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Using Nano - Selenium in Reducing the Negative Effects of High Temperature Stress on *Chrysanthemum morifolium* Ramat

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TUnder climate change, the escalating in temperature is considered a serious challenge threaten the agricultural production. This production includes food, feed and all other human needs. These higher temperatures represent heat stress, which cause physiological and biochemical damages in cultivated plants. Nano-selenium (nano-Se) has distinguished features enhancing the cultivated plants under stress. These features include the high bioactivity of nano-Se, which ameliorate the growth of different cultivated plants under different abiotic and biotic stresses such as salinity, drought, waterlogging and heat stress. Therefore, this study was carried out to investigate the ameliorative role of nano-Se on two sensitive chrysanthemum cultivars (i.e., Sensual and Francofone) under high temperature stress (up to 41.6°c). The growth of these cultivars, plant antioxidant system and floral attributes were investigated under graded nano-Se doses (*i.e.,* 50, 100, 150 and 200 mg nano-Se L^{-1}) during two successive growth seasons (*i.e.,* 2017 and 2018). The foliar application of nano-Se improved heat tolerance in chrysanthemum by enhancing the activities of antioxidant enzymes including catalase and peroxidase (up to 150 mg nano-Se L^{-1}) and decreasing polyphenol oxidase and electrolyte leakage up to 200 mg nano-Se L^{-1}. The nano-Se-treated cut chrysanthemum flowers under heat stress indicated the positive effects of nan-Se on improving of flower quality and the economic value. These results may confirm the definite roles of nano-Se in management the heat stress-induced adverse effects on not only cut chrysanthemum flowers but also other crops under climate change.

Keywords: Heat stress, Antioxidant enzyme, Polyphenol oxidase, Catalase, Peroxidase, Electrolyte leakage

Introduction

Climate change is considered one of the most important challenges facing our life in particular the agricultural production to feed many billion worldwide. This changing in climate represents in the escalating of atmospheric temperature leading to heat stress. This acceleration in temperature is projected to increase 1.8 to 4°C at the end of this century with average increase by 0.2°C per decade (Zafar et al., 2018). Heat stress is considered one of the most important abiotic stresses, which causes serious problems for cultivated plants including the damage in photosynthesis process, plant enzymatic activities, and its metabolism (Haghighi et al., 2014 and Du et al., 2018). In general, cultivated plants may generate a heat shock response within a few hours, when the surrounding temperature get be higher (> 10–15 °C) to avoid this stress (Du et al., 2018).

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is considered an economically flowering ornamental plant all over the world (Su et al., 2019) and a medicinal plant in China and Japan (Yang et al., 2018). The flowers of chrysanthemum have important biological activities like arylnaphthalene

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lignans and phenolic glycosides (Yang et al., 2018 and Peng et al., 2019) as well as neuro-protective and antioxidant activity (Han et al., 2019 and Yang et al., 2017&2019). Chrysanthemum also is considered the four major cut flowers worldwide representing a great source in the global cut flower industry (Du et al., 2018 and Dong et al., 2020). During all the year, this plant could be cultivated under different natural and artificial controlled environments (Kang et al., 2019). The stressful environments in particular heat stress on chrysanthemum might cause a reduction in shelf life of plants resulted from delaying the flowering and deteriorating in the quality of flowers (Kang et al., 2019). The production of this plant was handled by many investigators under different stresses such as drought stress (Deng et al., 2012 and Li et al. 2018), water stress (Hodaei et al., 2018), salinity (Gao et al., 2018), heat stress (Du et al., 2018; Qi et al., 2018 and Kang et al., 2019), UV-B radiation (Yang et al., 2018) and soil moisture regimes (Zhang et al., 2020). Chrysanthemum is severely restricted by heat stress due to its sensitivity to high temperatures, which mainly retards the plant growth causing defects in its flowers (Kang et al., 2019).

The humans and animals need in their growth and development several essential nutrients like selenium (Se). This micro-nutrient has distinguished roles in human health as reported by many studies (*e.g.,* Rayman, 2012; Roman et al., 2014; Schomburg, 2016; Vinceti et al., 2017; Dinh et al., 2018; Natasha et al., 2018 and Newman et al., 2019). Selenium can play many biological functions including antioxidant (Wang et al., 2017), antagonistic roles (Jin et al., 2018), immune-regulatory (Dinh et al., 2018) and potentials in cancer prevention (Tan et al., 2019). The main problem in selenium nutrition is the narrow range between the dietary deficiency (40 µg day^{-1}) and the toxic Se-concentration (400 µg day^{-1}) as reported by dos Reis et al. (2017). The role of Se in higher plant metabolism and its essentiality still needs more evidences (Pilon-Smits, 2019). Several reports confirmed that Se can stimulate the growth and plant quality at low applied concentration (Golubkina et al., 2018), improve the photosynthesis rate (Jiang et al., 2017 amd Zsiros et al., 2019), and strengthen the plant defense under stress (Bocchini et al., 2018; Hawrylak-Nowak et al., 2018; Singh et al., 2018 and Kolbert et al., 2019). On the other hand, nano –selenium (nano-Se) or selenium nanoparticles (Se-NPs) has also distinguished features including low toxicity and high bioactivity comparing with other Se-forms like selenate or selenite or selenomethionine (Djanaguiraman et al., 2018). In human and animal health, many applications have been reported also for nano-selenium including the nano-medicine and therapeutic applications (Chaudhary et al., 2016; Zhao et al., 2017; Hosnedlova et al., 2018; Khurana et al., 2019 and Song et al., 2020). Nano-Se has the ability to alleviate the stressful conditions on many cultivated plants such as heavy metal stress on rice (Hussain et al., 2020), NaCl stress on the growth and fruit quality of tomato (Morales-Espinoza et al., 2019), salinity stress on the yield of strawberry (Zahedi et al., 2019a), high-temperature stress on sorghum grains (Djanaguiraman et al., 2018), and disease stress on tomato (Quiterio-Gutiérrez et al., 2019). On the other hand, nano selenium has been used in ameliorating the growth of many cultivated plants under stressful conditions (*e.g.*, Morales-Espinoza et al., 2019), whereas there is no similar studies for chrysanthemum.

Therefore, this investigation is an attempt to focus on the ameliorative role of nano-Se in promoting the growth of chrysanthemum under heat stress.

Materials and Methods

Plant materials

This work was conducted in two successive seasons 2017 and 2018 in the nursery of El-Kenana Company located in Tanta, Gharbia Governorate, Egypt. This study was carried out to investigate the ameliorative role of biosynthetic nano selenium of two sensitive chrysanthemum cultivars (*i.e.*, Sensual and Francofone) under heat stress. The nano-Se was prepared in the Agricultural Microbiology Department, Soils, Water and Environment Research Institute (SWERI), Sakha Agricultural Research Station, Agriculture Research Center (ARC), Kafr El-Sheikh, Egypt. A biological procedure was used to prepare nano-selenium (50–200 nm) through using *Lactobacillus casei* according to Eszenyi et al. (2011). The cuttings of two sensitive chrysanthemum cultivars were imported from the Netherlands (Greneth Plants B.V. Company). These cuttings were cultured as mother plant to provide the source of cuttings during the two experimental seasons. The cuttings were collected from mother plants for rooting in April during the two seasons. The peat moss and perlite (1:1, v/v) was used for rooting of cuttings as a growth medium.

Greenhouse experiment

Rooted cuttings of two cultivars were cultured on 28 April 2017 and 3 May 2018 (in the first and second season, respectively) in greenhouse its dimension 9 m wide × 40 m long × 5 m height on rows with 1 m wide × 35 m long. This greenhouse had six rows. Before cultivating, all rows were covered with support net (64 wells/1 m). Each cultivar was cultured in three rows. Each row was sprayed with five nano-selenium doses (i.e., 0, 50, 100, 150 and 200 mg nano-Se L^{-1}).

The foliar nano-Se was applied three times starting with the first one after 20 days from planting (before forming of the flowering bud and at a plant length ranged from 30 to 35 cm as well as a rate of 500 ml nano-Se solution for 1 m^2, which includes 64 plants). The second and third doses of foliar nano-Se were applied after 40 and 60 days from sowing, the plant height was ranged from 67 to 75 and 90 to 100 cm using a dose of 800 and 1000 ml, respectively for 1 m^2. Each treatment for every cultivar had three replicates with 448 plants per each replicate. Cultivated plants were sprayed with nano-selenium after 15 days from culture for three times (one time for every month) during the growth season. All plants were managed (irrigation, fertilization and disease control) according to Taweesak et al. (2014). After opening the flowers (nearly 110 days from culture), plant height, leave number, vegetative weight, root weight and opened flower number were recorded.

Heat stress

The experiment carried out in Tanta during summer season from May to July in two successive years (i.e., 2017 and 2018). Concerning the optimum temperature for chrysanthemum flowering, it is cultivar-dependent and ranging between 17º – 22°C (Van Der Ploeg and Heuvelink 2006). However, the suitable temperature for other developmental traits is clear differences among cultivars and ranged between 12 to 28°C (Carvalho et al. 2002). In this study, the maximum of day temperature degree in Tanta during May, June and July 2017 were 37.3, 39.8 and 41.4°C, respectively. While, the maximum of day temperature degrees in Tanta during May, June and July 2018 were 39.5, 40.6 and 41.6°C, respectively (Table 1).

Determination of chlorophyll a and b contents

The amount of chlorophyll a and b in the fully expanded young leaves were determined by spectrophotometric analysis after opening the flowers. Chlorophyll was extracted from leaf tissue by grinding in a mortar with liquid nitrogen to a fine powder and adding 100 mg to a 2 mL Eppendorf tube. One mL of 80 % acetone was added and the powder was homogenized by inverting for 10 min in ice using a shaker. The absorbance was measured at 470, 649 and 665 nm using a Heliosepiclon spectrophotometer (GT 80^+, UK). Chlorophyll contents were calculated from the spectrophotometric data using the formulae of Lichtenthaler and Welburn (1983). There were three replicates within each treatment.

Measurement of enzyme activities

To determine the activities of antioxidant enzymes (i.e., catalase, peroxidase and polyphenol oxidase), 0.5 g of fully expanded young leaves was homogenized under liquid nitrogen with 1.5 ml of respective extraction buffer using pre-chilled mortar and pestle. The homogenate was filtered through four layers of cheesecloth and centrifuged at 22,000 ×g for 20 min at 4°C. The supernatant, which was re-centrifuged at 22,000 ×g for 20 min at 4°C, was used for the assays indicated next.

Catalase (CAT, EC 1.11.1.6) activity was measured by following the consumption of H_2O_2 at 240 nm (Aebi 1984). One ml reaction mixture

TABLE 1. Average temperature (°C) during the months of experiment according to the following website: https://en.tutiempo.net/climate/egypt.html

Months of the study	Maximum temp. (°C)	Minimum temp. (°C)	Average temp. (°C)	Maximum temp. (°C)	Minimum temp. (°C)	Average temp. (°C)
		2017			2018	
May	37.3	17.4	27.1	39.5	19.7	29.5
June	39.8	20.9	30.4	40.6	21.4	31.1
July	41.4	23.0	32.2	41.6	22.0	31.8

Temp. = temperature

contained 20 µg total protein, 50 mM sodium phosphate buffer (pH 7.0), and 10 mM H_2O_2. The reaction was initiated by adding the protein extract. For each measurement, the blank corresponds to the absorbance of the mixture at zero time and the actual reading corresponds to the absorbance after 1 min. One unit of CAT activity was defined as 0.01 decreases in absorbance at 240 nm per mg protein per minute.

Peroxidase (POX, EC 1.11.1.7) activity was determined according to procedure proposed by Hammerschmidt et al. (1982). The reaction mixture consisted of 2.9 ml of a 100 mM sodium phosphate buffer (pH 6.0) containing 0.25 % (v/v) guaiacol (2-methoxy phenol) and 100 mM H_2O_2. The reaction was started by adding 100 µl of crude enzyme extract. Changes in absorbance at 470 nm were recorded at 30 s intervals for 3 min. Enzyme activity was expressed as an increase in absorbance min^{-1} g^{-1} fresh weight.

Polyphenol oxidase (PPO; EC 1.10.3.1) activity was determined according to the method described by Malik and Singh (1980). The reaction mixture contained 3.0 ml buffered catechol solution (0.01 M), freshly prepared in 0.1 M phosphate buffer (pH 6.0). The reaction was started by adding 100 µl of crude enzyme extract. Changes in the absorbance at 495 nm were recorded at 30 s for 3 min. Enzyme activity was expressed as an increase in absorbance min^{-1} g^{-1} fresh weight.

Electrolyte leakage

Measurements were carried out as described by Szalai et al. (1996) with some modifications according to Dewir et al. (2015). Leaf discs of five treatments tissues were placed individually into 25 ml deionized water (Milli-Q 50, Millipore, Mass., USA). Flasks were shaken for 20 h at ambient temperature to facilitate electrolyte leakage from injured tissues. Initial electrical conductivity measurements were recorded for each vial using an Acromet AR20 electrical conductivity meter (Fisher Scientifc, Chicago, Il., USA). Flasks were then immersed in a hot water bath (Fisher Isotemp, Indiana, PA) at 80 °C for 1 h to induce cell rupture. The vials were again placed on the Innova 2100 platform shaker for 20 h at 21°C. Final conductivity was measured for each flask. Electrolyte leakage percentage was calculated as: (initial conductivity/final conductivity) × 100.

Total selenium analysis

The instrument of hydride generation atomic fluorescence spectroscopy was used in measuring the Se content in plant samples according to Dernovics et al. (2002) and Cabanero et al. (2004). The concentrated nitric acid was used in digesting the plant leaf samples at 100 °C for 30 min. Hydrogen peroxide (30%) was added after that to the mixture at 120 °C for 45 min in block digestion unit. The samples were completed with 3M HCl to 10 cm^3 after digestion and were filtered using filter paper (Macherey–Nagel 640 w). Se hydride was generated in a flow injection system for measuring with a flow rate of 1.5 mL/min (3 M hydrochloric acid) and a similar flow rate of the reductant solution (1.4 m/v % sodium tetra-hydroborate) were used to generate the Se hydride.

Statistical analyses

Experiments were set up in complete randomized block design in factorial with two factors (2 cultivars × five nano-selenium doses) and two-way ANOVA. Each treatment consisted of three replicates and each replicate was represented by 448 plants. The mean and ANOVA were calculated using SPSS (version 20) statistical program. The mean separations were carried out using Fisher's least significant difference test and significance was determined at $P \leq 0.05$.

Results and Discussion

Nano-Se and plant morphological characters under heat stress

The cut flowers like chrysanthemum could be used in the decorating purpose. The production of chrysanthemum flowers is mainly controlled by the common growth factors as well as the environmental conditions in particular the stresses (*e.g.,* salinity, drought and heat). The production of chrysanthemum flowers under heat stress has been reported by many researchers (e.g., Du et al. 2018; Qi et al. 2018; Kang et al. 2019). It could be overcome the heat stress using the conventional and molecular breeding approaches (Li et al., 2016) for getting the desire quality of cut flowers and flowering of chrysanthemum in suitable time (Kang et al., 2019). The role of nano selenium did investigate in enhancing the growth of chrysanthemum under heat stress based on our information. In this study, some plant morphological characters of two chrysanthemum cultivars investigated under heat stress and applied different doses of nano-Se (Tables 2 and 3). These characters include plant height, number of leaves per plant, the vegetative weight per plant, root weight and number of opening flowers during the two growing seasons. In general, all studies vegetative parameters were significantly impacted with the degrading nano-Se doses in both two seasons and cultivars. The highest values of these parameters were recorded after applying of 50 to 150 mg nano-Se L^{-1}. Francofone cultivar recorded the highest number of opening flowers (11.67 and 13.0) after applying only 50 mg nano-Se L^{-1}, whereas after 150 mg nano-Se L^{-1} for Sensual cultivar (12.66 and 13.34 in 2017 and 2018, respectively). That means Francofone cultivar is more efficiency than Sensual cultivar saving the 2/3 amount of applied nano-Se to harvest the same number of opining flowers under heat stress. No distinguished toxic symptoms were recorded after applying the highest nano-Se dose (200 mg nano-Se L^{-1}) on both chrysanthemum cultivars.

Based on the data in Tables 2 and 3, the role of nano-Se in promoting the vegetative and flowering growth of studied chrysanthemum cultivars is clear under heat stress. The impact of nano-Se may include the strengthening of plant defense system against heat stress and the increase in the opening flowers number under 50 mg nano-Se L^{-1} recorded around the double fold compared to the control for two cultivars. Sensual cultivar also is more tolerant to nano-Se comparing with Francofone cultivar.

TABLE 2. Response plant height and leaves number of two chrysanthemum cultivars to nano-selenium (Se-NPs) during two growing seasons

Cultivars	Applied Se-NPs doses (mg L^{-1})	Plant height (cm)		Number of leaves	
		2017	2018	2017	2018
Sensual	0	152	151	41.6	44.6
	50	153	156	40.6	45.6
	100	156	162	40.0	42.3
	150	152	160	50.0	50.3
	200	147	148	38.3	41.3
Francofone	0	139	125	107	97
	50	150	142	152	141
	100	152	148	105	104
	150	152	144	101	102
	200	146	139	129	115
LSD significance (0.05%)		7.06	5.22	7.87	5.44
Cultivars (Cv)		*	**	**	**
Se-NPs (Se)		**	**	**	**
Cv × Se		*	**	**	**

Notes: Significant (*), highly significant (**)

TABLE 3. Effect of nano-selenium (Se-NPs) treatments on vegetative weight (g), root weight (g) and opening flowers number of two chrysanthemum cultivars during two growing seasons

Cultivars	Applied Se-NPs dose (mg L^{-1})	Vegetative weight (g)		Root weight (g)		Number of opening flowers	
		2017	2018	2017	2018	2017	2018
Sensual	0	70.00	72.67	5.00	5.68	8.67	9.00
	50	78.34	84.33	6.34	7.34	9.34	10.34
	100	93.36	94.00	6.67	7.33	10.66	11.68
	150	85.67	85.65	5.00	6.34	12.66	13.34
	200	68.35	75.00	5.00	5.68	9.34	10.00
Francofone	0	91.68	84.37	5.00	5.66	6.68	6.67
	50	165	138.66	11.65	11.00	11.67	13.00
	100	99.34	92.35	7.67	8.34	9.35	10.35
	150	101.67	89.34	7.68	7.00	9.34	11.00
	200	132.66	115	13.34	9.35	11.36	12.68
LSD significance (0.05%)		13.24	5.79	2.94	2.28	1.45	1.12
Cultivars (Cv)		*	**	**	**	**	ns
Se-NPs (Se)		**	**	**	**	**	**
Cv × Se		*	**	**	**	**	**

Notes: Significant (*), highly significant (**), non-significant (ns)

Nano-Se and plant chlorophyll content

Under heat stress, the cultivated plants need to avoid the harmful effects of this stress, which impacts on almost the biological activities causing physiological, morphological and biochemical changes in plants (Song et al., 2014). The photosynthesis and chlorophyll contents are the most rapid physiological indicators compared to other indicators under stress on plants (Wu et al. 2019). In this study, the total chlorophyll content (a and b) in chrysanthemum cultivars increased by increasing the nano-Se doses up to 100 and 150 mg nano-Se L^{-1} for Francofone and Sensual cultivar, respectively under heat stress (Fig. 1). Concerning the total chlorophyll contents in chrysanthemum cultivars, Sensual cultivar again is seem to be more tolerant to nano-Se comparing with Francofone cultivar. Nano-Se might ameliorate the heat stress in chrysanthemum cultivars increasing the chlorophyll contents with protection under this stress. The dual effects of nano-Se could be distinguished in Fig. 1, where the positive role of nano-Se represents in promoting the chlorophyll function under heat stress, but the negative side may be appeared in the oxidative stress from high dose of selenium on plants. These results agree with Hussein et al. (2019) and Zahedi et al. (2019a), who found that nano-Se improved the total chlorophyll under infertile or sandy soils and salinity stress on groundnut and strawberry crop, respectively.

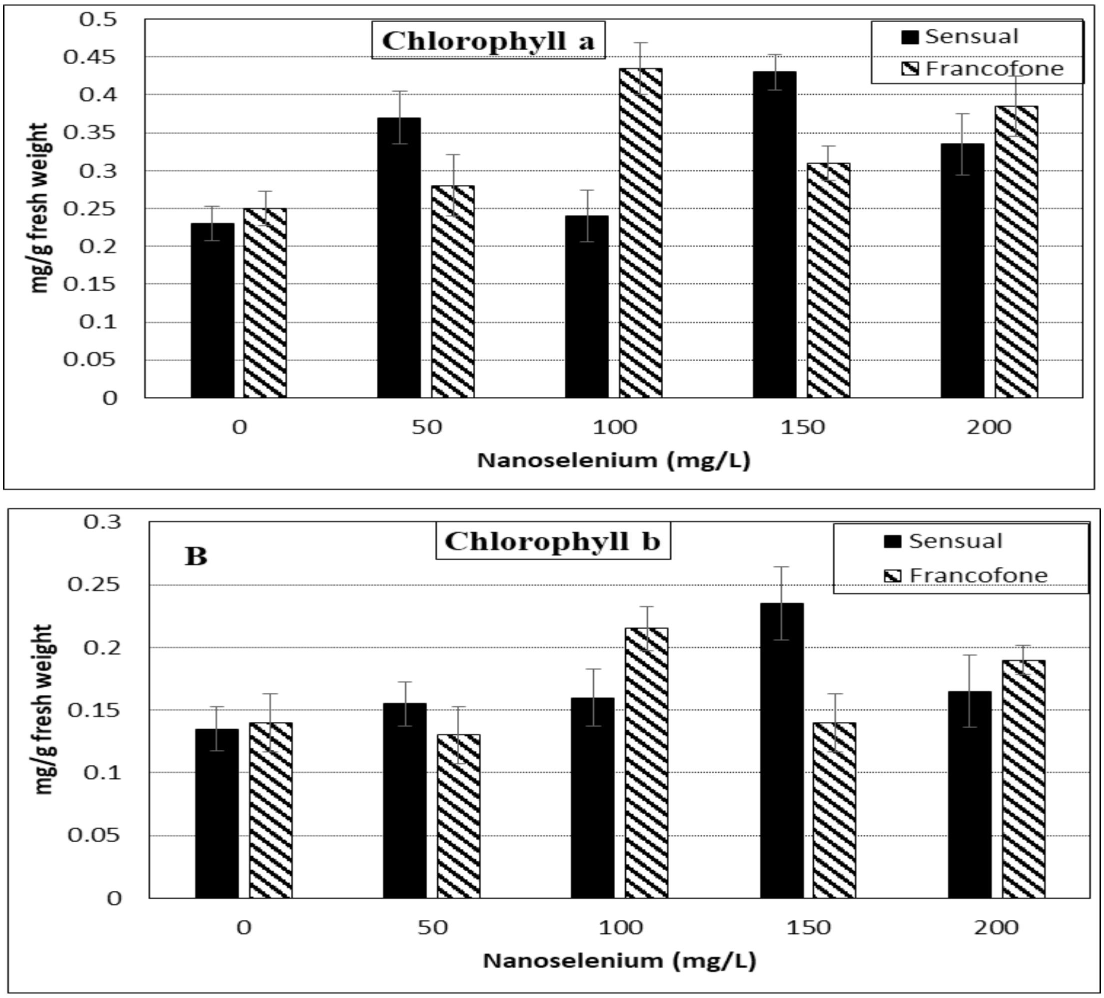

Fig. 1. Effect of nano-selenium treatments on chlorophyll a and b of two chrysanthemum cultivars (the data represent as a mean of the two growing seasons)

It could be concluded that, nano-Se treated chrysanthemum plants exhibited higher levels of both chlorophyll a and b than grown plants without nano-Se application under heat stress. The concentration of 100 and 150 mg L^{-1} nano-Se foliar spray successfully alleviated the adverse effect of heat stress for both chrysanthemum cultivars. This increase recorded for chlorophyll (a) by 180 and 200 % and (b) by 170 and 175 % content for Francofone and Sensual cultivar, respectively, relative to that of nano-Se untreated plants.

Nano-Se and plant antioxidant enzyme activities

Under stressful conditions, cultivated plants need to manage their defense system to avoid the harmful effects resulting from this stress. This defense includes the non-enzymatic (*e.g.,* flavonoids, carotenoids, ascorbate, glutathione, and tocopherols) and enzymatic antioxidants (*e.g.,* catalase, peroxidase, superoxide dismutase, glutathione reductase, and glutathione peroxidase). These plant antioxidants could overcome the oxidative damage, which result from the stress (Hasanuzzaman et al. 2019). Under heat stress, many plant biological activities will be suppressed such as the photosynthetic and respiration rate, the activities of hormonal and secondary metabolites (Zafar et al., 2018). Many plant enzymatic antioxidants also may be increased under heat stress like ascorbate peroxidase, glutathione reductase, and glutathione peroxidase (Hasanuzzaman et al., 2013). In this study, in chrysanthemum cultivars some enzymatic antioxidants including catalase (CAT) and peroxidase (POX) as well as polyphenol oxidase (PPO) and electrolyte leakage also were measured (Fig. 2). The highest mean values in enzymatic antioxidants in chrysanthemum peroxidase (40 and 85%) and catalase (60 and 80%) recorded at the level of 150 mg nano-Se L^{-1} for Sensual and Francofone cultivars, respectively over control. These results are in agreement with results of Sun et al. (2008), who reported that the applied exogenous Ca^{2+} activated oxygen scavenging enzymatic antioxidants (CAT and POX) of chrysanthemum under high temperature stress. The activity of peroxidase enzyme recorded nearly the same level at both control and 200 mg nano-Se L^{-1} for both cultivars referring to this nano-Se dose act as control and higher level of nano-Se need to be investigated. The nano-Se exhibited the highest increase in polyphenol oxidase enzyme activity up to 50 mg nano-Se L^{-1} relative to nano-Se untreated plants and then decreased for both cultivars. Concerning the electrolyte leakage, the potential role of nano-Se in lowering this activity recorded the minimum activity at 200 mg nano-Se L^{-1} for both cultivars. This decrease in electrolyte leakage values were 33 and 50 % for Francofone and Sensual cultivar, respectively.

It could be concluded that, cultivated plants under heat stress start their defense against this stress by excess generation of ROS (reactive oxygen species), which leads to oxidative stress on stressed plants. So, these stressed plants may produce some compatible solutes, which are able to organize proteins and modify the antioxidant system using enzymatic antioxidants like peroxidase and catalase. The general major effects of heat stress on different crops include the reduce the yield, leaf size, the rate of pollen and spikelet fertility of rice, decreased chlorophyll content, decrease enzyme activity and increase ROS content (Hasanuzzaman et al. 2013).

Nano-Se and plant selenium content

Nano-selenium has been attracted more attention in the last a few years due to its high stability, bioavailability and its low toxicity (Zahedi et al., 2019b). This distinguished and potential role of Se-nanoparticles under abiotic stress for cultivated plants has reported by many researchers but there is no any report included the impact of nano-Se on chrysanthemum cultivars under heat stress till now. In this study, the accumulation of Se in chrysanthemum leaves increased by increasing nano-Se doses up to 200 mg nano-Se L^{-1} for both cultivars (Fig. 3). This increase recorded 3 folds in case of Sensual cultivar, where was 20% for Francofone cultivar, but the highest accumulation rate of Se was found in Francofone cultivar. This result stated that the Sensual cultivar is more tolerant to nano-Se fertilization compared to Francofone cultivar and both cultivars did not exhibited any toxic symptoms. This study also referred to the used doses of nano-Se need to be higher to determine the toxic level of applied nano-Se on these chrysanthemum cultivars.

The previous results confirmed that Sensual cultivar had a high response to higher doses of nano-Se fertilization comparing with Francofone cultivar (Fig. 4; 150 and 50 mg nano-Se L^{-1} for both cultivars, respectively) under heat stress. This response represents in the higher recorded values in the cutting flowers number and the total chlorophyll content (*a* and *b*) for Sensual cultivar. It could be recommended that Sensual cultivar might suitable to cultivate and produce the cut chrysanthemum flowers under high doses of applied nano-Se or high soil contaminated with Se and under heat stress. On the other hand, Francofone cultivar reported in this study that, nearly the same production of cut chrysanthemum flowers (13.0) comparing with Sensual cultivar (13.34) but at nano-Se level of 50 and 150 mg nano-Se L^{-1}, respectively under heat stress. That means it could be produced the same amount of cut Francofone flowers using the 1/3 amount of applied nano-Se fertilizer.

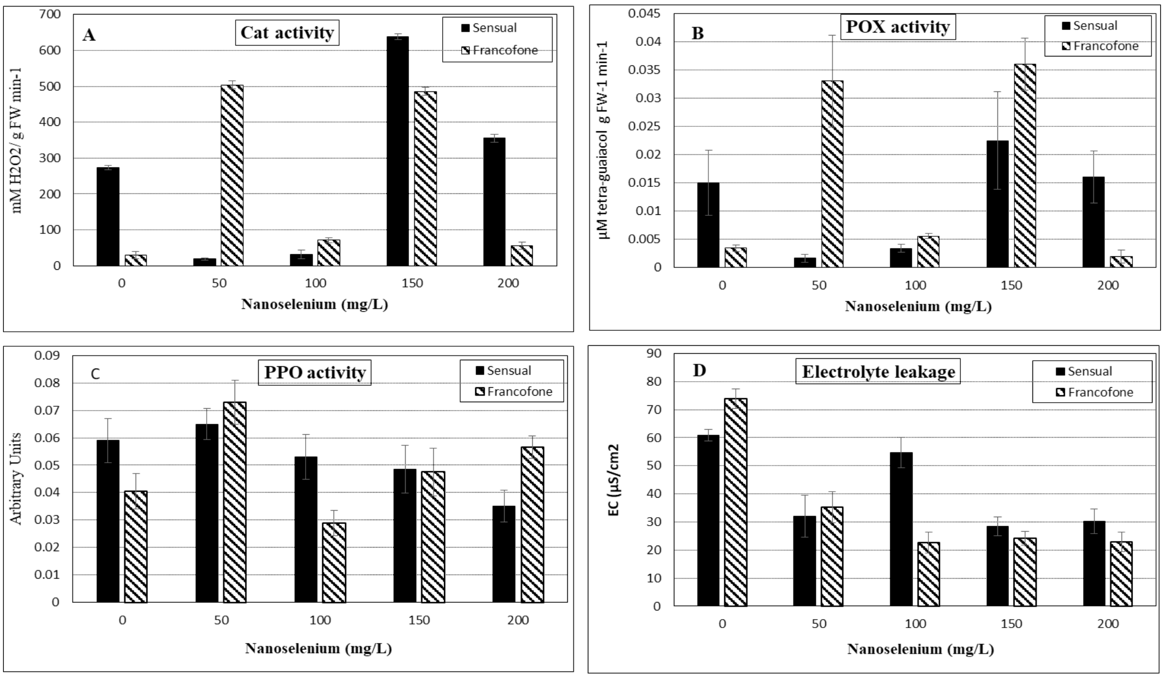

Fig. 2. Activity of antioxidant enzymes and electrolyte leakage in two chrysanthemum cultivars as treated by nano-selenium concentrations. A) catalase (CAT); B) peroxidase (POX); C) polyphenol oxidase (PPO); D) electrolyte leakage (the data represent as a mean of the two growing seasons)

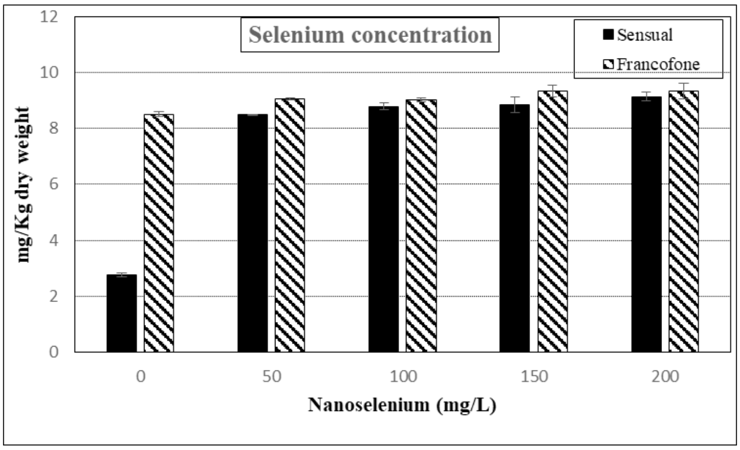

Fig. 3. Effect of foliar applied nano-selenium concentrations on different selenium concentration in leaves of chrysanthemum plants (the data represent as a mean of the two growing seasons)

Fig. 4. An overview for the two chrysanthemum cultivars, which they already used in this study, where (A) Sensual cultivar treated with 150 mg L-1 nano-selenium and (B) Francophone cultivar treated with 50 mg L-1 nano-selenium

This study opened the most important key question nowadays in Egypt: what is the expected of climate changes on cultivation timing of different crops? Is there an urgent need to establish a new schedule for the cultivating date of these crops under the changing in climate? It is reported that, an increase in the air temperature in Egypt and worldwide and this needs to re-draw the subjected scenario towards the new schedule for cultivation crops. The high temperature or heat stress is considered one of the most detrimental stresses. This study pointed that, heat stress is likely to happen during the upcoming in a few years will impact on the cultivation and production of different crops like chrysanthemum. This production of chrysanthemum may promote under nano-Se fertilization and definitely further investigations concerning cultivation and production of different cultivated crops under heat stress are needed.

Conclusion

Due to the distinguished and higher surficial activity of nano-Se comparing with other soluble Se-forms (mainly selenite and selenate), biological nano-Se is characterized by a higher solubility and mobility as well as being more biosafety and bioactivity properties. Nano-Se is considered nowadays a promising candidate for fertilization of different cultivated crops with Se under stress. The potential of nano-Se in production of chrysanthemum as a cut flower crop under arid and semi-arid conditions (around 40 ºC or heat stress) was studied using two sensitive cultivars to heat (i.e., Sensual and Francofone). This investigation showed that nano-Se has the ability to protect the studied two chrysanthemum cultivars from the adverse effects of heat stress by promoting photosynthetic process, enhancing the vegetative and floral growth, activating the antioxidant enzymes (i.e. catalase and peroxidase), polyphenol oxidase and electrolyte leakage in stressful plants. This study also confirmed that nano-Se (up to 100-150 mg nano-Se L^{-1}, depending on the cultivated cultivar) could be recommended to produce of cut chrysanthemum flowers under arid and semi-arid regions and other cultivated crops grown under heat stress. Therefore, the biological nano-Se could be used as a new candidate for diminishing the detrimental impacts of heat stress on chrysanthemum under arid and conditions.

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إستخدام النانوسيلينيوم فى خفض التأثيرات السلبية لإجهاد الحرارة العالية على نبات *Chrysanthemum morifolium* Ramat.

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يعتبر الإرتفاع فى درجات الحرارة تحديا خطيرا يهدد الإنتاج الزراعي في ظل تغير المناخ حيث يشتمل هذا الإنتاج على الغذاء والأعلاف وجميع الاحتياجات البشرية الأخرى. و تمثل درجات الحرارة المرتفعة هذه الإجهاد الحراري الذي يسبب أضرارًا فسيولوجية وكيميائية حيوية للنباتات المنزرعة. يتميز نانو سيلينيوم (nano-Se) بمميزات تساعد النباتات المزروعة تحت مثل هذه الضغوط. تشتمل هذه الميزات على الفاعاية الحيوية العالية للنانو - سيلينوم ، والتي تعمل على تحسين نمو النباتات المزروعة تحت الضغوط غير الحيوية والبيولوجية المختلفة مثل الملوحة والجفاف والأراضى الغدقة والإجهاد الحراري. و لذلك أجريت هذه الدراسة لاستقصاء الدور المحسّن للنانو سيلينيوم على صنفين حساسين Chrysanthemum morifolium تحت إجهاد درجات الحرارة العالية و التى وصلت إلى 41.6 درجة مئوية حيث تم تسجيل نمو هذه الأصناف ، وقياس مضادات الأكسدة النباتية وخصاص الأزهار تحت تأثير جرعات متدرجة من النانو سيلينيوم (هى 50 ، 100 ، 150 و 200 جزء فى المليون) خلال موسمي النمو على التوالي 2017 و 2018. حيث أدى الرش الورقي للنانو سيلينوم إلى تحسين التحمل الحراري عن طريق تعزيز نشاط إنزيمات مضادات الأكسدة بما في ذلك الكاتالاز والبيروكسيداز (ما يصل إلى 150 ميلي جرام نانو- سيلينوم) وتقليل تسرب البوليفينول وأكسيداز البوليتوليت حتى 200 ميلي جرام نانو سيلينوم. كما أشارت الدراسة الى ان الأزهار المقطوفة للنبات محل الدراسة والمعالجة بالنانو تحت الإجهاد الحراري إلى الآثار الإيجابية للنان سيلينيوم على تحسين جودة الأزهار والقيمة الاقتصادية. و اخيرا قد تؤكد هذه النتائج على الأدوار المحددة للنانو – سيلينيوم في إدارة الآثار الضارة الناجمة عن الإجهاد الحراري ليس فقط على زهور النبات المنزرعة بل وأيضًا على بعض المحاصيل الأخرى الخاضعة لتغير المناخ.