



Titanium dioxide nanoparticles increase resistance of *Lallemantia iberica* to drought stress due to increased accumulation of protective antioxidants

Niloufar Shoarian Sattari¹, Rashid Jamei^{2*}, Bahman Pasban Eslam³, Seyed Yahya Salehi Lisar⁴

1. Faculty of Natural Science, Urmia University International Pardis, Urmia, Iran

2. Biology Department, Faculty of Natural Sciences, Urmia University, Urmia, Iran

3. Crop and Horticultural Science Research Department, East Azarbaijan Agricultural and Natural Resources Research and Education Center, AREEO, Tabriz, Iran

4. Biology Department, Faculty of Natural Sciences, Tabriz University, Tabriz, Iran.

Abstract

This research was carried out to study the effect of TiO₂ and nano-TiO₂ on some chemical compounds in a medicinal plant *Lallemantia iberica* under water deficit conditions. The experiment was conducted as a factorial arrangement in a randomized complete block design with three replications. The first factor was the application of external materials as spraying with 3 levels of control, TiO₂ and nano-TiO₂, and the second factor was 100% (no stress), 75% (moderate stress) and 35% (severe stress) irrigation content. The results of measurements showed moderate stress and the use of nano-TiO₂ caused a significant increase in phenolic content, total flavonoid and antioxidant activity; severe drought stress significantly reducing these compounds. The effect of nano-TiO₂ on catalase and superoxide dismutase enzyme activity was also significant, and in the treatment of nano-TiO₂ and moderate stress, the highest activity level of these two enzymes was observed. Identification of phenolic compounds of the extracts by HPLC indicated the production of vanillic, ferulic acid and syringic acid by nano-TiO₂ spray application. Gallic acid was measurable in moderate and severe stress and caffeic acid in severe stress under the influence of nTiO₂. The sinapic acid and syringic acid contents increased under moderate stress and severe stress decreased them. Finally, it is concluded that nano-TiO₂ in comparison with TiO₂ reduces the effects of drought stress on *L. iberica* and can be used to produce this plant in water-deficit areas.

Keywords: Antioxidant activity, *Lallemantia iberica*, nano-TiO₂, phenolic compounds, drought stress

Shoarian Sattari, N., R. Jamei, B. Pasban Eslam and S.Y. Salehi Lisar. 2020. 'Titanium dioxide nanoparticles increase resistance of *L. iberica* to drought stress due to increased accumulation of protective antioxidants'. *Iranian Journal of Plant Physiology*, 10(4), 3343- 3354.

Introduction

Drought is one of the most important stress factors that affect the growth and development of

plants. Most plants respond to drought with physiological and metabolic mechanisms at the molecular, cellular, and organism levels (Osmolovskaya et al., 2018).

*Corresponding author

E-mail address: jamei.r96@gmail.com

Received: January, 2020

Accepted: August, 2020

Plants mainly have different mechanisms to confront the drought stress and adapt to drought stress by inducing a variety of physiological, biochemical and morphological responses (Mirzaee et al., 2013). A stress factor or combination of abiotic stresses causes an imbalance in cellular homeostasis due to the accumulation of reactive oxygen species such as H₂O₂ (Kaur et al., 2018). To eliminate reactive oxygen species and to protect homeostasis, plants have a single antioxidant defense system which includes antioxidant compounds (such as ascorbate, glutathione, phenolic compounds, etc.) and enzymes (such as superoxide dismutase, catalase, etc.) are involved in the ascorbate-glutathione cycle (Ahmad et al., 2018).

Flavonoids and other phenolic compounds are a large group of secondary metabolites that usually contain at least one hydroxyl group, most of which have antioxidant properties. These compounds are non-enzymatic antioxidants and have valuable anti-mutation, antimicrobial and anti-cancer properties (Ahmed et al, 2016). The concerted action of low molecular weight antioxidants, similar to polyphenols (Sgherri et al., 2003) and flavonoids (Hernandez et al., 2000), can effectively scavenge harmful radicals. Phenolic acids at high levels increase the activity of many antioxidant enzymes (Hegab and Abdelgawad, 2010).

Drought stress increases the activity of superoxide dismutase and catalase enzymes, although this activity depends on the susceptibility of different cultivars and the genetic potential of the species. (Masoumi et al., 2010). The role of these two enzymes in modulating the amount of oxygen free radicals has been proven and various researchers have considered superoxide dismutase as the primary defense mechanism against oxygen free radicals (Zandalinas et al., 2017). Research on safflower under drought stress to evaluate the activity of antioxidant enzymes showed an increase in catalase activity by increasing drought levels (Sirusmehr et al., 2015). The response of yield and composition of essential oils to water stress varies with duration and severity of stress. It is reported that the production of primary metabolites and the

yield of volatile oils may decrease when plants are under water stress (Panrong et al., 2006).

The Balangu (*Lallemantia iberica*) is an annual plant, herbaceous, short with opposite leaves, belonging to the Lamiaceae family and is distributed in southwest Asia and Europe (Ursu and Burcean, 2012). The results of the study by Khosravi Dehaghi et al. (2016) identify *L. iberica* as a valuable medicinal plant, containing substances responsible for various pharmacological activities. The antioxidant activity of compounds isolated from *L. iberica* also has been confirmed. Two sterols, β -sitosterol acetate, β -sitosterol, one triterpenic acid, ursolic acid, one polyphenol, rosmarinic acid and six flavonoids, Luteolin-7-O-glucoside, 4'-methoxy-luteolin-7-O-glucoside, apigenin-7-O-glucoside, Luteolin, diosmetin, apigenin were isolated from the ethyl acetate and methanol extracts of *L. iberica*. Despite the efforts made by agricultural scientists, crop yields and efficiency are still lower than their potential. This is due to the low yields of water and nutrients used by crops and the imposition of intense competition from pests and weeds on the plant.

Nanotechnology is a new scientific approach that can break down these barriers and is expected to increase crop yield and efficiency in the coming years and respond to the challenges of human food security. TiO₂ nanoparticles are among the most widely used nanomaterials but their interaction with plants has not been much investigated (Mutlu et al., 2018). Some studies have shown that the nano-TiO₂ application has positive and stimulating effects on plants (Ghosh et al., 2010); enhancing root and shoot growth (Haghighi and da Silva, 2014). A study on basil showed that the application of nano-TiO₂ under drought stress improved the negative effects of stress and increased catalase. The highest enzyme activity was at 0.03% TiO₂ concentration and irrigation regime at 40 and 70% of field capacity, indicating the effect of nano-TiO₂ at this concentration in reducing oxidative damage (Kiapour et al., 2015). TiO₂ nanoparticles increase plant resistance to environmental stresses and reduce free radicals (Hong et al., 2005). Lei et al. (2008) showed that nano anatase TiO₂ treatment can activate superoxide dismutase, catalase, ascorbate

peroxidase and glutathione peroxidase enzymes in spinach chloroplasts.

This study aimed to investigate the effects of TiO₂ nanoparticles application in comparison with TiO₂ under drought conditions on some biochemical properties of *Lallemantia iberica*.

Materials and Methods

Method of Cultivation and Treatment

The same seeds were planted after being sterilized with 3% sodium hypochlorite solution, on sandy loam soil. Irrigation up to the three-leaf stage was the same for all the treatments. After the three-leaf stage irrigation of control plants, according to field capacity, for moderate drought 75% and severe drought 35% was done. Stress was applied sixty days after planting to harvesting stage. The first treatment of plants was performed in the eight-leaf stage after the preparation of nano- TiO₂ anatase and bulk TiO₂ solutions (0.03% concentration). Distilled water replaced bulk and nano- TiO₂ solutions for spraying of control plants. The second treatment was performed in the flowering stage and the plant samples were harvested 14 days after the second treatment.

Determination Antioxidant Enzymes Activity

To measure antioxidant enzymes, 0.1 g fresh leaf tissue with 2 ml potassium phosphate buffer (50 mM) with pH = 7.8, after squashing, the extract was centrifuged at 10,000 g for 10 min and the upper phase of the solution was used for enzymatic assays.

Determination of Catalase (CAT; EC 1.11.1.6) activity

The catalase activity assay was performed based on a decrease in the absorbance of hydrogen peroxide at 240 nm. The reaction mixture consisted of 600 µl of potassium phosphate buffer (50 mM), 300 µl of hydrogen peroxide (33 mM) and 100 µl of enzyme extract. Adding hydrogen peroxide to the reaction mixture immediately initiated the reaction and the decrease in hydrogen peroxide uptake at 240 nm wavelengths every 30s was measured with a

spectrophotometer (UNIKO UV2100, USA) for 2 min. In order to calculate the enzymatic unit, the extinction coefficient of 40 mM⁻¹cm⁻¹ was used (Simon et al., 1974).

Determination of Superoxide dismutase (SOD; EC 1.15.1.1) Activity

To measure of superoxide dismutase activity, 3 ml reaction mixture consisted of 2650 µl of potassium phosphate buffer (50 mM), 200 µl of EDTA (0.1 mM) and potassium cyanide (0.3 mM), 100 µl of NBT (nitro blue tetrazolium) and 50 µl of the enzyme extract were mixed. The mixture was incubated at 350 µmolm⁻²s⁻¹ for 5 to 8 min then added 50 µl of riboflavin and kept again for 12 min at the same conditions. The absorbance at 560 nm wavelength was read by a spectrophotometer (Giannopolitis and Ries, 1977).

Preparation of Methanolic Extracts

To prepare the methanolic extract to measure the total phenol and flavonoid and antioxidant activity, 0.5 ml of methanol (80% v / v) was added to 0.5 g of plant tissue and centrifuged at 10,000 g for 15 min (Hahlbrock and Scheel, 1989).

Determination of Total Phenolic Content

The total phenolic content of the extracts was measured, using the Folin-Ciocalteu reagent (Meda et al., 2005). 100 µl of the extract solution was mixed with 100 µl of Folin-Ciocalteu reagent (50% V / V) and 2 ml of sodium carbonate solution (2%). The samples were kept at the environment temperature for 30 min after vortex. The absorbance of the solution was measured at 720 nm wavelength compared to the control. Gallic acid was used to draw the standard curve and the total amount of phenolic compounds in the extract was calculated using the standard curve equation. The results were expressed in mg Gallic acid /g of plant fresh weight.

Determination of Total Flavonoid Content

The aluminum chloride colorimetric method was used for the measurement of the total flavonoid

content with minor changes (Chang et al., 2002). The standard calibration curve of quercetin was used to determine the total flavonoid content in each sample. The amount of 1.5 ml of methanol (80%), 100 ml of aluminum chloride (10%), 100 ml of potassium acetate (1 M) and 2.8 ml of distilled water mixed with 500 µl of plant extract, then was incubated for 40 min at room temperature and the absorbance of the solution was measured at 415 nm compared to the control. The quercetin-free solution was used as control. The total flavonoid content of the extracts was measured by placing the samples in the standard curve equation and the total flavonoid content was expressed as mg quercetin equivalent (QE)/g of plant fresh weight.

Assay of DPPH Radical Scavenging

To determine the potency of the plant extract in trapping DPPH free radicals, 2 ml of the extract was mixed with 2 ml of methanolic DPPH solution (0.004%). The tubes were kept at room temperature and dark for 30 min. The absorbance of the samples was measured at 517 nm in comparison to the control. The control solution consisted of 2 ml DPPH and 2 ml methanol (80%). DPPH radical scavenging percent was calculated by the following equation: A control adsorbed control solution at 517 nm and A sample adsorbed sample at 517 nm (Miliauskas et al., 2004).

$$\% I = (A \text{ control} - A \text{ sample}) / A \text{ control} \times 100$$

Table 1.

Comparison of means effects of TiO₂ and nTiO₂ on Galic acid, Rutin, Caffeic acid and Hydroxy benzoic acid contents in *L.iberica* under drought conditions.

Treatment	Irrigation content	Galic acid µg/gDw	Rutin µg/gDw	Caffeic acid µg/gDw	Hydroxy benzoic µg/gDw
no treatment	100% FC	15.1a±0.46	14.01a±0.11	0±0	0±0
	75% FC	0c±0	14.02a±0.14	0±0	0±0
	35% FC	0c±0	0b±0	0±0	0±0
TiO ₂	100% FC	13.62b±0.44	13.99a±0.14	0±0	0±0
	75% FC	0c±0	13.98a±0.11	0±0	0±0
	35% FC	0c±0	0b±0	0±0	0±0
nTiO ₂	100% FC	13.44b±0.46	14.08a±0.12	0±0	0±0
	75% FC	13.6b±0.26	13.95a±0.15	0±0	0±0
	35% FC	13.68b±0.18	0b±0	3±1.73	0±0

In each column, the averages that have at least one letter in common do not differ significantly at the statistical level of 5%.

Determination of Phenolic Compounds of Extracts by HPLC

The amount and type of some phenolic compounds were determined by HPLC. For this purpose, the Knuer Model 2000 device with maxi-star k-1000 quadruple pumps and the C18 EUROSPHER-10 reverse-phase column with 25 cm length and 4.8 mm diameter and 5 µm particle size and a UV-Vis detector, set at 280 nm, was used. The mobile phase was water, acetic acid (2 %) and acetonitrile with a flow rate of 0.8 mm/min and an injection volume of 20 µl. The final calculation was done in µg/g of the dry weight of the plant.

Statistical Analysis

The results were statistically analyzed by SPSS software version 25. The mean comparisons were performed with Duncan's multiple range test and a randomized complete block design was used to compare the mean of interaction effects.

Results

Catalase Activity

The Results showed that nano-TiO₂ application and drought stress and their interaction with catalase were significant on the statistical level of 1%. The groupings of treatments showed that in the samples with nano-TiO₂ treatment under moderate stress the highest catalase content was observed (Fig.1A).

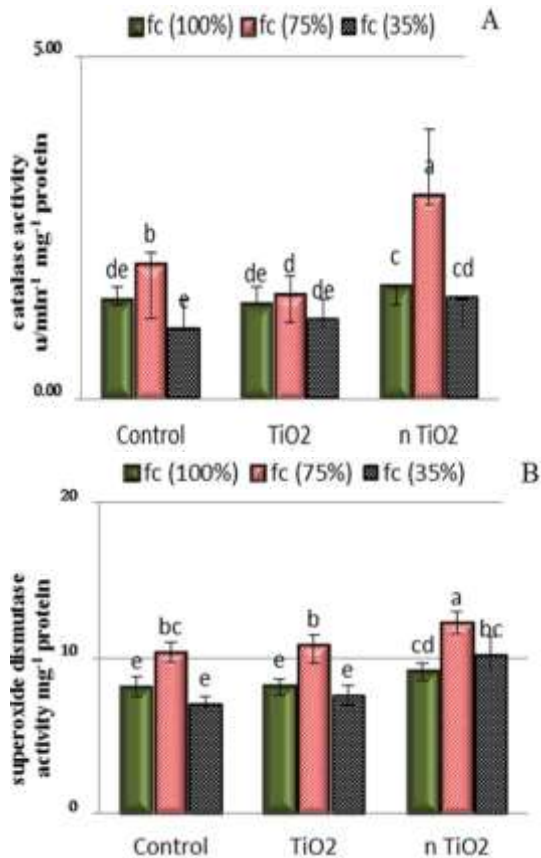


Fig. 1. Interaction effects of TiO₂ and nano-TiO₂ on catalase (A) and superoxide dismutase (B) in the shoot of *L. iberica* under drought stress. The averages with at least one letter in common did not differ significantly at the 0.05 level.

Superoxide Dismutase Activity

The results showed that the effect of nano-TiO₂, stress, and their interaction were significant at 0.01 level on superoxide dismutase activity. The highest level of superoxide dismutase was observed in samples with nano-TiO₂ with moderate stress (Fig. 1B).

Total Phenolic Content

The effects of nano-TiO₂, stress and TiO₂ + stress on total phenolic were significant at 0.01 level. In the samples treated with nano-TiO₂, under moderate stress, the highest total phenolic content was observed (Fig. 1IA). Based on the measurements, the total amount of phenolic compounds in the shoot of

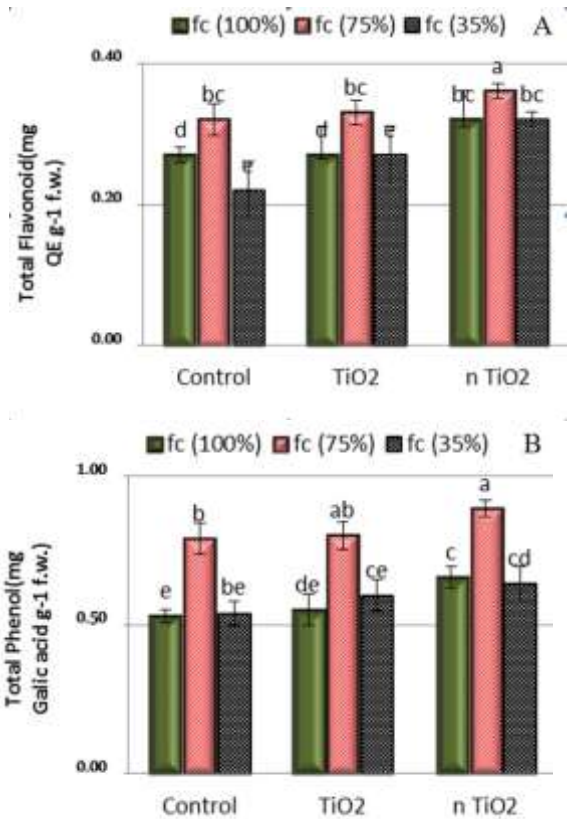


Fig. 2. Interaction effects of TiO₂ and nano-TiO₂ on total flavonoid (A) and total phenolic content (B) in the shoot of *L. iberica* under drought stress. The averages with at least one letter in common did not differ significantly at the 0.05 level.

L. iberica showed a significant increase with moderate drought stress, and the presence of nano-TiO₂ increased this trend, but severe stress (35% FC) reduced these compounds to moderate stress and the application of nano-TiO₂ partially prevented it from being reduced.

Total flavonoid Content

Total flavonoid content in the shoot was dependent on the soil moisture level and was observed at 75% of field capacity and this indicates the involvement of flavonoid in the response of the tested plants to soil water deficit. The lowest flavonoid content was observed at 35% of field capacity. Analysis of variance showed that the effect of nano-TiO₂ and the effect of stress on total flavonoids were significant on

the statistical level of 0.05 (Fig.IIB). Under such conditions, the application of TiO₂ nanoparticles caused flavonoid accumulation, indicating the activation of the antioxidant system in response to drought stress by nano-TiO₂. The presence of nano-TiO₂ in different irrigation regimes resulted in an increase in flavonoid content into the absence of nanoparticles.

Antioxidant Activity

Results showed that the effect of stress and the presence of nano-TiO₂ on antioxidant activity were significant at 0.05 level of probability. The DPPH test results showed an increase in free radical scavenging at moderate stress levels and foliar application of nano-TiO₂ improved this process (Fig.III).

Antioxidant Compounds Analysis by HPLC

The effect of stress and nano-TiO₂ on gallic acid was significant on statistical level of 1%. Gallic acid was measured despite moderate and even severe stress in the presence of nano-TiO₂ in the plant. However, in plants without nano-TiO₂ treatment, gallic acid was not observed. P-hydroxybenzoic acid was not found in the tested plants (Table 1).

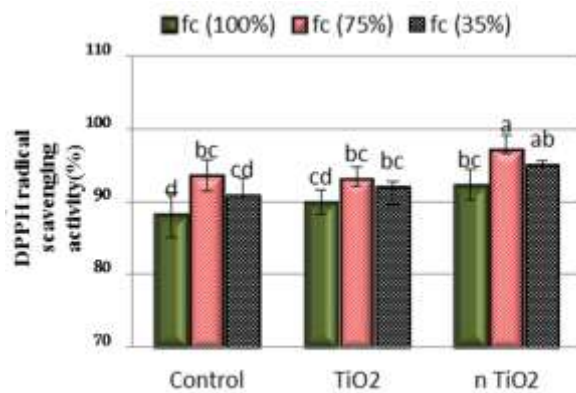


Fig. III. Interaction effects of TiO₂ and nano-TiO₂ on the activity of DPPH radical scavenging in the shoot of *L. iberica* under drought stress. The averages with at least one letter in common did not differ significantly at the 0.05 level.

Results showed that the effect of stress on the rutin with statistical level of 0.01 was significant but

the effect of TiO₂ was not significant. The stress and application of nano-TiO₂ on rutin, which had the highest molecular weight among the compounds studied and had less antioxidant power than the other compounds, had no significant effect. Rutin was not observed in plants under severe stress, possibly due to the effect of severe drought on the enzymes of its biosynthetic pathway (Table 1).

The results of HPLC analysis showed that vanillic acid was observed and measured only with nano-TiO₂ in the *L. iberica* plant. The effect of stress and nano-TiO₂ on vanillic acid was significant at 0.01 level; As the level of drought stress increased, its content decreased and the highest amount was observed in nano-TiO₂ treatment under non-stress conditions. (Table 2). The results indicated that the effect of stress and nano-TiO₂ on caffeic acid was not significant. Ferulic acid was only observed in nano-TiO₂-treated plants, and the effect of TiO₂ and the interaction of stress and TiO₂ was significant at 0.01 level (Table 2). Application of nTiO₂ and the effect of stress on sinapic acid were significant at 0.01 level and the amount of sinapic acid was increased in nano-TiO₂ treated plants compared to control plants and the highest level was observed in the presence of nano-TiO₂ in plants under moderate stress. Syringic acid was only measurable with the presence of nano-TiO₂ in comparison to untreated plants with TiO₂ and the results showed that the effect of stress and nTiO₂ was significant at 0.01 level on syringic acid. The highest content was similar to sinapic acid in nano-TiO₂ treated plants and observed under moderate stress (Table 2).

Discussion

Catalase had an effective role in signal transduction in defense and germination responses. Catalase is a titrimetric enzyme that breaks down hydrogen peroxide into water and oxygen. Catalase activity is essential to remove toxic hydrogen peroxide from various stresses and to avoid oxidative stress-related damage (He et al., 2014). Increased catalase activity in *Nigella sativa* under drought stress has been reported (Ghorbanli et al., 2011). Studies on barley (Karami and Sepehri, 2018) and broad bean under

Table 2.

Comparison of means effects of TiO₂ and nTiO₂ on vanilic, syringic, ferulic and sinapic acid contents in *L.iberica* under drought conditions.

Treatment	Irrigation content	Vanilic acid µg/gDw	Syringic acid µg/gDw	Ferulic acid µg/gDw	Sinapic acid µg/gDw
no treatment	100% FC	0c±0	0d±0	0±0	10.33d±0.63
	75% FC	0c±0	0d±0	0±0	32.56b±3.06
	35% FC	0c±0	0d±0	0±0	7.59e±0.89
TiO ₂	100% FC	0c±0	0d±0	0±0	5.85e±0.88
	75% FC	0c±0	0d±0	0±0	21.72c±3.32
	35% FC	0c±0	0d±0	0±0	0f±0
nTiO ₂	100% FC	6.24a±0.28	44.47b±3.52	29.92a±2.92	12.96d±0.24
	75% FC	2b±0.25	56a±1.55	29.8a±0.6	59.89a±3.89
	35% FC	1.93b±0.08	35c±5.2	29.68a±3.68	10.53d±1.06

In each column, the averages that have at least one letter in common do not differ significantly at the statistical level of 5%.

saline soil conditions (Abdel Latef et al., 2017) have also shown that the application of nano-TiO₂ increased catalase activity. According to the study on basil plant under drought stress, the highest catalase activity was observed under moderate drought stress at 0.03% concentration of nano-TiO₂, which is in agreement with the results of the present experiment. Investigation of the effect of TiO₂ nanoparticles on antioxidant enzymes in onions showed that the presence of nano-TiO₂ increased the activity of superoxide dismutase. The study on the *Spirodela polyrrhiza* showed that the activity of superoxide dismutase significantly increased with increasing n TiO₂ concentration. Increased superoxide dismutase activity can improve the antioxidant system to remove reactive oxygen species (Movafeghi et al., 2018). The increase of this enzyme in the leaf and root of *Mentha pulegium* has been shown under drought conditions (Niknam and Hassanpour, 2011).

Drought stress caused oxidative stress in the *L. iberica* and plant increased compounds that are mainly in the secondary metabolite group, especially phenolic compounds, to counteract damage induced by free radicals. In different plants, the content of these substances varies due to differences in drought resistance. According to the experiments by Morello et al. (2005), as the amount of olive tree irrigation increased, the total phenolics and PAL enzyme levels in the fruit decreased; therefore, the activity of this enzyme is strongly influenced by environmental conditions that play an important role in the control of total phenolic content. An important function of

phenols is their role in defense mechanisms (Solar et al., 2006). The phenolic content of cumin shoot under drought increased significantly (Bettaieb Rebey et al. 2012). In *Salvia officinalis*, total phenolic content increased in response to nano-TiO₂. The highest total phenolic content was observed in nano-exposed plants at concentrations of 100 and 200 mg / L, respectively (Ghorbanpour, 2015). Besides, Zhang et al. (2018) reported an increase in the amount of leaf phenolic content in *Populus tremula*. Flavonoid antioxidants have a protective effect during drought stress. Many flavonoids are active components of medicinal plants that play important roles in plants as physiologically active compounds, protective agents against stresses and uptake agents (Tattini et al., 2004). There is some evidence that flavonoids in plants play a role in reducing the effects of harmful radiation and toxic substances as well as the plant's response to stress through controlling auxin transport (Beveridge et al., 2007). The results showed that the presence of nTiO₂ increased the amount of flavonoid compounds. According to experiments on *Glycyrrhiza glabra* seedlings, the application of CuO and MgO nanoparticles caused flavonoid accumulation (Oloumi et al., 2015).

In a similar study that investigated the effect of drought stress on biochemical constituents and antioxidant activity of Cumin, four system models were used to investigate the effect of drought stress on antioxidant properties. DPPH test showed that the highest IC50 was in the control and the lowest in severe drought stress. But in the beta-carotene system model, the highest antioxidant activity was

related to moderate stress treatment (Bettaieb Rebey et al., 2012). In another study, Ahmed et al. (2012) investigated the effect of drought and salinity on total phenolic, flavonoid and antioxidant activities of *Lepidium sativum* and similar to the present study used DPPH test to evaluate antioxidant activity. The results showed that the amount of total phenol, total flavonoid and free radical scavenging activity increased under moderate stress and decreased in severe salinity and drought stress. In the presence of nano-TiO₂, the antioxidant activity of plant extracts is largely dependent on the concentration of phenolic compounds in the plant (Ghorbanpour, 2015). In the present study, it was found that the *L. iberica* was adapted to moderate drought, similar to *Cuminum cyminum* L. (Bettaieb Rebey et al., 2012).

Gallic acid with three hydroxyl groups showed good antioxidant activity related to the placement of the hydroxyl groups on the benzene ring. After deprotonisation, the gallic acid anion is formed which has a large effect on free radical scavenging. The results of research on the antioxidant power of benzoic acid and cinnamic acid derivatives showed that gallic acid has an antioxidant activity similar to caffeic acid and less than p-hydroxybenzoic acid (Velika and Kron, 2012). According to experiments performed by Szwajgier and Pielecki (2005), the measurement methods used strongly influence the results obtained. In the DPPH free radical scavenging method, vanillic acid has higher antioxidant power than sinapic and ferulic acid, with the same scavenging power. Caffeic acid also appeared weaker than sinapic and ferulic acid. Application of nano-TiO₂ in *L. iberica* produced vanillic acid which has higher antioxidant power than other compounds studied in the phenylpropanoid pathway. Caffeic acid, a polyphenol derived from hydroxycinnamic acid in the lignin biosynthesis pathway, leads to the conversion of ferulic acid and other structural components of lignin and conversion of caffeic acid to ferulic acid with the caffeate O-methyltransferase enzyme (2.1.1.68) (Boerjan et al., 2003). TiO₂ nanoparticles probably converted caffeic acid to ferulic acid by affecting the activity of this enzyme in *L. iberica* under both stress and drought stress conditions. Researches have shown that

sinapic acid esters regulate the inhibition of germination, growth, and dormancy break by abscisic acid in *Arabidopsis* with a negative feedback cycle (Bi et al., 2017). Nano-TiO₂ probably over-regulated the production of syringic acid, ferulic and sinapic acid by affecting the expression of biosynthesis pathway genes in phenylpropanoid compounds. Research on basil showed that the expression of 4-coumarate CoA ligase (EC6.2.1.12) and cinnamate-4-hydroxylase (EC1.14.14.91) genes in the phenylpropanoid pathway decreased under severe stress and the expression of cinnamyl alcohol dehydrogenase (EC 1.1.1.195) gene did not change (Abdollahi Mandoulakanet al., 2017). In a study by Ongphimai et al. (2013), there was a direct relationship between total phenolic content and total antioxidant activity in phytochemical extracts of different fruits. The highest phenolic content in fruits resulted in the highest antioxidant activity, indicating that phenols may be involved in total antioxidant activity in fruits. The inhibitory potency of different extracts largely depends on the number and position of the hydroxyl groups and the molecular weight of the phenolic compounds. In lower molecular weight phenolic compounds, hydroxyl groups are easily available (Jung et al., 2006). Some studies conducted to evaluate the antioxidant activity of benzoic acid and cinnamic acid derivatives have shown a relationship between structure and activity (Cuvelier et al., 2014). It was shown that the antioxidant potential depends on the replacement of the phenol ring with the hydroxyl groups at the ortho and para positions and the methylation of the phenol ring in the ortho position relative to the hydroxyl groups also increases activity. The researchers also noted that the higher antioxidant activity of the cinnamic acid derivatives compared to benzoic acid could be attributed to the presence of the CH-CH-COOH group in these molecules.

Phenylpropanoids are produced as secondary metabolites of plants during developmental stages in response to stressful conditions. Among the active components of the plant defense system, aromatic phenylpropanoids play an important role in protecting plant cells against drought and heat. Drought stress can be

suitable for the synthesis of phenolic compounds and indicates the regulation of the activity of related genes in response to drought. The increase in the accumulation of phenolic compounds, monoterpenes, alkaloids and other compounds is consistent with the activity of key genes in their biosynthetic pathway under severe stress and is established in recent studies (Yadav et al., 2013; Rastogi et al., 2014). It can be concluded that the phenylpropanoid pathway in *L. iberica* plant is more likely to produce sinapic acid, and the application of nano-TiO₂ induces the expression of genes involved in the production of some enzymes of this pathway to produce syringic acid, sinapic and ferulic acid.

Conclusion

This study showed that the foliar application of TiO₂ nanoparticles had a significant effect on some of *L. iberica*'s traits, which may indicate that titanium dioxide nanoparticles penetrate the leaf more readily than titanium dioxide particles. The data presented show that the application of titanium dioxide nanoparticles increased resistance of *L. iberica* to drought stress due to increased accumulation of protective antioxidants which in combination with increased antioxidant enzymes, total phenolic compounds, total flavonoids, antioxidant activity and produced some phenols under dry soil. Flavonoids and total phenolic compounds that were increased by moderate drought stress and the presence of nano-TiO₂ may indicate that *L. iberica* depends on large amounts of phenolic compounds to confront the negative effects of stress and antioxidant defense. Considering that *L. iberica* is cultivated for medicinal use and to achieve the maximum biological yield, irrigation regime (75% FC) with 0.03 concentration of titanium dioxide nanoparticles was identified as the best treatment in this study.

References

- Abdel Latef, A. A. H. A., A. K. Srivastava, M. S. A. El-sadek, M. Kordrostami and L-S.P. Tran.** 2017. 'Titanium Dioxide Nanoparticles Improve Growth and Enhance Tolerance of Broad Bean Plants under Saline Soil Conditions'. *Land Degradation and Development*, 29(4): 1065-1073.
- Abdollahi Mandoulakani, B., E. Eyvazpour and M. .** 2017. 'The Effect of Drought Stress on the Expression of Key Genes Involved in the Biosynthesis of Phenylpropanoids and Essential Oil Components in Basil (*Ocimum basilicum* L.)'. *Phytochemistry*, 139: 1-7.
- Ahmad, P., M.N. Alyemeni, M.A. Ahanger, L. Wijaya, P. Alam, A. Kumar and M. Ashraf.** 2018. 'Upregulation of Antioxidant and Glyoxalase Systems Mitigates NaCl Stress in *Brassica juncea* by Supplementation of Zinc and Calcium'. *Journal of Plant Interaction*, 13: 151–162.
- Ahmed, A.R., A.M.M. Gabr, H.M.A. AL-Sayed and I. Smetanska.** 2012. 'Effect of Drought and Salinity Stress on Total Phenolic, Flavonoids and Flavonols Contents and Antioxidant Activity in In vitro Sprout cultures of Garden cress (*Lepidium sativum*)'. *Journal of Applied Sciences Research*, 8(8): 3934-3942.
- Bettaieb Rebey, I., I. Jabri-Karoui, I. Hamrouni-Sellami, S. Bourgou, F. Limam and B. Marzouk.** 2012. 'Effect of Drought on the Biochemical Composition and Antioxidant Activities of Cumin (*Cuminum cyminum* L.) Seeds'. *Industrial Crops and Products*, 36(1): 238–245.
- Beveridge, C.B., U. Mathesius, R.J. Rose and P.M. Gresshoff,** 2007. 'Common Regulatory Themes in Meristem Development and Whole-Plant Homeostasis'. *Current Opinion in Plant Biology*, 10(1): 44-51.
- Bi, B., J. Tang, S. Han, J. Guo and Y. Miao,** 2017. 'Sinapic Acid or Its Derivatives Interfere with Abscisic Acid Homeostasis During *Arabidopsis thaliana* Seed Germination'. *BMC Plant Biology*, 17(1): 99-110.

- Boerjan, W., J. Ralph and M. Baucher.** 2003. 'Lignin Biosynthesis'. *Annual Review of Plant Biology*, 54: 519–546.
- Chang, C.C., M.H. Yang, H.M.Wen and J.C. Chern.** 2002. 'Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods'. *Journal of Food and Drug Analysis*, 10(3): 178-182.
- Cuvelier, M.E., H. Richard and C. Berset.** 2014. 'Comparison of the Antioxidative Activity of Some Acid-Phenols: Structure-Activity Relationship'. *Bioscience, Biotechnology, and Biochemistry*, 56 (2): 324-325.
- Ghorbanli, M., G.R. Bakhshi Khaniki, S. Salimi Elizei and M. Hedayati.** 2011. 'Effect of Water Deficit and Its Interaction with Ascorbate on Proline, Soluble Sugars, Catalase and Glutathione Peroxidase Amounts in *Nigella sativa* L.'. *Iranian Journal of Medicinal and Aromatic Plants*, 26(4): 466- 476.
- Ghorbanpour, M.** 2015. 'Major Essential Oil Constituents, Total Phenolics and Flavonoids Content and Antioxidant Activity of *Salvia officinalis* Plant in Response to Nano-Titanium dioxide'. *Indian Journal of Plant Physiology*, 20(3): 249–256. 13.
- Ghosh, M., M. Bandyopadhyay and A. Mukherjee.** 2010. 'Genotoxicity of Titanium dioxide (TiO₂) Nanoparticles at Two Trophic Levels: Plant and Human Lymphocyte'. *Chemosphere*, 8(10): 1253–1262.
- Giannopolitis, C.N. and S.K. Ries,** 1977. 'Superoxide Dismutase I. Occurrence in Higher Plant'. *Plant Physiology*, 59(2): 309-314.
- Haghighi, M. and J.A.Teixeira da Silva.** 2014. 'The Effect of N-TiO₂ on Tomato, Onion, and Radish Seed Germination'. *Journal of Crop Science and Biotechnology*, 17(4): 221-227.
- Hahlbrock, K. and D. Scheel.** 1989. 'Physiology and Molecular Biology of Phenylpropanoid Metabolism'. *Annual Review of Plant Physiology and Plant Molecular Biology*, 40: 347-369.
- He, H., D. Vidigal, I.B. Snoek, S. Schnabel, H. Nijveen, H. Hilhorst and L. Bentsink.** 2014. 'Interaction Between Parental Environment and Genotype Affects Plant and Seed Performance in *Arabidopsis*'. *Journal of Experimental Botany*, 65(22): 6603-6615.
- Hegab, M. M. and H. Abdelgawad.** 2010. 'Methanol Extract Potential of Field Bindweed (*Convolvulus arvensis* L.) for Wheat Growth Enhancement'. *International Journal of Botany*, 6(3): 334-342.
- Hernandez, J.A., A. Jimenez, P. Mullineaux and F. Sevilla.** 2000. 'Tolerance of Pea (*Pisum sativum* L.) to Long-Term Salt Stress Is Associated with Induction of Antioxidant Defenses'. *Plant Cell Environment*, 23(8): 853–862.
- Hong, F., J. Zhou, C. Liu, F. Yang, C. Wu, C. Zheng and P. Yang,** 2005. 'Effect of Nano-TiO₂ on Photochemical Reaction of Chloroplasts of Spinach'. *Biological Trace Element Research*, 105(1-3): 269–279.
- Jung, C.H., H.M. Seog, I.W. Choi, M.W. Park and H.Y. Cho,** 2006. 'Antioxidant Properties of Various Solvent Extracts from Wild Ginseng Leaves'. *LWT- Food Science and Technology*, 39 (3): 266-274.
- Karami, A. and A. Sepehri.** 2018. 'Nano Titanium Dioxide and Nitric Oxide Alleviates salt Induced Changes in Seedling Growth, Physiological and Photosynthesis Attributes of Barley'. *Zemdirbyste-Agriculture*, 105(2):123-132.
- Kaur, H., G. Sirhindi, R. Bhardwaj, M.N. Alyemeni, K.H.M. Siddique and P. Ahmad.** 2018. '28-Homobrassinolide Regulates Antioxidant Enzyme Activities and Gene Expression in Response to Salt- and Temperature-Induced Oxidative Stress in *Brassica juncea*'. *Scientific Reports*, 8(1): 8735.
- Khosravi-Dehaghi, N., A.R. Gohari, S.S. Sadat-Ebrahimi, H. Naghdi-Badi and Y. Amanzadeh,** 2016. 'Phytochemistry and Antioxidant Activity of *Lallemantia iberica* Aerial Parts'. *Research Journal of Pharmacognosy*, 3(3): 27-34.
- Kiapour, H., P. Moaveni, D. Habib, and B. Sani,** 2015. 'Evaluation of the Application of Gibberellic Acid and Titanium Dioxide

- Nanoparticles under Drought Stress on Some Traits of Basil (*Ocimum basilicum* L.)'. *International Journal of Agronomy and Agricultural Research*, 6(4): 138-150.
- Lei, Z., M.Y. Su, X.C. Wu, C.X. Qu, L. Chen, H. Huang, X.Q. Liu and F.S. Hong.** 2008. 'Antioxidant Stress Is Promoted by Nano-Anatase in Spinach Chloroplasts under UV-Beta radiation'. *Biological Trace Element Research*, 121(1): 69-79.
- Masoumi, H., M. Masoumi, F. Darvish, J. Daneshian, G. Nourmohammadi and D. Habibi,** 2010. 'Change in Several Antioxidant Enzymes Activity and Seed Yield by Water Deficit Stress in Soybean (*Glycine max* L.) Cultivars'. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 38(3): 86- 94.
- Meda, A., C.E. Lamien, M. Romito, J. Millogo and O.G. Nacoulma,** 2005. 'Determination of the Total Phenolic, Flavonoid and Proline Contents in Burkina Fasan honey, as well as Their Radical Scavenging Activity'. *Food Chemistry*, 91(3): 571-577.
- Miliauskas, G., P.R.Venskutonis and T.A. van Beek,** 2004. 'Screening of Radical Scavenging Activity of Some Medicinal and Aromatic Plant Extracts'. *Food Chemistry*, 85(2): 231-237.
- Mirzaee, M., A. Moieni and F. Ghanati,** 2013. 'Effects of Drought Stress on the Lipid Peroxidation and Antioxidant Enzyme Activities in Two Canola (*Brassica napus* L.) Cultivar'. *Journal of Agricultural Science and Technology*, 15(3): 593-602.
- Morello, J.R., M.P. Romero, T. Ramo and M.J. Motilva,** 2005. ' Evaluation of L-Phenylalanine Ammonia-Lyase activity and Phenolic Profile in Olive Drupe (*Olea europaea* L.) from Fruit Setting Period to Harvesting Time'. *Plant Science*, 168(1): 65-72.
- Movafeghi, A., A. Khataee, M. Abedi, R. Tarrahi, M. Dadpour and F. Vafaei,** 2018. 'Effects of TiO₂ Nanoparticles on the Aquatic Plant *Spirodela polyrrhiza*: Evaluation of Growth Parameters, Pigment Contents and Antioxidant Enzyme Activities'. *Journal of Environmental Sciences* 64: 130-138.
- Mutlu, F., F. Yurekli, B. Mutlu, F. Bilge Emre, F. Okusluk and O. Ozgul,** 2018. 'Assessment of Phytotoxic and Genotoxic Effects of Anatase TiO₂ Nanoparticles On Maize Cultivar by Using RAPD analysis'. *Fresenius Environmental Bulletin* 27 (1): 436-445.
- Niknam, V. and H. Hassanpour.** 2011. 'Effects of Penconazole and Water Deficit Stress on Physiological and Antioxidative Responses in Pennyroyal (*Mentha pulegium*L.)'. *Acta Physiologiae Plantarum* 34(4): 1537–1549.
- Oloumi, H., R. Soltaninejad and A. Baghizadeh,** 2015. 'The Comparative Effects of Nano and Bulk Size Particles of CuO and ZnO on Glycyrrhizin and Phenolic Compounds Contents in *Glycyrrhiza glabra* L. Seedlings'. *Indian Journal of Plant Physiology*, 20(2): 157–161.
- Ongphimai, N., S. Lilitchan, K. Aryasuk, A. Bumrungpert and K. Krisnangkura,** 2013. 'Phenolic Acids Content and Antioxidant Capacity of Fruit Extracts from Thailand. *Chiang. Mai Journal of Science*, 40(4): 636-642.
- Osmolovskaya, N., J. Shumilina, A. Kim, A. Didio, T. Grishina, T. Bilova, O.A. Keltsieva, V. Zhukov, I. Tikhonovich, E. Tarakhovskaya, A. Frolov and L.A. Wessjohann.** 2018. Methodology of Drought Stress Research: Experimental Setup and Physiological Characterization. *International Journal of Molecular Sciences*, 19(12): 4089.
- Panrong, C., L. Chunyan and L. Kebin.** 2006. 'Aromatic Constituents in Fresh Leaves of *Lingtou dancong* Tea Induced by Drought Stress'. *Frontiers of Agriculture in China*, 1(1): 81-84.

- Rastogi, S.h., R. Kumar, C.S. Chanotiya, K. Shanker, M.M. Gupta, D.A. Nagegowda and A.K. Shasany.** 2013. '4-Coumarate: CoA Ligase Partitions Metabolites for Eugenol Biosynthesis'. *Plant Cell Physiology*, 54(8): 1238-1252.
- Sgherri, C., E. Cosi and F. Navaro-Izzo.** 2003. 'Phenols and Antioxidative Status of *Raphanus sativus* Grown in Copper Excess'. *Physiologia Plantarum*, 118(1): 21-28.
- Simon, J.R., D.L. Martin and M. Kroll.** 1974. 'Sodium-dependent efflux and exchange of GABA in synaptosomes', *Journal of Neurochemistry*, 23(5): 981-991.
- Sirousmehr, A., J. Bardel and S. Mohammadi.** 2015. 'Changes of Germination Properties, Photosynthetic Pigments and Antioxidant Enzymes Activity of Safflower as Affected by Drought and Salinity Stresses'. *Journal of Crop Ecophysiology*, 8(4): 517-533.
- Solar, A., M. Colaric, V. Usenik and F. Stampar,** 2006. 'Seasonal Variations of Selected Flavonoids, Phenolic Acids and Quinines in Annual Shoots of Common Walnut (*Juglans regia* L.)'. *Plant Science*, 170(3): 453-461.
- Szwajgier, D., J. Pielecki and Z. Targonski.** 2005. 'Antioxidant Activities of Cinnamic and Benzoic Acid Derivatives'. *Acta Scientiarum Polonorum Technologia Alimentaria*, 4(2): 129-142.
- Tattini, M., C. Galardi, P. Pinelli, R. Massai, D. Remorini and G. Agati.** 2004. 'Differential Accumulation of Flavonoids and Hydroxycinnamates in Leaves of *Ligustrum vulgare* under Excess Light and Drought Stress'. *New Phytologist*, 163(3): 547-561.
- Ursu, B. and I. Borcean.** 2012. 'Researches Concerning the Sowing Technology at *Lallemantia iberica* F.et M.'. *Research Journal of Agricultural Science*, 44(1): 168-171.
- Velika, B. and I. Kron.** 2012. 'Antioxidant Properties of Benzoic Acid Derivatives Against Superoxide Radical'. *Free Radicals and Antioxidants*, 2(4): 62-67.
- Yadav, R., R. Sangwan, F. Sabir and A. Srivastav.** 2013. 'Effect of Prolonged Water Stress on Specialized Secondary Metabolites, Peltate Glandular Trichomes, and Pathway Gene Expression in *Artemisia annua* L'. *Plant Physiology and Biochemistry*, 74: 70-83.
- Zandalinas, S. I., D. Balfagón, V. Arbona and A. Gómez-Cadenas,** 2017. 'Modulation of Antioxidant Defense System Is Associated with Combined Drought and Heat Stress Tolerance in Citrus'. *Frontiers in Plant Science*, 8: 953.
- Zhang, Y., V. Virjamo, N. Sobuj, W. Du, Y. Yin, L. Nybakken, H. Guo and R. Julkunen-Tiitto.** 2018. 'Sex-related responses of European aspen (*Populus tremula* L.) to combined stress: TiO₂ nanoparticles, elevated temperature and CO₂ concentration'. *Journal of Hazardous Materials*, 352: 130-138

