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RESEARCH ARTICLE

ANTIOXIDANT CAPACITY AND TOTAL PHENOLS CONTENT CHANGES OF BROCCOLI SPROUTS AFTER EXOGENOUS SUPPLY WITH NANO SELENIUM

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ABSTRACT

Background: The relation between the minerals content of plants and human health benefits was proven by previous data from literature. Selenium is an essential trace element for human health due to its different biological activities.

Objective: Our aim was to investigate the effects of nano-selenium (NSe) supply on broccoli sprouts from point of view of growth parameters, total phenols content and antioxidant capacity of selenium-enriched broccoli sprouts.

Method: The NSe particles were produced by chemical reduction of $NaHSeO_3$ with glucose. Physicochemical characteristics of NSe was performed by UV-Vis spectroscopy and Dynamic Light Scattering. Broccoli seeds were germinated in the plastic boxes, sprinkled every day with different concentrations of nano-selenium solution (10, 50 and 100 mg/L) for 9 days. The length of shoots and roots were measured, and the total phenols content was determined by Folin-Ciocalteu method, while antioxidant capacity was evaluated by DPPH assay.

Results: By NSe supply, the growth parameters of broccoli sprouts were not affected compared with the control sample. Total phenol content of shoots was not affected by treatment with NSe, but 50 and 100 mg/L NSe supply increased the total phenols content of roots compared with the control. The antioxidant capacity of shoots was increased significantly (P=0.05) in all the samples treated with NSe. With respect to the roots, only the treatment with 10 mg/L significantly increased the antioxidant capacity.

Conclusion: Using NSe particles as fertilizer, selenium-enriched broccoli sprouts can be obtained, with positive effects on human health.

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INTRODUCTION

Broccoli is an important source of bioactive compounds, and it is considerate a functional food. Data evidence sustain that Phenolic compounds, green pigments and flavonoid compounds possess a great antioxidant activity and that they may have an influence on prevention of chronic diseases, especially cancer. *(Villarrea and Iacobo, 2016).* The importance of selenium for human health has been suggested in a number of studies, although, due to the fact that this mineral has narrow safety margins, the controversies regarding supplementation still remain. Also, the epidemiologic data is far from conclusive (Piekarska *et al*., 2014). Proper intake of selenium was shown to be associated with reduced risk of prostate, skin, colorectal, liver, mammary, and lung cancers (Clark, 1998; Lener *et al*., 2013; Reid *et al*., 2002; Yoon *et al*., 2001; Zen and Combs, 2008; Zlowcka-Perlowska *et al*., 2012).

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The aim of this experimental work was to observe the effect of different concentrations of selenium nanoparticles on plant germination and its relation to the growth parameters of *Brassica oleracea* sprouts.

MATERIALS AND METHODS

The research was conducted in the Biotechnological, Chemical and Biochemistry laboratories in The College of Environmental Protection of the University of Oradea. The plant material, the broccoli seeds (*Brassica oleracea var. cymosa*), were purchased from the Agrosel Company and certified as professional products. Seeds were presented in plastic casseroles, 50 seeds/casserole (in duplicates – 8 casseroles for each nanoparticle). The growing substrate used for the seedlings was the filter paper. The seeds were sprinkled twice a day with different concentrations of NS elenium solution (10, 50 and 100ppm). The NSe particles were produced by chemical reduction of $NaHSeO_3$ with glucose.

After each treatment, the control group was sprinkled with pure water. The experiments continued for 8 days (1-02.-08.02 2016).

Determination of germination capacity and biometrics measurements

The germination was carried out in the (phytotron) with controlled temperature (25^0Celsius) and photoperiod (16 h of light and 8 h in the dark). The germination capacity was determined at 48 hours from starting the experiments, when the radicle was approximately 2 mm long or more. To determine the percent of germination (%) the following formula was used:

Germination (%) =number of germinated seeds x 50 /total number seeds

At the final of experiment (after 8 days), stems and roots were separated, and their length/weight were measured immediately after harvest. Values were calculated in gram per 20 seedlings.

Statistical findings

All the data were processed by one-way analysis of variance (ANOVA) (P = 0.05). Mean value differences were analysed with Tukey's test (P = 0.05). Total phenolic content were determined by the Folin Ciocalteu method(Singleton *et al.*1999, *Vicas et al. 2011*), 10-fold diluted (100 µl), broccoli extracts were mixed with 1700 µl distilled water and 200µl Folin-Coocalteau reagent (freshly diluted 1:10,v/v).After 3 minutes, 1 ml of 15% sodium carbonate was added. The samples were incubated at room temperature for 2 hours. After absorbance was measured at 765 nm, the calibration curve was performed with the spectrophotometer mini UV- Vis Schimatzu, the calibration curve was performed against gallic acid over a range of 0.05-0.25mg/ml, the result was expressed in mg equivalents of gallic acid (AGE) 100ml.

A simple and inexpensive method to measure the antioxidant capacity of plant extracts is the use of 2,2-Diphenyl-1-picrylhydrazyl free radical(DPPH**)**. The DPPH method was determined according to the method described by *Vicas et al. 2011.*In few,2,8 ml of 80 µM concentration solution was introduced into the cuvette spectrophotometer, 200 µl of extract was added to the reagent, and the reaction was monitored at 515 nm for 5 minutes. The percentage of neutralization by apple juice was calculated using the following equation:

The percentage of inhibition of $DPPH\ (\%) = [(A_0 - A_s) \times 100] / A_0$

RESULTS AND DISCUSSION

Germination samples were calculated at 48 hours post-experiment; it was determined by counting not germinated seeds in relation to the total number of seeds. The percent of germination (%) of broccoli seeds encountered a growth of different concentrations of nano selenium (10, 50 and 100 ppm) that are presented in Fig. No.1, Fig. No.2 and Fig. No.3 Under NSe treatment, the broccoli germination was not affected when the different concentrations of NSe were used compared with control sample (Fig.No.1). Regarding to the roots weight, the broccoli sprouts recorded a decrease comparative with control.

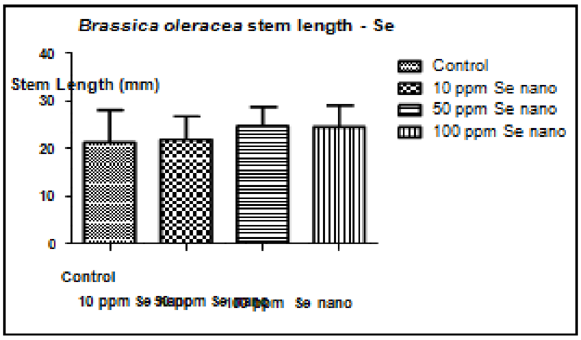

Figure 1. ***Brassica oleracea*** **sprout stem length**

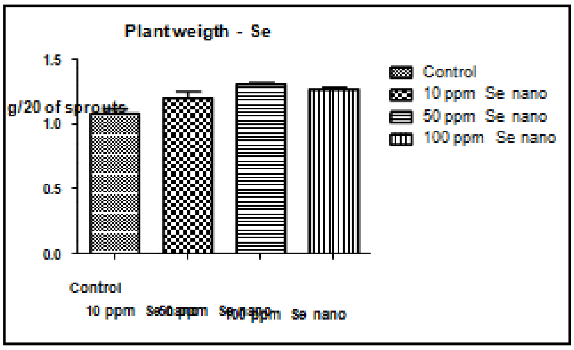

Figure 2. ***Brassica oleracea*** **sprout stem weigth**

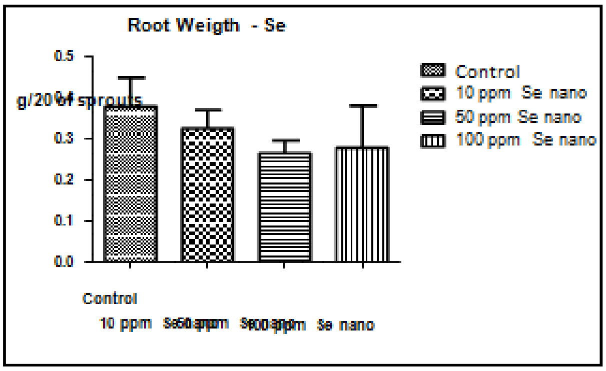

Figure 3. ***Brassica oleracea*** **sprout root weigth.**

Some *Brassica* vegetables have been designated as selenium accumulators because of their ability to absorb large amounts of inorganic form of selenium (Piekarska *et al*., 2014). Inorganic form of selenium absorbed may be transformed into various forms. Because selenium and sulphur share the same uptake and assimilation pathways in plants, selenium can be incorporated into any organo-sulphur compound (Ramos *et al*., 2011). The data obtained after quantification of the polyphenolic compounds were statistically processed and the meanings obtained are shown in Fig. No. 4

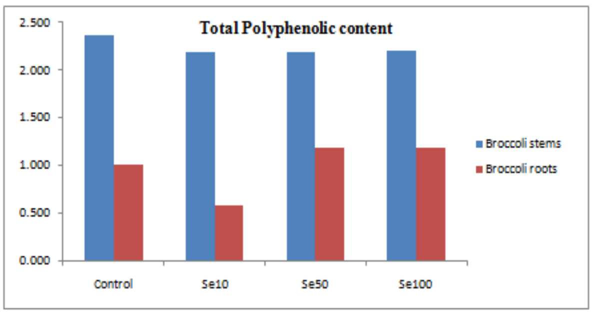

Fig 4. ***The total content of polyphenolic compounds in the roots and stems***

There were no significant statistical differences between the experimental variants studied. In contrast, significant differences were observed between the blank and the sample 10 ppm sample. Among the variants that were sprayed with selenium solutions of different concentrations, significant differences in total polyphenol content in the root were obtained between the 10-ppm sample and the 50-ppm sample, and between the 10-ppm sample and the 100-ppm sample. The ability to remove the DPPH by broth stems and roots of the four experimental variants is presented in Fig. No. 5.

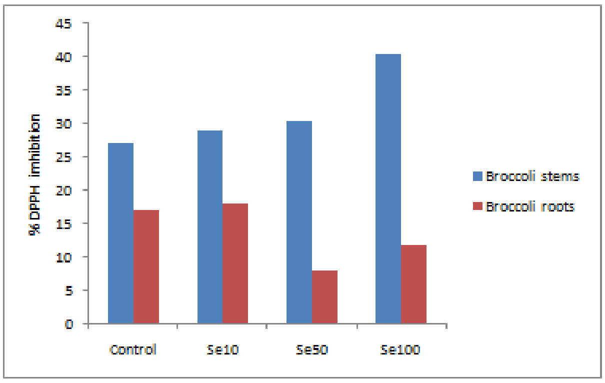

Fig. No. 5 The antioxidant capacity of broccoli germ stem extracts and the antioxidant capacity of broccoli germ root extracts

The highest DPP removal capacity was shown in the experimental variant of the 100-ppm sample (40.44%) from the broccoli strain. From a statistical point of view, significant differences were noted between all experimental variants, both in the case of stem extracts and in the case of root extracts. By comparing the antioxidant capacity of strains and broccoli germs, it is observed that the stems have an antioxidant capacity almost double that of the root.

Conclusion

In this present study, the broccoli seeds watered with NSe particles proofed to be an efficient way of enriching *Brassica* sprouts with this regarding element without a visible impact on plant physiology. Brassica sprouts and their biological effects in human health suggest that NSe-enriched sprouts may be recommended as a good and safe means for combating selenium deficiencies in the diet.

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