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Metabolic and Enzymatic Responses of *Calendula officinalis* L. to Foliar Application of Spermidine, Citric Acid and Proline under Drought Stress in a Post Harvest Condition

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ABSTRACT

The first experiment was performed to study the effect of foliar application of spermidine, citric acid and proline (0, 50 and 100 mg L^{-1}) on some metabolites and enzymatic activities of *Calendula officinalis* L. under drought stress (No stress: Control, 25, 50, and 75% field capacity). The second experiment was performed to study the effect of foliar application of spermidine, citric acid and proline (0, 50 and 100 mg L^{-1}) on the post-harvest shelf life of flowers (beginning of the experiment, 5, and 10 days post-harvest). The experiments were factorial in a completely randomized design with 3 replications. All treatments had a significant effect on the measured variables. In the first experiment, FC 25% reduced all traits, FC 75% increased carotenoid, carbohydrate, phenol, flavonoid, protein, Peroxidase (POD) and Superoxide Dismutase (SOD) activities. The highest vitamin C was observed in the control FC. Also, foliar application of 100 mg L^{-1} proline, increased carotenoid, carbohydrate and phenol, 50 mg L^{-1} proline increased protein content. Also, 100 mg L^{-1} of spermidine increased flavonoid and 100 mg L^{-1} of citric acid increased vitamin C, SOD and POD activities. In the second experiment, all evaluated traits were reduced ten days after harvest, the highest post-harvest life was for 100 mg L^{-1} of citric acid (9.7 days) and the lowest was for the control (5.3 days). The study results showed that application of 100 mg L^{-1} spermidine, citric acid and proline with FC 75% improved biochemical properties, nutritional traits, and post-harvest life of Pot marigold.

Keywords: Medicinal and ornamental plants, Pot marigold, Shelf life.

INTRODUCTION

Pot marigold (*Calendula officinalis* L.) is considered as an important medicinal and ornamental plant of the Asteraceae family and has a wide range of pharmacological effects such as anti-inflammatory, wound-healing, antibacterial, antitumoral and immunostimulatory effects (Sedghi *et al.*, 2012). The flowers of this plant are edible and their dried and fresh petals are used as a spice in a variety of foods (Leonti, 2012).

Unlike cut flowers, edible flowers are cut from the petiole of the stem, thus exposing them to additional stresses. Edible flowers continue respiration and their shelf life is reduced (Kou *et al.*, 2012). Today, due to the global approach to increase the shelf life of horticultural products and the tendency to consume fresh products, the use of healthy compounds to maintain the products is necessary.

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Drought stress, as a limiting factor in plant growth, in addition to reducing yield, disrupts plant physiological processes, increases production of Reactive Oxygen Species (ROS), resulting in oxidative stress in plants (Pal *et al*., 2015). Plants use different strategies to deal with drought stress. Also, external application of various compounds such as organic solutions and minerals is a solution for increasing drought resistance in plants (Ashraf *et al*., 2011). These compounds include spermidine, citric acid, and proline.

Spermidine belongs to plant polyamines and increases membrane consolidation in stress condition. They also co-exist with ethylene, a common precursor called S-adenosylmethionine, through which they compete with ethylene synthesis and are known as anti-aging and anti-stress compounds (Sood and Nagar, 2008). Foliar application of spermidine, under salinity stress, increased the activity of catalase, ascorbate peroxidase and guaiacol peroxidase enzymes in *Panax ginseng* (Parvin *et al*., 2014).

Citric acid plays an important role in the Krebs cycle as a source of carbon and energy, membrane stability and activation of carrier enzymes, carbohydrate metabolism and transfer, as well as the positive role of antioxidants in chelating free radicals and stimulation of plant growth (Da Silva, 2003). Citric acid application in *Helianthus annuus* L., increased the protein and activity of antioxidant enzymes (Mujahid *et al*., 2017).

Proline is an amino acid that exists both freely and in the structure of proteins. The role of proline as osmolyte, abductor of ROS, stabilization of protein structure and protection of cells from stress damage has been reported (Szabados and Savoure, 2009). Application of proline on *Foeniculum vulgare* Mill. increased carotenoid, polyphenol, carbohydrate of *Foeniculum vulgare* Mill. (Gholami Zali and Ehsanzadeh, 2018)

Pot marigold can be used as a suitable edible flower for large commercial surfaces. It contains useful and healthy compounds and can be used as a new product to increase the health of the community and be included in the daily consumption of households. However, short shelf life limits its commercial usage, so, it is important to evaluate the nutritional value and its storage capacity. The purpose of conducting this study was to investigate the effect of foliar application of spermidine, citric acid and proline on some metabolites and enzymatic activities of *Calendula officinalis* L. under drought stress and in a post-harvest condition.

MATERIALS AND METHODS

Plant Materials and Treatments

In the first experiment, the effect of foliar application of spermidine, citric acid and proline (0, 50 and 100 mg L^{-1}) on some metabolites and enzymatic activities of marigold was studied under drought stress (no stress, 25, 50, and 75% field capacity). In the second experiment, the effects of foliar application of spermidine, citric acid, and proline (0, 50, and 100 mg L^{-1}) on nutritional value and shelf life (beginning of the experiment, 5, and 10 days post harvest) of marigold were investigated in a greenhouse in northern Iran (latitude 53.44 °N, 36.45 °E, and 15 m above sea level), in 2018.

Pot marigold was grown in a greenhouse at 25/15°C day/night temperature and 14/10 hours day/night photoperiod. At the beginning, a pre-test was performed to determine the treatments and concentrations on some pots. Approximately 3 weeks after planting the seeds in the appropriate bed, the seedlings were transferred to a pot (15 Size) (diameter 28 cm and height 30 cm) that contained a mixture of soil, sand and leaf composts (1:1:1). In general, 216 plants were used in this experiment with no mortality record. Drought stress treatments were applied at six visible leaves stage. To determine FC, some pots were saturated and

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water was allowed to drain out from the bottom of the pots. The pot surface was covered with aluminum foil to prevent evaporation. The weight of pots was measured each day until it was constant. Thereafter, the soil of each pot was mixed and then some soil was removed to record its wet weight. To evaluate the soil dry weight, it was placed in the oven at 72°C for 24 hours. Field capacity was determined using the following equation (Mohammadi *et al*., 2020):

FC= (WW-DW/DW)×100.

Where, WW: Soil Wet Weight and DW: Soli Dry Weight)

The spray solutions of spermidine, citric acid and proline (Sigma, Germany) were prepared based on distilled water. Foliar application was done at three stages with intervals of about 20 days including six visible leaves, complete tillering, and emergence of the first bud. Then, sampling was done at the appropriate stage of harvesting.

In order to evaluate the effect of foliar application of spermidine, citric acid and proline on changes of some metabolites and enzymatic activity in a post-harvest condition, the Pot marigold flowers were stored at 4°C. (standard temperature for storing agricultural and food products in temperate regions). The pots were irrigated with tap water at control FC.

Physiological Measurements

Carotenoid was determined using Sarvandi *et al*. (2020) method, the absorbance was read with spectrophotometer at 470 nm and expressed in mg g^{-1} FW (Fresh Weight) petal.

Protein percentage was determined by Du Preez and Bale (2008) methods. The SOD activity was measured according to the method of Acemi *et al.* (2018). Absorbance was read at wavelength of 560 nm and expressed in nmole g^{-1} FW petal. The method of Sedghi *et al*. (2012) was used for POD enzyme assay and absorbance was read at wavelength of 470 nm and expressed in nmole g^{-1} FW petal. Total phenol was determined by Aliyari *et al*. (2020) method and absorbance was read at 765 nm by spectrophotometer. The results were reported as mg (gallic acid) g^{-1} FW petal.

Total flavonoid was measured by using Chang *et al*. (2002) method and the absorbance was read at wavelength of 415 nm and expressed in mg (quercetin) g^{-1} FW petal. Carbohydrates were measured according to the method of Jin *et al.* (2015) method and absorbance was measured at wavelength of 625 nm and expressed in mg (glucose) g^{-1} DW (Dry Weight) petal. Vitamin C was determined by Shams Najafabadi *et al*. (2020) method and expressed in mg (ascorbic acid) $100g^{-1}$ FW.

In order to measure post-harvest life, the flowers were kept at 4°C. When the petals lost their turgor, the flower life ended and the results were reported in days (Ezhilmathi, 2007).

Statistical Analysis

Two factorial experiments were performed in a completely randomized design with 3 replications. The data were analyzed using SAS software. The comparison of the means was done by Duncan's multiple range at 1 and 5% levels, and the graphs were drawn using the Excel software (Roosta *et al.*, 2020).

RESULTS

The results of the first experiment showed that the effect of drought and foliar application was significant on carotenoid, SOD, POD activity, phenol, carbohydrate at 1% level, and for protein, flavonoid and vitamin C at 5% level.

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The results of the second experiment also showed that the effect of foliar application and time was significant on carotenoid, phenol, flavonoid, carbohydrate, post-harvest life, SOD and POD at 1%, and for vitamin C and protein at 5% level.

Carotenoid

In the first experiment, the highest carotenoid (1.20 mg g^{-1} FW) was for the control irrigation and 100 mg L^{-1} of proline and the lowest (0.68 mg g^{-1} FW) was for 25% FC treatment (Figure 1). Also, Figure 2 shows that carotenoid content in the second experiment decreased during the post-harvest days. The highest carotenoid (1.20 mg g^{-1} FW) was related to 100 mg L^{-1} of proline at the beginning of the experiment and the lowest (0.57 mg g^{-1} FW) was for 50 mg L^{-1} of spermidine at the 10^{th} day.

Protein

In the first experiment, the highest protein (7.03%) was for the control FC and 50 mg L^{-1} of proline, and the lowest (1.30%) was for 25% FC (Figure 1). Also, Figure 2 shows that the protein content in the second experiment increased slightly during the first post-harvest days and declined five days after harvest. The highest protein (7.26%) was related to 50 mg L^{-1} of proline five days after harvest and the lowest (4.63%) was for control on the 10^{th} day.

Superoxide Dismutase

In the first experiment, the highest SOD activity (97.06 nmol g^{-1} FW) was for 75% FC and 100 mg L^{-1} of citric acid and the lowest (27.54 nmol g^{-1} FW) was for 25% FC (Figure 3). Also, Figure 4 shows that the activity of SOD increased in the second experiment, up to the fifth day after harvest, and then the enzyme activity decreased. The highest SOD activity (71.27 nmol g^{-1} FW) was related to 50 mg L^{-1} of citric acid on the fifth day after harvest and the lowest activity (31.21 nmol g^{-1} FW) was for the control on the 10^{th} day.

Peroxidase

In the first experiment, the highest POD activity (253.22 nmol g^{-1} FW) was related to 75% FC and 100 mg L^{-1} citric acid, and the lowest (52.74 nmol g^{-1} FW) was related to, 25% FC (Figure 3). Figure 4 also shows that POD activity in the second experiment increased up to the fifth day after harvest, and then the enzyme activity decreased. The highest POD activity (218.44 nmol g^{-1} FW) was for 100 mg L^{-1} of citric acid on the fifth day after harvest, and the lowest (75.41 nmol g^{-1} FW) was for the control on the 10^{th} day.

Phenol

In the first experiment, the highest phenol (140.43 mg GAE g^{-1} DW) belonged to 75% FC and 100 mg L^{-1} of proline and the lowest (49.75 mg GAE g^{-1} DW) was for 25% FC (Figure 5). Figure 6 also shows that phenol content in the second experiment decreased during the post-harvest days. The highest phenol (67.76 mg GAE g^{-1} FW) was for 100 mg L^{-1} of proline at the beginning of the experiment and the lowest (23.61 mg GAE g^{-1} FW) was for the control on the 10^{th} day.

Flavonoid

In the first experiment, the highest flavonoid (23.51 mg QE g^{-1} DW) was related to 75% FC and 100 mg L^{-1} of spermidine and the lowest (17.26 mg QE g^{-1} DW) was related to 25% FC (Figure 5). Also, Figure 6 shows that flavonoid in the second experiment declined during the post-harvest days. The highest flavonoid (15.81 mg QE g^{-1} FW) was for 100 mg L^{-1} of spermidine at the beginning of the

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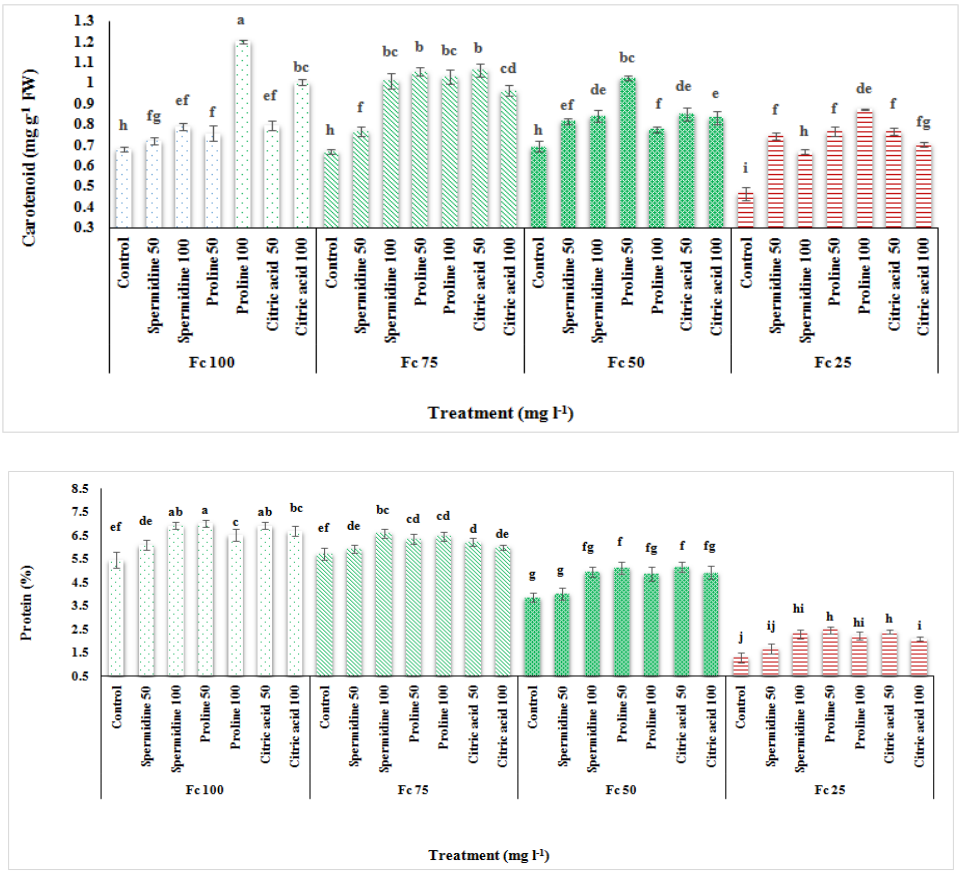

Figure 1. Effect of drought stress and foliar application of spermidine, citric acid and proline on carotenoid and protein. Values are mean±SD. Values marked by different letters are significantly different (P< 0.05).

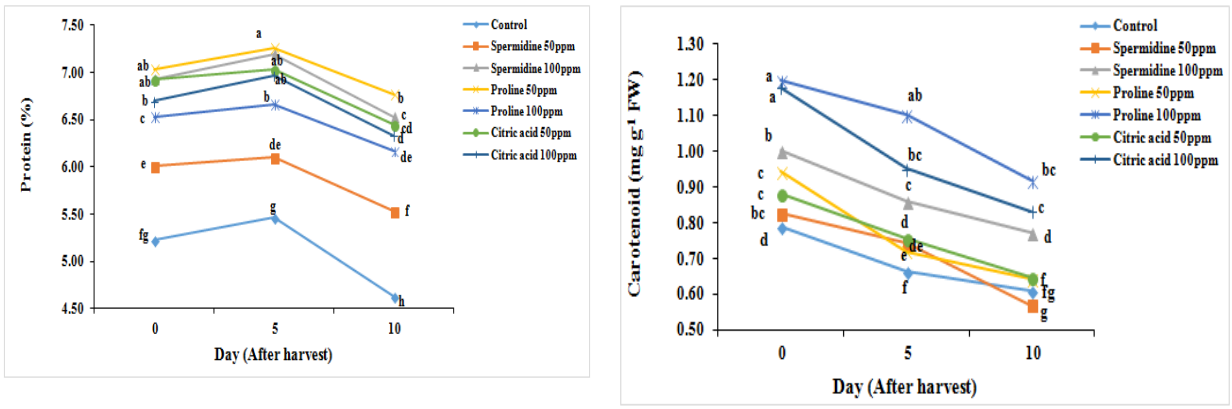

Figure 2. Changes in petals carotenoid and protein during the post-harvest days. Values are mean±SD. Values marked by different letters are significantly different (P< 0.05).

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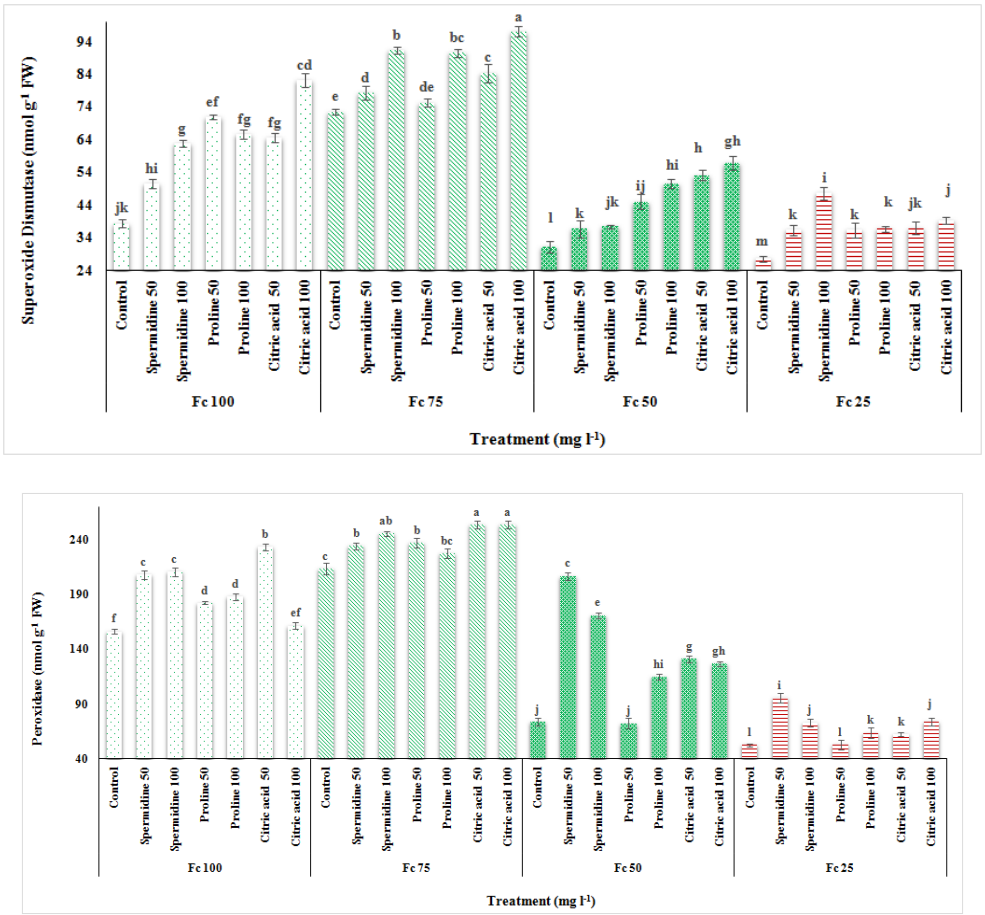

Figure 3. Effect of drought stress and foliar application of spermidine, citric acid and proline on SOD and POD activity. Values are mean±SD. Values marked by different letters are significantly different (P< 0.05).

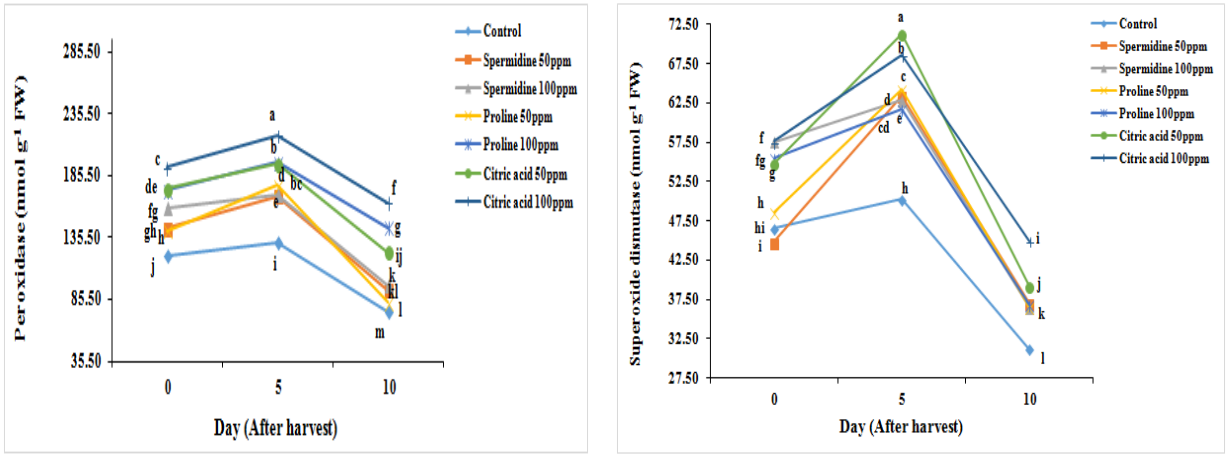

Figure 4. Changes in petals SOD and POD contents during the post-harvest days. Values are mean±SD. Values marked by different letters are significantly different (P< 0.05).

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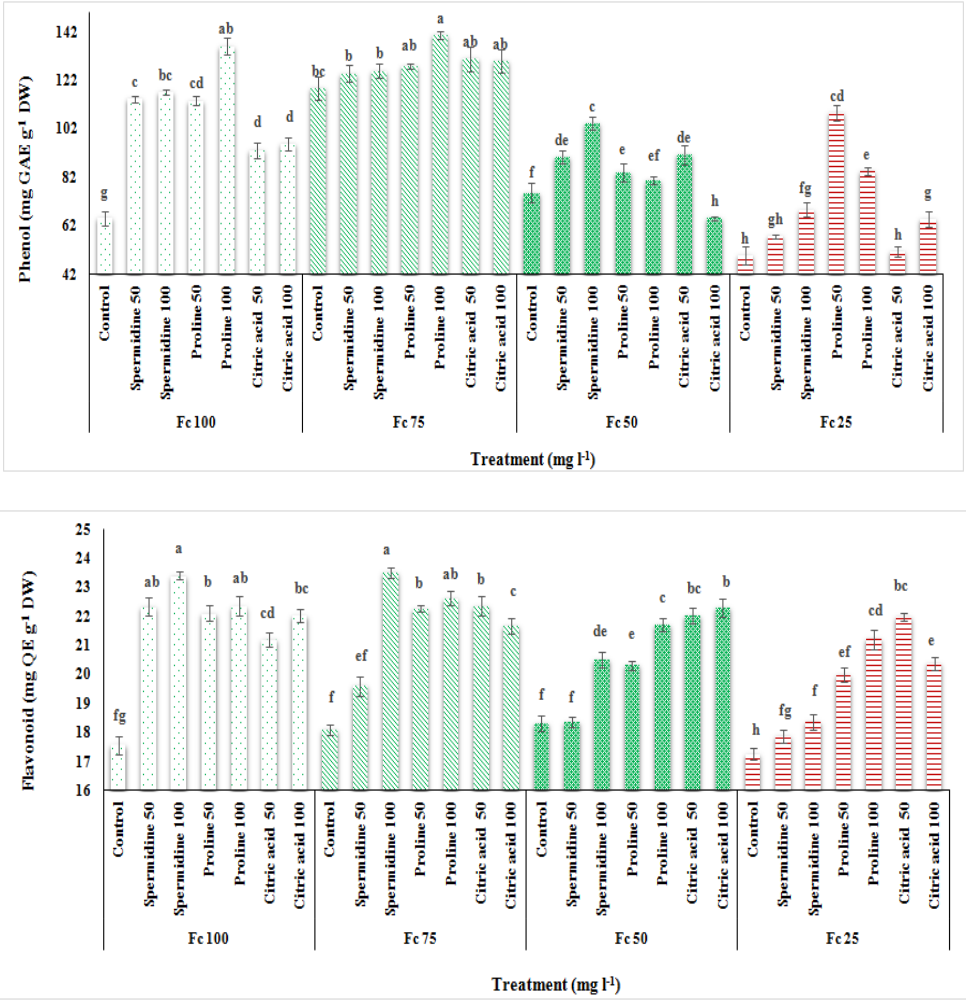

Figure 5. Effect of drought stress and foliar application of spermidine, citric acid, and proline on phenol and flavonoid. Values are mean±SD. Values marked by different letters are significantly different (P< 0.05).

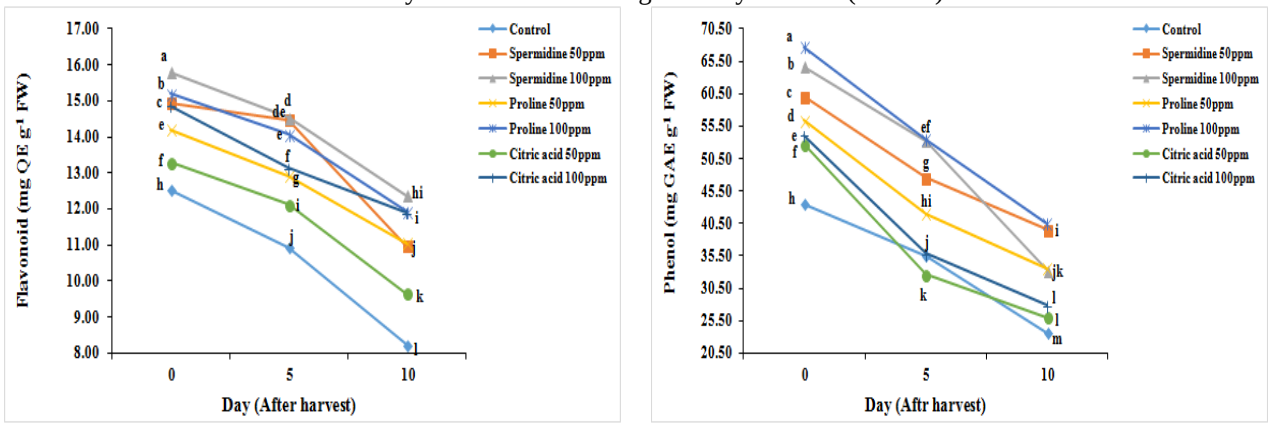

Figure 6. Changes in petals phenol and flavonoid content during the post-harvest days. Values are mean±SD**.** Values marked by different letters are significantly different (P< 0.05).

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experiment and the lowest (8.23 mg QE g^{-1} FW) was for the control on the 10th day.

Total Carbohydrate

In the first experiment, the highest carbohydrate (16.96 mg GLUC g^{-1} DW) was related to 75% FC and 100 mg L^{-1} of proline and the lowest (9.23 mg GLUC g^{-1} DW) was related to 25% FC (Figure 7). Also, Figure 8 shows that carbohydrate in the second experiment declined during the post-harvest days. The highest rate of carbohydrate at the begining day of experiment (274.79 mg GLUC g^{-1} FW) was for 100 mg L^{-1} of proline and the lowest (19.59 mg GLUC g^{-1} FW) was for the control on the 10th day.

Vitamin C

In the first experiment, the highest amount of vitamin C (6.81 mg AA 100 g^{-1} FW) was related to the control and 100 mg L^{-1} of citric acid and the lowest (2.38 mg AA 100 g^{-1} FW) was related to 25% FC (Figure 7). Figure 8 also shows that vitamin C decreased in the second experiment during the post-harvest days. The highest amount of vitamin C (4.43 mg AA 100 g^{-1} FW) was for 100 mg L^{-1} of citric acid at the beginning of the experiment and the lowest (0.11 mg AA 100 g^{-1} FW) was for the control on the 10th day.

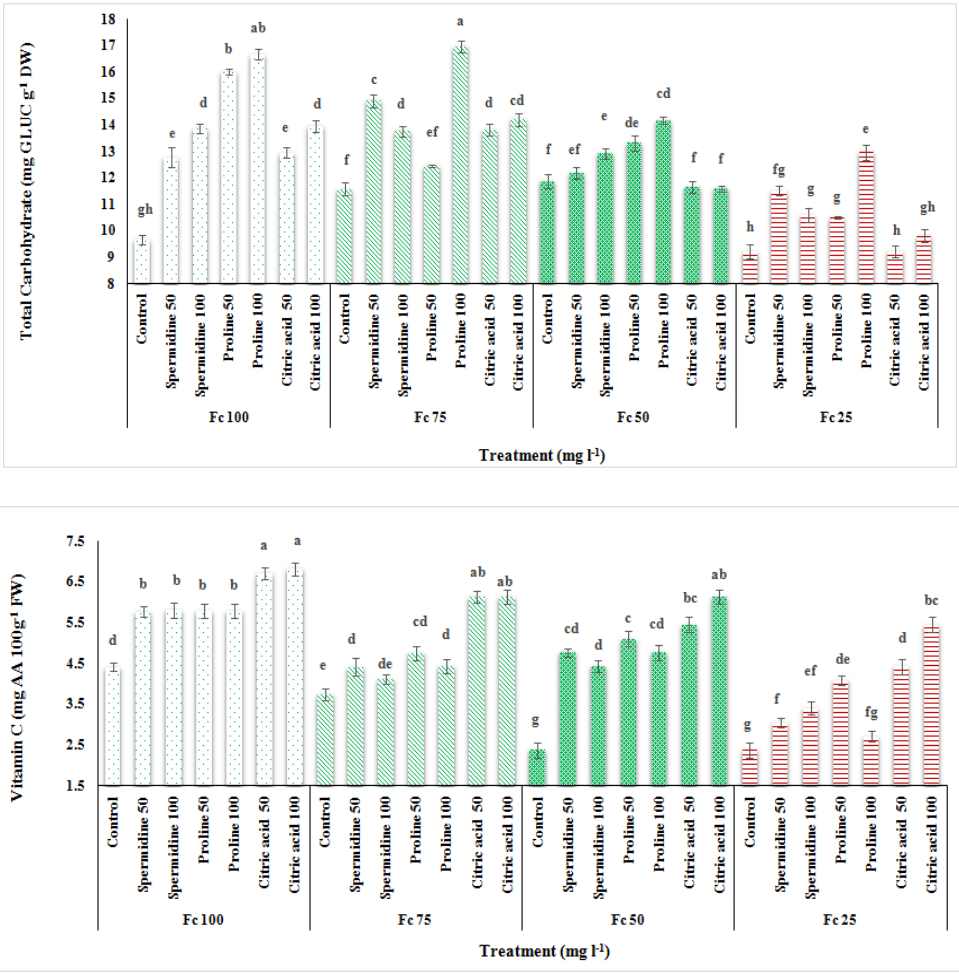

Figure 7. Effect of drought stress and foliar application of spermidine, citric acid, and proline on carbohydrate. Values are mean±SD. Values marked by different letters are significantly different (P< 0.05).

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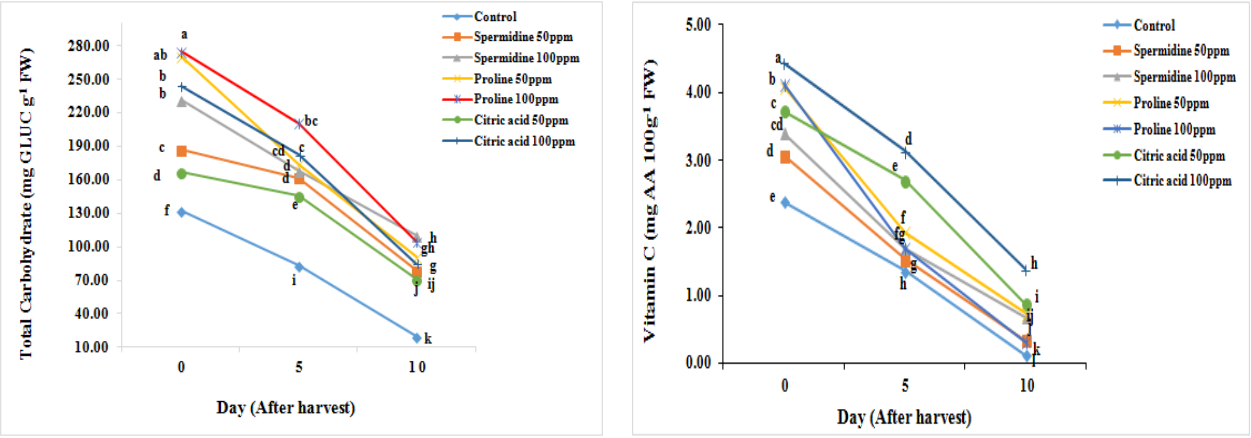

Figure 8. Changes in petals carbohydrate and vitamin C during the post-harvest days. Values are mean±SD. Values marked by different letters are significantly different (P< 0.05).

Post-Harvest Life

The highest post-harvest life (9.6 days) was related to 100 mg L^{-1} of citric acid and the shortest (5.3 days) was related to the control (Figure 9).

DISCUSSION

Drought stress causes accumulation of ROS and can lead to oxidative stress in plants (Song *et al*., 2008). Also, in terms of metabolic changes, plant aging occurs as a result of oxidative processes resulting from the production of ROS (Ohe *et al*., 2005). Numerous studies have reported that different compounds such as organic solutions reduce the effects of oxidative stress (Ashraf *et al*., 2011).

The results of the present study showed that under severe drought stress and during the post-harvest days, petal carotenoid content was reduced and could not play its protective role. The carotenoids reduction can be due to stimulation of ionic stress, a reduction in photosynthetic level, an increase in the production of oxygen radicals, peroxidation of these pigments and

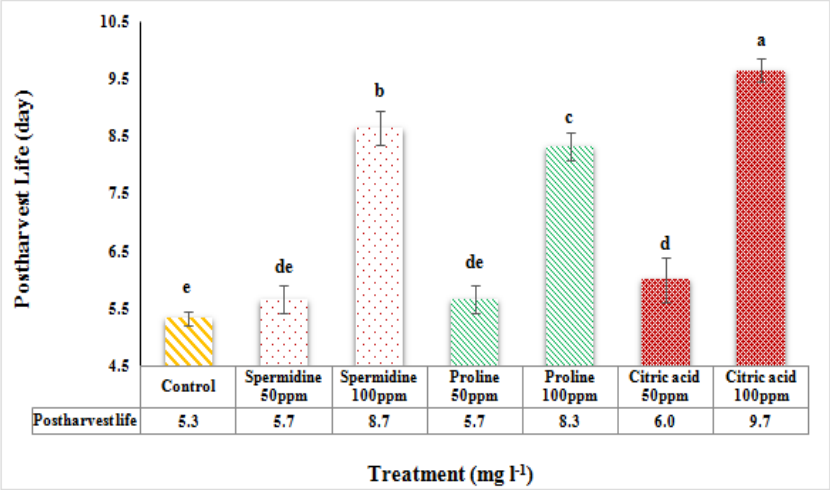

Figure 9. Effect of foliar application of spermidine, citric acid and proline on post-harvest life. Values are mean±SD. Values marked by different letters are significantly different (P< 0.05).

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chemical degradation of genes related to their biosynthetic path (Idrees *et al*., 2010). Kozminska *et al.* (2017) stated that salt stress in pot marigold reduces carotenoid content. However, the results of Jafarzadeh *et al*. (2013) showed that with increasing stress to relatively high stress levels, the content of photosynthetic pigments decreased, and increased again in severe stress. Proline application enhanced the biosynthesis and protection of photosynthetic pigments further, probably due to the effect of proline stimulation on photosynthetic pigments because of the stability of the active site of the enzymes (Ali *et al*., 2013). That is in agreement with the results reported by Gholami Zali and Ehsanzadeh (2018) about the application of proline on *Foeniculum vulgare* Mill.

In general, under drought stress and aging of petals, expression of genes encoding intracellular proteases is induced, causing protein degradation, nitrogen re-mobilization and subsequently synthesis of soluble matter. Hence, reducing the protein content under the above conditions will reduce the protein synthesis as well as the accumulation of free amino acids such as proline and increase the activity of the protein degrading enzymes (Naeemi *et al*., 2012). Increase in protein levels and then decrease during the post-harvest period can be due to an increase in amino acids caused by the breakdown of enzymes and a decrease in metabolic activity in the plant and, after that, the proteins in the plant begin to hydrolyze and reduce the amount of protein (Jalili Marandi, 2012). In this study, proline application increased protein. Proline can play a role in hydration of the layer of phospholipids, react with hydroxyl groups, thereby preventing the degradation of membrane proteins and phospholipids, maintaining membrane stability and increasing plant resistance to biological and non-biological stresses (Vendruscolo *et al*., 2007). The study results of Naghizadeh *et al*. (2019) on *Dracocephalum moldavica* L. and Farahbakhsh and Pasandipour (2017) on *Lawsonia inermis* L. were consistent with the above results.

Pandey *et al*. (2010) stated that the reduction in SOD and POD activities under severe stress indicates the ability to purify the plant cells, and since the measurement of enzyme activity is a result of synthesis and degradation, any reduction in enzyme activity under stress can be attributed to a reduction in synthesis or an increase in their degradation. Taiz and Zeiger (2006) stated that the level of response to drought depended on species, metabolic state, developmental stages, time, and intensity of stress. The plant storage at 4°C produces ROS such as superoxide radical. These radicals act as signals that activate SOD (Tajvar *et al*., 2011). SOD activity increases the amount of hydrogen peroxide, which is a secondary signal and activates peroxidases and other antioxidant enzymes (Tajvar *et al*., 2011). Citric acid, by acidifying the medium, provides optimal conditions for the activity of SOD and POD enzymes, thereby eliminating free radicals and enhancing the defensive system of a plant (Yoruk *et al*., 2005). It was consistent with the results of Tian *et al*. (2012) on *Tagetes erecta* L. and Abd Elbar *et al*. (2019) on *Thymus vulgaris* L.

The phenol content protects plants from cells dehydration by increasing osmotic potential and/or regulation of redox potential and removal of ROS under stress conditions (Redha *et al*., 2012). However, phenol reduction under severe stresses indicates that these conditions have a negative impact on plant antioxidant properties (Toberman *et al.*, 2008). The cause of a reduction in phenols during the post-harvest period may also be related to chemical and enzymatic changes such as oxidation of phenolic compounds by phenol oxidases, glycoside hydrolysis by glycosidases and polymerization of free phenolic compounds (Remorini *et al*., 2008). In this experiment, exogenous proline increased the amount of phenolic compounds, stimulating proline along with the pentose phosphate path to produce $NADH_2$ for anabolic paths,

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including phenolic path and antioxidant responses (Shetty and Wahlqvist, 2004). It was consistent with the study results of Gholami Zali and Ehsanzadeh (2018), *Foeniculum vulgare* Mill. and Gharibi *et al*. (2016) on *Achillea pachycephala* L.

Flavonoids, due to their antioxidant role, directly inhibit oxidative stress by inducing reductive reactions and indirectly by iron chelating (Seyoum *et al*., 2006). It is likely that with increasing drought stress and petal aging, stimulation of other antioxidant mechanisms of plants, flavonoids were reduced. Because, when the plant is exposed to stress, a large amount of ROS such as hydroxyl radical, superoxide anion and hydrogen peroxide are produced. Most plants activate enzymatic systems to remove these radicals, so, it can be concluded that flavonoids were applied before the enzyme system acts, but, with increasing stress, the enzymatic system was activated and flavonoids decrease slightly (Jubany-Mari *et al*., 2010). Also, spermidine increased flavonoids. Spermidine can stimulate the activity of antioxidant systems and increase resistance to stress in plants (Yiu *et al*., 2009). The results of this experiment were consistent with the results of Habibi (2018) on *Aloe vera* and Ali *et al*. (2007) on *Origanum majorana* L.

In this experiment, the increase in soluble carbohydrate under mild stress was due to its role in cell osmotic potential regulation and the reduction in soluble carbohydrate under severe stress may be due to the consumption of sugars for the synthesis of metabolites such as proline in the shoot (Sodaiezadeh *et al*., 2016). Generally, oxidative processes that occur in post-harvest decrease carbohydrates contents during senescence (Cavasini *et al.*, 2018). Proline application increased carbohydrate, as the plant did not need sugar to synthesize these compounds and, as a result, the sugar content increased in this treatment (Gholami Zali and Ehsanzadeh, 2018). The study results were consistent with those of Abd-Elhamid *et al*. (2016) on *Trigonella foenum-graecom* L. and Farhoudi *et al*. (2014) on *Matricaria recutita* L.

In this experiment, ascorbate reduction under severe stress could be due to direct degradation of ascorbate by O_2 or other ROS, as well as the use of ascorbate for the synthesis of Zeaxanthin and reproduction of alpha-tocopherol (Sharma *et al*., 2014). Also, ascorbic acid acts as a cofactor for ACC oxidase to produce ethylene. Therefore, its value declined with time and during storage (Spinardi, 2005). Citric acid increased vitamin C levels. Citric acid protects the cell membrane and cellular contents, including vitamin C, due to its antioxidant properties, as well as by reducing pH and acidifying the medium, it inhibits ACC synthetase activity, thereby preventing ethylene production and vitamin C reduction (Ruoyi *et al*., 2005). The study results were consistent with Sharma *et al*. (2014) on *Cucumis melo* L.

By reducing pH and acidifying the medium, citric acid inhibits the activity of synthase in the alkaline medium, thereby preventing ethylene production, reducing respiration and, finally, enhancing post-harvest life (Spinardi, 2005). It was consistent with the results of Eidyan *et al*. (2014) on *Polianthes tuberosa* L.

Based on the studies, it could be concluded that foliar application of spermidine, citric acid, and proline can be used as efficient compounds for increasing the quality and quantity of medicinal plants metabolites and can be used purposefully for increasing the efficiency of medicinal plants production.

In general, it can be stated that *Calendula officinalis* L. with a strong enzymatic system can remove free radicals and prolong life under drought stress as well as during post-harvest days. In this experiment, foliar application of 100 mg L^{-1} citric acid increased the activity of SOD, POD, vitamin C and postharvest life. Carotenoids, carbohydrates, and phenol increased with 100 mg L^{-1} proline. Fifty mg L^{-1} proline and 100 mg L^{-1} spermidine increased protein and flavonoid, respectively. As a result, in order

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to obtain the highest phytochemical properties as well as post-harvest life of this valuable plant with respect to the medicinal and nutritional value of *Calendula officinalis* L., irrigation at 75% FC with the foliar application at 100 mg L^{-1} concentrations of spermidine, citric acid and proline is recommended.

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پاسخ‌های متابولیکی و آنزیمی گیاه همیشه‌بهار(*Calendula officinalis* L.) به محلول‌پاشی اسپرمیدین، اسید سیتریک و پرولین، تحت تنش خشکی پس از برداشت

س. سروری، ا. دانائی، خ. همتی، و ع. ر. لادن مقدم

چکیده

این تحقیق در قالب دو آزمایش انجام گرفته است. آزمایش اول به منظور بررسی اثر محلول‌پاشی، اسپرمیدین، اسید سیتریک و پرولین (صفر، 50 و 100 mg/L) بر متابولیت‌ها و فعالیت‌های آنزیمی گیاه همیشه‌بهار (*Calendula officinalis* L.) تحت تنش خشکی (بدون تنش(شاهد)، 25، 50 و 75 درصد ظرفیت زراعی) و آزمایش دوم به منظور اثر محلول‌پاشی اسپرمیدین، اسید سیتریک و پرولین (صفر، 50 و 100 mg/L) بر ارزش تغذیه‌ای و عمر پس از برداشت گل‌ها (شروع آزمایش، 5 و 10 روز)، انجام شد. هر دو آزمایش بصورت فاکتوریل در قالب طرح آماری کاملاً تصادفی با 3 تکرار اجرا گردید. نتایج حاصل از این آزمایشات نشان داد، تمام تیمارها بر متغییرهای اندازه‌گیری شده اثر معنی‌دار داشتند. در آزمایش اول، تنش 25٪ ظرفیت زراعی، کلیه صفات به جز فعالیت آنزیم پلی‌فنل اکسیداز را کاهش داد، اما تنش 75٪ ظرفیت زراعی، موجب افزایش کارتنوئید، کربوهیدرات کل، فنل، فلاونوئید، پروتئین، فعالیت آنزیم‌های پراکسیداز و سوپراکسید دیسموتاز شد. بالاترین ویتامین ث در بین سطوح خشکی در شاهد مشاهده شد. همچنین محلول‌پاشی گل‌ها با پرولین 100 mg/L، میزان کارتنوئید، کربوهیدرات و فنل و پرولین 50 mg/L، میزان پروتئین را افزایش داد. اسپرمیدین 100 mg/L، میزان فلاونوئید و اسید سیتریک 100 mg/L، میزان ویتامین ث، فعالیت آنزیم‌های سوپراکسید دیسموتاز و پراکسیداز را افزایش داد. در آزمایش دوم، تمام صفات مورد ارزیابی، پس از ده روز از برداشت، کاهش یافتند، بیشترین عمر پس از برداشت در تیمار اسید سیتریک 100 mg/L (9/7 روز) و کمترین در تیمار شاهد (5/3روز) بود. نتایج این تحقیق نشان داد، کاربرد اسپرمیدین، اسید سیتریک و پرولین با غلظت 100 mg/L به همراه سطح آبیاری 75٪ ظرفیت زراعی موجب بهبود ویژگی‌های بیوشیمیایی و صفات تغذیه‌ای و عمر پس از برداشت همیشه‌بهار شد.

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