Journal of Plant Process and Function, Vol. 8, No. 34, Year 2020

Effects of ZnO NPs on phenolic compounds of rapeseed seeds under salinity stress

Torfeh Akhavan Hezaveh[1], Latifeh Pourakbar[1*], Fatemeh Rahmani[1], Hadi Alipour[2]

[1] Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran
[2] Department of Plant Breeding and Biotechnology, Urmia University, Urmia, Iran
(Received: 28/02/2019-Accepted: 02/08/2019)

Abstract

In this study, rapeseed Okapi cultivar was treated under three NaCl levels (0, 50, and 100 mM) and four Zinc oxide nanoparticle (ZnO NPs) concentrations (0, 20, 40, and 80 mgL^{-1}) at flowering stage. The experiment was performed in a completely randomized design (CRD) in the greenhouse of Biology Department of Urmia University, in 2015. Results showed that NaCl (100 mM) significantly enhanced total phenolic, flavonoid anthocyanin and phosphorus contents and increased ability to scavenge the DPPH radical scavenging activity. Treatment with ZnO NPs (80 mgL^{-1}) significantly elevated total phenolic and decreased anthocyanin, phosphorus and zinc contents and seed weight. Foliar application of ZnO NPs ameliorated the negative impacts of NaCl by reduction in phenolic, anthocyanin and phosphorus contents and elevation in seed oil content (20.82%) and seed weight (79.28%) at 50 mM salinity and 20 mgL^{-1} of ZnO NPs compared to NaCl (50 mM) alone. In conclusion, foliar application of ZnO NPs at 20 mgL^{-1} is recommended to farmers as replacement for fertilizers to alleviate the adverse effects of salinity stress.

Keywords: Antioxidant activity, *Brassica napus*, Salt stress, Secondary metabolites

Introduction

Rapeseed (*Brassica napus* L.), the third most important oil crop after soybean and palm, is cultivated mainly for oil, human consumption and biodiesel production all over the world. There has been a large increase in the area of rapeseed grown in Iran over the past decade. It is a popular oilseed crop in the world because of its ability to adapt to climate change, suitability for mechanization, and low labor requirements (Dekamin *et al*., 2018). Although rapeseed produces maximum yield under normal soil and environmental conditions, however like other important crops, its growth, grain yield and oil production are markedly reducing due to environmental stresses such as drought, salinity, low and high temperature and nutrient deficiency or excess. *Brassica napus* is well known for its enriched secondary metabolites, especially for phytochemicals with antioxidant activity (Wang *et al*., 2018).

Salinity stress is a major constraint to crop production in many areas of the world. It affects nearly 20% of the world's cultivated areas and about half the world's total irrigated lands (Aslam *et al*., 2017). Soils with high salt concentration have a low water potential zone; consequently, it is difficult for the plant to absorb water and nutrients. In other words, saline soils expose plants to osmotic stress (Agarwal *et al*., 2013). Salinity stress increases secondary metabolites such as phenolic acid, flavonoid, and anthocyanin and enzymes activity which play important role against oxidative stress caused due to salinity (Rezayian *et al*., 2018).

Zn is an important cofactor in essential bio catalytic enzymes including oxidoreductases, transferases, hydrolases, ligases, and isomerases (Auld, 2001). Nowadays, nanoparticles (NPs) are considered ideal for delivering Zn fertilizers to plants (Prasad *et al*., 2012) and suitable for plants against abiotic stresses (Elsakhawy *et al*., 2017). ZnO NPs is one of the most frequently used NPs in food packaging and medicine. The increased popularity of using Zn in fertilizers and pesticides is also commissioned due to its normal request as a micronutrient in the body (Latef *et al*., 2017).

To the best of our knowledge, no attention has been paid to evaluate role of ZnO NPs in alleviation of salinity stress. Therefore, a greenhouse experiment was conducted to study accumulation of phenolic, DPPH radical scavenging activity flavonoid, anthocyanin contents in seeds of rapeseed under application of NaCl stress and ZnO NPs. In addition, Zn and P contents of the seed oil and seed weight were measured.

Material and Methods

Seeds of rapeseed, Okapi cultivar were obtained from Agricultural Breeding and Technology Center, West

*Corresponding Author, Email: L.pourakbar@urmia.ac.ir

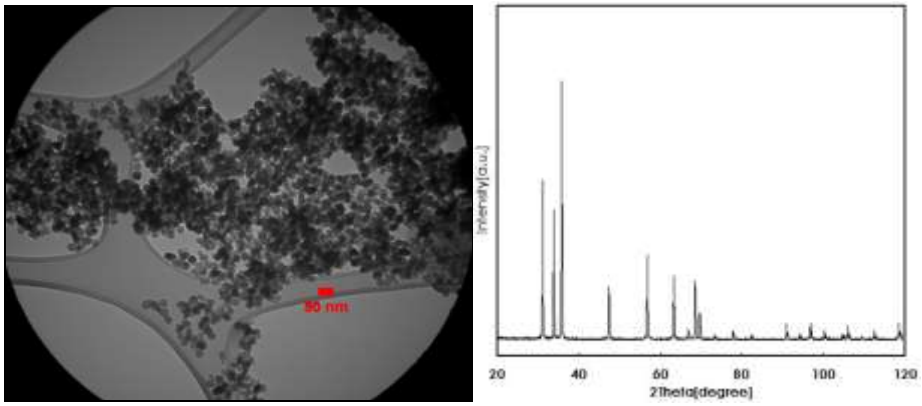

Figure 1- Graph and images by microscope SEM (Scanning Electron Microscope).

Azerbaijan in 2015. Greenhouse experiments were conducted during growing seasons in 2015 to investigate the effect of salinity stress and ZnO NPs foliar applications on biochemical responses. ZnO NPs with determined specifications were purchased from Pishgaman-e-Nano-materials Iranian Company (Mashhad). ZnO NPs was first suspended in de-ionized water using an ultrasonic device (100 W and 40 KHz for 30 minutes) (Prasad *et al*., 2011). The pH was determined 6.7 after dispersion. Figure 1 shows SEM (Scanning Electron Microscope) images of ZnO NPs. The particles of ZnO NPs were in white color and almost spherical morphology with diameter of 10-30 nm, 99% purity and 5.60 g/cm^3 density. Soil samples were collected from 0 to 30 cm depth of a farmland before planting. Physical and chemical properties of soil texture components were analyzed according to Hydrometer method (Beretta *et al*., 2014) and the obtained results are shown in Table 1. After filling pots (35 cm diameter and 20 cm depth) with perlite/soil mixture (1:2), ten seeds were planted in each pot. At four-leaf stage, three plants with strongest growth were selected from each pot and the remaining plants were discarded. The pots were kept in a greenhouse for 4 weeks at 20±3 °C and 18±3 °C during days and nights, respectively, with a photoperiod of 16/8 hrs. Then, plants were put in an upright refrigerator at 4±2 °C for vernalization. A photoperiod of 10 hrs. was provided using cool-white fluorescent lights delivering 50µmol s^{-1}m^{-2} PAR (Photosynthetically active radiation). After vernalization for 10 weeks, plants were kept in a cold room (13±2 °C) for 72 hrs. Light was provided by a 150-W incandescent bulb and cool-white fluorescent tubes with overall delivery of 15 µmol s^{-1}m^{-2} PAR. Then, the plants were taken back to the original greenhouse where they treated with different concentrations of NaCl and ZnO NPs at flowering stage according to a completely randomized design (CRD) with three replications.

The experiment was performed at three salinity (NaCl) levels (0, 50, and 100 mM) (Bybordi *et al*., 2010) and four ZnO NPs concentrations (0, 20, 40, and 80 mgL^{-1}) (Latef *et al*., 2017). As soon as the first flowers opened, salinity stress was applied. The plants were subjected to three sets of salinity treatments through irrigation with a nutrient solution containing 0, 50, and 100 mM (NaCl) every day until water was drained from the bottom of the pot. The ZnO NPs was applied once a day at 7:00 AM for a period of 21 days (Khoshgoftarmanesh *et al*., 2016).

Preparation of extracts: Dried seeds (0.5 g) were extracted with methanol, chloroform and ethyl acetate using the soaking method for 48 hrs., shaken for 30 min, filtered through anhydrous sodium sulfate, and vacuum-evaporated. After filtration and solvent evaporation, extracts were stored in sealed vials at 4 °C until biological testing.

Total phenolic content: Total phenolic content was measured in methanolic extract of seed by the Folin-Ciocalteu method (Malgorzata and Aleksander, 2016). One milliliter of methanol extract was mixed with 5 ml of 10% Folin- Ciocalteu's reagent dissolved and 4 mL sodium carbonate solution (7.0 %). The mixture was kept for 2 hrs. before its absorbance was measured at 765 nm. Gallic acid was used as a standard for the calibration curve (Rezayian *et al*., 2018). After 2 hrs., absorbance at λ_{max} 725 nm was measured against a reagent blank using a UV-Vis spectrophotometer (HALO XB-10). Results were expressed as total phenols in micromoles of gallic acid equivalents (GAE) per gram of fresh weight.

DPPH free radical scavenging activity: Briefly, a 0.1 mM solution of DPPH in methanol was prepared and 0.3 ml of this solution was added 0.5 ml of samples at different concentrations (10-80 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then، the absorbance was measured at 517 nm by spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity (Ak and Gulcin, 2008). The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect % = (1- (AS/AC)) ×100

Where AC is the absorbance of the control and AS is the absorbance in the presence of samples or standards (Elmastas *et al*., 2006). The scavenging activity was reported as percentage decrease in absorbance with time.

Table 1- Some of physical and chemical properties of soil

Texture	Clay	Sandy	Silt	EC dSm^{-2}	Mn	Cu	Fe	Zn	K	P	N	PH
	%				mgL^{-1}				mg/100g soil			
Loam-sand	15	53	32	1.1	0.6	0.2	7.6	1	15	8.5	14	7.6

Table 2- The Zn^{+2} content of ZnO NPs suspension

Concentration ZnO NPs mgL^{-1}	20	40	80
Zn (mgL^{-1})	0.55±0.8	0.89±1.4	1.45±0.5

Flavonoid content: Flavonoid content was determined using spectrophotometric method (Quettier *et al*., 2000). One ml of methanol extract, 3 ml of sodium acetate, and 1 ml of aluminum chloride solution were mixed and the absorbance was measured at 445 nm after 2.5 hrs. (Malgorzata and Aleksander, 2016).

Anthocyanin content: To determine the anthocyanin content, 100 mg of fresh flower tissue was extracted in 1 mL of methanol containing 0.3% (v/v) HCl, following overnight incubation in the dark at 4 °C with shaking at 150 rpm. The extract was centrifuged at 10500 g for 30 min (Malgorzata and Aleksander, 2016).

Ion content measurement: To determine the role of dissolved metal ions in causing phytotoxicity, ZnO NPs suspensions at (0, 20, 40, and 80 mgL^{-1}) were centrifuged at 15,000 rpm for 30 min after dispersal. Then, the supernatant was filtered through 0.2µm glass filters, and the content of Zn element was analyzed by ICP-OES method (730-ES Varian company, USA) at Sharif Industrial Laboratory Service Center (206 nm) (Table 2).

Elemental analysis: Seeds for nutrient's determination were first ignited at 600 °C, and then dissolved in 5 mL of 33% HNO3 and added into separate conical flasks. Then, 3-5 ml of concentrated H_2SO_4 and 30% H_2O_2 (each) were added occasionally. The continuous heating (70-80 °C) further allowed the complete decomposition of the organic matters until obtaining clear solutions. The whole contents of the flasks were evaporated and the semidried mass dissolved in a small amount (approximately 5 mL) of deionized water, filtered through Whatman 42 paper (Sigma-Aldrich), and made up to a final volume of 25 mL in volumetric flasks with 2N HNO_3 (Horwitz, 2002). Finally, the concentration of Zn was determined using an atomic absorption spectrophotometer (model Aa680, Shimadzu, Japan) with three replications. Total phosphorus content was obtained by complete digestion of the samples (500 mg FW, in triplicate) with nitric acid (100%) and perchloric acid (100%) (1:1, v/v) and subsequent colorimetric quantification using the molybdate method (Gomes *et al*., 2017).

Oil extraction: First, the samples were completely powdered in mortar, and each extraction process was performed with 10 ml of diethyl ether each time. Finally, the extraction solvents of each stage were mixed together with the inert nitrogen gas in a solvent and the remaining oil was weighed to obtain the oil content.

Seed weight: Seeds weight of each pot was measured by scale.

Statistical analysis of data: The data were tested for normality of residuals using the Kolmogorov-Smirnov's test. Analysis of variance (ANOVA) was performed using PROC GLM in SAS 9.2. Means were separated using Duncan's multiple range test (DMRT) and each average was presented with the standard error. Significant differences between means were denoted with letters and different letters denote statistical significance at $P<0.05$.

Results

Total phenolic content: Phenolic content of seeds showed an increasing trend in a salt concentration dependent manner (Figure 2A). The highest induction was observed at NaCl (100 mM) with 28.45% elevation compared to the control plants. The foliar application of ZnO NPs (20 mgL^{-1}) had a significant decreasing effect (10.71 %) on phenolic content while the ZnO NPs (80 mgL^{-1}) increased it (31.22%) as compared to the control plants. Application of ZnO NPs (80 mgL^{-1}) and NaCl (100 mM) enhanced total phenolic content of seed (48.76%) as compared to the control plants (Figure 2A).

DPPH radical scavenging activity: DPPH radical scavenging activity significantly increased (48.65%) at NaCl (100 mM) exposure as compared to the control plants (Figure 2B). The foliar application of ZnO NPs (up to 80 mgL^{-1}) had a significant incremental trend (12.91%) on DPPH radical scavenging activity of seed compared to the controls. Application of ZnO NPs at all concentrations with NaCl (50 and 100 mM), non-significantly declined DPPH radical scavenging capacity as compared to salinity (50 and 100 mM) alone.

Flavonoid content: Total flavonoid content was elevated up to 27.76% in NaCl (100 mM) treated plants (Figure 2C). The foliar application of ZnO NPs (20 mgL^{-1}) significantly enhanced leaf flavonoid content (16.66%) compared to the controls. However, flavonoid content of seed significantly decreased (13.09%) by application of both NaCl (100 mM) and ZnO NPs (20 mgL^{-1}). The lowest flavonoid level was observed at combined application of NaCl (100 mM) and ZnO NPs (20 mgL^{-1}) (Figure 2C).

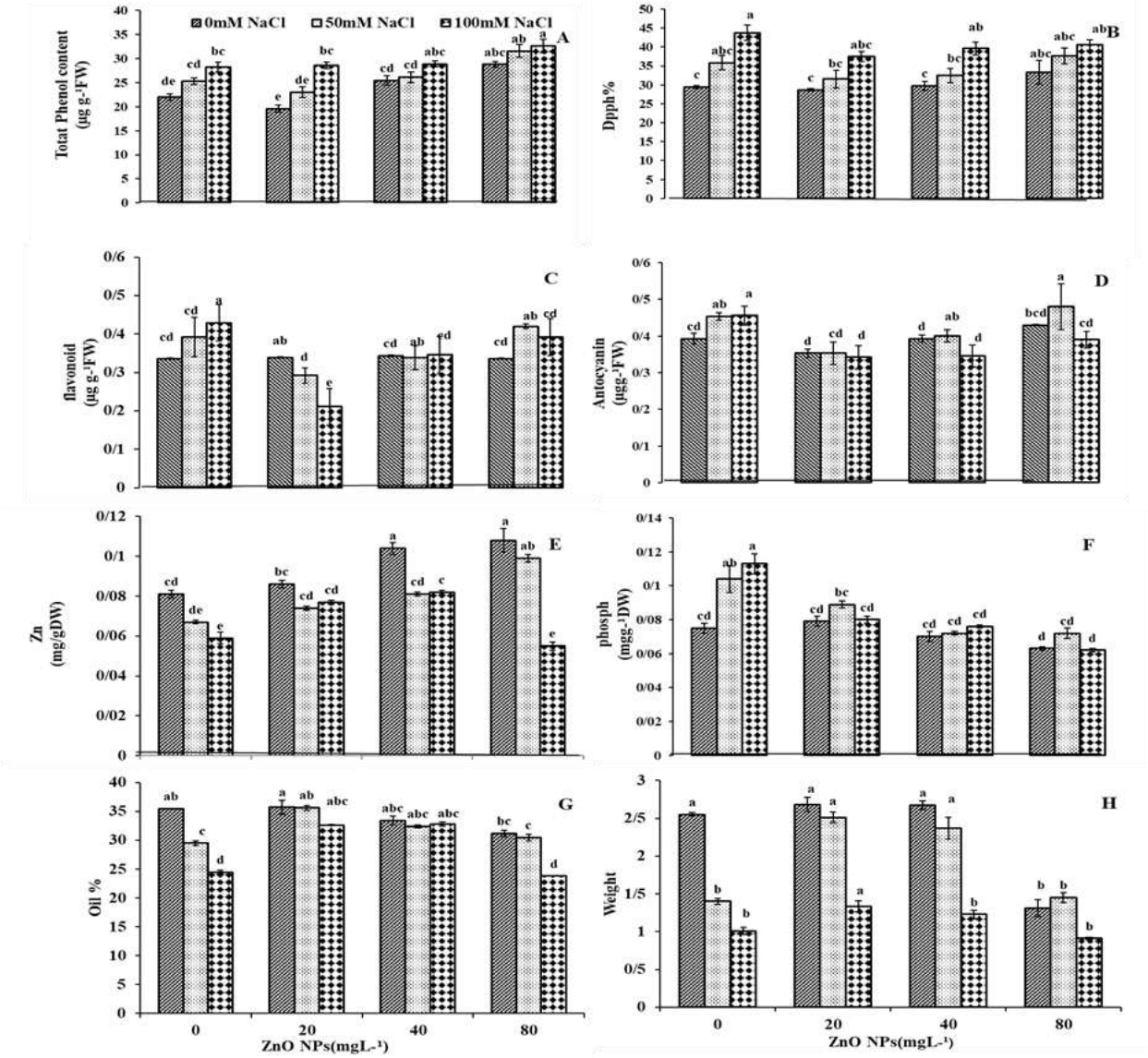

Figure 2- Effects of different concentrations of NaCl, and ZnO NPs on (A) Total Phenol content; (B) DPPH%; (C) Flavonoid; (D) Anthocyanin; (E) Zn Content; (F) P Content (G) Percentage of Oil; (H) Seed weight per pot. Means followed by different letters differ significantly according to Duncan's multiple range test (P<0.05)

Anthocyanin content: The anthocyanin content of seeds increased (16.03%) at NaCl (100 mM) stress (Figure 2D). However, anthocyanin content was lowered at ZnO (20 and 40 mgL^{-1}) but later enhanced by further increase of ZnO NPs concentration (80 mgL^{-1}). In addition, application of NaCl (50 mM) with ZnO NPs (80 mgL^{-1}) showed the maximum anthocyanin content (22.13%) compared to the control plants. foliar applicatio of ZnO NPs at levels of 20, 40 and 80 mgL^{-1} reduced the amount of anthocyanin at salinity stress of 100 mM (Figure 2D).

Zn content: The Zn content of seeds declined (27.16%) at NaCl (100 mM) (Figure 2E). rapeseed plants showed higher accumulation of Zn at 20, 40 and 80 mgL^{-1} of ZnO NPs application. The highest Zn content was obtained at salinity (0 mM) and 80 mgL^{-1} ZnO NPs (33.33%) (Figure 2E). However, salinity stress (100 mM) and ZnO NPs (80 mgL^{-1}) significantly reduced the content of Zn (32.09 %) to minimum level as compared to the control plants.

Phosphorus content: P content was enhanced by increasing salinity level as compared to the control plants (Figure 2F). However, P contents of seed did not show any meaningful change by the foliar application of ZnO NPs alone. The co-application of NaCl (100 mM) with ZnO NPs (20, 40, and 80 mgL^{-1}) decreased P content of seed compared to the imposing salinity (100 mM) alone (Figure 2F) with the highest reduction (50%) related to NaCl (100 mM) and ZnO NPs (80 mgL^{-1}), compared to the salinity (100 mM) alone.

Percentage of oil: Salinity relatively reduced the percentage of oil (Figure 2G). The highest decrease was observed under NaCl (100 mM) with 10.97% decline as compared to the control plants. The foliar application of ZnO NPs at a concentration of 20 and 40 mgL^{-1} prevented the reduction of oil content due to increased

salt stress. However, the oil percentage was significantly decreased (32.87%) at NaCl (100 mM) and ZnO NPs of 80 mgL^{-1} as compared to the control plants (Figure 2G).

Seed weight: Seed weight was strongly decreased by salinity stress. The highest reduction (34.83%) was observed under salinity (100 mM) compared to the control plants (Figure 2H). The foliar application of ZnO NPs at concentrations of 20 and 40 (mgL^{-1}) under salinity stress of 50 mM prevented seed weight loss, but by increasing salinity level up to 100 mM, seed weight decreased significantly. The highest reduction (34.83%) was observed under salinity (100 mM) compared to the control plants. The foliar application of ZnO NPs (0, 20, and 40 mgL^{-1}) at NaCl (100 mM) on rapeseeds did not show any significant impact. However, a significant decrease of 48.62% of seed weight was detected at 80 mgL^{-1} ZnO NPs in comparison with the control plants NaCl (0 mM) + ZnO NPs (0 mgL^{-1}). The foliar application of rapeseed plants with ZnO NPs at concentrations of 20 and 40 mgL^{-1} with NaCl (50 mM) increased the seed weight compared to the application of NaCl (50 mM) alone (Figure 2H). However, co-treatments of NaCl (100 mM) with ZnO NPs (80 mgL^{-1}) caused weight loss (64.31%) compared to the control plants.

Discussion

In the present study, phenolic contents were increased under salinity stress and application of ZnO NPs (Figure 2A). These enhancements might be due to higher expression of genes in phenolic biosynthetic pathway. The role of Zinc in the application of carbon to produce (through enhanced carbohydrates) phenolic compounds in shikimic acid cycle and acetate could be one of the reasons of this increase (Misra *et al*., 2006). Phenolic compounds play the most important role in non-. Enzymatic system. When plant experiences metal stress, phenolic compounds can act as metal chelators or ROS scavengers (Michalak, 2006). Zn has critical effect in homeostasis and oxidative stress with great association to photosynthesis. Although it has been demonstrated that the application of NPs ZnO affects the physiology and biochemistry of plants, there are still generalized debates and ambiguities regarding the effects on the secondary metabolism of the plant (Abdal Dayem *et al*., 2017). Mainly, the effect of NPs on the phenolic compounds, since these molecules play important roles in the yield and adaptation of the plant, in response to biotic and abiotic stresses (Abdal Dayem *et al*., 2017). Therefore, it is a priority to understand the integral functioning of the secondary metabolism of the plant, in response to the application of NPs as a possible generator of oxidative stress.

The degree of cellular oxidative damage in plants exposed to abiotic stress is controlled by the capacity of the plants to produce antioxidant agents. The DPPH assay provides basic information on the antiradical activity of the extracts. The actual reaction that is taking place between the DPPH stable radical and the antioxidant (AH) is DPPH. The radical that is formed (A.) in general is less reactive, depending on the structure of the molecule, or it can follow a radical interaction to create a stable molecule (Huang *et al*., 2005). By analyzing the antioxidant activity of the different extracts under different levels of salinity, it was possible to demonstrate that they all had the capacity to scavenge DPPH free radicals (Figure 2B). Zn protects membrane proteins and lipids against free radicals which could explain elevation of total antioxidants (DPPH) by foliar application of ZnO (Marreiro *et al*., 2017). Furthermore, Zn acts as a cofactor in the enzymatic system and purifies free radicals which can also be attributed to increase total antioxidant capacity by foliar application of Zn (Marreiro *et al*., 2017). Results of the previous study revealed that ZnO NPs could act as a free radical scavenger (Kumar *et al*., 2014). The free radical scavenging capacity of ZnO NPs may be due to the transfer of electron density located at oxygen to the odd electron located at nitrogen atom in DPPH (Das *et al*., 2013). Antioxidants could interfere with the oxidation process induced by various stress through acting as oxygen scavengers, therefore the tolerance to salinity stress might be correlated with an increase in the antioxidant potential (Zhou *et al*., 2018).

Flavonoid accumulation was observed after treatment with NaCl (100 mM) (Figure 2C) as a part of the adaptive reaction to salt stress (Sarker *et al*., 2018). Flavonoids decrease the production of ROS through suppression of singlet oxygen and inhibition of enzymes that generate ROS (Mierziak *et al*., 2014). Our results indicated that concomitant application of NaCl (100 mM) + ZnO NPs (20, 40, and 80 mgL^{-1}) reduced flavonoid content (Figure 2C). This reduction may be due to intolerance of nanoparticles at high concentrations. Modulation of metal homeostasis plays an important role in their bioactivity. It has been shown that dietary flavonoids may affect the homeostasis, transportation and uptake of Zn (Wei and Guo, 2014). Flavonoids are frequently induced by abiotic stress and play a role in plant protection (Mierziak *et al*., 2014). Accumulation of flavonoids due to NaCl may indicate that rapeseed relied on large amounts of flavonoid to cope with harmful impacts of NaCl. Plants respond to NaCl stress by simulating phenylalanine ammonia lyase (PAL) (Gao *et al*., 2008) which is involved in the phenylpropanoid pathway enhancing phenolic production (Lim *et al*., 2012). One reason for flavonoid content induction is restriction of photosynthetic electron transfer during stress causing metabolic changes in the plant. Flavonoids make membranes resistant to oxidative factors by reducing their fluidity and prevention of free radical's release (Mierziak *et al*., 2014).

Our results in response to salt stress indicate elevation in anthocyanin content (Figure 2C). In accordance to our findings, anthocyanin accumulation,

often occurs in response to environmental stresses (Khavari-Nejad *et al*., 2008). Anthocyanin accumulation is also a part of the adaptive reaction to salt stress. The result of this study showed an increase in anthocyanin content after treatment with NaCl (50 and 100 mM). The increase in secondary metabolites and anthocyanin accumulation is associated with increasing plant defense system and thought to enhance ROS scavenging efficiency, thereby improving *B. napus* tolerance (Chutipaijit, *et al*., 2011). Different varieties accumulate different kinds and contents of anthocyanin in different tissues (Guo and Ling, 2015).

The co-application of ZnO NPs (80 mgL^{-1}) with NaCl (50 mM) increased anthocyanin of seeds (Figure 2D). Higher anthocyanin contents in response to Zn supply can be justified by reciprocal aspects. It is still unknown whether the induction of anthocyanin is only a response to stress or a defensive system against biological damage (Asad *et al*., 2015). However, higher anthocyanin level can decrease oxidative stress (Elisia *et al*., 2007). One of the factors indicating that heavy metals (e.g. Zn) lead to oxidative stress is an increase in the amount of anthocyanin (Posmyk *et al*., 2009). Increase in anthocyanin content indicates its protective role to cope with stress and possibly reduce the damage caused by salinity (Li *et al*., 2011).

Zn is an active element in biochemical processes and has chemical and biological interactions with some other elements (Mousavi *et al*., 2012). Our data are in agreement with earlier reports on decline in Zn accumulation under salinity stress (Hussein *et al*., 2018). Saleh and Maftoun (2008) offered that, ZnO NPs should be added to saline soils especially those with low in available Zn. Mateos-Naranjo *et al*. (2008) demonstrated that Zn affects tissue phosphorus concentrations of *S. densiflora*. In this study, foliar application of ZnO NPs resulted in elevation of Zn in seed. Zinc shortage also resulted in a high accumulation of P (Figure 2F). P is the most important element interfering with Zn uptake by plants. Metabolism defects can appear in plant cells due to Zn and P imbalance; therefore, by increasing Zn concentration, P functions are impaired at specific positions of the cells (Mousavi *et al*., 2012). These findings suggested that Zn nutrition can directly affect P uptake mechanisms in plants.The highest ZnO NPs (80 mgL^{-1}) level resulted in the lowest decrease of P. Mateos-Naranjo *et al.* (2008) reported that Zn affects the tissue concentrations of P.

Salt stress is one of major problems of brassica plants in arid and semi-arid regions, which deteriorates the quality and quantity of oil seed production (Mbarki *et al*., 2018). Our results complied with those obtained by Bybordi *et al*. 2010 and Javadi *et al*. 2014. Salinity (50 mM) and ZnO NPs (20 and 40 mgL^{-1}) (Figure 2G) co-application had appreciable effects on oil constituents of rapeseed. Similar results were obtained under Zn application (10 kg ha^{-1}) and salinity (8 dS m^{-1}) in rapeseed plant (Ebrahimian *et al*., 2017).

As shown in Figure 2H, a significant decrease of seed weight was observed following the application of different NaCl levels. The decrease of seed weight might be due to the impaired development and small size of grains under salinity, because of the reduction in water uptake and photosynthesis as well as imbalanced nutrition (Sabagh *et al*., 2019). Decrease in seed weight can be due to prevention of assimilate transport to the seeds and decrease in growth during seed filling stage (Bybordi *et al.*, 2010) or changing photosynthetic materials in order to coping with salty stress effects. Other researchers such as Ashraf and MacNilly (2004) also have reported a decline in grain yield in the Brassica family under salinity conditions. Previous results demonstrated that application of ZnO NPs had positive effects on seed weight of soybean (Seyed Sharifi, 2016). The reason for the seed weight increase under salinity and ZnO NPs compared to only salinity, may be related to Zn which increases photosynthesis rate and improves leaf area survival (Baybordi and Mamedov, 2009). Zn affects auxin (growth regulator) biosynthesis positively; which in turn can promote mineral absorption, cell division and thus enhance plant growth under salinity (Hussein *et al*., 2018). However, at high concentration, toxic effects have been created. In spite of increasing percentage of oil content and seed weight under 20 mgL^{-1} of ZnO NPs, percentage of oil content and seed weight decreased due to increasing ZnO NPs concentration to 80 mgL^{-1}. Therefore, it can be concluded that seed weight and content of oil have similar alteration pattern under salt stress and ZnO NPs.

Conclusion

In this research, we demonstrated that physiological and biochemical changes can increase the tolerance against salinity stress due to application of ZnO NPs. This is the first record on the phytochemistry of the rapeseed under ZnO NPs at flowering stage under greenhouse growth conditions. According to the results of the present study, ZnO NPs at optimal concentration (20 mgL^{-1}) resulted in a significant increase in the amount of seed weight and oil under salinity stress. Thus, 20 mgL^{-1} ZnO NPs concentration can be considered to be the optimum Zn concentration for alleviating salinity stress in rapeseed. We believe that our findings would contribute to our knowledge regarding the interactions of ZnO NPs with plant systems under salinity stress. In our study, the tolerance of seeds to high salinity (100 mM) treatments coincided with the seed enrichment in total phenols paralleled with a decrease in the amount of seed oil and weight. The results show concomitant stimulations in both phenolic biosynthesis and antioxidant activity in seed tissues when exposed to NaCl salinity.

References

Abdal Dayem, A., Hossain, M. K., Lee, S. B., Kim, K., Saha, S. K., Yang, G. M., Choi, H. Y. and Cho, S. G. (2017) The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. International Journal of Molecular Sciences 18:.120-141.

Agarwal, P. K., Shukla, P. S., Gupta, K. and Jha, B. (2013) Bioengineering for salinity tolerance in plants: state of the art. Molecular Biotechnology 54: 102-123.

Ak, T. and Gulcin, I. (2008) Antioxidant and radical scavenging properties of curcumin. Chemico-Biological Interactions 174: 27-37.

Asad, S. A., Muhammad, S., Farooq, M., Afzal, A., Broadley, M., Young, S. and West, H. (2015) Anthocyanin production in the hyperaccumulator plant *Noccaea caerulescens* in response to herbivory and zinc stress. Acta Physiologiae Plantarum 37: 1-9.

Ashraf, M. and McNeilly, T. (2004) Salinity tolerance in brassica oilseeds. Critical Reviews in Plant Science 23: 157-174.

Aslam, M., Ahmad, K., Akhtar, M. A. and Maqbool, M. A. (2017) Salinity stress in crop plants: effects of stress, tolerance mechanisms and breeding strategies for improvement. Journal of Agriculture and Basic Sciences 2: 70-85.

Auld, D. S. (2001) Zinc coordination sphere in biochemical zinc sites. In Zinc Biochemistry, Physiology, and Homeostasis Springer, Dordrecht 14: 271-313.

Baybordi, A. and Mamedov, G. (2009) Evaluation of application methods efficiency of zinc and iron for canola (*Brassica napus* L.). Notulae Scientia Biologicae 1: 17-26.

Beretta, A. N., Silbermann, A. V., Paladino, L., Torres, D., Bassahun, D., Musselli, R. and Garcia-Lamohte, A. (2014) Soil texture analyses using a hydrometer: modification of the Bouyoucos method. Ciencia e Investigación Agraria 41: 263-271.

Bybordi, A., Tabatabaei, S. J. and Ahmadev, A. (2010) Effects of salinity on fatty acid composition of canola (*Brassica napus* L.). Journal of Food Agriculture and Environment 8: 113-115.

Chutipaijit, S., Cha-um, S. and Sompornpailin, K. (2011) High contents of proline and anthocyanin increase protective response to salinity in *Oryza sativa* L. spp.'indica'. Australian Journal of Crop Science 5: 1191-1198.

Das, D., Nath, B. C., Phukon, P. and Dolui, S. K. (2013) Synthesis of ZnO nanoparticles and evaluation of antioxidant and cytotoxic activity. Colloids and Surfaces B: Biointerfaces 111: 556-560.

Dekamin, M., Barmaki, M., Kanooni, A. and Meshkini, S. R. M. (2018) Cradle to farm gate life cycle assessment of oilseed crops production in Iran. Engineering in Agriculture, Environment and Food 11: 178-185.

Ebrahimian, E., Bybordi, A. and Seyyedi, S. M. (2017) How nitrogen and zinc levels affect seed yield, quality, and nutrient uptake of canola irrigated with saline and ultra-saline water. Communications in Soil Science and Plant Analysis 48: 345-355.

Elisia, I., Hu, C., Popovich, D. G. and Kitts, D. D. (2007) Antioxidant assessment of an anthocyanin-enriched blackberry extract. Food Chemistry 101: 1052-1058.

Elmastaş, M., Dermirtas, I., Isildak, O. and Aboul-Enein, H. Y. (2006) Antioxidant activity of S-carvone isolated from spearmint (*Mentha Spicata* L. Fam Lamiaceae*).* Journal of Liquid Chromatography and Related Technologies 29: 1465-1475.

Elsakhawy, T., Omara, A. E. D., Alshaal, T. and El-Ramady, H. (2017) Nanomaterials and plant abiotic stress in agroecosystems. Environment, Biodiversity and Soil Security 2: 73-94.

Gao, S., Ouyang, C., Wang, S., Xu, Y., Tang, L. and Chen, F. (2008) Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. Plant Soil Environ 54: 374-381.

Gomes, M. A. D. C., Pestana, I. A., Santa-Catarina, C., Hauser-Davis, R. A. and Suzuki, M. S. (2017) Salinity effects on photosynthetic pigments, proline, biomass and nitric oxide in *Salvinia auriculata* Aubl. Acta Limnologica Brasiliensia 29: 1-13.

Guo, H. and Ling, W. (2015) The update of anthocyanins on obesity and type 2 diabetes: experimental evidence and clinical perspectives. Reviews in Endocrine and Metabolic Disorders 16: 1-13.

Horwitz, A.V. (2002) Outcomes in the sociology of mental health and illness: Where have we been and where are we going? Journal of Health and Social Behavior 43: 143-151.

Hussein, M. M. and Abou-Baker, N. H. (2018) The contribution of nano-zinc to alleviate salinity stress on cotton plants. Royal Society Open Science 5: 1-11.

Huang, D., Ou, B. and Priop, R. L. (2005) The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry 53: 1841-1856.

Javadi, N., Abas, F., Hamid, A. A., Simoh, S., Shaari, K., Ismail, I. S., Mediani, A. and Khatib, A. (2014) GC-MS-based metabolite profiling of *Cosmos caudatus* leaves possessing alpha-glucosidase inhibitory activity. Journal of Food Science 79: 1130-1136.

Khavari-Nejad, R. A., Bujar, M. and Attaran, E. (2008) Ecophysiology of High Salinity Tolerant Plants. Springer, Dordrech.

Khoshgoftarmanesh, A., Torabian, S. and Zahedi, M. (2016) Effect of foliar spray of zinc oxide on some antiox idant enzymes activity of sunflower under salt stress. Journal of Agricultural Science and Technology 18: 1013-1025.

Kumar, B., Smita, K., Cumbal, L. and Debut, A. (2014) Green approach for fabrication and applications of zinc oxide nanoparticles. Bioinorganic Chemistry and Applications 1-7.

Latef, A. A. H. A., Alhmad, M. F. A. and Abdelfattah, K. E. (2017) The possible roles of priming with Zno nanoparticles in mitigation of salinity stress in Lupine (*Lupinus termis*) plants. Journal of Plant Growth Regulation 36: 60-70.

Li, C., Wang, P., Wei, Z., Liang, D., Liu, C., Yin, L., Jia, D., Fu, M. and Ma, F. (2011) The mitigation effects of exogenous melatonin on salinity induced stress in *Malus hupehensis*. Journal of Pineal Research 53: 298-306.

Lim, J. H., Park, K. J., Kim, B. K., Jeong, J. W. and Kim, H. J. (2012) Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. Food Chemistry 135: 1065-1070.

Malgorzata, N. K. and Aleksander, S. (2016) Changes of phenolic content in rapeseed during preliminary drying. Journal of Oilseed Brassica 1: 33-38.

Marreiro, D. D. N., Cruz, K. J. C., Morais, J. B. S., Beserra, J. B., Severo, J. S. and de Oliveira, A. R. S. (2017) Zinc and oxidative stress: current mechanisms. Antioxidants 6: 1-9.

Mateos-Naranjo, E., Redondo-Gomez, S., Cambrolle, J., Luque, T. and Figueroa, M. E. (2008) Growth and photosynthetic responses to zinc stress of an invasive cordgrass *Spartina densiflora*. Plant Biology 10: 754-762.

Mbarki, S., Sytar, O., Zivcak, M., Abdelly, C., Cerda, A. and Brestic, M. (2018) Anthocyanins of coloured wheat genotypes in specific response to salt stress. Molecules 23: 1518.

Mierziak, J., Kostyn, K. and Kulma, A. (2014) Flavonoids as important molecules of plant interactions with the environment. Molecules 19: 16240-16265.

Misra, A., Dwivedi, S., Srivastava, A. K., Tewari, D. K., Khan, A. and Kumar, R. (2006) Low iron stress nutrition for evaluation of Fe-efficient genotype physiology, photosynthesis, and essential monoterpene oil (s) yield of (*Ocimum sanctum*). Photosynthetica 44: 474-477.

Michalak, A. (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies 15: 523-530.

Mousavi, S. R., Galavi, M. and Rezaei, M. (2012) The interaction of zinc with other elements in plants: a review. International Journal of Agriculture and Crop Sciences 4: 1881-1884.

Mohsenzadeh, S. and Moosavian, S. S. (2017) Zinc sulphate and nano-zinc oxide effects on some physiological parameters of *Rosmarinus officinalis*. American Journal of Plant Sciences 8: 2635-2649.

Mousavi, S. R., Galavi, M. and Rezaei, M. (2012) The interaction of zinc with other elements in plants: a review. International Journal of Agriculture and Crop Sciences 4: 1881-1884.

Posmyk, M. M., Kontek, R. and Janas, K. M. (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotoxicology and Environmental Safety 72: 596-602.

Prasad, K. N., Hassan, F. A., Yang, B., Kong, K. W., Ramanan, R. N. and Azlan, A. (2011) Response surface optimisation for the extraction of phenolic compounds and antioxidant capacities of underutilized Mangifera pajang Kosterm. Food Chemistry128: 1121-1127.

Prasad, T. N. V. K. V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K. R. and Pradeep, T. (2012) Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of Plant Nutrition 35: 905-927.

Quettier, D. C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., LuyCkx, M. C., Cayin, J. C., Baillul, F. and Trotin, F. (2000) Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. Journal of Ethnopharmacology 72: 35-42.

Rezayian, M., Niknam, V. and Ebrahimzadeh, H. (2018) Differential responses of phenolic compounds of *Brassica napus* under drought stress. Plant Physiology 8: 2417-2425.

Saleh, J. and Maftoun, M. (2008) Interactive effects of NaCl levels and zinc sources and levels on the growth and mineral composition of rice. Journal of Agricultural Science and Technology 10: 325-336.

Sabagh, A. E., Hossain, A., Barutcular, C., Islam, M. S., Ratnasekera, D., Kumar, N., Meena, R. S., Gharib, H. S., Saneoka, H. and da Silva, J. A. T. (2019) Drought and salinity stress management for higher and sustainable canola (*Brassica napus* L.) production: A critical review. Australian Journal of Crop Science13: 88-110.

Sarker, U., Islam, M. T. and Oba, S. (2018) Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals and antioxidant activity in *Amaranthus tricolor* leaves. PloS one13: 1-18.

Seyed Sharifi, R. (2016) Application of biofertilizers and zinc increases yield, nodulation and unsaturated fatty acids of soybean. Zemdirbyste-Agriculture 103: 251-258.

Wang, Y., Meng, G., Chen, S., Chen, Y., Jiang, J. and Wang, Y. P. (2018) Correlation analysis of phenolic contents and antioxidation in yellow-and black-seeded *Brassica napus*. Molecules 23: 1815-1830.

Wei, Y. and Guo, M. (2014) Zinc-binding sites on selected flavonoids. Biological Trace Element Research 161: 223-230.

Zhou, Y., Tang, N., Huang, L., Zhao, Y., Tang, X. and Wang, K. (2018) Effects of salt stress on plant growth, antioxidant capacity, glandular trichome density, and volatile exudates of *Schizonepeta tenuifolia* Briq. International Journal of Molecular Sciences 19: 252-267.