

Journal of Ornamental Plants www.jornamental.iaurasht.ac.ir ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

Research Paper DOR: https://dorl.net/dor/20.1001.1.22516433.2021.11.2.5.4

Selenium Improves Physiology, Biochemistry, and Quality Characteristic of Flowers in Ivy Geranium (*Pelargonium peltatum* L.)

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Received: 15 January 2021 Accepted: 15 January 2021

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Selenium (Se) is an essential microelement for humans and a beneficial element for plants. Recently, biofortification with Se has emerged as a key practice to increase plant quality. The aim of present study was to investigate the postharvest responses of *Pelargonium peltatum* L. to the application of Se in soil. The Se was used as sodium selenite (Na₂SeO₄) in soil application of 20, 40, and 60 μ M distilled water. The results showed that flower weight, relative water content (RWC), anthocyanin, and vitamin C decreased over the storage time. Malondialdehyde (MAD) increased by progressing the time of storage. Total phenolic content (TPC) and total flavonoid content (TFC) were significantly enhanced at early days of storage and then decreased up to the end of storage on days 4 and 6. Se significantly improved flower weight, RWC, anthocyanin, vitamin C, TPC, and TFC entire the storage. Although, Se was effective in enhancing the quality of *P. peltatum* L., there was no significant difference between 40 and 60 μ M Se in all traits. Therefore, this experiment suggests using 40 μ M Se to alleviate adverse effects induced by postharvest time growth on productivity of *P. peltatum* L.

Keywords: Anthocyanin, Biochemical properties, Postharvest, Sodium selenite.

Abstrac

INTRODUCTION

Phytotherapy by edible flowers has been used since the earliest times (Pinela *et al.*, 2017). Recently, a new trend has emerged, mainly in the widespread use of flowers. The main task of plants in nature is to grow fruits and seeds. Therefore, flowers may contain insect repellents or toxic substances produced by plants to prevent their loss (Egebjerg *et al.*, 2018). It is worth noting that not all flowers used in herbal medicine are edible. Some of them contain substances that have a very strong effect on the human body and may be toxic (Kristanc and Kreft, 2016). For hundreds of years, edible flowers have been used for flavoring and decoration in the culinary arts. Early reports indicate that the Romans used flowers to cook, as did Chinese, Middle Eastern, and Indian cultures (Pinela *et al.*, 2017).

Pelargonium (*Pelargonium peltatum*) is a genus of 400 species distributed in temperate and humid regions of the world. This species has an annual production value of US \$ 2.5 billion, ranking third among ornamental plants. This plant is also a medicinal plant, recently introduced as an edible flower. It has been widely used in Iran to treat a wide range of diseases such as bronchitis and sinusitis (Abedini-Aboksari *et al.*, 2018).

Nutrient supply is the most important component for growth and productivity (Mohiti *et al.*, 2011; Mohammadi Torkashvand and Kaviani, 2014; Mohammadi Torkashvand *et al.*, 2016; Abedini-Aboksari *et al.*, 2016; Mehrabani *et al.*, 2016). Most of the plants require 17 essential elements. Selenium (Se) is considered to be a beneficial nutrient but it has not been shown to be essential (Hartikainen, 2005; El-Ramady *et al.*, 2016). Se concentrations in plants are significantly related to its status in human dietary (Feng *et al.*, 2013). Low Se content in humans is globally concern for humans. Loss of Se causes epilepsy and immunodeficiency and decreases fertility. Se has significant role in immunity system with anti-ageing and anticancer effects (Du *et al.*, 2019).

Literature review has shown the useful effects of Se on postharvest response different plants (Aqaei *et al.*, 2020). For instance, Lu *et al.* (2020) showed improved antioxidant enzymes, RWC, and MDA under Se application in garlic leaves *Lilium longiflorum* cut flower. The improvement of tomato ripping during storage period under Se was reported by Puccinelli *et al.* (2019). However, there is no investigation about Se on nutritional and biochemical values of edible flowers during postharvest storage. Therefore, the present study was conducted to assess the effect of Se concentrations on flower weight, physiological and biochemical properties of *P. peltatum* L. as an edible flower. We hypothesized that Se would be effective in improving the flower quality during storage time. By evaluating the possibility of the utilization of Se in alleviating undesirable effects induced over the storage, the findings of this study help to suggest the best concentration of Se and also the appropriate time of storage time of *P. peltatum* L.

MATERIALS AND METHODS

Plant material

The cuttings of *P. peltatum* L. were obtained from a commercial grower of Netherland. They were planted in cultivation beds containing coco peat, perlite, rice husk, cattle manure, and sandy soil (30, 30, 20, 10, and 10%). Table 1 shows the soil properties in the experiment. The pot experiment was conducted in a greenhouse with a photoperiod of 16/8 (lightness/darkness) and relative humidity of 65%-80% at a commercial greenhouse in Golzar, Pakdasht of Iran. In total, we used 96 experimental pots with a top diameter of 19 cm and height of 10 cm.

Experimental design and treatment details

The factorial experiment was carried out based on a completely randomized design (CRD) with three replications in 2018. Se was used as sodium selenite (Na₂SeO₄) in the soil application of 20, 40, and 60 μ M. After 4 leaves developmental stage, the plants were treated with Se concen-

рН	EC (dS/m)	OC (%)	N (%)	P (mg/kg)	K (mg/kg)	Cd (mg/kg)	Sand (%)	Silt (%)	Clay (%)
8.1	0.85	1.2	0.98	16	275	0.10	22	45	32

Table 1. The soil characteristics for Pelargonium peltatum L.

trations four times during the experiment in the 10 day intervals. At the flowering stage, the plants were harvested and kept at the storages times as day 0, day 2, day 4, and day 6. At the end of experiment, the samples were sent to University of Tehran for further physiological and biochemical analysis.

Relative water content (RWC) measurement

The developed leaves were used to measure RWC according to the method of Dhopte and Manuel (2002) as follows:

$$RWC = \frac{(FW - DW)}{(SW - DW)} \times 100$$

Where, FW is fresh weight, SW is leaf weight after soaking for 24 hours at room temperature.

Determination of ascorbic acid (vitamin C)

To determine ascorbic acid, 0.2 g of flower plants were homogenized in 1 ml of distilled water and then shaken at 4°C overnight. The solution was centrifuged (12000 rpm) for 10 min at 4°C and the supernatant was directly used for ascorbic acid assay (Quarrie *et al.*, 1988).

Determination of anthocyanins

The amount of anthocyanin after proper dilution was measured at 530 and 657 nm with a spectrophotometer (Shimadzu, Tokyo, Japan) as described by Sankhla *et al.* (2003).

Malondialdehyde (MAD) concentration

To measure the MAD content, phosphate buffer was used to extract the samples and centrifuged at 14,000 rpm for 30 min. Subsequently, thiobarbituric acid (0.5% w/v) containing 20% w/v trichloroacetic acid was added to the mixture. After experiencing the hot water bath for 30 min, the extract was immediately cooled on ice, and finally centrifuge at 10,000 rpm for 10 min. The samples were read at wavelengths of 532 and 600 nm (Heath and Packer, 1969).

Determination of total phenolic content (TPC)

The Folin-Ciocalteu reagent was chosen to determine TPC spectrophotometrically (Medina, 2011). 0.1 mL Folin-Ciocalteu reagent was mixed with 4 m Na₂CO₃ solution. Then 0.5 ml solution of each plant extract or gallic acid was added to the mixture. The mixtures were placed at room temperature for 15 min. After this period, the absorbance of the samples was measured by a Lambda 45-UV / Visible spectrophotometer at 765 nm. The standard curve was prepared by concentrations of 0, 50, 100, 150, 200, 250 mg L⁻¹ gallic acid. TPC was calculated as equivalent to mg of gallic acid (GAE) per gram of dry weight.

Determination of total flavonoid content (TFC)

The flavonoid levels were measured by aluminum chloride colorimetric method (Zhishen

et al., 1999). First, 0.1 ML of 10% aluminum chloride was mixed with 0.1 ml of potassium acetate (1 M), and then 2.8 mL of distilled water was added. In the next step, 0.5 ml of each extract solution mixed with 1.5 ml of ethanol was added to a mixture of aluminum chloride, potassium acetate and water. The final mixture for each extract (5 ml) was placed at room temperature for 30 min. Then the adsorption of the reaction mixture at 415 nm was measured by a Lambda 45-UV / Visible spectrophotometer. The TPC was calculated and expressed as equivalent to mg quercetin per gram of dry weight.

Statistical analysis

The data (n=3) were subjected to one-way analysis of variance (ANOVA) and using the SAS software package for Windows (SAS, version 9.3, SAS Institute, Cary, NC). Duncan's multiple range tests showed the comparison of mean values. The data were statistically investigated at 5% probability level.

RESULTS AND DISCUSSION

Flower weight

The changes of flower weight over time under Se application are presented in Fig. 1. It decreased over the time after harvesting, but increased by Se application. There was no significant difference between initial time (day 0) and second day of postharvest; however, the significant reduction of flower weight was obtained in days 4 and 6 compared to day 0. In non-Se treatment, a 40 % decline of flower weight was observed in the end of experiment compared to the first time. Se significantly increased flower weight as 40 and 60 μ M was significantly stronger in increasing the flower weight compared to other treatments.

Relative water content (RWC) and malondialdehyde (MDA)

RWC decreased over time and increased by Se application. It ranged from 53% in day 6 and non-Se treatment to 80.3% in day 2 and 40 μ M Se. The plants which were remained by days 4 and 6 had lower RWC as compared to control. However, RWC increased by progressing Se up to 40 μ M (Fig. 2a). Although, MDA increased over time of harvesting, it was remained unchanged under Se application. Under non-Se application, day 6 increased MDA by 60% compared to day 0 (Fig. 2b).

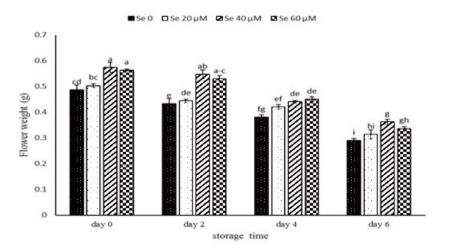


Fig. 1. Flower weight under selenium concentrations during storage time. Values are means \pm standard error of mean (SEM) of three replications (n= 3). Different letters show statistically significant differences among treatments at P<0.05.

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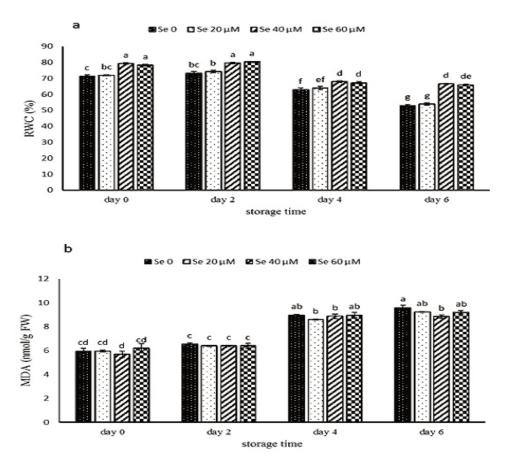


Fig. 2. Relative water content (RWC) and malondialdehyde (MDA) under selenium concentrations during storage time. Values are means \pm standard error of mean (SEM) of three replications (n= 3). Different letters show statistically significant differences among treatments at P<0.05.

Vitamin C and anthocyanin

By progressing the time of storage, vitamin C concentration decreased. The Se was effective in improving vitamin C content. It differed from 0.53 mg g⁻¹ FW at day 6 and non-Se treatment to 1.35 mg g⁻¹ FW at day 0 and 20 μ M Se (Fig. 3a). Like vitamin C, anthocyanin showed the decline in days 4 and 6. Although, all concentrations of Se increased anthocyanin content compared to control, there was no significant difference between 40 and 60 μ M Se (Fig. 3b). Anthocyanin in petals of flowers increased in day 2 and then decreased in plants experiencing days 4 and 6 (Fig. 3b).

Total phenolic content (TPC) and total flavonoid content (TFC)

The changes of TPC and TFC over time under Se are presented in Fig. 4. We observed the higher TPC in day 2 with 40 or 60 μ M Se compared to other treatments. The remarkable reduction of TPC was obtained in flowers experiencing the maximum time of storage (day 6). Under non-Se application, the 45% reduction of TPC was reported on day 6 when compared to day 2. In addition, TFC increased on day 2 and then decreased on days 4 and 6. However, Se was effective in improving the TFC. The concentration of TFC differed from 4.33 mg QA g⁻¹ DW on day 6 with non-Se use to 9.36 mg QA g⁻¹ DW on day 2 and 40 μ M Se.

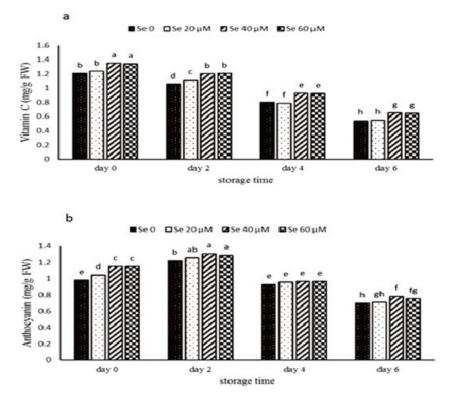


Fig. 3. Vitamin C and anthocyanin contents under selenium concentrations during storage time. Values are means \pm standard error of mean (SEM) of three replications (n= 3). Different letters show statistically significant differences among treatments at P<0.05.

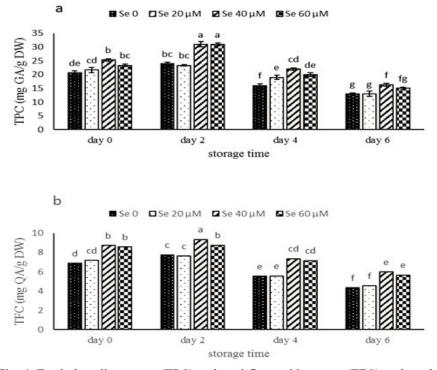


Fig. 4. Total phenolic content (TPC) and total flavonoid content (TFC) under selenium concentrations during storage time. Values are means \pm standard error of mean (SEM) of three replications (n=3). Different letters show statistically significant differences among treatments at P<0.05.

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Moderate level of Se (40 μ M) was effective in plant weight. This concentration of Se, probably by increasing the starch content in chloroplasts, increase plant growth and protect the cell membrane of these plants due to antioxidant properties against lipid peroxidation (Aqaei *et al.*, 2020). According to our results, concentration of Se more than 40 μ M Se was not suggested due to no significant difference in all traits even it showed negative impact in some traits. So, it can be concluded that more than 60 μ M Se can change the membrane permeability to Na, K, and Ca which impair respiration and water uptake (Mozafariyan *et al.*, 2017). Similarly, other authors have also found a positive effect of Se application on growth and yield of snapdragon flowers (Tognon *et al.*, 2016), spinach (Golubkina *et al.*, 2017), and tobacco (Jiang *et al.*, 2015) under preand postharvest treatments.

RWC decreased over time of storage, which further increased the production of MDA and H₂O₂ (Lu et al., 2020). By progressing the time, the oxidative damage to P. peltatum L. was induced by enhancing the production of MDA and reactive oxygen species (ROS). However, RWC was considerably increased by Se application up to 40 µM. It is well addressed that Se can protect plants from water loss (Alyemeni et al., 2018). At a high Se concentration (60 µM), we found that compared with the lowest concentration, RWC tended to decrease at day 6 of storage. High concentrations of Se are harmful to plants, resulting in reduced water content and physiological processes (Ekanayake et al., 2015). Lu et al. (2020) reported the improvement of RWC and MDA under moderate Se concentration. Excess Se was addressed to be toxic to plants by increasing ROS production, further exacerbating oxidative damage to plants (Silva et al., 2018). The permeability of the cell membrane, expressed as a relative leakage rate, can reflect the degree of senescence and cell damage, as well as the integrity of the cell membrane structure of the harvested plant (Lu et al., 2020). The production of superoxide anions and the accumulation of ROS increase to modify the balance between ROS and its scavenging system. Excessive ROS production promotes membrane peroxidation (MDA) followed by severe cell membrane damage and increased membrane permeability (Andrade et al., 2018; Tofighi Alikhani et al., 2021).

Vitamin C is usually used for evaluating nutritional quality and flavor in edible plants (Molmann *et al.*, 2015). As shown in Fig. 3, contents of vitamin C and anthocyanin exhibited a downward tendency with increasing storage time. However, Se showed the positive impact on vitamin C and anthocyanin. It could be concluded that Se treatment could effectively inhibit the decreases of vitamin C and anthocyanin and subsequently maintain better quality of *P. peltatum* L. It is well documented that Se contains properties that make it a unique element relative to other metals and metalloids. It occurs in both organic and inorganic forms, which are differentially toxic and is an essential element for most organisms (Silva *et al.*, 2018). Se prompts antioxidant capacity of plant to improve the quality and resistance to biotic and abiotic stresses.

TPC and TFC increased slightly in the second day of storage, followed by a decline on days 4 and 6. This might due to the further biosynthesis of flavonoid as antioxidant for plant protection in the first days after harvest, presumably triggered as a reaction to stress (Rezaei Nejad *et al.*, 2020). Lu *et al.* (2020) showed the slight increase of TPC and TFC at early days of storage and them they decreased over time, which are strongly in agreement with our results. Se increased the TPC and TFC due to its function on synthesis of antioxidants. Hence, the sufficient Se level was essential for the synthesis of antioxidant compounds and consequently enhanced the antioxidant activity of *P. peltatum* L. From the above results, it could be concluded that Se treatment could effectively inhibit the degradation of anthocyanin, vitamin C, and flavonoid in *P. peltatum* L.

CONCLUSION

The present study attempted to find the best concentrations of selenium (Se) on the edible flowers quality of *P. peltatum* L. under postharvest condition. It observed that there was no signif-

icant change in the quality of plants in early time of storage (day 0 - day2), and then the adverse effects of storage we appeared. The 40 μ M Se was a positive effect on plant quality by increasing flower weight, vitamin C, RWC, TPC, and TFC. *P. peltatum* L. Therefore, it can be concluded that moderate Se concentration can alleviate the adverse effects of late storage time by improving the physiological and biochemical attributes.

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Razmavar, Z., Naderi, R., abdossi, V., Ladanmoghadam, A., Nematollahi, F. 2021. Selenium improves physiology, biochemistry, and quality characteristic of flowers in Ivy Geranium (*Pelargonium peltatum* L.). *Journal of Ornamental Plants*, 11(2), 99-108. URL: http://jornamental.jaurasht.ac.ir/article 682861 d3153c718547c46dc9c3e1189d967458.pdf



How to cite this article: