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Effects of TiO_2 nanoparticles and water-deficit stress on morpho-physiological characteristics of dragonhead (*Dracocephalum moldavica* L.) plants

Hamid MOHAMMADI[1], Mohammad ESMAILPOUR[2], Ali GHERANPAYE[1]

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ABSTRACT

Water-deficit stress is the most important environmental factors limiting plant growth, and production. Nano-titanium dioxide (nano anataseTiO_2) can have various profound effects on the crop physiological, biochemical and morphological characteristics. In the present research, the influences of different concentrations ofTiO_2 nanoparticles (NPs) (0, 10 and 40 ppm) and water-deficit stress on Dragonhead (*Dracocephalum moldavica* L.) were investigated in a factorial experiment based on randomized complete block design with three replications. Results showed that under normal irrigation, foliar application of 10 ppm TiO_2 NPs increased plant shoot dry mass and essential oils content. Under water-deficit stress condition, plants treated with 10 ppm TiO_2 NPs had more proline and much less H_2O_2 and malondialdehyde content as compared to untreated plants. Therefore, it can be concluded that proper concentration of TiO_2 NPs probably can be used as an exogenous stimuli for improvement of shoot growth and essential oil content in plants. Furthermore, water-deficit stress-induced damages such as oxidative stress and membrane damage can be ameliorated by foliar application of TiO_2 NPs at appropriate concentrations.

Key words: aromatic plants, drought stress, malondialdehyde, reactive oxygen species,TiO_2NPs

IZVLEČEK

UČINKI NANO DELCEV TiO_2 IN SUŠNEGA STRESA NA IZBRANE MORFOLOŠKE IN FIZIOLOŠKE LASTNOSTI MOLDAVSKE KAČJEGLAVKE (*Dracocephalum moldavica* L.)

Sušni stres je eden izmed najpomembnejših okoljskih dejavnikov, ki omejujejo rast in produktivnost rastlin. Uporaba nano delcev titanovega dioksida (TiO_2 nano anataza) ima lahko velike učinke na fiziološke, biokemične in morfološke lastnosti gojenih rastlin. V tej raziskavi je bil v naključnem faktorskem bločnem poskusu preučevan vpliv različnih koncentracij nano delcev TiO_2 (0, 10 in 40 ppm) in sušnega stresa na moldavsko kačjeglavko (*Dracocephalum moldavica* L.) v treh ponovitvah. Rezultati so pokazali, da sta se suha masa in vsebnost eteričnih olj povečali pri normalnem namakanju in listnem dodajanju 10 ppm TiO_2 nano delcev. V razmerah sušnega stresa so vsebovale rastline, ki so bile tretirane z 10 ppm TiO_2 več prolina in mnogo manj H_2O_2 in malonildialdehida v primerjavi z netretiranimi rastlinami. Na osnovi tega bi lahko zaključili, da bi z uporabo primerne koncentracije nano delcev TiO_2 kot zunanjega stimulanta, lahko izboljšali rast poganjkov in vsebnost eteričnih olj pri tej rastlini. Poškodbe kot sta oksidativni stres in poškodovanost membran, ki nastanejo zaradi sušnega stresa, bi lahko ublažili s foliarno aplikacijo primerne koncentracije nanodelcev TiO_2.

Ključne besede: aromatične rastline, sušni stres, malondialdehid, reaktivne oblike kisika, TiO_2 nano delci

1 INTRODUCTION

Dragonhead (*Dracocephalum moldavica* L.), a perennial aromatic herb belonging to Lamiaceae family, has antioxidative properties and can be used as food and cosmetic related preservatives

[1] Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran

[2] Department of Medicinal and Aromatic plants, Jahrom Universtiy, Jahrom, Iran. *Corresponding Author's email: hm34476@yahoo.com ; hmohammadi@azaruniv.edu

(Dastmalchi et al., 2007). It is also known as medicinal herb which is used for treatment of stomach and liver disorders. Dragonhead shoot contains various secondary metabolites such as phenolic compounds (flavonoids), essential oils and etc. which are responsible for medicinal characteristics of this plant (Dastmalchi et al., 2007; Yang et al., 2014). Production and accumulation of these metabolites depends highly on the plant growing conditions such as available soil water (Selmar and Kleinwachter, 2013; Yousefzadeh et al., 2013).

Drought stress is widespread and upcoming limiting environmental factor which adversely affects crop production especially in drought prone areas. The most common plant responses to drought stress are stomata closure and overproduction of different types of secondary metabolites in order to prevent water losses and oxidative damage, respectively (Serraj and Sinclair, 2002). Upon stomata closure, CO_2 supply to Calvin cycle is severely declined which in turn can finally result in reduced biomass production. It has been reported that severe drought stress significantly reduced root and shoot dry mass, leaf chlorophyll pigments and leaf relative water content (RWC) of Dragonhead plants (Alaei et al., 2013). Under severe drought stress condition, plant cells undergo oxidative damage due to production of Reactive Oxygen Species (ROS). Parts of these free radicals may be detoxified by antioxidant enzyme such as super oxide dismutase producing H_2O_2. However, ROSs may attack the phospholipids of the cell membrane causing lipid peroxidation and electrolyte leakage. In this case, malondialdehyde (MDA) which is one of the final products of lipid peroxidation can be considered as an evaluation factor for membrane damage. Concentration of compatible organic solutes such as proline which contributes to stabilizing sub-cellular structures and osmotic adjustment in cell cytosol are also generally enhanced in plants suffering drought stress (Ashraf and Foolad, 2007). Besides, an increased synthesis of essential oils in response to drought stress is reported in aromatic plants (Kleinwachter et al., 2015). These metabolites may contribute to prevent damage caused by free radicals. However, drought stress-related increase in the concentration of essential oils may be compensated by the related loss in biomass, resulting in almost the same overall essential oil content in both drought-stressed and well-watered plants (Selmar and Kleinwachter, 2013).

TiO_2 nanoparticles (NPs) have various profound effects on the crop physiological, biochemical and morphological characteristics (Mishra et al.2014). Exogenous application of TiO_2 NPs during spinach growth stage promoted chlorophyll formation, Rubisco activase activity and photosynthetic rate which finally lead to increase in plant dry mass (Gao et al., 2008). It is also reported that foliar application of TiO_2 NPs increased seed yield of cowpea (*Vigna unguiculata* (L.) Walp.), possibly due to increased photosynthetic rate (Owolade et al., 2008). Also, activity of antioxidant enzymes such as catalase and peroxidase has been boosted in response to TiO_2NPs application. As a result, accumulation of MDA lessened due to induction of plant antioxidant systems (Lei et al., 2008). In addition, low concentration of TiO_2 NPs reliably improved resistance of chickpea genotypes to cold stress and also alleviated cold-induced damages via activating defense mechanisms (Mohammadi et al., 2013). Therefore, TiO_2 has opened new and interesting horizon for plant physiologists in order to improve plant performance even under stress conditions. Since, the effects of TiO_2 NPs may not be the same in all environmental conditions and would be vary between different plant species and applied concentrations (Feizi et al., 2012). Thus, in the present research the influences of TiO_2 NPs concentrations on morpho-physiological and biochemical characteristics of dragonhead, as an aromatic and medicinal plant, were investigated under both water stress and non-stress conditions.

2 MATERIALS AND METHODS

2.1 Characterization and scanning electron microscopy (SEM) image of nano TiO_2

Nano TiO_2 (namely nano-anatase) was provided from the Nanomaterials Pioneers Company, NANOSANY (Mashhad, IRAN). The provided pack was characterized by laboratory analytical methods. The specific surface area (SSA) of nano TiO_2 was approximately 200-240 m^2g^{-1}, with a pore size of 0.1 ml g and purity of >99 %. The size of TiO_2 NPs were specified by Scanning Electron Microscope (SEM), and estimated to be 10-25 nm (Figure 1). The crystal characteristics of nano TiO_2 particles were determined by X-Ray Diffraction (XRD) method (XPert PRO MPD, PANalytical) in the 2θ range of 30°-120° operated at a voltage of 40 kV and a current of 40 mA with Cu Kα radiation. The XRD analytical procedure revealed that employed nanoTiO_2 particles were all exhibited in the anatase form (Figure 2).

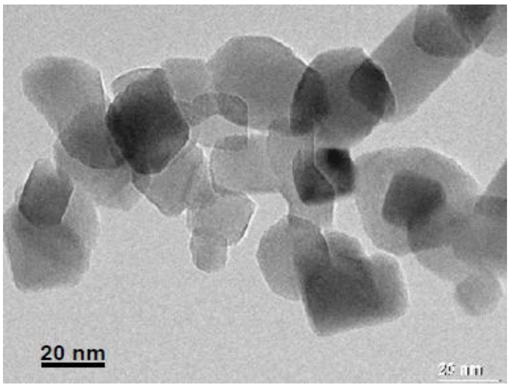

Figure 1: SEM micrograph of TiO_2 NPs. Average size of the nanoparticles was 10-25 nm

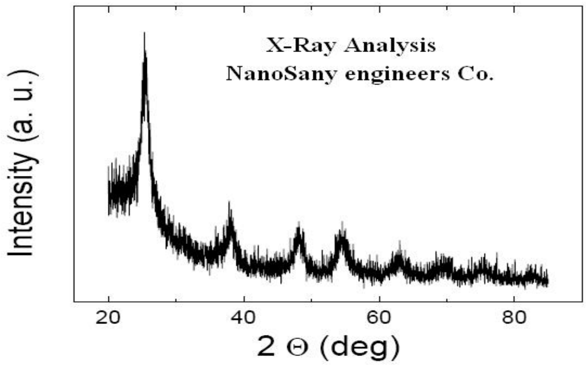

Figure 2: XRD pattern of TiO_2 NPs. XRD measurement showed that used TiO_2 NPs were all in the anatase phase

2.2 Plant materials, treatments and experimental setup

Dragonhead (*Dracocephalum moldavica* L.) is distributed in the north and northwestern parts of Iran, especially in the western parts of Azarbaijan province, and Albourz Mountains. Landrace Dragonhead seeds were prepared from Urmia University, Iran. Seeds were surface sterilized with 1 % sodium hypochlorite (NaOCl) for 5 min and then were washed three times, soaked in distilled water for 10 min. Ten seeds were directly grown in pots, containing approximately 4 kg of soil comprising a mixture of clay, silt and sand in the ratios of 4.5, 71.5 and 24 percent, respectively, with an electric conductivity (EC) of 1.52 dSm^{-1} and pH of 7.2. The concentrations of total N, P, and K were 0.07 %, 12.8 mg kg^{-1}, and 7.4 mg kg^{-1}, respectively. At the 3-4 leaf stage, plants were thinned to five per pot. Natural light supplemented with fluorescent lamps was provided in the greenhouse for 16 h per day with an irradiance of 250 $\mu molm^{-2}s^{-1}$, and temperature of 28/18 ˚C (day/night).

A factorial experiment based on randomized complete block design was carried out with three replications. Treatments were NPs of TiO_2 solutions (three levels), and water stress (two levels). NPs of TiO_2 solutions were prepared at concentrations of 0, 10 and 40 mgl^{-1} with filtered, double-distilled water. Working solutions were made by vigorous vortexing (using ultrasonic) when required. Plants were exposed to water stress at the initiation of flowering stage with daily weighting pots. Plants were irrigated every two days to achieve field capacity (FC) and 50 % of FC by pressure plate set, for control and water-deficit conditions, respectively. At the beginning of flowering stage, 50 ml of TiO_2 NPs were sprayed on the plant shoots in each pot for three successive days using hand atomizer. The plants sprayed with the same volume of distillated water were considered as control. Leaf samples of each treatment were taken at complete flowering stage and were separated into two parts. Part of each sample was immediately frozen in liquid nitrogen for two minutes and then stored at -70 ˚C for all measurements such as plastid pigments, MDA, proline and H_2O_2 contents. The other part was shade dried for a week and then used for extraction of essential oils. Plant morphological traits including shoot dry mass (SDM), root dry mass (RDM), stem branch number (SBN) and plant leaf number (PLN) were recorded at full flowering stage.

2.3 Plant physiological parameters assays

2.3.2 Leaf relative water content determination

RWC was determined according to Barr and Weatherley (1962):

RWC (%) = (FM-DM)/(TM-DM) 100

Where: FW: fresh mass; DW: dry mass; TW: turgor mass.

2.3.2 Plastid pigment measurements

Chlorophyll (Chl) and carotenoids were extracted from 0.5 g of the youngest fully expended fresh leaves by grounding them in 0.5 ml of acetone (80 % V/V). The absorption was recorded at 645 nm (Chl*a*), 663 nm (Chl*b*) and 470 nm (carotenoids) in a spectrophotometer (PG Instrument LTD T80+UV/VIS. Photosynthetic pigment contents were calculated from the following equations as described by Lichtenthaler and Wellburn (1983).

Chl*a* ($mg.g^{-1}FM$) = $11.75 \times A_{663} - 2.35 \times A_{645}$

Chl*b* ($mg.g^{-1}FM$) = $18.61 \times A_{645} - 3.96 \times A_{663}$

Carotenoids ($mg.g^{-1}FM$) = $4.69 \times A_{470} - 0.268 \times (20.2 \times A_{645} + 8.02 \times A_{663})$

2.3.3 Determination of H_2O_2 content

Hydrogen peroxide (H_2O_2) content in the leaves of Dragonhead plant was specified according to Velikova et al. (2000). Briefly, fresh tissues of leaves (0.5 g) were homogenized in an ice bath with 5 ml of TCA (0.1 % w/v). The homogenate was centrifuged at 12,000 × g for 15 min. Then 0.5 ml of the supernatant was supplemented to 0.5 ml of 10 mm potassium phosphate buffer (pH 7.0) and 1 ml of 1 m KI. Finally, the absorbance of the supernatant was recorded at 390 nm in a spectrophotometer (PG Instrument LTD $T80^+$ UV/VIS). The content of H_2O_2 was estimated by comparison with a standard calibration curve previously made by various H_2O_2 concentrations.

2.3.4 Determination of the MDA content

The level of MDA content (as an end product of lipid peroxidation) was assessed according to Heath and Packer (1968).Briefly, 0.5 g of fresh tissues of leaves were homogenized in 5 ml of 0.1 % (w/v) TCA solution and centrifuged at 12,000 × g for 15 min at 25 °C. Then, 2 ml of supernatant was added to 2 ml of 0.6 % (w/v) TBA. The mixture was incubated at 95 °C for 30 min, then cooled on ice and then samples were centrifuged at 4,000 × g for 20 min. Thereafter, the absorbance of supernatant was recorded at 532 nm.

The MDA content was calculated based on its extinction coefficient of 155 mM^{-1} cm^{-1}.

2.3.5 Determination of the proline content

Proline contents in leaf tissue were measured by the Bates et al. (1973) method. Fresh leaf material (0.5 g) was homogenized in 10 ml of 3 % aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. 2 milliliter of the supernatant was mixed with 2ml of acid ninhydrin and 2ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 ^{o}C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a spectrophotometer (PG Instrument LTD T80+UV/VIS). Appropriate proline standards were included for the calculation of proline in the samples.

2.3.6 Determination essential oil yield

Shade-dried aerial parts of the plants (50 g) were subjected to hydro-distillation for 4 h using a Clevenger type apparatus to extract essential oils (Sefidkon et al., 2004).

2.4 Statistical analysis

The data were analyzed using the SAS statistical software. A factorial experiment based on randomized complete block design was carried out with three replicates (n = 3). Duncan's Multiple Range Test (P < 0.01) was used to compare the means.

3 RESULTS AND DISCUSSION

3.1 Foliar application of TiO_2NPs under normal irrigation

Analysis of variance (ANOVA) showed that 10 ppm foliar application of TiO_2 NPs had various effects on morphology and physiology of dragonhead plants. Under normal irrigation, RDM, PLN, SBN and RWC of the plants sprayed with 10 ppm TiO_2 didn't changed, while their SDM increased significantly in comparison with untreated plants (Table 1, 2). However, application of 40 ppm TiO_2 decreased RDM, PLN, SBN, SDM and RWC (Table 2). Increase in spinach dry mass and mung bean (*Vigna radiata* L.) shoot and root length in response to TiO_2 NPs has been reported (Raliya et al., 2015). It has been shown that foliar applied NPs can enter the leaves through stomatal openings and then are translocated to various tissues via the symplast and/or apoplast (cell wall and intercellular space) pathways (Larue et al., 2012).When located inside the photosynthetic cells, TiO_2 could increase Rubisco activase activity and even its mRNA expression (Gao et al., 2008; Ma et al., 2008) and promote photosynthesis rate (Zhang et al., 2008), the possible reasons behind increase in shoot dry mass of treated dragonhead plants. In addition, NPs can sequester nutrient elements on their surface and serve as a nutrient stock to the plants (Navarro et al., 2008). Also, TiO_2 NPs *per se* may act as nano-nutrient fertilizer to improve biomass production by stimulating plant metabolic activities (Raliya et al., 2015). In the other hand, TiO_2 NPs induce production of hydroxyl radicals. These radicals have been described as potential cell wall-loosening agent by unspecific cleavage of polysaccharides (Larue et al., 2012). The TiO_2 NPs which are passed through apoplast in an appropriate concentration would thus possibly loosen cell wall structure indirectly which may leads to stimulate cell enlargement and growth of the treated plant. Also, it has been demonstrated that high concentrations of the NPs which are toxic for the plants may have positive effects on physiological performance of plants in low concentrations (Khodakovskaya and Lahiani, 2014).

Table 1: Analysis of variance (ANOVA) for studied traits in treated dragonhead plants with TiO_2 nanoparticles under water-deficit stress

Traits	Mean squares for source of variation				
	block	Factor a (TiO_2)	Factor b (Water deficit)	Interaction a×b	Error
Stem branch Number	1.16 ns	38.00 **	138.88 **	14.88 **	0.3
Leaf number	56.00 ns	68085.50 **	99755.55 **	19590.05 **	105.73
Root dry mass	0.00001 ns	0.1375 **	0.5408 **	0.0337 **	0.0002
Shoot dry mass	0.04 ns	5.53 **	15.01 **	0.89 **	0.02
RWC	1.05 ns	226.05 **	329.38 **	77.72 **	1.32
Chlorophyll *a*	0.0003 *	0.208 **	0.011 **	0.024 **	0.000006
Chlorophyll *b*	0.00004 **	0.0285 **	0.0008 **	0.0046 **	0.000001
Total chlorophyll	0.0006 **	0.3912 **	0.0186 **	0.0493 **	0.00001
Carotenoid	0.0002 **	0.1299 **	0.0126 **	0.0155 **	0.000004
Shoot MDA contents	0.0005 **	0.2467 **	3.0258 **	0.1686 **	0.00005
Shoot H_2O_2 contents	0.0005 ns	3.5325**	14.8512 **	1.4541 **	0.0102
Shoot proline contents	7.79 *	49.97 **	424.27 **	220.11 **	1.53
Essential oil yield	0.0007 ns	1.5840 **	0.0005 **	0.0369 **	0.0073

*,** Significant at the 0.05 and 0.01 probability level, respectively. ns Not significant.

Table 2: Mean comparison of physio-morphological and biochemical traits in treated dragonhead plants with TiO_2 nanoparticles under water-deficit stress

Traits	TiO_2 (ppm)					
	Untreated (0)		10		40	
	Normal irrigation	Water deficit	Normal irrigation	Water deficit	Normal irrigation	Water deficit
Stem branch Number	15.33^a	7.33^c	15.33^a	13.33^b	12.67^b	6.00^d
Leaf number	418.33^a	137.67^d	427.00^a	350.00^b	220.00^c	131.00^d
Root dry mass (g)	0.65^a	0.15^c	0.67^a	0.47^b	0.44^b	0.10^d
Shoot dry mass (g)	3.47^b	0.77^f	4.53^a	3.00^c	2.70^d	1.46^e
RWC (%)	76.00^a	60.67^c	77.67^a	68.33^b	61.33^c	60.33^c
Leaf chlorophyll *a* (mg g^{-1} FM)	0.77^a	0.57^d	0.40^f	0.44^b	0.30^c	0.31^e
Leaf chlorophyll *b* (mg g^{-1} FM)	0.30^a	0.23^b	0.15^d	0.19^c	0.13^e	0.12^f
Total chlorophyll (mg g^{-1} FM)	1.078^a	0.809^b	0.561^d	0.641^c	0.446^e	0.442^e
Leaf carotenoids (mg g^{-1} FM)	0.66^a	0.49^b	0.36^d	0.39^c	0.30^e	0.28^f
Shoot MDA contents (nmolg^{-1}FM)	0.13^f	1.16^b	0.19^e	0.63^c	0.31^d	1.31^a
Shoot H_2O_2 contents (µmolg^{-1}FM)	0.73^d	3.05^b	0.67^d	1.35^c	1.32^c	3.77^a
Shoot proline contents (µmolg^{-1}FM)	25.54^f	39.57^b	27.93^e	47.01^a	34.37^c	30.40^d
Essential oil yield (v/w) %	1.59^b	1.69^b	1.89^a	1.98^a	1.02^c	0.85^d

Values with the same lower case letters in a row within subspanner heading are not significantly different at $P < 0.05$.

In the current study, plants treated with 10 ppm TiO_2 interestingly produced more essential oils than that of control plants (Table 1, 2). Essential/volatile oil produced in dragonhead is valuable and applicable secondary metabolite since it possesses antibacterial, antimicrobial, and antioxidant activities (Dastmalchi et al., 2007). To the best of our knowledge, this study is the first report demonstrating TiO_2 NPs *per se* have stimulating effect on essential oils production. This promising influence may be due to the fact that improved essential oils synthesis may contribute to prevent damages caused by free radicals triggered to some extend by TiO_2. In the other hand, increase in SDM of plants treated with 10 ppm TiO_2 (explained above) can be the other reason underling improved plant essential oil content. In general, drought stress can also induce essential oil production in the aromatic plants. But the point is that growth and biomass of the most plants would decline concomitantly, and consequently no essential oil content may increase generally in the plants exposed to drought stress (Selmar and Kleinwachter, 2013). Therefore, present result may be the first step for future investigations on the use of TiO_2 NPs as exogenous stimuli for improvement of essential oil production in dragonhead and possibly the other medicinal plants as well.

Data analysis showed that MDA and H_2O_2 content were increased very slightly in response to 10 ppm application, showing small production of ROSs (Table 2). However, application of 40 ppm TiO_2 were increased leaf MDA and H_2O_2 content as classical markers of oxidative stress more than 2-fold in comparison with untreated plants (Table 1, 2). Genotoxicity and cytotoxicity of high concentration of TiO_2 NPs have been approved in plants. Formation of MDA as a consequence of lipid peroxidation is reported to be as a possible reason for the genotoxic potential of TiO_2 NPs (Ghosh et al., 2010). The current findings indicate that the effects of TiO_2 NPs are highly concentration dependent and can be adverse and cytotoxic to the plant if the concentration exceeded the special threshold.

In the present study, leaf chlorophyll content (*a* and *b*) and carotenoid pigments were significantly reduced in response to 10 ppm TiO_2 application (Table 2). Despite of photosynthetic pigment degradation, SDM did not decrease and even increased in response to 10 ppm TiO_2, as previously discussed. Our data suggest that chlorophyll may be associated with growth, but that other factors could also be important. One of the explanations for this observation is that TiO_2 NPs probably enhanced rate of light independent reactions of photosynthesis, since these NPs can significantly promote Rubisco activase activity and its mRNA expression (Ma et al., 2008; Gao et al., 2008). Rubisco activase plays an important role in the regulation of photosynthesis and over-expression of this enzyme increased CO_2 assimilation (Yamori et al., 2012). In the other hand, TiO_2 can significantly improve photochemical activity of photosystem II and promote energy transfer within this photosystem (Su et al., 207). Other positive effects of TiO_2 NPs on various growth aspects of plants (described above) can also be attributed to this observation. Negative effect of TiO_2 NPs on chlorophyll *a* concentration during the early growth stages of algae (*Picochlorum* sp.) has been also reported (Hazeem et al., 2016). However, there are other diverse reports showing TiO_2 NPs have no effect on chlorophyll and carotenoid content in bread wheat (Larue et al., 2012) and even promoted chlorophyll formation in spinach (Gao et al., 2008) and mung bean (Raliya et al., 2015). These different observations may be derived from the notion that NPs have different effects on different plants. Also, the size of used NPs and even application condition may be the source of various observations in different research (Larue et al., 2012).

3.2 Foliar application of TiO_2 NP under water-deficit stress

Water-deficit stress *per se* was the only factor which influenced on the measured traits when no NPs were applied under water-deficit stress. In this condition, morphological and physiological traits were affected (Table 1). Under water-deficit stress, SDM, RDM and leaf number of 10 ppm treated plants compared to untreated ones were less negatively affected by drought stress (Table 2). Also, water-deficit stressed-10 ppm treated plants still retained more photosynthetic pigments in comparison to that of counterpart treated plants under normal irrigation (Table 2). All together, these evidence shows that TiO_2 NPs probably triggers diverse reactions in different compartments within the cell.

Leaf RWC is a good indicator of plant water-status and even is a relevant screening tool for drought-tolerance (Teulat et al., 2003). In the present research, RWC has been reduced significantly in water deficit stressed-untreated dragonhead plants (Table 1, 2). It shows that transpiration rate exceeded water supply from roots to leaves and the plants sensed drought. It has been reported that significant reduce in leaf RWC of dragonhead was observed just under severe drought stress condition (Alaei et al., 2013). In this condition stomata closure and cell osmotic adjustment are the plants main strategies to prevent water losses and to cope with the cell dehydration. Following stomata closure, CO_2 as main substrate for the photosynthetic carbon reduction cycle in the chloroplast would be barely accessible to leaf mesophyll cells and the cycle would runs at the lowest rate. Therefore $NADP^+$ which is the final acceptor of excited electrons produced from the photosynthetic electron transport chain would remain in its reduced form (i.e. NADPH-H^+). In this condition, excited electrons would be transferred to oxygen, generating superoxide radicals (Selmar and Kleinwachter, 2013). These radicals are very reactive and would enter numerous further reactions and thereby generate various types of ROS, which would finally destroy the entire photosynthetic apparatus. The first defense line against ROS is dismutation of two superoxide molecules to hydrogen peroxide (H_2O_2) and oxygen by superoxide dismutase (SOD) (Melchiorre et al., 2009).

In the present study, H_2O_2 content of water-deficit stressed plants has been increased more than 4-folds, indicating parts of superoxide radicals were scavenged by SOD (Table 2). However, leaf MDA content which is used as a biomarker to measure the level of oxidative stress has also tremendously increased indicating that lipid peroxidation has occurred in water-deficit stressed-untreated plants. Crucial site of cellular injury due to drought stress is lipid peroxidation of the membranes as a result of ROS production. There is a close relation between ROS formation and oxidative stress-induced damages to cell membranes (Nazari et al., 2012). ROS degrades polyunsaturated membrane lipids, forming MDA. The degree of lipid peroxidation can be estimated by the amount of MDA in tissues. Less MDA production, more cell membrane integrity. In the present study, MDA and also H_2O_2 content of the plants treated with 10 ppm TiO_2 was interestingly much less than that of untreated plants under water-deficit condition (Table 2). It demonstrates that stress-induced damages were ameliorated as a result of 10 ppm TiO_2 application. This finding is in line with the work of Mohammadi et al. (2013) who have reported that 5 ppm TiO_2 reduced MDA production and membrane electrolyte leakage index in the leaves of chickpea under cold stress condition. Decline in MDA content of the plants treated with low concentration of TiO_2 has been attributed to stabilized composition and improved physical properties of their membranes (Mohammadi et al. 2013). TiO_2 NPs can also improve the activities of antioxidant enzyme systems such as superoxide dismutase, catalase, and ascorbate peroxidase (Lei et al., 2008). This may be the other reason behind reduced lipid peroxidation and improved membrane integrity under drought stress. However, 40 ppm treated and water-deficit stressed plants had maximum amount of H_2O_2 and MDA content (Table 2). This suggests that 40 ppm TiO_2 NPs indirectly caused to excessive generation of superoxide radicals resulting in increased lipid peroxidation and oxidative stress. This evidence can be attributed to both toxic concentration and photocatalitic properties of TiO_2. That was probably why chlorophyll and carotenoid pigments along with proline and even essential oil contents were also reduced severely (Table 2). It is not clear that TiO_2 caused to degrade pre-existing pigments or inhibited their production at transcriptom level. Generally, 40 ppm TiO_2 could not alleviate adverse effects of water-deficit stress on plant growth parameters.

The other strategy that plants take to cope with cell dehydration due to drought stress is accumulation of compatible organic solutes such as proline. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures in cell cytosol (Ashraf and Foolad, 2007). In the present research, leaf proline content of drought stressed dragonhead plants increased in comparison with control plants under normal irrigation (Table 2). In the other hand, 10 ppm treated plants had significantly much more leaf proline and higher relative water content compared with untreated plants under water-deficit stress (Table 2). This shows that TiO_2 not only can improve activity of antioxidant enzymes but also can induce synthesis of proline which is one of the osmolytes responsible for maintaining cell turgor under drought stress condition.

In the current research essential oils of untreated plants were not increased significantly in response to water-deficit stress (Table 1, 2). That was probably because their shoot dry mass has been severely decreased in this condition. In this case, no significant changes in oil content of German chamomile (*Matricaria recutita* L.) in response to various drought intensities were reported (Baghalian et al., 2011). Furthermore, overall content of terpenoids in *Melissa officinalis* L., *Nepeta cataria* L. and *Salvia officinalis* L. were decreased under drought stress condition (Manukyan, 2011). Therefore, drought stress may have no overall positive effect on escalating essential oil content in some plants. In this study, we found that application of nano TiO_2 at specific concentrations can increase essential oil content of dragonhead plants during normal and water-deficit stress conditions.

According to our data, leaf photosynthetic pigments such as chlorophyll (*a* and *b*) and carotenoids were reduced significantly in water-deficit stressed plants in comparison with counterpart plants under normal irrigation (Table 2). Degradation of these light receiving pigments would reduce photosynthetic rate and subsequently biomass production. That was probably why stem dry mass (SDM) and root dry mass (RDM) in the present study were severely declined in response to water-deficit stress. Morphological traits such as plant leaf number (PLN) and stem branch number (SBN) were also reduced. TiO_2 NPs is a photocatalist which can strongly absorb photosynthetically active radiation (PAR) and is capable of undergoing electron transfer reactions. When located in thylakoid membrane, proper concentration of TiO_2 can accelerate energy transfer from chlorophyll *b* and carotenoid to D1/D2/Cyt b559 complex within photosystem II reaction center. This would obviously lead to enhancement of oxygen evolution and generation of additional excited electrons (Su et al., 2007). Under normal environmental condition, it may proliferate photosynthetic capacity and hence promote growth of some plants (Hong et al., 2005). However, under drought stress condition when stomata are tightened and the most electron acceptors are in reduced form, production of these excessive excited electrons due to presence of TiO_2 can result in huge production of free radicals and photo oxidative damages.

4 CONCLUSIONS

Since inward flows of CO_2 to leaf mesophyll cells would be diminished upon stomata closure, drought stress-related increase in essential oil content is usually concomitant with decrease in growth and biomass production resulting in no overall changes of essential oils content in aromatic herbs. In the present research, 10 ppm foliar application of TiO_2 NPs significantly increased shoot dry mass and essential oil content of dragonhead plants under normal irrigation and water-deficit stress. If further improved, it seems that proper concentration of TiO_2 NPs can be a good candidate to be used as exogenous stimuli for boosting essential oils content in aromatic dragonhead plants without decrease in shoot biomass under both watering condition. However, the effect of TiO_2 NPs on quality or components of essential oils needs to be more clarified. MDA and H_2O_2 content of the dragonhead plants treated with 10 ppm TiO_2 were significantly much less than that of untreated plants under drought stress condition. Moreover, the treated plants not only had much more proline content but also maintained more shoot and root dry mass, leaf relative water content, leaf number and stem branches compared with untreated plants under these conditions. These evidences indicate that proper concentration of TiO_2 NPs can alleviate drought stress-induced oxidative damages and have potential to stabilize morphological characteristics of the plants under drought stress condition. In the other hand, from the results obtained, it can be concluded that foliar treatment of dragonhead plant with nano TiO_2 at high concentrations could increase deleterious effects of drought stress on physiological processes through changing the levels of MDA and H_2O_2 and stability of plastid pigments. Therefore, extensive use of TiO_2 NPs in order to lessen adverse effects of drought stress even in the case of food crops worth to be more investigated in detail. Besides, the influence of TiO_2 NPs residues on the environment and human health should also be considered and elucidated.

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