# SALINITY AND SELENIUM NANOPARTICLES EFFECT ON ANTIOXIDANT SYSTEM AND MALONDIALDEHYDE CONTENT IN *OCIMUM BASILICUM* L. SEEDLINGS

**LĂCRĂMIOARA OPRICĂ*[1], OLGA MOLCHAN[2], MARIUS-NICUŞOR GRIGORE[1]**

Received: 30th of November 2018 / Revised: 3rd of December 2018
Accepted: 28th of December 2018 / Published: 31st of December 2018

**Keywords:** *Ocimum basilicum*, selenium nanoparticles, salinity, antioxidant system

**Abstract:** Selenium (Se) is an essential nutritional element and its presence has a crucial role in fortification of crops. The aim of the work was to investigate the possible use of selenium nanoparticles (SeNPs) in the mitigation of salinity stress on basil (*Ocimum basilicum* L.) seedlings. Non-enzymatic compounds, enzymatic activity and malondialdehyde (MDA) as indicator of lipid peroxidation products were quantified under the influence of SeNPs and two concentrations of NaCl (50 mM and 100 mM) applied singular or combined. The activity of antioxidant enzymes in *O. basilicum* seedlings was generally lower in the control regardless of the treatment applied singular or combined. The same decreasing trend was noted in the case of total polyphenols and MDA content. On the other hand, both combined treatments stimulated the soluble protein content in relation to the single variant, the increase being more pronounced at 50mM + SeNPs in respect with the singular treatment.

## INTRODUCTION

Sal stress conditions are known to affect plants physiological and biochemical potential, which in turn affect crops primary and secondary metabolism (Hebbara et al., 2003; Hendawy and Khalid, 2005). Salinity is one of the world environmental and agricultural constraints to crop production that not only delays, but also decreases germination of most crops. Lower levels of salinity delay germination, whereas higher levels reduce the final percentage of seed germination (Ghoulam and Fares, 2001). High saline sodic condition affects field and horticultural crops performance particularly in arid and semi-arid regions of the world (Grigore et al., 2014; Grigore and Toma, 2017). On the other hand salinity hinders plant development and diminishes plant growth.

The sweet or common basil, *O. basilicum* L. is the most important species of the genus *Ocimum* which belongs to *Lamiaceae* family. Basils ("king of the herbs"), especially the sweet basil, are commonly used in gastronomy and oral health care (Baritaux et al. 1992; Khatri et al., 1995; Sajjadi, 2006). Although traditionally used as a medicinal herb to treat various conditions, basil has a long history as a culinary herb due to its foliage, which adds a distinctive flavour to many foods (Labra et al., 2004). Since these plants are quite rich in essential oils, they are commonly produced for economic purposes such as diuretic in pharmaceutics or as fragrance in cosmetics (Caliskan et al., 2017). Basil is popular among consumers year-round and controlled environmental conditions enabling stable production worldwide (Park et al., 2016, Oprica, 2016).

Selenium (Se) is an essential nutritional element, but excessive Se can be toxic to animals and humans. Selenium can exist in the (+6), (+4), (0), and (-2) oxidation states, the major feature of Se chemistry that affects the Se solubility and movement in nature. In waters, dissolved inorganic Se is normally present as (+6) selenate ($SeO_4^{2-}$) and as (+4) selenite ($SeO_4^{2-}$) (Long et al., 1990). However it is first removed from soils by plants and soil microorganisms, which can take it into their proteins and produce volatile forms such as dimethyl selenide (El-Ramady Hassan et al., 2014). The obtained SeNP are reported as novel compounds with excellent antioxidant properties and lower cellular toxicity compared with other selenospecies (Estevez et al., 2014). In addition, results of Heba and Ibrahim, (2014, indicate that Se pretreatment enhanced tolerance of wheat plants against drought oxidative stress through modulation of the plant's antioxidant system.

Due to the distinctive properties of Se nanoparticles (SeNP), there is inquisitiveness in its synthesis for nanotechnology applications. SeNP have been exploited for medical purposes such as antimicrobial, antifungal and anticancer. The nanoform of Se can be used for various applications due to its advantages over the bulk form such as low toxicity, better reactivity, and low dosage (Chhabria and Desai, 2016).

Studies have highlighted that Se can offset the damaging effects of abiotic stress, such as drought (Hasanuzzaman and Fujita, 2011), High-Temperature (Djanaguiraman et al., 2010) and heavy metals (Tedeschini et al., 2015, Kumar et al., 2012), UV-B (Yao et al., 2013, Mostafa and Hassan, 2015), salinity (Ardebili et al., 2014, Diao et al., 2014). Using antioxidants up-regulation and its enzymes, Se exposure has been linked at optimal levels to the reduction of

various reactive oxygen species (ROS) (Feng et al., 2013), inhibition of uptake and translocation of heavy metals, changing in heavy metals speciation and rebuilding of cell membrane, chloroplast structures as well as recovery of the photosynthetic system.

On the other hand, Hussein and Abou-Baker, (2018), showed that the foliar application of nano-Zn led to mitigate the adverse effect of salinity of the cotton plants which were irrigated with diluted seawater. In addition, the results of Shalaby et al., (2017), showed that Se addition mitigated the detrimental effect of salinity on lettuce growth and its development.

Therefore in this context, the aim of this study was to report a novel approach in using Se-NPs under abiotic stress. The study also provides an estimation of the response of enzymatic and non-enzymatic antioxidant defense system in *O. basilicum* to Se-NPs application under salinity condition.

## MATERIAL AND METHODS

### 2.1. Experimental design of plant materials and growth conditions

Study of *Ocimum basilicum* seedlings tolerance to salinity and responses to SeNPswas conducted in laboratory conditions, based on completely randomized design with three replications. Sweet basil seeds used in the present experiments were supplied from Agricultural Research and Development Station, Secuieni Neamt. Intact seeds, which were homogeneous and identical in size and colour, and free from wrinkles, were chosen. These seeds were sterilized with 10% sodium hypochlorite for 30 seconds and then were washed with sterile distilled water.

### 2.2. Treatment of biological material

Twenty-five seeds of *Ocimum basilicum* were placed for 4 hours in distilled water solutions and 100µM Selenium nanoparticles (SeNPs) solution (which was prepared at our partner V.F.Kuprevich Institute of Experimental Botany of the National Academy of Sciences of Belarus).
After application of this pre-treatment, the seeds were placed in Petri dishes and watered with water for 7 days. Treatments with selenium nanoparticles and NaCl solution (50 mM, 100 mM) were applied singular or combined, on the sweet basil seedlings, resulting the following variants from Table 1. Biochemical analyses were performed on 14-day-old *Ocimum basilicum* seedlings.

Table 1. Description of SeNPs and saline solution treatments applied singular or combined

| Variants | Treatment |
|---|---|
| Control | Water only |
| SeNPs | Watered only with SeNPs solution |
| 50 mM NaCl | Watered only with saline solution |
| 50mM NaCl + SeNPs | Watered with saline solution and nanoparticle solution (1: 1) |
| 100 mM NaCl | Watered only with saline solution |
| 100mM NaCl + SeNPs | Watered with saline solution and nanoparticle solution (1: 1) |

### 2.3. Oxidative stress index

*Malondialdehyde (MDA)* as indicator of lipid peroxidation products was quantified in enzymatic extracts according to the method described by Hodges et al. (1999). One ml of the enzymatic supernatant was mixed with 2 ml of 0.5% thiobarbituric acid (TBA) solution (in 10% TCA). The mixture was kept at 95 °C for 60 min, and cooled at room temperature, then centrifuged at 12.000 rpm for 10 min to remove the interfering substances. Absorbance was read at 532 nm using UV–Vis spectrophotometer.

### 2.4. Antioxidant system

### 2.4.1. Non enzymatic constituent

*Total polyphenol content assay*

The total polyphenols content was determined by using a modified Folin-Ciocalteau method Singleton et al., (1999). The absorbance of resulting bleu-colored solution was read at 765 nm after two hours, against the blank (distilled water). The amount of the total polyphenolic content was expressed as mg gallic acid equivalent (mg GAE/g DW) ($R^2$ = 0.99). Three readings were taken for each sample and the result averaged.

## 2.4.2. Antioxidant enzymes

### *Preparation of enzyme extracts*

16-days old seedlings sample was homogenized with 0.1 M phosphate buffer (pH=7.5). After that the homogenates were centrifuged at 15,000 × g for 15 min at 4°C, and the supernatants were used for enzyme assays.

*2.4.2.1. Superoxide dismutase (SOD) activity* was estimated by recording the decrease in absorbance of superoxide-nitroblue tetrazolium complex by the enzyme (Winterbourn et al., 1975). About 3 ml of reaction mixture, containing 0.1 ml of 1.5 mM nitroblue tetrazolium (NBT), 0.2 ml of 0.1 M EDTA, 2.55 ml of 0.067 M potassium phosphate buffer, and 0.01 ml of enzyme extraction, were taken in test tubes in duplicate from each enzyme sample. One tube without enzyme extract was taken as control. The reaction was started by adding 0.05 ml of 0.12 mM riboflavin and placing the tubes below a light source of 215 W florescent lamps for 5 min. The reaction was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed maximal color. A non-irradiated complete reaction mixture, which did not develop color, served as blank. Absorbance was recorded at 560 nm and 1 unit of enzyme activity was taken as the quantity of enzyme that reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

2.4.2.*2. Peroxidase (POD) activity* was determined spectrophotometrically by measuring the oxidation of o-dianisidine (3, 30-dimethoxybenzidine) at 540 nm (Möller and Ottolenghi, 1966) with slight modification. The reaction was started by adding 0.1 $H_2O_2$ 0.05% on mixture reaction containing 0.2 ml of enzyme extraction, 0.8 ml distilled water and 1.5 ml 1% o-dianisidine. After 5 min. the reaction was stopped with 2.5 ml $H_2SO_4$ 50%. One unit of POD activity was expressed as the amount of enzyme that produced a change of 1.0 absorbance per min.

*2.4.2.3. Catalase (CAT) activity* was measured according to the method described by Sinha, (1942). Briefly, the assay mixture consisted of 0.4 ml phosphate buffer (0.01 M, pH 7.0), 0.5 ml hydrogen peroxide (0.16 M) and 0.1 ml enzymatic extract in a final volume of 3.0 ml. About 2 ml dichromate acetic acid reagent was added in 1 mL of reaction mixture, boiled for 10 min, cooled. Changes in absorbance were recorded at 570 nm. CAT activity was expressed as the amount of enzyme needed to reduce 1 µmol of $H_2O_2$ per min. The activity of these enzymes (SOD, POD and CAT) was expressed as unit per mg proteins (U/mg protein).

The determination of soluble protein content was determined according to Bradford method (1976) with bovine serum albumin as standard. Thus, this assay is refers to the binding of Coomassie Brilliant Blue G-250 at aromatic amino acid radicals and measuring the colour at 595nm.

**Statistical analysis.** All experiments were carried out with three independent repetitions and the results were expressed as the mean values ± standard deviation (STDEV).

## RESULTS AND DISCUSSIONS

In the conducted study, heterogeneous responses regarding the growth (Fig.1) and activity of antioxidant defense system determined on *O. basilicum* 14 days old seedlings were obtained as a result of saline treatments with different concentrations of NaCl and a solution of 100 µM SeNPs (Figure 2, 3 , 4).

● All treatments, singular and combined applied to *O. basilicum* seedlings determined a decrease in SOD activity in comparison with plants control. There is about a half (45%) reduction in SOD activity in SeNPs treatment applied to seedlings (Figure 2). Singular treatments with 50mM NaCl indicated a comparable SOD activity like in the control. Interestingly, this enzyme activity was reduced by half (46%) in 100mM NaCl treatments. Enrichment of medium of basil seedlings with SeNPs reduced the SOD activity compared with control with 46% (50mM+SeNPs) and 56% (100mM+SeNPs).

● Catalase, enzyme involved in the removal of reactive oxygen species (ROS), converts $H_2O_2$, which results from reaction catalyzed by SOD, in $H_2O$ and $O_2$ in peroxisomes. Treatments applied to the species *O. basilicum* lead to an extremely low activity in singular treatment with

SeNPs, the decrease being of 83% compared to control (Figure 3). Combined treatments determined an increase comparatively with simple treatment (both with SeNPs and 50 mM NaCl) in the case of 50mM + SeNPs. Interestingly, in the 100mM saline treatment and combined with SeNPs, CAT activity showed very close values.

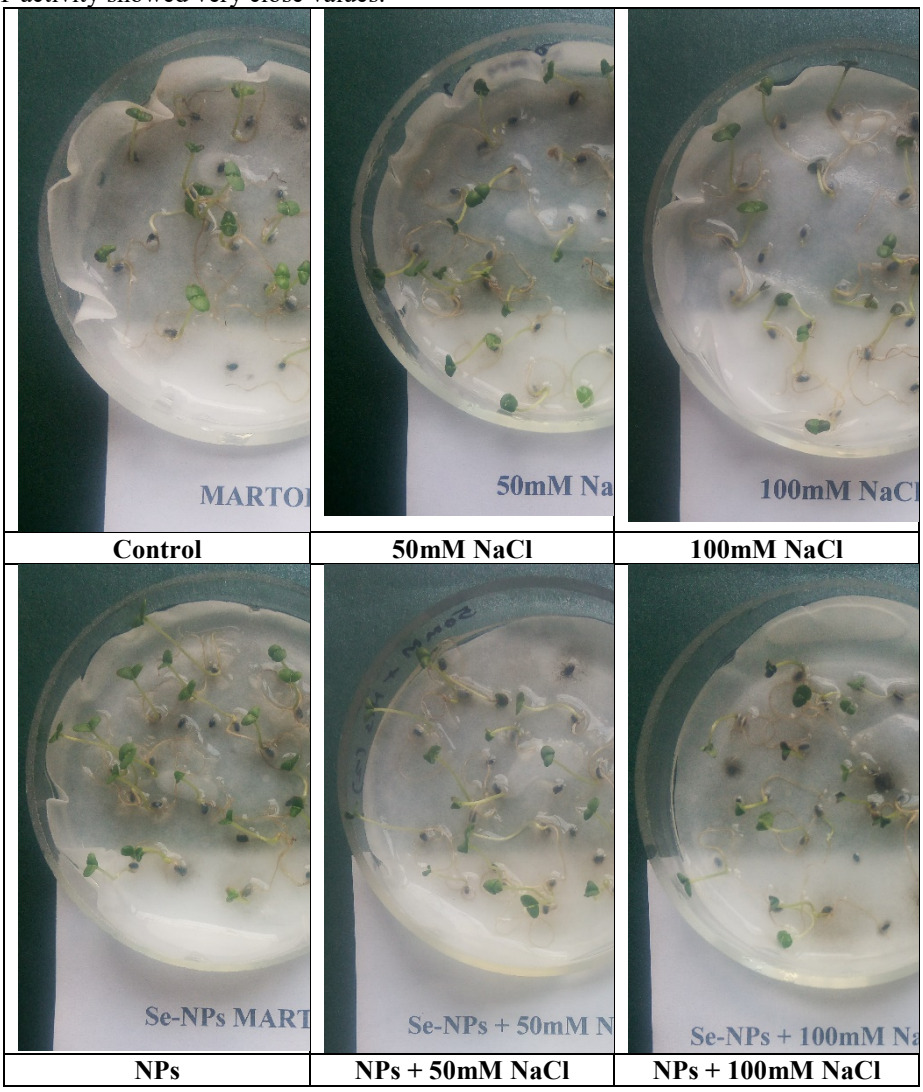


**Fig. 1.** Effect of treatment of Se NPS and NaCl solution (singular or combined) in 14-day-old *Ocimum basilicum* seedling

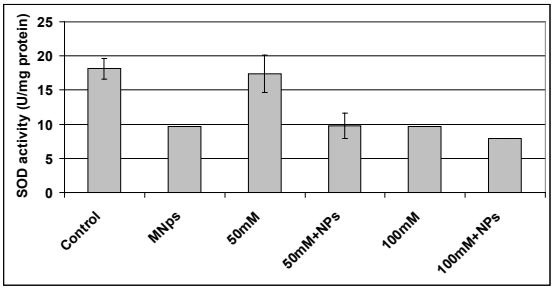


Fig. 2. SOD activity in 14-days-old *Ocimum basilicum* seedlings

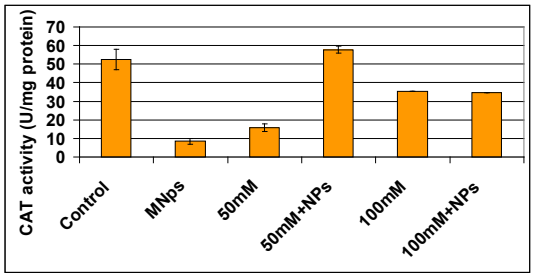


Fig. 3. CAT activity in 14-days-old *Ocimum basilicum* seedlings

● Peroxidase is involved in the oxidation of many substrates in the presence of $H_2O_2$ with the production of $H_2O$: $AH_2 + H_2O_2 \rightarrow A + 2H_2O$. This enzyme is stimulated by the accumulation of $H_2O_2$, being able to remove this toxic compound. Compared to CAT, POD has an increased affinity for $H_2O_2$ but has a low processing rate. The obtained results indicate a decrease with 28% in POD activity in *O. basilicum* seedlings treated with SeNPs compared to the control. The combined treatment 50mM NaCl + SeNPs indicates stimulation of POD activity compared to 50mM NaCl singular treatment and with the plants control (with 51%). In contrast, treatment combined with 100mM NaCl + SeNPs reveals a relatively similar activity to singular treatment and an increased compared to control (48%) (Figure 4).

● Saline and SeNPs treatments applied singular and combined determined a stimulation of soluble protein content in basil seedlings compared to the plant control. Thus, singular SeNPs treatment improved the stimulation of soluble protein content with 51%. Both combined treatments stimulated the soluble protein content in relation to the single ones, the increase being more pronounced at 50mM + SeNPs compatively with the singular treatment (Figure 5). On the other hand, the highest amount of protein was obtained when applying 100mM + SeNPs treatments. It can be seen the SeNPs role in alleviate the adverse effect of salinity in terms of increasing the soluble proteins content.

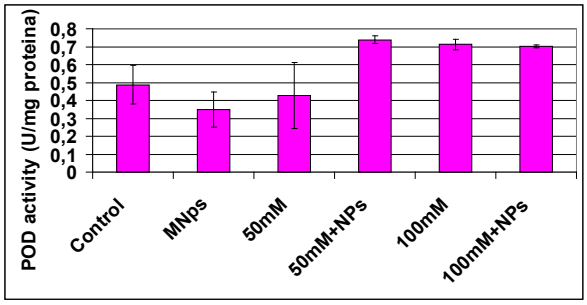


Fig. 4. POD activity in 14-days-old *Ocimum basilicum* seedlings

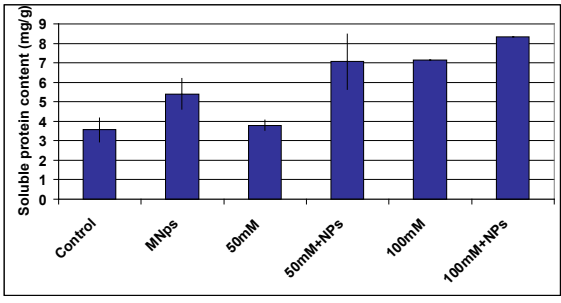


Fig. 5. Soluble protein content in 14-days-old *Ocimum basilicum* seedlings

● Lipid peroxidation induced by free radicals is also important in cell membrane damage. The level of lipid peroxidation, measured by the MDA content, was considered an indicator of salinity induced oxidation and useful for determining the salt tolerance of plants (Hernandez and

Almansa, 2002). Data of MDA content in 14-days-old *O. basilicum* seedlings in the presence of SeNPs and salinity are depicted in Figure 5. Therefore, singular SeNPs treatment applied to *O. basilicum* caused a slight decrease (10%) in MDA content as opposed to control. The lowest NaCl concentration applied singular leaded to the MDA content very similar to the control. In addition, both concentrations of NaCl in combination with SeNPs determined a decrease in MDA content compared to singular treatments (Figure 6).

● High antioxidant activity is reported from different medicinal plants, and phenolic compounds and flavonoids are the usual antioxidants present in them. Figure 7 showed a decrease of total polyphenol content in *O. basilicum* seedlings after the applied of treatments.

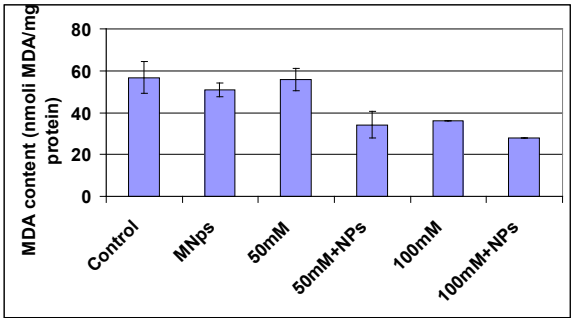


Fig. 6. MDA content in 14-days-old *Ocimum basilicum* seedlings

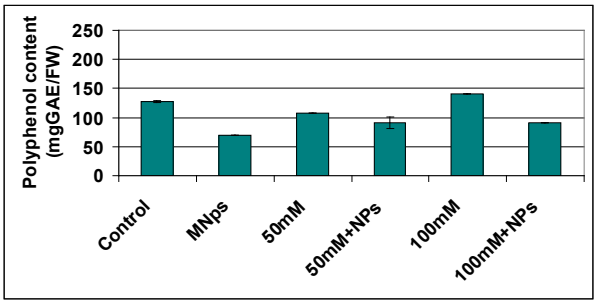


Fig. 7. Polyphenol content in 14-days-old *Ocimum basilicum* seedlings

Basil seedlings treated with singular SeNPs indicated a half-reduced (45%) polyphenol content compared to the plant control. Combined treatments with SeNPs and two NaCl concentrations applied determined a decrease in polyphenol content in relation to the corresponding saline treatments; the decrease being more pronounced at 100mM + SeNPs versus 100mM NaCl (140,71 mgGAE/FW and 90,7464, respectively).

## CONCLUSIONS

Singular treatments with SeNPs determined in 14-days-old *O. basilicum* seedlings, a decrease in the activity of antioxidant enzymes (SOD, CAT, POD), in total MDA and total polyphenols compared to the plant control. Furthermore, the same treatment stimulated the soluble protein content.

Combined treatments with SeNPS and different concentrations of NaCl have resulted in a decrease in SOD activity in basil seedling as compared to the corresponding single treatments. The same downward trend was noted for MDA and total polyphenols contents when saline stress was alleviate by applying SeNPs. On the other hand, the protein content was stimulated by the application of combined treatments, the mitigating salinity being more pronounced at low NaCl concentration.

## REFERENCES


- **Ardebili, N.O., Saadatmand, S., Niknam, V., Khavari-Nejad, R.A.,** (2014): The alleviating effects of selenium and salicylic acid in salinity exposed soybean. Acta Physiol Plant 36:3199–3205.
- **Baritaux O., Richard H., Touche J., Derbesy M.,** (1992): Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. Flavour and fragrance, J., 7, 267-271
- **Bradford, M.M.,** (1976): A rapid and sensitive method for microgram quantities of protein utilizing the principle of protein-dye binding, An. Biochem., 72, 248–254.
- **Caliskan, O., Kurt, D., Temizel, K.E., Odabas, M. S.**, (2017): Effect of Salt Stress and Irrigation Water on Growth and Development of Sweet Basil *(Ocimum basilicum L.)*. Open Agriculture, 2, 589-594.
- **Chhabria S., Desai K.,** (2016): Selenium Nanoparticles and Their Applications, Chapter in *Encyclopedia of Nanoscience and Nanotechnology* (Edited by H. S. Nalwa), American Scientific Publishers
- **Diao, M., Long, Ma, Jianwei, W., Cui, J., Fu, A., Liu, H-Y.,** (2014): Selenium promotes the growth and photosynthesis of tomato seedlings under salt stress by enhancing chloroplast antioxidant defense system. J Plant Growth Regul 33:671–682.
- **Djanaguiraman, M., Prasad, P.V.V., Seppanen, M**, (2010): Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol. Biochem. 48, 999−1007.
- **El-Ramady, Hassan R, Domokos-Szabolcsy Eva, Abdalla Neama A., Alshaal Tarek A, Shalaby Taek A, Sztrik Attila, Prokisch Joszef, Fari Miklos,** (2014), Selenium and nano-selenium in agroecosystems, Environmental Chemistry Letters, 12 (4), 495-510, 1610-3653.
- **Estevez H, Garcia-Lidon J.C., Luque-Garcia J.L., Cmara C.,** (2014): Effects of chitosan-stabilized selenium nanoparticles on cell proliferation, apoptosis and cell cycle pattern in HepG2 cells: comparison with other selenospecies. Colloid Surf B., 122:184–93.
- **Feng, R., Wei, C., Tu, S.,** (2013): The roles of selenium in protecting plants against abiotic stresses. Environ Exp Bot 87:58–68
- **Ghoulam C., K. Fares,** (2001): Effect of salinity on seed germination and seedling growth of sugar beet (*Beta vulgaris* L.). Seed Sci. Technol., 29: 357-364.
- **Grigore MN, Ivanescu L., Toma C.,** (2014): Halophytes. An integrative anatomical study. Springer, Cham, Heidelberg, New York, Dordrecht, London.
- **Grigore MN, Toma C.,** (2017): Anatomical Adaptation of Halophytes. A review of Classic Literature and Recent Findings, Spronger International Publishing.
- **Hasanuzzaman, M., Fujita, M.,** (2011): Selenium pre-treatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biol. Trace Elem. Res., 143, 1758−1776
- **Heba, M. Ibrahim**, **(**2014): Selenium pretreatment regulates the antioxidant defense system and reduces oxidative stress on drought-stressed wheat (*Triticum aestivum* L.) Plants, Asian Journal of Plant Sciences,**13** (3): 120-128.
- **Hebbara, M., Rajakumari, G.R., ravishankari, G. and Raghavaiah, C.V.** (2003): Effect of salinity stress on seed yield through physiological parameters in sunflower genotypes. Helia 26(39): 155-160.
- **Hendawy, S.F., Khalid, K.A.** (2005): Response of sage (*Salvia officinalis* L.) plants to zinc application under different salinity levels. Journal of Applied Sciences Research 1(2): 147-155.
- **Hernández, JA, Almansa, MS.,** (2002): Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves, Physiol Plant., 115(2):251-257.
- **Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K.**, (1999): Improving the thiobarbituric acid reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta,* 207**,** 604–611.
- **Hussein, MM, Abou-Baker, NH.** (2018): The contribution of nano-zinc to alleviate salinity stress on cotton plants. R. Soc. Open Sci., 1-15.
- **Khatri, L.M., Nasir, M.K.A., Saleem, R., Noor, F**., (1995): Evaluation of Pakistani sweet basil oil for commercial exploition. Pakistan J. Sci. Ind. Res., 38, 281-282
- **Kumar, M., Bijo, A., Baghel, R. S., Reddy, C., Jha, B.,** (2012): Selenium and spermine alleviate cadmium induced toxicity in the red seaweed Gracilaria dura by regulating antioxidants and DNA methylation. Plant Physiol. Biochem., 51, 129−138.

- **Labra, Mariangela, Miele, Bernardetta, Ledda, Fabrizio, Grassi, Mauro, Mazzei, Francesco, Sala,** (2004): Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars Massimo, Plant Science 167, 725–731.
- **Long, R.H.B., Benson, S.M., Tokunaga, T.K., Yee, A.** (1990): Selenium Immobilization in Pond Sediment at Kesterson Reservoir. J. Environ. Qual.,19, 302-311.
- **Möller, K.M., Ottolenghi, P.,** (1966): The oxidation of o-dianisidine by $H_2O_2$ and peroxidase at neutral pH. Compts. Rend. Trav. Lab., Carlsberg, .35, 369–389.
- **Mostafa, E.M., Hassan, A.M.A.** (2015): The ameliorative effect of selenium on *Azolla caroliniana* grown under UV-B stress. Phytoprotection 95:20–26.
- **Oprica L,** (2016): Metaboliti secundari din plante. Origine, Structura si functii. Editura Universitatii Alexandru Ioan Cuza Iasi.
- **Sajjadi ,S.E., (**2006): Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. Daru, 4(3), 128-130
- **Shalaby, T., BayoumiY., Alshaal, T., Elhawat, N., Sztrik, A., El-Ramady, H.,** (2017): Selenium fortification induces growth, antioxidant activity, yield and nutritional quality of lettuce in salt-affected soil using foliar and soil applications, Plant and soil, v.421 no.1-2 pp. 245-258.
- **Singleton V., Orthofer R., Lamuela-Raventós R. M.,** (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods in Enzymology, 299, 152-178.
- **Sinha, A. K.,** (1972): Colorimetric assay of catalase. Anal. Biochem., **47**:389-394.
- **Tedeschini, E., Proietti, P., Timorato, V., D'Amato, R., Nasini, L., Dei, Buono, D., Businelli, D., Frenguelli, G.** (2015): Selenium as stressor and antioxidant affects pollen performance in Olea europaea. Flora.
- **Winterbourn, C, Hawkins, R, Brian, M, Carrell, R.,** (1975): The estimation of red cell superoxide dismutase activity. J. Lab. Clin. Med., 85: 337.
- **Yao, X., Jianzhou, C., Xueli, H., Binbin, L., Jingmin, L., Zhaowei, Y.** (2013): Effects of selenium on agronomical characters of winter wheat exposed to enhanced ultraviolet-B. Ecotoxicol Environ Saf 92:320–326.


**Acknowledgements**
We would like to acknowledge Professors Olga MOLCHAN who provided the selenium nanoparticles with which we designed this experiment.

[1]"Alexandru Ioan Cuza "University, Faculty of Biology, 20A Carol I Bd., 700506 Iasi, Romania
[2]"V.F.Kuprevich Institute of Experimental Botany of the National Academy of Sciences of Belarus, 220030, Akademicheskaya str., 27, Minsk

*Corresponding author: lacramioara.oprica@uaic.ro