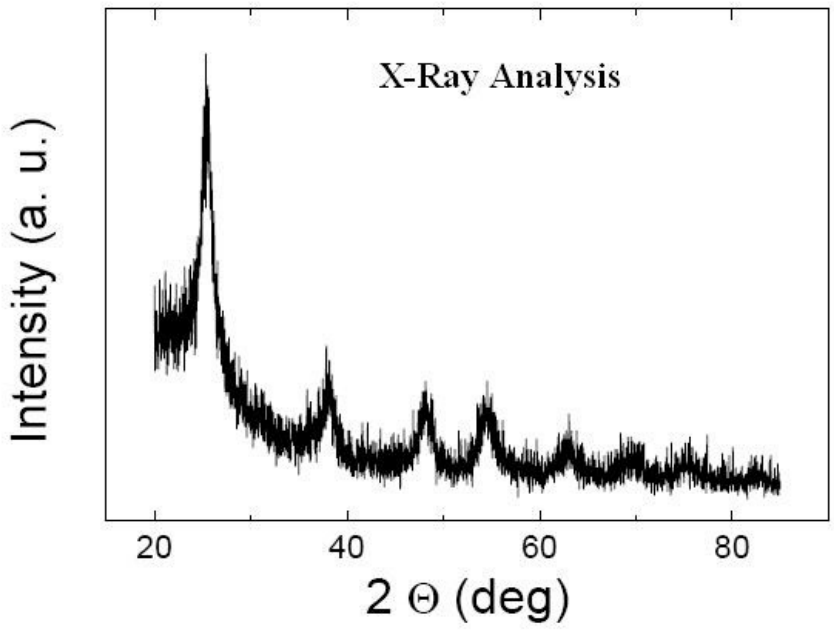

Figure 2: XRD (X-ray diffraction) pattern of Titanium oxide (TiO_2). Nanopowder (TiO_2 anatase)- size: 10-15 nm- purity: 99%- surface area: 200-240 m^2 g^{-1}- pH: 6-6.5- bulk density: 0.24 g cm^{-3}- true density: 3.9 g cm^{-3}- color: white

2.2 Plant growth conditions and treatments

The experiment was carried out in greenhouse conditions (25 °C day/17 °C night temperature, natural light 16 h light: 8 h dark and 75% relative humidity). Henbane seeds generally have low germination rate under normal laboratory conditions. Therefore, seeds were treated with 250 mg L^{-1} gibberellic acid (GA_3) for 48 h at room temperature (25 ± 0.5 °C) for breaking dormancy and accelerating germination. After that seeds were surface-sterilized in 70% ethanol for 2 min and then in 25% commercial bleach (containing 6% sodium hypochlorite) for 10 min and finally rinsed with sterile distilled water. Subsequently, seeds were placed in petri dishes on two layers of filter paper (Whatman No.1) moistened with 4 ml distilled water. After 3 days, 90% of seeds germinated steadily. After germination, individual, healthy and uniform seedlings (when they had three true leaves) were transplanted into experimental pots (25 cm diameter and 30 cm deep, containing 8 kg soil). The physical and chemical characteristics of employed soil are given in table 1. Deionised water was used to prepare 0, 20, 40 and 80 mg l^{-1} NT and BT solutions. Then, three month-old plants at flowering stage were treated with 50 ml of employed solutions. Both sides of the leaves and stems i.e., whole foliage of the plants were sprayed with equal amounts of 50 ml aqueous solution of NT and BT by hand atomizer. Control plants were only treated with deionised water. The study was set up as completely randomized design with three replicates. All pots were harvested at the end of flowering stage and subsequently plant dry matter was weighted with a precision of 0.0001 g scale and was finely powdered in an electronic blender for enzymes assays and alkaloids extraction.

2.3 Antioxidant enzymes assays

A crude enzyme extract was prepared by homogenizing 0.5 gram of powdered leaf sample in extraction buffer containing 0.5% Triton X-100 and 1% polyvinyl pyrrolidone in 100 mM potassium phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged and the supernatant was used for the following enzyme assays.

2.3.1. Superoxide dismutase (SOD, EC 1.15.1.1)

SOD activity was determined according to Beauchamp and Fridovich (1971). The reaction mixture contained 1.17×10^{-6} mol l^{-1} riboflavin, 0.1 mol l^{-1} methionine, 2×10^{-5} mol l^{-1} KCN and 5.6×10^{-5} mol l^{-1} nitroblue tetrazolium (NBT) salt dissolved in 3 ml of 0.05 mol l^{-1} sodium phosphate buffer (pH 7.8). 3 ml of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity is expressed in U mg^{-1} protein. (U = change in 0.1 absorbance h^{-1} mg^{-1} protein under assay conditions).

2.3.2. Catalase (CAT, EC 1.11.1.6)

CAT activity was assayed according to the method of Chandlee and Scandalios (1984). The assay mixture contained 2.6 ml of 50 mmol l^{-1} potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mmol l^{-1} H_2O_2 and 0.04 ml of enzyme extract. Changes in the absorbance were read at 240 nm. The enzyme activity was expressed in U mg^{-1} protein (U=1mM of H_2O_2 reduction min^{-1} mg^{-1} protein). The enzyme protein was estimated by the method of Bradford (1976).

2.3.3. Peroxidase (POX, EC 1.11.1.7)

POX activity was determined by the method of Kumar and Khan (1982). Assay mixture of POX contained 2 ml of 0.1 mol l^{-1} phosphate buffer (pH 6.8), 1 ml of 0.01mol.L^{-1} pyrogallol, 1 ml of 0.005 mol l^{-1} H_2O_2 and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 ml of 2.5 mol l^{-1} H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 mol l^{-1} H_2SO_4 at zero time. The activity was expressed in U mg−1 protein. One U is defined as the change in the absorbance 0.1 min^{-1} mg^{-1} protein.

2.4 Alkaloid extraction

Leaf samples were air dried, grinded into fine powder and sieved with laboratory mesh (size 30, mesh opening 545 μm). A subsample of one gram

from each samples was added to appropriate volume of $CHCl_3$: MeOH: NH_4OH 25%, (15:5: 1), sonicated for 20 min and then kept at water bath (40 ºC) for one hour. Subsequent sample preparation and alkaloids extraction were based essentially on the method described by Kamada *et al.,* (1986).

2.4.1. Alkaloid analysis and quantification

Alkaloids extracted were identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. GC analysis was performed using a GC system equipped with a flame ionization detector (FID) and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Injector and detector temperatures were set at 220 and 290 °C, respectively. The column temperature was initially kept at 50 °C for 5 min, then gradually increased to 300 ºC at a rate of 3 ºC/min and maintained for 3 min. The flow rate of gas He was 0.8 ml/min. Then, 1 µL of extract was directly injected into the gas chromatograph. Each extraction was replicated three times and the compound percentages are the means of the three replicates. GC-MS analysis was carried out on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, USA) fitted with a fused silica HP-5MS capillary column (30m×0.25mm×0.25µm). Oven temperature was programmed from 50 ºC to 285 ºC at 3 ºC/min, and helium was used as carrier gas (0.8 mL/min), Mass spectra were obtained in an Agilent 5973 system operating in electron impact mode (EIMS) at 70 eV, coupled to an GC system. The identification of alkaloids was based on the comparison of their GC retention time and mass spectra (MS) data with their standards substances (HYO. HCl and SCO. HBr, Merck). The total tropane alkaloids (HYO + SCO) yield was quantified by both alkaloid content and dry weight; Total alkaloid yield (g $plant^{-1}$) = Alkaloid content (g kg^{-1}) × Plant dry weight (g $plant^{-1}$).

2.5 Statistical analysis

The data were subjected to ANOVA based on a completely randomized design (CRD) with three replications and were analyzed by SAS and MSTAT-C program, and probabilities of significance were used to test for significance among treatments and interactions, and the Duncan's multiple range test ($p \leq 0.05$) was used to compare means. Values obtained were expressed as mean ± SD (standard deviation) from three replications (n=3) of each treatment.

3 RESULTS

3.1 Plant biomass and antioxidant enzymes status

Analysis of variance showed that the most measured traits of this study have been significantly ($P \leq 0.05$) affected by NT levels. Mean comparison of data revealed that increasing titanium dioxide concentration especially at nano-sized (10-15 nm) up to 40 mg l^{-1} significantly improved the plant dry weight to 42% compared to the unexposed control plants (table 2). However, there were no significant differences among BT levels on plant biomass production. The maximum and minimum plant biomass, 7.53 and 3.24 g $plant^{-1}$, were obtained in NT treated plants at 40 and 80 mg l^{-1}, respectively. There were noticeably differences in antioxidant enzymes activities among the employed treatments. SOD activity increased with NT and BT levels, and TiO_2 application played a significant role in adjusting the enzyme activity (Fig 3 and 4). SOD activity increased with increasing TiO_2 concentration in both nano-sized and bulk treatments. On the other hand, the highest SOD activity was observed at the highest NT and BT supply. CAT activity increased with NT application up to 20 mg l^{-1} and then decreased compared to other NT level, whereas, BT at all concentrations enhanced the CAT activities (Fig 3).

Table 1: The physical and chemical characteristics of soil used in current experiment

Characteristic	Quantity	Characteristic	Quantity
Soil texture	Sandy loam	CEC (Cmol(c)kg^{-1})	11.23
Clay(%)	14.32	total nitrogen(%)	0.051
Silt(%)	16	available phosphate ($mgkg^{-1}$)	9.12
Sand(%)	69.68	available potassium ($mgkg^{-1}$)	175
pH	7.0	Fe ($mgkg^{-1}$) *	8.4
EC (dS/m)	1.04	Mn ($mgkg^{-1}$) *	10.15
$CaCO_3$%	5.82	Cu ($mgkg^{-1}$) *	0.84
OC%	0.81	Zn ($mgkg^{-1}$) *	0.52
SP%	28.2		

* DTPA-Extractable

Table 2: Mean values for plant biomass, major tropane alkaloids including hyoscyamine (HYO) and scopolamine (SCO) content and yield (mean ± S.D., n=3) in *H. niger* plants treated with different nano-sized TiO_2 (NT) and bulk (BT) levels

Treatment ($mg\ l^{-1}$)	Biomass ($g\ plant^{-1}$)	Alkaloid content ($g\ kg^{-1}$)		Alkaloid yield ($g\ plant^{-1}$)		Total alkaloids yield ($g\ plant^{-1}$)
		HYO	SCO	HYO	SCO	
Control	4.32±0.14^{c}	0.168±0.006^{e}	0.084±0.013^{ef}	0.725±0.014^{f}	0.362±0.021^{f}	1.087±0.025^{g}
NT 20	5.81±0.22^{b}	0.216±0.004^{c}	0.126±0.004^{a}	1.254±0.012^{b}	0.732±0.012^{b}	1.986±0.022^{b}
NT 40	7.53±0.16^{a}	0.252±0.005^{b}	0.114±0.005^{b}	1.897±0.014^{a}	0.858±0.014^{a}	2.755±0.014^{a}
NT 80	3.24±0.15^{d}	0.286±0.003^{a}	0.114±0.003^{b}	0.926±0.016^{c}	0.434±0.011^{e}	1.360±0.012^{d}
BT 20	4.35±0.18^{c}	0.176±0.005^{d}	0.108±0.002^{c}	0.765±0.011^{e}	0.469±0.013^{d}	1.234±0.021^{e}
BT 40	5.91±0.21^{b}	0.142±0.008^{f}	0.098±0.009^{de}	0.839±0.021^{d}	0.579±0.015^{c}	1.418±0.022^{c}
BT 80	4.41±0.19^{c}	0.161±0.009^{e}	0.105±0.003^{cd}	0.710±0.019^{f}	0.463±0.021^{d}	1.173±0.027^{f}
LSD	0.17	0.008	0.005	0.016	0.012	0.023

Means in each column with similar letters are not significantly ($p \leq 0.05$) different through the Duncan's multiple range test

The maximum CAT activity was observed in BT at 80 mg l^{-1} treatment. With regard to the effects of NT and BT on adjusting CAT activity, low NT and high BT application significantly increased CAT activity up to 50% compared to untreated control plants. However, POX activity significantly increased under employed NT up to 40 mg l^{-1}, and then decreased with NT concentration (Fig 3). Application of the high NT and BT concentrations significantly decreased POX activity; however, the final value was not lower than that of control in NT treated plants. However, the highest and the lowest POX activity were observed in plants exposed to NT at 40 mg l^{-1} and control, respectively. Generally, all tested enzymes activities were higher in NT treated plants that those of BT except CAT activity at 80 mg l^{-1}.

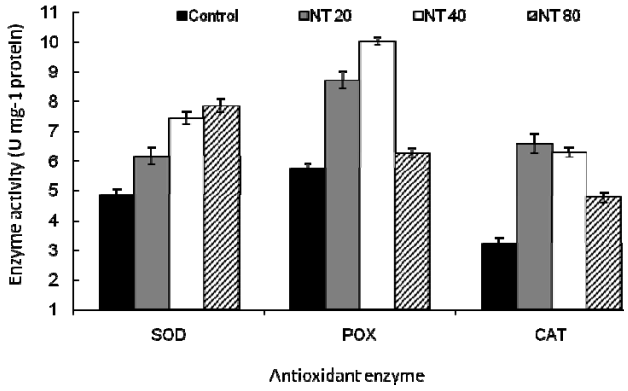

Figure 3: Influence of Nano-sized titanium dioxide (NT) concentrations (0, 20, 40 and 80 mg l^{-1}) on superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) activities in *H. niger* plants. Values are given as mean ± S.D., (n=3)

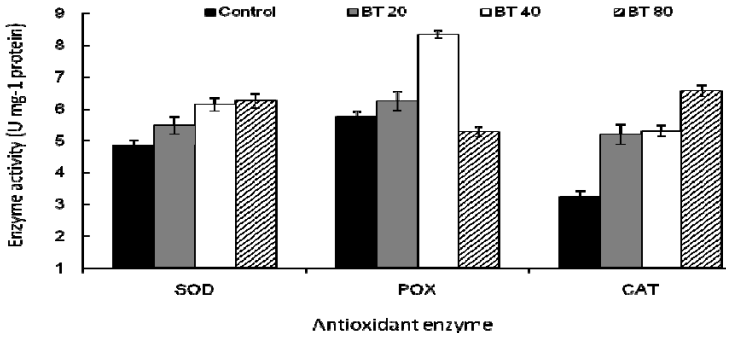

Figure 4: Influence of bulk titanium dioxide (BT) concentrations (0, 20, 40 and 80 mg l^{-1}) on superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) activities in *H. niger* plants. Values are given as mean ± S.D., (n=3)

3.2. Alkaloids biosynthesis

The results demonstrated that the applied treatments affected the shoot HYO and SCO content of black henbane plants (table 2). At high NT concentration (80 mg l^{-1}), the highest HYO content (0.286 g kg^{-1}) was obtained. By contrast, low NT concentration, 20 mg l^{-1}, resulted in high SCO content (126 g kg^{-1}). The lowest content of HYO (142 g kg^{-1}) and SCO (0.087 g kg^{-1}) were observed in plants treated with BT at 40 mg $l^{-1}$and control groups, respectively. The yield of both HYO and SCO in black henbane plants was increased with NT application at 40 mg l^{-1} as presented in table 2. However, the minimum HYO (0.710 g $plant^{-1}$) and SCO (0362 g $plant^{-1}$) yield were recorded with application of the highest BT dose and unexposed control plants, respectively. The largest total alkaloids (HYO+ SCO) yield (2.755 g $plant^{-1}$) was achieved in medium NT application (40 mg l^{-1}) mainly because of high dry weight under this situation in comparison with the other treatments (table 2).

4 DISCUSSION

Nowadays, nanoparticles happen to interest, mostly because of their possible use in varied technologies. They can be defined as objects ranging in size from 1-100 nm that because of their size may differ in the properties from the bulk materials. This can result from the high surface to volume ratio that increases their reactivity, the ability to penetrate cell membranes and possible biochemical activity. Application of nanotechnology is now widely distributed overall

the life, especially in agricultural systems. Nanoparticles because of their physicochemical characteristics e.g., large surface area to volume ratio, ability to engineer electron exchange and highly surface reactive capabilities, are among the potentially candidates for modulating the redox status and changing the growth, performance and quality of plants (Mukherjee and Mahapatra, 2009).

The ninth most abundant element and the second most abundant transition metal in the earth's crust is titanium element (about 6.32 ppm). Metal oxide nanoparticles, represented by titanium dioxide (TiO_2), is of great technological importance in the field of heterogeneous catalysis for catalytic support of a wide variety of metals (Biener *et al.,* 2005). The most important effects of TiO_2 compounds on plants are enhancement of the yield of various crops (~10-20%); an improvement of some essential element contents in plant tissues; an increase in the peroxidase, catalase, and nitrate reductase activities in plant tissues; and an enhancement of the chlorophyll content in paprika (*Capsicum anuum* L.) and green alga (*Chlorella pyrenoidosa*) (Hruby *et al.,* 2002). In our current work, different responses of the examined traits to various nano-sized TiO_2 dosages could be due to the following principal factors that previously reported by many researchers: concentration of nanoparticles, particle size and specific surface area, physicochemical properties of nanoparticles, plant species, plant age/life cycle stage, growth media conditions, nanoparticles stability, and dilution agent.

In our current experiment, NT treated *H. niger* plants at proper concentration caused higher biomass production than that of bulk and the control untreated plants. Whereas, at the highest NT concentration caused no positive impacts, when compared to the control, indicating the potential toxicity of NT particles with this adverse effect. Yin *et al.,* (2011) mentioned that increasing nanosilver concentration caused a decrease in plant root growth, which indicate an increase in phytotoxicity of nano particles. Also, in our present research activity of antioxidant enzymes, SOD, POX and CAT were affected differently under various employed NT and BT treatments. In both NT and BT treated plants, there was an increase in activity of SOD and POX at certain concentrations, however, a significant decrease was observed for CAT activity at the highest NT concentration when compared to the other NT and BT treatments.

According to Priyadarshini *et al.,* (2012) Nano-silver particles decreased H_2O_2 production and increased the efficiency of redox reactions. And also they reported that higher concentration of nano-silver enhanced the activity of H_2O_2 metabolizing enzymes.

It is well known that SOD is an enzyme that catalyzes the conversion of the O^{-2} to O_2 and H_2O_2 (Hafis *et al.,* 2011). Enhanced SOD activity of leaves under employed treatments may be interpreted as a direct response to augmented O^{-2} formation. It is previously suggested that the overexpression of SOD, if this is accompanied by increment of H_2O_2 scavenging mechanisms like POX and CAT, has been considered as a strategy to cope with oxidative damage (Kohler et al., 2006). Our results also indicated significant role of NT, particularly application of moderate levels (40 $mg.L^{-1}$), provides a protective mechanism by increasing the activity of defense enzymes. Similar result was reported by Krishnaraj *et al.,* (2012) that high activity of CAT and POX were recorded from leaf samples of plants subjected to nanosilver treatment, implying less ROS formation, resulting in less toxicity to the plants. They also reported that CAT and POX are enzymes that plays major role in ensuring protection against oxidative damage in plants exposed to nanosilver particles treatments. Lei *et al.,* (2008) stated that nanoparticles (TiO_2) declined oxidative damage in spinach chloroplast by increasing APX, SOD, POX, and CAT activity. It is suggested that combined reduction of APX, SOD and CAT activity resulted in high generation of intercellular ROS concentrations, which may be directly or indirectly be involved in the lipid peroxidation, senescence and cell death of plant (Debasis *et al.,* 2007).

In our research, the decrease in antioxidant enzymes activity observed in control plants may be directly attributed to lower secondary metabolites, HYO and SCO, biosynthesis. The content of alkaloids in plants could be increased through genetic and or environmental manipulations. However, not much information is available on the

effect of nano-sized material impacts on the content of tropane alkaloids in *H. niger* plants. Here, HYO was found as a main alkaloid in the aerial parts of black henbane plants.

5 CONCLUSION

Our results suggest that nano-sized titanium dioxide particles in proper levels may act as elicitor for physiological and biochemical responses and tropane alkaloids biosynthesis pathway in *H. niger* plants. In addition, low NT concentration showed enhancement on the production of plant HYO and SCO yield.

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