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Original Article

The Response of Plant Growth and Physio-biochemical Properties Inedible Flowers of *Pelargonium peltatum* L. to Soil Applied Potassium and Selenium

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Abstract

Since edible flowers have been widely used to treat different diseases, new management practices are required to improve their productivity. Potassium (K) and selenium (Se) as important nutrients can influence plant quality and quantity. The purpose of present study was to investigate the effect of soil applied potassium (K) and selenium (Se) on plant growth, flower quality, and some physiological and biochemical properties of *Pelargonium peltatum* (L.) L'Hér. In a completely randomized design (CRD). K was used as potassium nitrate (KNO_3) in the concentrations of 2, 4, and 6 mM and Se was applied as sodium selenite (Na_2SeO_4) in soil application of 20, 40, and 60 µM. The results showed that the highest flower number was obtained at 5 mM K. Shoot fresh weight (SFW) significantly increased by both 6 mM K and 40 µM Se. Flower longevity was improved with increasing K concentration so that the highest flower longevity was obtained at 6 mM. The highest relative water content (RWC) and total chlorophyll (Chl.) content were observed at 4 mM K. In contrast, the lowest RWC was observed when plants were supplied with 60 µM Se. Anthocyanin and malondialdehyde (MAD) significantly increased with K and Se concentrations. Although total phenolic compound (TFC) in all concentrations of K and Se was higher than control, there was no significant difference between K and Se levels. Ascorbic acid and phenylalanine ammonia-lyase **(**PAL) activity increased with K and Se application, in which its highest amounts were observed at 6 mM K. *P. peltatum* was identified as Se accumulator because of more than 100 mg Se/kg DW in the leaves when treated with Se levels. Heat map analysis represented that 4 and 6 mM K were obviously distinguished from other treatments. This experiment suggests using 4-6 mM K and 40 µM Se to improve growth and productivity of *P. peltatum*.

Keywords: Sodium selenite, Potassium nitrate, Flower longevity, Phenolic contents, Antioxidant capacity

Introduction

Herb flowers have been used in phytotherapy for centuries. Fresh flowers from the earliest times were used primarily to decorate and aromatise the rooms. Recently, a new trend has been observed consisting in the widespread use of mainly fresh flowers for consumption. The main task of plants in nature is to grow fruits and seeds. Flowers may therefore contain repellents or poisonous substances produced by the plant to prevent their loss [1,2]. It is worth noting that not all flowers used in herbal medicine are edible. Some of them contain substances that act very strongly on the human body and can be poisonous [3]. Edible flowers have been used in culinary arts for flavor and garnish for hundreds of years. Early reports indicate that Romans used flowers in cooking, as did Chinese, Middle Eastern and Indian cultures. Ivy geranium *(Pelargonium peltatum* (L.) L'Hér.) is a medicinal plant that was recently introduced as edible flower. It has been widely used in Iran to treat a wide range of diseases such as bronchitis and sinusitis [4]. The preliminary phytochemical screening of ivy geranium shows that the n-hexane and methylene chloride fractions are rich in sterols and triterpenes, while

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ethyl acetate and n-butanol fractions are rich in flavonoids and tannins [5]. The main essential oil components of *P. peltatum* have been reported as β-myrcene, β-pinene, and α-pinene [5], all of which are monoterpene hydrocarbons with various medical attributes [6]. In addition, the antibacterial [7], antifungal [8], and anti-inflammatory [5] properties have been reported for essential oils of ivy geranium.

Nutrient supply is the most important component for growth and productivity [5]. Most of the plants require 17 essential elements including Potassium (K), which is more effective in promoting the plant growth and yield and also required in large quantity [9]. It is important in plant metabolisms such as cells synthesis, enzyme activation, and protein production [10,11]. K deficiency leads to decline in plant growth and yield [11]. In edible flowers, K could be accumulated in leaves and be useful for its consumers [2]. Selenium (Se) is considered to be a beneficial nutrient but it has not been shown to be essential [12,13]. Se concentrations in plants are significantly related to its status in human dietary [14]. 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 [15].

Plants alter the primary and secondary metabolites under external stimulators such as foliar application. Primary metabolites are involved in growth, development, and reproduction of the organism. The primary metabolite is typically a key component in maintaining normal physiological processes; thus, it is often referred to as a central metabolite [16]. Primary metabolites are typically formed during the growth phase as a result of energy metabolism, and are deemed essential for proper growth. Secondary metabolites are typically organic compounds produced through the modification of primary metabolite synthases. Secondary metabolites do not play a role in growth, development, and reproduction like primary metabolites do, and are typically formed during the end or near the stationary phase of growth. Many of the identified secondary metabolites have a role in ecological function, including defense mechanism(s), by serving as antibiotics and by producing pigments [17].

Previous studies have shown the positive effects of K and Se on plant growth and yield. Most investigations have documented K as the most important nutrient for plant growth and development [16,17]. Osakabe *et al.* [18] revealed improved plant growth of Arabidopsis when K was used. In addition, Se has been used to improve the physiology and growth of some plant species. For instance, Astaneh *et al*. [19] showed improved chlorophyll and carotenoid contents under Se application in garlic leaves. Motesharezadeh *et al*. [20] showed that Se treatment improved plant growth and Se concentration in leaves of alfalfa. Higher chlorophyll and phenol contents in rice seedlings were obtained by du *et al*. [21] at lower Se levels (15-75 mg/l). However, there is no investigation about the K and Se on plant nutrition of edible flowers. Therefore, the present study was conducted (a) to assess the effect of K and Se concentrations on plant growth (shoot and root biomass), flower longevity and number, (b) to evaluate the chlorophyll, malondialdehid (MAD) , and water contents under K and Se concentrations, and (c) to determine the K and Se effects on phenol and flavonoid contents, ascorbic acid and PAL activities of *P. peltatum* as an edible flower.

Material and Methods

Preparation of *P. peltatum* (L.) L'Hér.

The cuttings of *P. peltatum* were obtained from a commercial grower of Netherland. They were planted in a media containing coco peat, perlite, rice husk, cattle manure, and sandy soil (30, 30, 20, 10, and 10%) (Table 1). 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, there were used 54 experimental pots with a top diameter of 19cm, a base diameter of 13 cm base, and a height of 10 cm. Table 1 shows the soil properties for *P. peltatum*

Experimental design and treatment details

The pot experiments were conducted in a completely randomized design (CRD) with three replications in 2018. Soil applied K as potassium nitrate (KNO_3) was in the concentrations of 2, 4, and 6 mM and Se was used as sodium selenite (Na_2SeO_4) in the soil application of 20, 40, and 60 µM. After 4-leaf developmental stage, the plants were treated with K and Se concentrations four times during the experiment in the 10 day intervals.

At the flowering stage, the plants were harvested and transferred to University of Tehran for physiological, biochemical, and minerals analysis.

Table 1 The soil characteristics for *P. peltatum* (L.) L'Hér.

pH	EC	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

Chlorophyll Measurement

Total chlorophyll content was measured according to the method of Arnon [22]. 0.1 g of leaf samples were mixed with 3 mL of 80% acetone and the final volume of extract was increased to 15 mL. The extract was then centrifuged at 5000×g for 10 min. A spectrophotometer (Shimadzu UV-160) was used to measure the absorbance at 645 nm and 663 nm.

Relative Water content (RWC) Measurement

The RWC of leaves was calculated as a percentage according to the method of Dhopte and Manuel [23] as follows:

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

Where, FW is fresh weight, SW is leaf weight after soaking for 24 hours at room temperature and DW is leaf dry weight after drying for 24 h at 75 °C.

Determination of Ascorbic Acid

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 (12 000 *g*) for 10 min at 4 °C and the supernatant was directly used for ascorbic acid assay [24].

Malondialdehyde (MAD) Concentration

To determine the MAD content, the samples were extracted with phosphate buffer and centrifuged at 14,000 rpm for 30 min. After that, the thiobarbituric acid (0.5% w/v) containing 20% w/v trichloroacetic acid was added to the mixture. The samples were placed in a hot water bath for 30 min and then were immediately cooled with ice and finally centrifuged at 10,000 rpm for 10 min. Samples were read at 532 and 600 nm wavelengths [25].

PAL Activity Measurement

The activity of PAL was measured by monitoring the production of t-cinnamic acid at 290 nm [2]. The reaction mixture included enzyme extract, 50 mmol Tris-HCl buffer (pH 8.7), and 20 mmol L-phenylalanine. Incubation was at 30 °C, and the reaction was stopped by the addition of 0.5 mL 10% trichloroacetic acid. The mixture was measured at 290 nm after 30 min. One unit of enzyme activity was defined as the amount of enzyme causing the decrease in absorbance of 0.01 per min. PAL activity was expressed as enzyme units per gram fresh weight (U/g FW).

Determination of Total Phenolic Content (TPC)

Folin–Ciocalteu reagent was selected to measure TPC spectrophotometrically [26]. 0.125 mL of the extracted solution was mixed with 0.5 mL distilled water. Then, 0.25 mL of FIVC reagent with 1.25 mL of 7% sodium carbonate was added to the sample and kept at room temperature for 5 min. The resulting mixture was distilled with distilled water to a volume of 3 mL and kept at room temperature for 90 min. Finally, the absorbance of the samples was measured at 760 nm. TPC was calculated by the standard curve of Gallic acid (GA) and reported in mg/g dry weight.

Determination of Total Flavonoid Content (TFC)

The flavonoid levels were measured by aluminum chloride colorimetric method [27]. Briefly 0.5 mL of extract solution with 1.5 mL of 95% ethanol, 0.1 mL of aluminum chloride 10%, 0.1 mL of 1 M potassium acetate were mixed with 2.8 mL of distilled water. The mixture vortexed for 10 s and left to stand at 25 ºC for 30 min. The absorbance of the mixture was read at 415 nm. Quercetin concentrations (0 to 1200 µg/mL) were prepared and linear fit was used for calibration of the standard curve.

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. Heat map was designed by clustered image map (CIM) miner software.

Results

Flower Number

Flower number significantly was influenced by K application ($P \leq 0.01$), but Se did not change it ($P \geq 0.5$). The highest flower number (5 flowers) was obtained at 4 mM K and it decreased to 4.67 flowers when the plants treated with 6 mM K (Table 2).

Shoot Fresh Weight (SFW) and Root Volume

SFW and root volume significantly influenced by both K and Se levels ($P \leq 0.01$). 6 mM K and 40 µM Se increased SFW by 14% and 11%, respectively, compared to control (Table 2). The greatest root volume (7.87 cm^3) was observed in plants treated with 40 µM Se. The concentration of 40 µM Se increased root volume by 22% compared to non-treated plants (Table 2).

Flower Longevity

Se and K significantly changed flower longevity ($P \leq 0.01$). Flower longevity was improved with increasing K levels so that the highest flower longevity was obtained at 6 mM to be 8.66 days (Fig. 1). Although all concentrations of Se induced higher flower longevity compared to control, there was no significant difference between the Se levels (20, 40, and 60 µM).

Relative Water Content (RWC) and Chlorophyll (Chl) Content

The concentrations of K and Se significantly affected RWC and chlorophyll content ($P \leq 0.01$). RWC increased up to 4 mM and decreased at 6 mM of K. A 25% increase of RWC was observed at 4 mM K in comparison with control (Fig. 2a). However, a significant reduction of RWC was observed when plants were supplied with 60 µM Se (Fig. 2a). Total chlorophyll content in plants treated with 4 mM K increased by 23% compared to control (Fig. 2b).

Anthocyanin and Malondialdehid (MAD)

Anthocyanin and MAD were changed under K and Se levels ($P \leq 0.01$). Compare to control, the increases of 27, 60, and 48% were observed with the use of 2, 4, and 6 mM K. Se improved anthocyanin content by 16, 36, and 12 % with 20, 40, and 60 µM, respectively, compared to control (Fig. 2c). MAD remained without any significant change by K application up to 4 mM, but it was increased at 6 mM. The highest MAD (8.3 µ mol/g FW) accumulated in plants treated with 60 µM Se (Fig. 2d).

Total Phenolic Content (TPC) and Flavonoid Content (TFC)

K and Se significantly influenced TPC and TFC ($P \leq 0.01$). The 4 mM K increased TPC by 33% in comparison with control (Fig. 3a). A significant decline of TPC was found at high concentrations of K and Se compared to their lower levels. Although TFC in all concentrations of K and Se was higher than control, there was no significant difference between K and Se levels (Fig. 3b).

Vitamin C and Phenylalanine Ammonia-lyase (PAL)

Vitamin C and PAL were changed under K and Se levels ($P \leq 0.01$). Vitamin C increased with K and Se application, in which its highest amount was observed at 6 mM K (Fig. 3c). Compared to control, there were significant increases of PAL activity with all concentrations of K and Se. In plants treated with 6 mM K, PAL activity increased by 61% compared to control plants (Fig. 3d).

Leaf Selenium (Se) and Potassium (K) Contents

Leaf Se and K concentrations are shown in Figure 4. Leaf Se content remained uncaged with K application up to 4 mM, but it decreased at 6 mM K. The highest leaf Se (211 mg/kg DW) was obtained in plants supplied with 60 µM Se. Leaf K was significantly increased when plants treated with K application and its highest value was observed at 6 mM K application to be 41.33 mg/g DW. However, leaf K content decreased with progressing in Se application.

Heat Map Analysis

According to meat map analysis, three distinguished clusters have been identified for the treatments as cluster 1: 4 and 6 mM K, cluster 2: 60 µM Se, and cluster 3: control, 2 mM K, 20 and 40 µM Se. The relative weight of traits varied from blue (minimum) to red (maximum). The physiological and biochemical traits showed a high concentration (red value) at 4 mM K and 40 µM Se treatments.

Table 2 The effect of K and Se concentrations on flower number, shoot fresh weight (SFW), and root volume. Small letters within each column show mean comparison by Duncan's multiple range test at a 5% probability level.

Treatment	Flower number	SFW (g)	Root volume (cm^3)
Control	2.33±0.45 b	87.67±1.63 e	6.13±0.49 d
2 mM K	3.33±0.48 b	90.40±1.55 de	7.10±0.29 bc
4 mM K	5.00±0.81 a	94.87±0.87 a	7.50±0.24 ab
6 mM K	4.67±0.47 a	99.30±1.33 bc	6.43±0.20 cd
20 µM Se	2.67±0.46 b	93.43±1.88 cd	6.73±0.16 cd
40 µM Se	3.33±0.47 b	97.30±1.48 ab	7.87±0.32 a
60 µM Se	2.67±0.47 b	89.40±1.83 e	7.63±0.33 ab

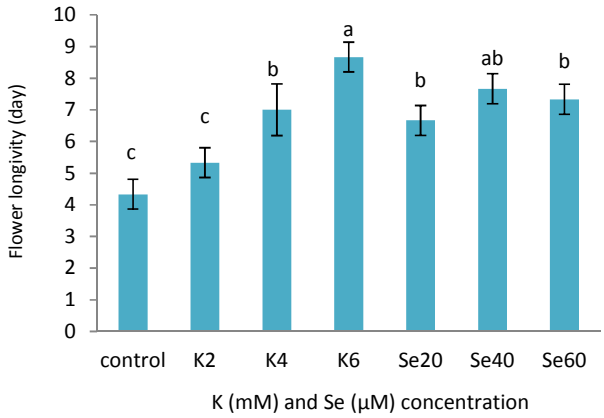
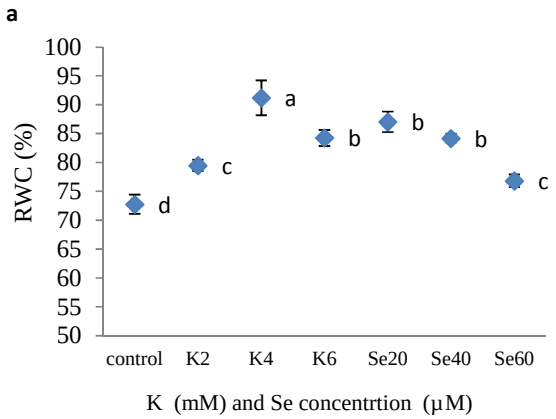
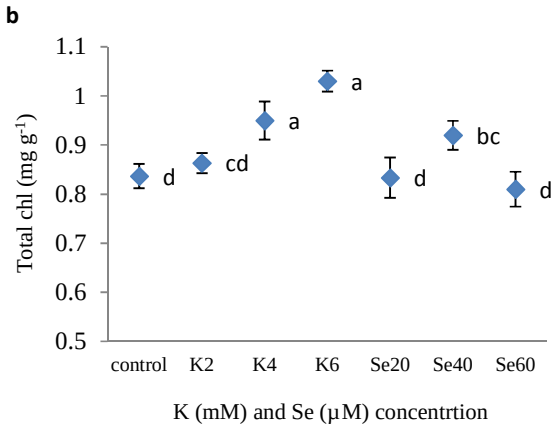
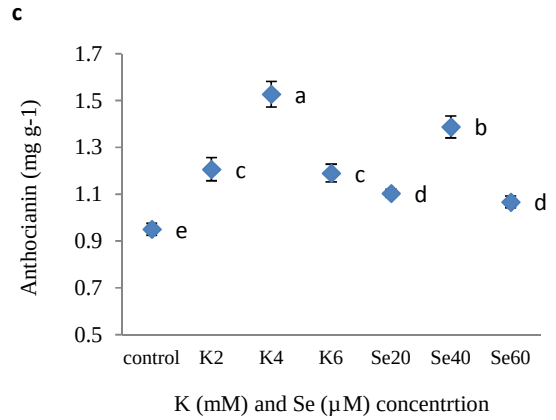


Fig. 1 The effect of K and Se concentrations on flower longevity. Small letters within each column show mean comparison by Duncan's multiple range test at a 5% probability level.







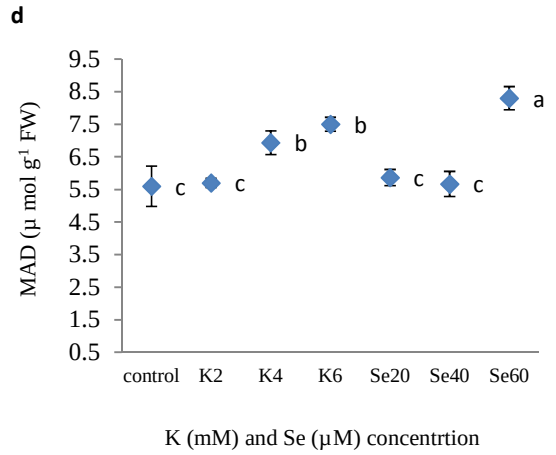
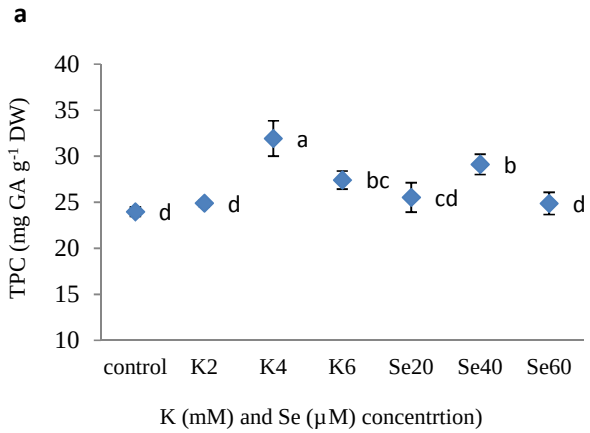


Fig. 2 The effect of K and Se concentrations on relative water content (RWC) (a), Total chlorophyll (Chl.) content (a), anthocyanin (c), and malondialdehid (MAD) concentrations (d). The values are represented with mean ±SD, n=3. Small letters show mean comparison



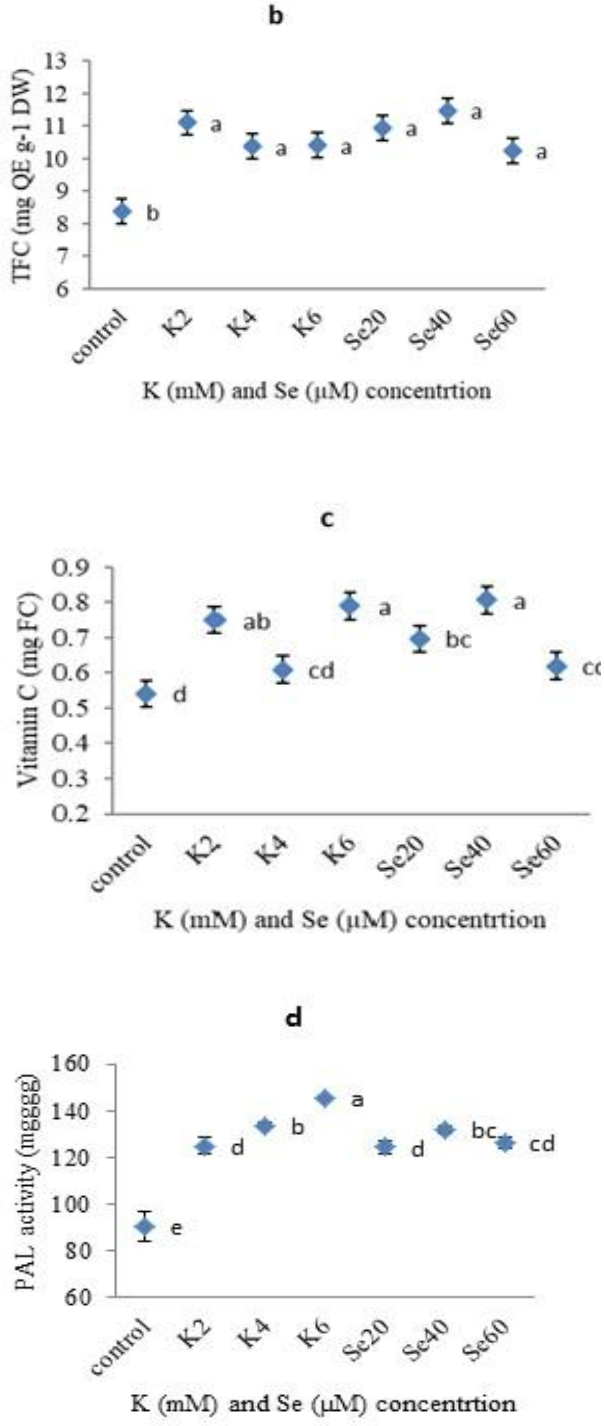


Fig. 3 The effect of K and Se concentrations on total phenolic content (TPC) (a), total flavonoid content (TFC) (b), Ascorbic acid (c), and PAL activities (d). The values are represented with mean ±SD, n=3. Small letters show mean comparison by Duncan's multiple range test at a 5% probability level.

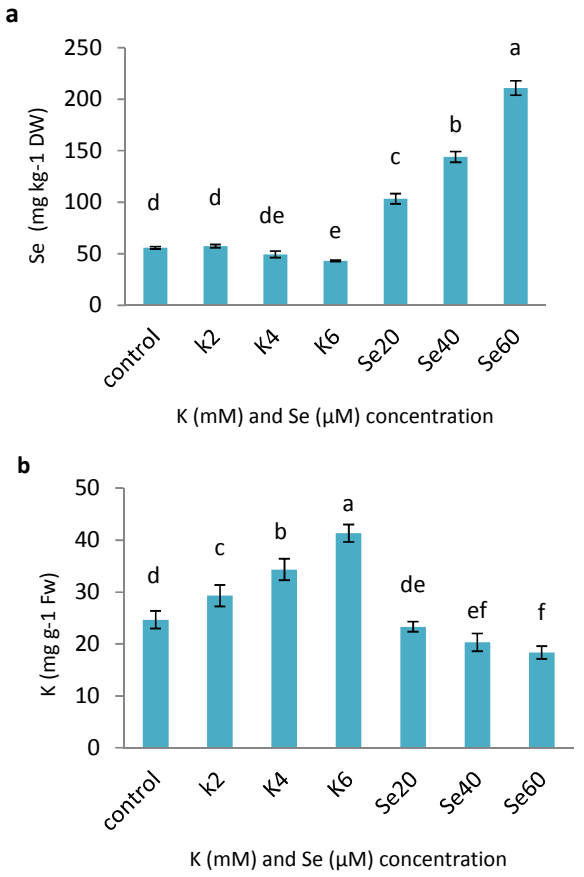


Fig. 4 The effect of K and Se concentrations on leaf Se (a) and K (b) content. The values are represented with mean ±SD, n=3. The values are represented with mean ±SD, n=3. Small letters show mean comparison by Duncan's multiple range test at a 5% probability level.

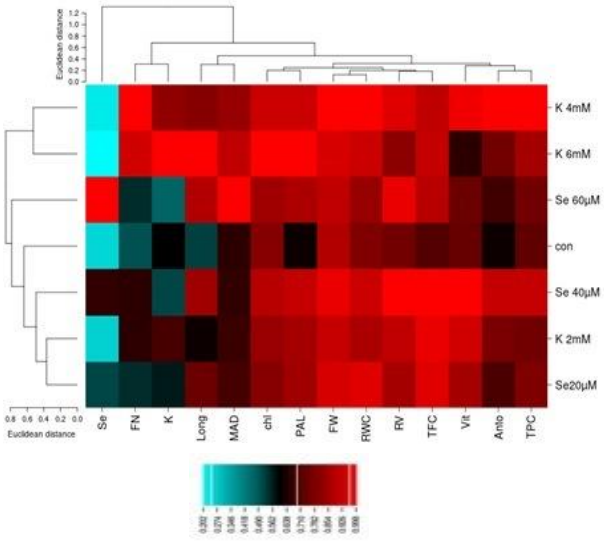


Fig. 5 Heat map analysis for the traits under Se and K concentrations.

Discussion

Low and medium levels of K (up to 4 mM) and Se (up to 40 µM) increased plant yield. K due to its significant role in photosynthesis and development of plant can significantly influence plant growth and development. K controls the function of stomata in photosynthesis and transpiration such as maintenance of plant turgor, photophosphorylation, and enzyme activation [9]. Low concentrations 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 [29]. Decrease in plant biomass with high Se concentration can be due to changes in membrane permeability to Na, K, and Ca which impair respiration and water uptake [29]. Similarly, other authors have also found a positive effect of Se application on plant yield of potato, rye grass (*Lolium perenne* L.), lettuce, and mustard (*Brassica rapa* L.) [30,31].

Increased flower longevity was observed with increasing K concentration. Although Se is not an essential element for higher plants [32], we obtained increased flower longevity with Se concentrations compared with control. A possible explanation for the increase in flower longevity may be due to the increase in antioxidant activity which confers more efficient photosynthesis [31]. Most works have reported a decrease in chlorophyll content under Se exposure [33,34]. However, Mozafariyan *et al*. [28] showed increased Chl content at medium Se concentration, which are in accordance with our results. Low concentrations of Se by protecting chloroplast enzymes and improving the efficiency of photosystem II increase the content of photosynthetic pigments [35]. Decreased Chl content at 60µM Se appears to be due to decreased leaf area and also the Se use in replacement of magnesium in chlorophyll structure and the negative effect of high Se accumulation on the porphobilinogen synthase [36].

RWC of the leaf tissue are considerably increased by K application up to 4 mM. It has been reported that K could protect the plant against water loss conditions [37]. In high concentration of Se (60 µM), we found a decline of RWC compared to its lower concentration. The high Se concentration act as a poisonous condition for plant and it results in decreased water content and physiological processes [38]. In our study, heat map analysis supported our findings by distinguishing the 60 µM Se with its significant effects on all plant traits.

TPC and TFC increased with K and Se concentrations particularly 40 µM. 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 [39]. K prompts antioxidant capacity of plant to improve the quality and resistance to biotic and abiotic stresses. Therefore, in our study K concentrations significantly improved secondary metabolites. Nossier [5] showed the high antioxidant capacity of *P. peltatum* due to high amount of valuable phenolic compounds.

Increased ascorbic acid and PAL activity were reported after K and Se application. Increased ascorbic acid was reported with K application by Iqbal *et al.* [40]. K has an important role in transport of essential ingredients for ascorbic acid synthesis and also due to close relationship between carbohydrate metabolism and formation of ascorbic acid [41]. Djanaguiraman *et al*. [42] showed increased antioxidant enzymes activities in sorghum leaves with Se application.

K in higher levels inhibited Se uptake and it also Se had this function for K uptake. Based on Se accumulation, plants are classified into non-Se accumulators (<100 mg kg^{1-} DW), Se-accumulators (100-1000 mg/kg) and Se hyper-accumulators (>1000 mg/kg DW) [43]. Therefore, *P. peltatum* is considered as a Se-accumulator plant based on our findings. In terms of human health, *P. peltatum* Selenium uptake by humans via food crops is a critical exposure pathway for Se [13]. Therefore, Se accumulation in edible plant parts is an important factor with respect to human health. Generally, vegetables are more active in accumulating Se in relative to fruits [43].

Conclusions

The present study attempted to find the best concentrations of potassium (K) and selenium (Se) on the edible flowers quality of *P. peltatum.* It showed that 4 to 6 mM is the best range of K concentration to increase the yield, flower longevity, and antioxidant capacity of the plants. For Se application, there was a positive effect on plant quality and quantity by increasing the Se to 40 µM. However, 6 µM Se caused negative effects of plant growth and development by accumulating more than 200 mg/kg DW of leaves. In addition, the K is significantly more effective in improving the plant quality and quantity compared to Se application. *P. peltatum* is identified as Se-accumulator edible plant when it is subjected to Se levels, therefore, it could be more attention for consuming in this condition.

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Author contribution: The initial draft and data analysis was performed by Zohreh Razmavar. Other authors participated in the design and revise of manuscript. All authors read and approved the final manuscript.

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